Non-glutamate Type Pyrrolo[2,3-*d*]pyrimidine Antifolates. II.[1] Synthesis and Antitumor Activity of N^5-Substituted Glutamine Analogs

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The glutamic acid moiety of *N*-[4-[3-(2,4-diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]benzoyl]-L-glutamic acid (1b, TNP-351) and related compounds was replaced with some N^5-substituted glutamines. Antifolates (4A—S) were effectively prepared by coupling pyrrolo[2,3-*d*]pyrimidine carboxylic acids (11a, b) with some properly protected N^5-substituted glutamine derivatives (10A—S), which were prepared by coupling Boc-Glu-OMe (7) with various amines (8A—S) using a suitable condensing reagent, followed by hydrolysis. The inhibitory effects of the resulting products on dihydrofolate reductase (DHFR), thymidylate synthetase (TS) and the growth of murine fibrosarcoma Meth A cells in culture were examined. All N^5-substituted glutamine analogs (4A—S) inhibited DHFR much more strongly than TNP-351 and some analogs exhibited the same potent growth inhibition of Meth A cells as TNP-351. Some typical analogs (4Bb, 4Db, 4F, 4Oa) were also examined for inhibitory effects on the growth of methotrexate (MTX)-resistant human CCRF-CEM cells in culture and for *in vivo* antitumor activities against murine leukemia and solid tumors. MTX-resistant cells, with a defect in transport and decreased polyglutamylation activity, showed little cross resistance to the analog (4Oa) having a tetrazole moiety as a substituent of glutamine, which exhibited potent antitumor activities. These results demonstrate that the antifolate analogs (4) with N^5-substituted glutamine in place of glutamic acid are novel potent DHFR inhibitors with activity against MTX-resistant tumors. The potent antitumor activity of these analogs (4) may result from their effective uptake *via* reduced folate carrier in combination with their potent inhibition of DHFR.

Key words TNP-351; pyrrolo[2,3-*d*]pyrimidine antifolate; N^5-substituted glutamine containing pyrrolo[2,3-*d*]pyrimidine antifolate; dihydrofolate reductase inhibition; antitumor activity; methotrexate-resistant tumor

The antifolate methotrexate (MTX) has been clinically used for treating acute lymphocytic leukemia and choriocarcinoma for more than 30 years. In view of the intracellular transport and polyglutamation of MTX and the inhibition of target enzymes by its polyglutamates, it had been thought that the glutamic acid moiety of MTX was essential to the action of MTX.[2] However, Rosowsky *et al.*[3] and Piper *et al.*[4] reported that some MTX derivatives (**3**) with glutamic acid γ-amide in place of glutamic acid were more efficiently transported into cells *via* the reduced folate carrier-mediated transport system (RFC) and more active against MTX-resistant tumor cells in culture than MTX. These findings suggested that modifications of the glutamic acid moiety might lead to novel antifolates.

We recently reported that *N*-{4-[3-(2,4-diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]benzoyl}-L-glutamic acid (**1b**, TNP-351), a novel dihydrofolate reductase (DHFR) inhibitor characterized by a pyrrolo[2,3-*d*]-pyrimidine ring, showed potent antitumor activities against solid tumor cells *in vitro*[5] and *in vivo*.[6] Biological evaluations of TNP-351 demonstrated that TNP-351 was more efficiently taken up into tumor cells and much more quickly converted to its polyglutamates than MTX,[6] and that the formed polyglutamates were potent inhibitors of aminoimidazolecarboxamide ribonucleotide transformylase,[7] in addition to DHFR.[8] As an extension of our research on TNP-351, we have made modifications of the glutamic acid moiety in TNP-351, as shown in Fig. 1. We previously reported the replacement of the α- or γ-carboxyl group of the glutamic acid in TNP-351 by a 5-substituted tetrazole ring, an isosteric group with acidic properties similar to those of a carboxyl group.[1] The replacement of the α-carboxyl group on the glutamic acid with a tetrazolyl group (**2a**) caused little change in the inhibitory activity of DHFR, but resulted in a decrease of growth-inhibitory activity against Meth A cells. On the other hand, the conversion of the γ-carboxyl group to a tetrazolyl group (**2b**) led to an increase of DHFR-inhibitory activity and also retained the potent growth inhibition against Meth A cells. These findings suggested that an α-carboxyl group plays an important role in transport *via* the RFC, and that the γ-carboxyl group moiety is an important for tight binding to DHFR. Therefore, we designed N^5-substituted glutamine analogs (**4**) of TNP-351 and the related compound (**1a**) as novel non-glutamate type antifolates, which were expected to be transported effectively into cells *via* the RFC due to existence of the α-carboxyl group and to be strong inhibitors of DHFR because of optimization of the γ-carboxyl group moiety of the glutamic acid residue.

In this paper, we describe the preparation of novel pyrrolo[2,3-*d*]pyrimidine antifolates **4** with N^5-substituted glutamine in place of glutamic acid, together with their inhibitory effects on DHFR, thymidylate synthetase (TS), the growth of murine Meth A cells and MTX-resistant human CCRF-CEM cells in culture and their *in vivo* antitumor activity against some murine tumors.

Chemistry

As illustrated in Chart 1, our synthetic method for N^5-substituted glutamine-containing antifolates (**4A—S**) consists of the condensation of properly protected N^5-substituted glutamines (**10A—S**) and pyrrolo[2,3-*d*]pyrimidine carboxylic acids (**11a, b**)[9] and subsequent remov-

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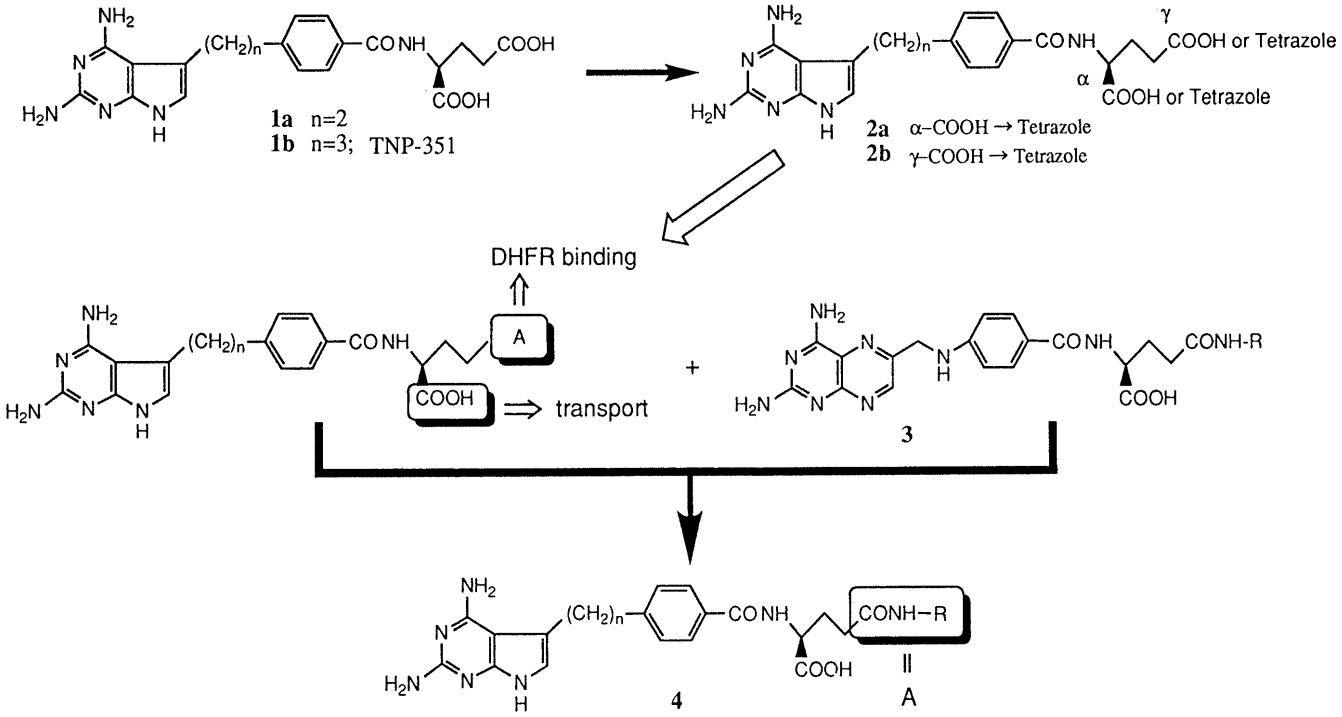


Fig. 1

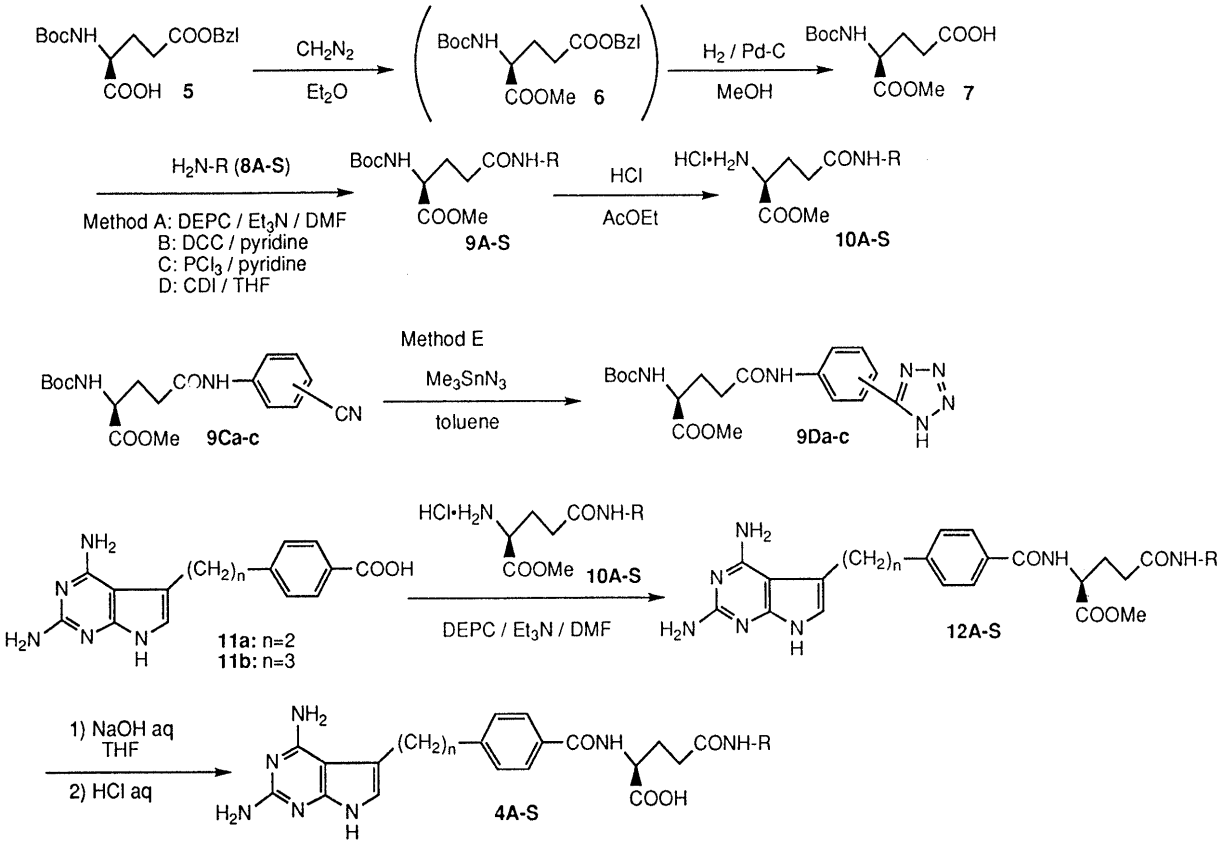
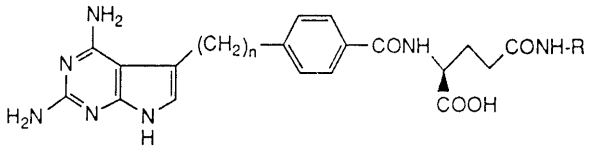


Chart 1

al of the protecting groups. Therefore, we needed an effective preparation of various N^5-substituted glutamine derivatives (**9**A—S). The glutamine derivatives (**9**A—S) were prepared using a coupling reaction of various amines (**8**A—S) and Boc–Glu–OMe (**7**), which was derived from commercially available Boc–Glu(OBzl) (**5**) by esterification with diazomethane followed by catalytic hydrogenation. In the coupling reaction, we used condensing reagents appropriate for the nucleophilicity of the amines (**8**A—S) used. In the case of highly reactive amines such as amino acids and *meta*-substituted anilines, we chose a diethyl phosphorocyanidate (DEPC) as the condensing reagent (method A). With decrease of the nucleophilicity of the amines, we used more powerful condensing reagents

Table 1. Preparation and *in Vitro* Activities of N^5-Substituted Glutamine-Containing Antifolates **4**



Compd.	*n*	R	mp (°C)	Formula	SIMS (MH^+)	IC_{50} (μM)		
						DHFR	TS	Meth A
4Aa	2	Ph-3-$B(OH)_2$	>270 (dec.)	$C_{26}H_{28}N_7O_6B\cdot 2.0H_2O$	545	0.014	>80	0.0014
4Ab	3	Ph-3-$B(OH)_2$	>270 (dec.)	$C_{27}H_{30}N_7O_6B\cdot 2.5H_2O$	559	0.22	>80	0.0049
4Ba	2	Ph-2-COOH	192—195	$C_{27}H_{27}N_7O_6\cdot 2.9H_2O$	546	0.0090	>80	0.0054
4Bb	2	Ph-3-COOH	205—206	$C_{27}H_{27}N_7O_6\cdot 3.1H_2O$	546	0.0082	43	0.00080
4Bc	3	Ph-3-COOH	191—193	$C_{28}H_{29}N_7O_6\cdot 2.5H_2O$	560	0.073	63	0.0068
4Bd	2	Ph-4-COOH	208—210	$C_{27}H_{27}N_7O_6\cdot 1.8H_2O$	546	0.061	33	0.0019
4Ca	2	Ph-2-CN	219—221	$C_{27}H_{26}N_8O_4\cdot 4.0H_2O$	527	0.018	60	0.021
4Cb	2	Ph-3-CN	187—188	$C_{27}H_{26}N_8O_4\cdot 3.0H_2O$	527	0.065	>80	0.0072
4Cc	2	Ph-4-CN	194—195	$C_{27}H_{26}N_8O_4\cdot 3.5H_2O$	527	0.018	>80	0.0025
4Da	2	Ph-2-Tet	207—209	$C_{27}H_{27}N_{11}O_4\cdot 1.85H_2O$	570	0.0087	35	0.0093
4Db	2	Ph-3-Tet	187—189	$C_{27}H_{28}N_{11}O_4$ [a]	570	0.016	>80	0.0010
4Dc	2	Ph-4-Tet	221—223	$C_{27}H_{28}N_{11}O_4$ [a]	570	0.012	>80	0.0045
4Ea	2	Ph-2-OH	182—183	$C_{26}H_{27}N_7O_5\cdot 2.6H_2O$	518	0.028	>80	0.0015
4Eb	2	Ph-3-OH	187—189	$C_{26}H_{27}N_7O_5\cdot 2.0H_2O$	518	0.0088	>80	0.0019
4Ec	2	Ph-4-OH	196—198	$C_{26}H_{27}N_7O_5\cdot 2.7H_2O$	518	0.016	>80	0.0016
4F	2	Ph-3-CH_2COOH	195—196	$C_{28}H_{29}N_7O_6\cdot 4.2H_2O$	560	0.0099	27	0.0010
4G	2	Ph-3-COOH-4-OH	222—224	$C_{27}H_{27}N_7O_7\cdot 2.6H_2O$	562	0.012	72	0.0031
4H	2	Ph-3-COGlu	218—220	$C_{32}H_{34}N_8O_9\cdot 3.0H_2O$	675	0.015	13	0.0028
4I	2	Ph-3-COOH-6-F	202—204	$C_{27}H_{26}N_7O_6F\cdot 3.0H_2O$	564	0.0065	18	0.0012
4J	2	Ph-3,4-OCH_2O–	185—186	$C_{27}H_{27}N_7O_6\cdot 1.8H_2O$	546	0.019	>80	0.0031
4K	2	Ph-4-SBzl	167—168	$C_{33}H_{33}N_7O_4S\cdot 2.0H_2O$	624	0.14	>80	0.29
4La	2	2-Napht-3-COOH	235—237	$C_{31}H_{28}N_7O_6\cdot 1.6H_2O$	596	0.021	75	0.031
4Lb	2	2-Napht-4-COOH	208—211	$C_{31}H_{29}N_7O_6\cdot 4.0H_2O$	596	0.0065	39	0.0020
4Lc	2	2-Napht-5-COOH	211—212	$C_{31}H_{29}N_7O_6\cdot 2.0H_2O$	596	0.0052	32	0.0018
4M	2	5-Benzotriazol	212—214	$C_{28}H_{28}N_8O_4\cdot 2.0H_2O$	541	0.010	>80	0.017
4Na	2	3-Py-5-COOH	214—216	$C_{26}H_{26}N_8O_6\cdot 3.0H_2O$	547	0.0070	19	0.0014
4Nb	2	2-Py-5-$COONH_4$	260—262	$C_{26}H_{29}N_9O_6\cdot 2.6H_2O$	nd	0.0091	18	0.0050
4Oa	2	Tet	207—209	$C_{21}H_{24}N_{11}O_4$ [a]	494	0.0087	31	0.00089
4Ob	3	Tet	197—198	$C_{22}H_{26}N_{11}O_4$ [a]	508	0.10	64	0.0014
4Pa	2	2-Thiazol-4-CH_2COOH	203—204	$C_{25}H_{26}N_8O_6S\cdot 2.4H_2O$	567	0.010	13	0.0010
4Pb	3	2-Thiazol-4-CH_2COOH	189—191	$C_{26}H_{28}N_8O_6S\cdot 2.0H_2O$	581	0.067	61	0.0081
4Q	2	Gly	188—190	$C_{22}H_{25}N_7O_6\cdot 2.7H_2O$	484	0.018	>80	0.0023
4R	2	*tert*-Leu	200—203	$C_{26}H_{33}N_7O_6\cdot 4.1H_2O$	540	0.011	>80	0.013
4Sa	2	Phe	180—181	$C_{29}H_{31}N_7O_6\cdot 2.5H_2O$	574	0.017	27	0.0051
4Sb	3	Phe	164—166	$C_{30}H_{33}N_7O_6\cdot 2.0H_2O$	588	0.26	48	0.0093
TNP-351						0.37	99	0.0006

Tet, 1*H*-tetrazol-5-yl; Napht, naphthyl; Py, Pyridyl; *a*) Exact MS (FAB), [MH^+]; nd, not determined.

such as dicyclohexylcarbodiimide (DCC) and 1,1-carbonyl diimidazole (CDI) (methods B and D, respectively). In the case of amines with very low nucleophilicity, such as anilines with an electron-withdrawing substituent at the 2 or 4 position, we utilized the phosphazo method with phosphorous trichloride (PCl_3) in pyridine, as reported by Ueki *et al.*[10] (method C). They reported that two molar equivalents of amine was necessary in order to obtain the products in good yield. We reexamined the reaction conditions and found that the addition of PCl_3 to a mixture of amine and carboxylic acid (1 : 1) in pyridine gave the condensed product in excellent yield. This reaction was thought to proceed *via in situ* generation of the acid chloride. We also prepared the tetrazole-containing compounds (**9D**) by 1,3-cycloaddition reaction of the corresponding nitriles (**9C**) to trimethyltin azide in toluene (method E).

The desired antifolates (**4**) were prepared by condensing the glutamine analogs (**10**) prepared above with pyrrolo[2,3-*d*]pyrimidine carboxylic acids (**11a, b**) in the presence of DEPC and Et_3N, followed by alkaline hydrolysis (Table 1).

Biological Activity and Discussion

Inhibitory activities of the prepared antifolates (**4A—S**) against DHFR, TS and murine fibrosarcoma Meth A cell growth were examined in comparison with those of TNP-351 (**1b**), a parent drug with a glutamic acid residue (Table 1).

All of the prepared N^5-substituted glutamine analogs (**4A—S**) were found to be much more potent DHFR inhibitors than TNP-351 (**1b**), and strongly inhibited the growth of Meth A cells. Although their TS-inhibitory activity was comparatively weak, we found a tendency for TS inhibition to be enhanced by converting a benzene ring as a substituent of glutamine to a naphthalene or a

Table 2. Inhibitory Effects of the N^5-Substituted Glutamine-Containing Antifolates on MTX-Resistant CCRF-CEM Cell Lines

Compound	IC_{50} (μM)			
	S (wild)	R1 (DHFR↑)	BO (transport↓)	30/6 (FPGS↓)
		Resistant (mutant/wild)		
4Bb	0.0040	0.10	0.10	0.0016
		25.0	25.0	0.40
4Db	0.0064	0.24	0.31	0.0025
		37.5	48.4	0.39
4F	0.0039	0.078	0.079	0.0010
		20.0	20.3	0.26
4Oa	0.0027	0.10	0.014	0.0011
		37.0	5.2	0.41
MTX	0.010	0.46	2.0	0.0096
		46.0	200.0	0.96

Table 3. *In Vivo* Antitumor Activity of N^5-Substituted Glutamine-Containing Antifolates

	Meth A		Colon 26		P388	
	Dose (mg/kg)	T/C (%)	Dose (mg/kg)	T/C (%)	Dose (mg/kg)	T/C (%)
4Aa	4	34	16	34[a]	4	37
			6	58[b]		
4Bb	8	25	16	27[a]	4	21
			6	58[b]		
4Db	4	29	8	62[a]	8	23
			6	67[b]		
4Oa	2	35	2	58[a]	2	10
			3	23[a]		
4Pa	8	48	16	19[a]	6	18
TNP-351	8	21	4	43[b]	4	33

a) CDF1 mice. *b*) BALB/c mice.

heteroaromatic ring such as pyridine, tetrazole or thiazole. In the case of pteridine antifolates such as MTX, it is reported that the replacement of the γ-carboxyl group with other groups such as tetrazole,[11] *tert*-butyl ester[12] and amides[3,4] does not greatly affect the inhibition of DHFR, whereas the conversion of the glutamate moiety of the pyrrolo[2,3-*d*]pyrimidine antifolates to a γ-tetrazole-containing glutamic acid[1] or N^5-substituted glutamine led to much more potent inhibition of DHFR. The difference in binding to DHFR between pteridine and pyrrolo[2,3-*d*]pyrimidine antifolates is very interesting. With regard to the length of the spacer between the pyrrolo[2,3-*d*]pyrimidine ring and the benzene ring, the ethylene analogs (**4Bb**, **4Oa**) inhibited DHFR and Meth A cell growth more potently than the trimethylene analogs (**4Bc**, **4Ob**). As for the substituent of the glutamine, an aromatic ring containing an acidic functional group (**4A**—**P**) was found to be better than an aliphatic chain, such as a dipeptide analog (**4Q**—**S**). Among **4A**—**P**, we found a tendency for antifolates with an acidic group such as carboxylic acid (**4B**, **F**—**I**, **L**, **N**, **P**), boric acid (**4A**), tetrazole (**4O**) or phenol (**4C**) on the substituent of the glutamine to exhibit strong inhibition of DHFR and more potent growth-inhibitory activity. These acidic functional groups may play some role in efficient transport *via* the RFC, an anion exchange transporter, and in binding to the polyglutamate-binding region of DHFR. In particular, the analogs (**4Oa**) having a tetrazole as a substituent of the glutamine showed the strongest inhibitory activities against Meth A cell growth *in vitro*.

Inhibitory effects of some typical compounds (**4Bb**, **4Db**, **4F**, **4Oa**) on the growth of MTX-sensitive and resistant CCRF-CEM cells in culture were examined in comparison with those of MTX in order to test whether the prepared antifolates were active against MTX-resistant tumor cells. The MTX-resistant cell lines used were CCRF-CEM R_1,[13] CCRF-CEM R_{BO}[14] and CCRF-CEM $R_{30/6}$,[15] whose resistances to MTX were attributed to an increase of DHFR level, a defective transport through the RFC and a decrease of polyglutamylation by FPGS, respectively. As shown in Table 2, the CCRF-CEM R_1 cells with an increase of DHFR level were found to show cross-resistance to the non-glutamate antifolates as well as MTX, while the CCRF-CEM R_{BO} cells with defective transport were generally much less resistant to them than to MTX, and the CCRF-CEM $R_{30/6}$ cells with decreased polyglutamylation were also sensitive. The tetrazole analog (**4Oa**) exhibited the most potent growth inhibition, and the CCRF-CEM R_{BO} cells were only weakly resistant (5-fold resistant) to **4Oa**. These findings are consistent with previous studies[16] showing that some non-glutamate derivatives of MTX such as PT523[3b,16] are effective against MTX-resistant cells with defective transport or with decreased polyglutamylation. The inhibition of DHFR is thought to be a primary mode of action of these compounds for the following reasons; 1) they have two amino groups on the pyrimidine ring[17] and strongly inhibit DHFR with IC_{50} values of 1—10 nM, whereas they are weak TS inhibitors with IC_{50} values of $>10\,\mu$M, 2) they can not be converted by FPGS to their polyglutamates, which could more potently inhibit other folate-requiring enzymes with increasing number of glutamate moieties[2] and 3) the DHFR-increased resistant cells were cross-resistant to them as well as MTX.

In vivo antitumor activities of the compounds (**4Bb**, **4Db**, **4Oa**, **4Pa**), which exhibited strong *in vitro* inhibition of Meth A cell growth, were examined using Meth A, Colon 26 and P388 transplanted mice, and the results are shown in Table 3. The non-glutamate antifolates (**4Bb**, **4Db**, **4Oa**, **4Pa**) exhibited strong antitumor activities against all tumors, tending to show more potent anti-leukemia activity than TNP-351. Among them, the tetrazole analog (**4Oa**) exhibited the highest potency. These findings are consistent with the potent DHFR inhibition and effective uptake through the RFC.

We previously suggested that it might be possible to obtain novel DHFR inhibitors by replacing the γ-carboxyl group of glutamic acid with other appropriate groups while leaving the α-carboxyl group intact. As described above, we have indeed found novel potent DHFR inhibitors by designing non-glutamate type antifolates (**4**) with an N^5-substituted glutamine analog in place of glutamic acid.

Experimental

Melting points were determined on a Yanagimoto micro melting

point apparatus without correction. IR spectra were obtained on a JASCO IR-810 spectrometer. ^{1}H-NMR spectra were recorded on a Varian Gemini-200 spectrometer; chemical shifts are given in ppm with tetramethylsilane as an internal standard, and coupling constants (J) were measured in hertz (Hz). Secondary ion mass spectra (SIMS) were determined on a Hitachi M-80B instrument. High-resolution mass spectra (HR-MS) and fast atom bombardment mass spectra (FAB-MS) were measured on a JEOL JMS-AX505W instrument. Column chromatography was carried out using silica gel 60 (E. Merck, Darmstadt, Germany).

***N*-(*tert*-Butyloxycarbonyl)-L-glutamic Acid α-Methyl Ester (Boc-Glu-OMe) (7)** A solution of CH_2N_2 in Et_2O was added to a stirred solution of *N*-(*tert*-butyloxycarbonyl)-L-glutamic acid γ-benzyl ester (**5**, 15.0 g) in Et_2O (30 ml) until a yellow color persisted. After decomposition of excess CH_2N_2 by adding AcOH, the mixture was concentrated *in vacuo*. The residue was dissolved in MeOH (50 ml) and hydrogenated over 10% Pd–C (5.0 g) at room temperature for 15 h. The catalyst was removed and the solution was concentrated *in vacuo* to give **7** (11.0 g, 95%) as a colorless syrup. ^{1}H-NMR ($CDCl_3$) δ: 1.45 (9H, s), 1.85—2.05 (1H, m), 2.08—2.30 (1H, m), 2.44 (2H, d, J=8.6), 2.48 (1H, dd, J=6.8, 3.0), 3.76 (3H, s), 4.36 (1H, m), 5.19 (1H, br d, J=8.2).

Synthesis of Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-substituted-L-glutaminate (9A—S). Method A: General Procedure Et_3N (17.5 mmol) was added to a stirred solution containing **7** (5.7 mmol), amine (**8**, 6.5 mmol), and DEPC (5.7 mmol) in dry *N*,*N*-dimethylformamide (DMF, 12 ml) and the mixture was stirred at room temperature for 2 h then concentrated *in vacuo*. The residue was chromatographed on silica gel using $CHCl_3$–MeOH (15 : 1) as the eluent to give **9**A, **9**Bb, **9**Ea—c, **9**G, **9**H, **9**J, **9**Q, **9**R, or **9**S in 35—100% yield.

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-[3-(dihydroxyboryl)phenyl]-L-glutaminate (9A) IR (neat): 3340, 2950, 1735, 1665, 1600, 1545, 1430, 1200, 1050, 1020 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (9H, s), 2.00 (1H, m), 2.25 (1H, m), 2.43 (2H, t, J=7.0), 3.72 (3H, s), 4.35 (1H, m), 5.48 (1H, m), 6.25 (1H, br s), 7.30 (1H, m), 7.40—7.65 (2H, m), 7.87 (1H, br s), 8.58 (1H, br s).

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-(3-ethoxycarbonylphenyl)-L-glutaminate (9Bb) IR (neat): 3300, 2980, 1745, 1720, 1600, 1530, 1450, 1405, 1390, 1365, 1305, 1270, 1105, 1050, 1020 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.38 (3H, t, J=7.2), 1.46 (9H, s), 1.85—2.10 (1H, m), 2.20—2.40 (1H, m), 2.48 (2H, m), 3.74 (3H, s), 4.37 (3H, q, J=7.2), 5.41 (1H, br d, J=7.6), 7.39 (1H, t, J=7.8), 7.78 (1H, d, J=7.8), 7.95 (1H, br d, J=7.8), 8.12 (1H, br s), 8.84 (1H, br s).

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-(2-hydroxyphenyl)-L-glutaminate (9Ea) IR (neat): 3325, 2980, 2930, 2850, 1740, 1710, 1655, 1600, 1525, 1500, 1455, 1395, 1365, 1310, 1280, 1245, 1220, 1165, 1100, 1050, 1030, 850, 750 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.48 (9H, s), 1.91 (1H, m), 2.35 (1H, m), 2.56 (2H, t, J=5.4), 3.76 (3H, s), 4.39 (1H, m), 5.45 (1H, d, J=8.2), 6.86 (1H, dt, J=7.0, 1.6), 7.00—7.20 (3H, m), 9.22 (1H, s), 9.29 (1H, s).

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-(3-hydroxyphenyl)-L-glutaminate (9Eb) IR (neat): 3325, 2980, 2930, 2850, 1740, 1710, 1655, 1605, 1535, 1510, 1445, 1395, 1245, 1220, 1165, 1100, 1050, 1030, 850 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.38 (9H, s), 1.40—2.20 (2H, m), 2.38 (2H, t, J=7.4), 3.63 (3H, s), 3.98 (1H, m), 6.41 (1H, d, J=6.6), 6.93 (1H, d, J=6.6), 7.04 (1H, t, J=6.6), 9.22 (1H, s), 7.17 (1H, s), 7.29 (1H, d, J=8.2), 9.33 (1H, s), 9.76 (1H, s).

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-(4-hydroxyphenyl)-L-glutaminate (9Ec) IR (neat): 3400, 3250, 3040, 2950, 2925, 2600, 1745, 1655, 1605, 1550, 1515, 1440, 1240, 835 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.12 (2H, m), 2.63 (2H, dd, J=7.0, 6.0), 3.73 (3H, s), 4.08 (1H, m), 7.76 (2H, d, J=9.2), 7.82 (2H, d, J=9.2), 8.61 (3H, br s), 10.71 (1H, s).

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-(3-ethoxycarbonyl-4-hydroxyphenyl)-L-glutaminate (9G) IR (neat): 3300, 2980, 1745, 1720, 1680, 1510, 1490, 1370, 1290, 1215, 1165, 1085, 1030 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.40 (3H, t, J=7.0), 1.47 (9H, s), 1.80—2.05 (1H, m), 2.20—2.50 (3H, m), 3.74 (3H, s), 4.35 (1H, m), 4.40 (2H, q, J=7.0), 5.40 (1H, br d, J=7.8), 6.94 (1H, d, J=8.8), 7.62 (1H, dd, J=8.8, 2.6), 8.11 (1H, d, J=2.6), 8.61 (1H, br s), 10.69 (1H, s).

Diethyl *N*-[3-(N^2-*tert*-Butyloxycarbonyl-O^1-methyl-γ-L-glutamylamino)benzoyl]-L-glutamate (9H) IR (neat): 3320, 2975, 2925, 1740, 1710, 1630, 1620, 1600, 1530, 1450, 1390, 1365, 1270, 1100, 1060, 1040 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.22 (3H, t, J=7.0), 1.33 (3H, t, J=7.0), 1.45 (9H, s), 2.00—2.40 (4H, m), 2.46 (4H, m), 3.74 (3H, s), 4.12 (2H, q, J=7.0), 4.23 (2H, q, J=7.0), 4.37 (1H, m), 4.78 (1H, m), 5.40 (1H, d, J=7.8), 7.06 (1H, m), 7.39 (1H, t, J=7.6), 7.53 (1H, d, J=7.0), 7.88 (1H, d, J=7.6), 7.96 (1H, br s), 8.81 (1H, br).

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-(3,4-methylenedioxyphenyl)-L-glutaminate (9J) IR (neat): 3320, 2975, 1740, 1710, 1690, 1505, 1490, 1450, 1370, 1240, 1210, 1160, 1040 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s), 1.80—2.00 (1H, m), 2.20—2.45 (3H, m), 3.74 (3H, s), 4.35 (1H, m), 5.36 (1H, br d, J=7.6), 5.94 (2H, s), 6.74 (1H, d, J=8.4), 6.90 (1H, dd, J=8.4, 2.0), 7.29 (1H, d, J=2.0), 8.44 (1H, br s).

Methyl *N*-(N^2-*tert*-Butyloxycarbonyl-O^1-methyl-γ-L-glutamyl)glycinate (9Q) IR (neat): 3320, 2975, 2925, 1740, 1710, 1620, 1530, 1450, 1390, 1365, 1270, 1105, 1050, 1020 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.89 (3H, t, J=7.0), 1.38 (9H, s), 1.64—2.05 (2H, m), 2.21 (2H, t, J=7.4), 3.63 (3H, s), 3.79 (2H, d, J=6.0), 3.93 (2H, m), 4.08 (2H, q, J=7.0), 4.10 (1H, m), 7.28 (1H, d, J=7.2), 8.27 (1H, t, J=6.0).

Methyl *N*-(N^2-*tert*-Butyloxycarbonyl-O^1-methyl-γ-L-glutamyl)-L-*tert*-leucinate (9R) IR (neat): 3330, 2975, 2925, 1740, 1710, 1625, 1535, 1450, 1390, 1365, 1270, 1100, 1050, 1020 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.92 (9H, s), 1.38 (9H, s), 1.72 (1H, m), 1.89 (1H, m), 2.28 (2H, m), 3.62 (6H, s), 3.96 (1H, m), 4.17 (1H, d, J=8.6), 7.22 (1H, d, J=7.8), 8.04 (1H, d, J=8.6).

Methyl *N*-(N^2-*tert*-Butyloxycarbonyl-O^1-methyl-γ-L-glutamyl)-L-phenylalaninate (9S) IR (neat): 3330, 2975, 2925, 1740, 1710, 1620, 1600, 1530, 1450, 1390, 1360, 1305, 1270, 1105, 1050, 1020 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.44 (9H, s), 1.80—2.00 (2H, m), 2.10—2.35 (3H, m), 3.13 (2H, m), 3.73 (6H, s), 4.20 (1H, m), 4.88 (1H, m), 5.22 (1H, br d, J=7.4), 6.31 (1H, br d, J=6.2), 7.09—7.35 (5H, m).

Method B: General Procedure A solution containing **7** (4.0 mmol), the amine (**8**, 4.0 mmol), and DCC (4.08 mmol) in dry pyridine (10 ml) was stirred for 16 h at 120 °C and the mixture was concentrated *in vacuo*. The residue was chromatographed on silica gel using AcOEt–*n*-hexane (1 : 3) as the eluent to give **9**Ba, **9**Bc, or **9**Cb in 58—84% yield.

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-(4-ethoxycarbonylphenyl)-L-glutaminate (9Bc) IR (neat): 3330, 2975, 2925, 1740, 1710, 1600, 1530, 1450, 1405, 1390, 1365, 1305, 1270, 1105, 1050, 1020, 855, 770, 695, 660 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.39 (3H, t, J=7.2), 1.47 (9H, s), 1.92 (1H, m), 2.33 (1H, m), 2.48 (2H, t, J=5.6), 3.75 (3H, s), 4.37 (1H, m), 4.37 (2H, q, J=7.2), 5.40 (1H, d, J=6.8), 7.69 (2H, d, J=8.8), 8.02 (2H, d, J=8.8), 9.04 (1H, s).

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-(2-methoxycarbonylphenyl)-L-glutaminate (9Ba) IR (neat): 3305, 2970, 2950, 2925, 1745, 1700, 1600, 1590, 1525, 1450, 1430, 1390, 1360, 1310, 1295, 1260, 1160, 1080, 1050, 1025, 960, 860, 755, 700 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.42 (9H, s), 2.00—2.45 (2H, m), 2.56 (2H, dt, J=7.8, 3.4), 3.75 (3H, s), 3.93 (3H, s), 4.37 (1H, m), 5.23 (1H, d, J=7.6), 7.08 (1H, dd, J=8.2, 7.4), 7.54 (1H, dd, J=8.2, 7.8), 8.03 (1H, d, J=7.8), 8.69 (1H, d, J=7.4), 11.09 (1H, s).

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-(3-cyanophenyl)-L-glutaminate (9Cb) IR (neat): 3330, 2990, 2940, 2855, 2240, 1740, 1700, 1650, 1605, 1590, 1545, 1485, 1450, 1430, 1395, 1365, 1305, 1285, 1255, 1210, 1165, 1055, 1025, 890, 860, 795, 755 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.49 (9H, s), 1.10—2.20 (2H, m), 2.20—2.60 (2H, m), 3.76 (3H, s), 4.36 (1H, m), 5.45 (1H, d, J=8.0), 7.39 (1H, dt, J=9.0, 1.8), 7.43 (1H, t, J=9.0), 7.83 (1H, dt, J=9.0, 1.8), 8.04 (1H, s), 9.33 (1H, br s).

Method C: General Procedure i) An amine (**8**, 4.0 mmol) was added to a stirred solution of PCl_3 (2.0 mmol) in dry pyridine (2 ml) and the mixture was stirred for 30 min at room temperature, then a solution of **7** (4.0 mmol) in dry pyridine (4 ml) was added. The reaction mixture was stirred for 3 h at 45 °C and concentrated *in vacuo*. The residue was dissolved in $CHCl_3$ (20 ml) and the solution was successively washed with 2% aqueous AcOH, saturated aqueous $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by recrystallization or silica gel chromatography to give **9**.

ii) PCl_3 (2.0 mmol) was added dropwise to a stirred solution of the amine (**8**, 4.0 mmol) and **7** (4.0 mmol) in dry pyridine (4 ml) at 0 °C under an argon atmosphere and the mixture was stirred at room temperature for 1 h. After removal of the solvent, the residue was dissolved in AcOEt (20 ml) and the solution was successively washed with 2% aqueous AcOH, saturated aqueous $NaHCO_3$ solution and brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by recrystallization or silica gel chromatography to give **9**Ca, c, **9**F, **9**I, **9**K, **9**La—c, **9**Na, b, or **9**P in 64—96% yield.

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-(2-cyanophenyl)-L-gluta-

minate (9Ca) IR (KBr): 3380, 3280, 3230, 2980, 2930, 2220, 1740, 1715, 1700, 1600, 1580, 1525, 1475, 1445, 1435, 1400, 1390, 1365, 1355, 1290, 1250, 1220, 1210, 1160, 1090, 1050, 1025, 980, 870, 780, 755, 680, 610, 560, 550, 495 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.45 (9H, s), 2.03 (1H, m), 2.32 (1H, m), 2.57 (2H, dt, J=8.0, 2.4), 3.78 (3H, s), 4.41 (1H, m), 5.29 (1H, d, J=8.0), 7.19 (1H, t, J=7.8), 7.60 (2H, m), 8.22 (1H, br s), 8.35 (1H, d, J=8.8).

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-(4-cyanophenyl)-L-glutaminate (9Cc) Without further purification, compound **9**Cc was treated with a 4 N solution of HCl in AcOEt to give **10**Cc.

Methyl N^2-(4-Cyanophenyl)-L-glutaminate Hydrochloride (10Cc) IR (KBr): 3420, 3240, 3170, 3090, 2925, 2850, 2220, 1745, 1670, 1595, 1530, 1445, 1405, 1380, 1310, 1250, 1175, 840, 550 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.10 (2H, m), 2.46 (2H, t, J=7.0), 3.74 (3H, s), 4.06 (1H, m), 6.61 (2H, d, J=8.8), 7.36 (2H, d, J=8.8), 8.59 (3H, br s), 9.87 (1H, br s).

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-(3-methoxycarbonylmethylphenyl)-L-glutaminate (9F) IR (KBr): 3330, 2980, 2950, 2940, 1740, 1720, 1700, 1610, 1600, 1550, 1520, 1495, 1440, 1365, 1300, 1255, 1210, 1160, 950, 920, 895, 860, 780, 720, 690 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s), 1.95 (1H, m), 2.27 (1H, m), 2.42 (2H, t, J=6.6), 3.62 (2H, s), 3.70 (3H, s), 3.75 (3H, s), 4.37 (1H, m), 5.37 (1H, d, J=7.2), 7.03 (1H, d, J=7.2), 7.27 (1H, t, J=7.2), 7.48 (1H, d, J=7.2), 7.56 (1H, s), 8.52 (1H, s).

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-(6-fluoro-3-methoxycarbonylphenyl)-L-glutaminate (9I) IR (KBr): 3300, 2995, 2950, 1750, 1730, 1690, 1675, 1615, 1530, 1485, 1445, 1425, 1390, 1370, 1325, 1300, 1290, 1245, 1225, 1200, 1170, 1120, 1065, 1000, 990, 780, 760, 670, 630 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.38 (9H, s), 1.70—2.15 (2H, m), 2.50 (2H, m), 3.64 (3H, s), 3.85 (3H, s), 4.03 (1H, m), 7.26 (1H, d, J=8.0), 7.38 (1H, dd, J=10.6, 8.6), 7.73 (1H, ddd, J=8.0, 4.8, 2.2), 8.60 (1H, dd, J=7.8, 2.2), 9.88 (1H, s).

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-(4-benzylthiophenyl)-L-glutaminate (9K) IR (KBr): 3360, 3270, 2980, 2950, 1735, 1690, 1655, 1585, 1520, 1495, 1450, 1435, 1395, 1370, 1340, 1310, 1280, 1250, 1220, 1160, 1090, 1030, 1005, 810, 780, 715, 690 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.38 (9H, s), 1.65—2.15 (2H, m), 2.39 (2H, t, J=7.4), 3.63 (3H, s), 4.01 (1H, m), 4.14 (2H, s), 7.15—7.35 (8H, m), 7.50 (2H, d, J=8.8), 9.90 (1H, s).

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-(3-methoxycarbonyl-2-naphthyl)-L-glutaminate (9La) IR (KBr): 3375, 3300, 2980, 2950, 1750, 1700, 1660, 1630, 1580, 1550, 1515, 1485, 1450, 1430, 1390, 1370, 1355, 1340, 1290, 1210, 1160, 1130, 1075, 1050, 1030, 1000, 890, 780, 750, 480 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.39 (9H, s), 1.80—2.25 (2H, m), 2.45 (2H, t, J=7.4), 3.66 (3H, s), 3.92 (3H, s), 4.05 (1H, m), 7.35 (1H, d, J=7.6), 7.51 (1H, t, J=8.2), 7.63 (1H, t, J=8.2), 7.90 (1H, d, J=8.2), 8.04 (1H, d, J=8.2), 8.61 (1H, s), 8.66 (1H, s), 10.57 (1H, s).

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-(4-methoxycarbonyl-2-naphthyl)-L-glutaminate (9Lb) IR (KBr): 3330, 2975, 2950, 1745, 1720, 1690, 1620, 1600, 1585, 1550, 1500, 1455, 1435, 1420, 1390, 1365, 1300, 1240, 1200, 1160, 1150, 1130, 1050, 1020, 790, 750 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.38 (9H, s), 1.70—2.20 (2H, m), 2.49 (2H, t, J=8.0), 3.65 (3H, s), 3.95 (3H, s), 4.05 (1H, m), 7.29 (1H, d, J=7.8), 7.54 (2H, m), 7.91 (1H, m), 8.30 (1H, d, J=2.0), 8.51 (1H, d, J=2.0), 8.67 (1H, s), 10.30 (1H, s).

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-(5-methoxycarbonyl-2-naphthyl)-L-glutaminate (9Lc) IR (KBr): 3350, 2970, 2950, 2920, 2850, 1740, 1715, 1625, 1600, 1580, 1545, 1500, 1465, 1455, 1435, 1365, 1270, 1250, 1200, 1160, 1130, 1070, 1050, 1020 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.48 (9H, s), 2.00 (1H, m), 2.35 (1H, m), 2.53 (2H, t, J=5.8), 3.75 (3H, s), 4.00 (3H, s), 4.42 (1H, m), 5.40 (1H, d, J=7.0), 7.47 (1H, t, J=8.4), 7.54 (1H, dd, J=7.0, 2.2), 7.99 (1H, dt, J=8.4, 1.4), 8.11 (1H, dd, J=7.4, 1.4), 8.48 (1H, s), 8.89 (1H, d, J=9.6).

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-(5-methoxycarbonyl-3-pyridyl)-L-glutaminate (9Na) IR (KBr): 3330, 2980, 2950, 1730, 1585, 1540, 1455, 1420, 1390, 1365, 1305, 1250, 1225, 1165, 1110, 1050, 1020, 1000, 765, 700 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.38 (9H, s), 1.75—2.20 (2H, m), 2.39 (2H, t, J=7.4), 3.64 (3H, s), 3.89 (3H, s), 4.04 (1H, m), 7.29 (1H, d, J=8.0), 8.63 (1H, t, J=2.0), 8.75 (1H, d, J=2.0), 8.88 (1H, d, J=2.0), 10.36 (1H, s).

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-(5-methoxycarbonyl-2-pyridyl)-L-glutaminate (9Nb) IR (KBr): 3340, 3260, 3230, 3150, 3030, 3000, 2980, 2970, 1745, 1720, 1695, 1585, 1540, 1455, 1440, 1420, 1390, 1370, 1295, 1245, 1210, 1180, 1160, 1120, 1080, 780 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.38 (9H, s), 1.70—2.20 (2H, m), 2.56 (2H, t, J=7.0), 3.63 (3H, s), 3.86 (3H, s), 4.01 (1H, m), 7.26 (1H, d, J=7.6), 8.19 (1H, d, J=8.7), 8.28 (1H, dd, J=8.7, 2.2), 8.83 (1H, d, J=2.2), 10.87 (1H, s).

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-[4-(methoxycarbonylmethyl)-thiazol-5-yl]-L-glutaminate (9P) IR (KBr): 3270, 2980, 2950, 1740, 1710, 1690, 1550, 1435, 1390, 1365, 1350, 1275, 1210, 1160, 1050, 1020, 955, 850, 750 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.38 (9H, s), 1.70—2.20 (2H, m), 2.46 (2H, t, J=7.2), 3.61 (3H, s), 3.63 (3H, s), 3.69 (2H, s), 3.99 (1H, m), 5.29 (1H, d, J=8.0), 6.96 (1H, s), 7.27 (1H, s).

Method D: General Procedure A solution containing **7** (4.0 mmol) and CDI (4.4 mmol) in dry tetrahydrofuran (THF) (30 ml) was stirred at 60 °C until generation of CO_2 gas ceased, and then Et_3N (4.5 mmol) and amine (**8**, 4.0 mmol) were added. The reaction mixture was refluxed for 5 h and concentrated *in vacuo*. A 10% aqueous solution of citric acid was added to the residue and the resulting precipitate was collected by filtration, washed with water and dried *in vacuo* to give **9**O or **9**M in 96 or 74% yield, respectively.

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-(1*H*-tetrazol-5-yl)-L-glutaminate (9O) IR (KBr): 3340, 3260, 3220, 2980, 1740, 1695, 1680, 1620, 1590, 1530, 1370, 1240, 1210, 1165 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.37 (9H, s), 1.50—2.20 (4H, m), 3.62 (3H, s), 4.00 (1H, m), 7.29 (1H, d, J=7.6), 11.92 (1H, br s).

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-(1*H*-benzotriazol-5-yl)-L-glutaminate (9M) IR (KBr): 3320, 2975, 1740, 1710, 1690, 1630, 1600, 1550, 1510, 1500, 1450, 1390, 1365, 1250, 1220, 1160, 1055, 750 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.48 (9H, s), 1.90—2.15 (1H, m), 2.30—2.50 (1H, m), 2.62 (2H, t, J=6.4), 3.74 (3H, s), 4.42 (1H, m), 5.54 (1H, br d, J=6.8), 7.27 (1H, dd, J=9.0, 1.6), 7.91 (1H, d, J=9.0), 8.62 (1H, br s), 9.49 (1H, br s).

Method E: General Procedure A solution containing methyl N^2-(*tert*-butyloxycarbonyl)-N^5-cyanophenyl-L-glutaminate (**9**C, 3.0 mmol) and Me_3SnN_3 (5.0 mmol) in toluene (10 ml) was stirred for 24 h at 85 °C and concentrated *in vacuo*. The residue was dissolved in $CHCl_3$ (50 ml) and the solution was successively washed with 1 N HCl and brine, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel using $CHCl_3$–MeOH–H_2O (65 : 25 : 4) as the eluent to give **9**Da—c in 62—91% yield.

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-[2-(1*H*-tetrazol-5-yl)phenyl]-L-glutaminate (9Da) IR (KBr): 3370, 3130, 3080, 2975, 2940, 1740, 1680, 1620, 1590, 1545, 1520, 1485, 1440, 1410, 1390, 1365, 1330, 1300, 1280, 1250, 1210, 1160, 1060, 995, 750 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 1.42 (9H, s), 2.05—2.50 (2H, m), 2.64 (2H, t, J=7.2), 3.77 (3H, s), 4.33 (1H, t, J=5.4), 5.78 (1H, d, J=7.0), 7.23 (1H, dt, J=7.6, 1.0), 7.52 (1H, dt, J=7.6, 1.0), 7.90 (1H, d, J=7.6), 8.63 (1H, d, J=7.6), 11.12 (1H, s). SIMS m/z: 405 (MH^+).

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-[3-(1*H*-tetrazol-5-yl)phenyl]-L-glutaminate (9Db) IR (KBr): 3240, 2970, 2920, 2860, 2775, 2740, 2630, 1740, 1705, 1675, 1610, 1570, 1520, 1485, 1440, 1385, 1360, 1345, 1305, 1270, 1240, 1200, 1155, 1055, 1020, 860, 790, 750, 740, 680 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.38 (9H, s), 1.65—2.20 (2H, m), 2.46 (2H, t, J=7.4), 3.65 (3H, s), 4.03 (1H, m), 7.31 (1H, d, J=8.0), 7.51 (1H, t, J=8.0), 7.68 (1H, d, J=8.0), 7.70 (1H, t, J=8.0), 8.41 (1H, s), 10.18 (1H, s).

Methyl N^2-(*tert*-Butyloxycarbonyl)-N^5-[4-(1*H*-tetrazol-5-yl)phenyl]-L-glutaminate (9Dc) IR (KBr): 3350, 3300, 3020, 2980, 2950, 2860, 2770, 1730, 1680, 1620, 1600, 1525, 1430, 1395, 1365, 1345, 1320, 1300, 1285, 1260, 1225, 1165, 1055, 1010, 880, 850, 755, 700, 530 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.43 (9H, s), 2.06 (1H, m), 2.22 (1H, m), 2.50 (2H, t, J=7.4), 3.76 (3H, s), 4.31 (1H, m), 5.99 (1H, d, J=7.2), 7.75 (2H, d, J=8.4), 7.96 (2H, d, J=8.4), 9.81 (1H, s).

Methyl N^2-[4-[3-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]benzoyl]-N^5-[3-(dihydroxyboryl)phenyl]-L-glutaminate (12Ab). Typical Procedure A 4 N HCl solution of AcOEt (5 ml) was added to a solution of **9**A (867 mg) in CH_2Cl_2 (2 ml), and the mixture was stirred at room temperature for 10 min and then concentrated *in vacuo* to give crude methyl N^5-[3-(dihydroxyboryl)phenyl]-L-glutaminate hydrochloride (**10**A, 720 mg). Et_3N (910 mg) was added dropwise to a stirred solution of 4-[3-(2,4-diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]-benzoic acid[9] (**11**b, 700 mg), **10**A, and DEPC (560 mg) in dry DMF (15 ml) under ice cooling. The reaction mixture was stirred at room temperature for 2 h, then concentrated *in vacuo*, and the residue was dissolved in $CHCl_3$. The $CHCl_3$ solution was successively washed with H_2O and brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was chromatographed on silica gel using $CHCl_3$–MeOH (10 : 1) as the eluent to give **12**Ab (1.03 g, 80%) as a colorless solid. IR (KBr): 3340, 2950, 1735, 1665, 1645, 1610, 1545, 1430, 1200, 720 cm^{-1}.

^{1}H-NMR (DMSO-d_6) δ: 1.75—2.30 (4H, m), 2.46 (2H, m), 2.50 (2H, m), 2.71 (2H, m), 3.67 (3H, s), 4.47 (1H, m), 5.39 (2H, br s), 5.96 (2H, br s), 6.43 (1H, s), 7.24 (1H, t, J=8.2), 7.31 (2H, d, J=8.2), 7.45 (1H, d, J=6.2), 7.69 (1H, d, J=7.4), 7.81 (1H, s), 7.83 (2H, d, J=8.2), 7.98 (2H, s), 8.71 (1H, d, J=7.4), 9.85 (1H, d, J=4.4), 10.41 (1H, br s). SIMS m/z: 573 (MH^+).

The following compounds **12**A—S were similarly prepared by removal of the Boc group of 9A—S (1.1 mmol) and subsequent condensation of pyrrolo[2,3-*d*]pyrimidinecarboxylic acid[9] (**11**a, b, 1 mmol) and methyl N^5-substituted glutaminate hydrochloride (**10**A—S) using DEPC (1.5 mmol) and Et_3N (4 mmol).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-[3-(dihydroxyboryl)phenyl]-L-glutaminate (12Aa) IR (KBr): 3335, 2950, 1735, 1665, 1645, 1605, 1545, 1430, 1200, 735 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 2.10—2.45 (2H, m), 2.56 (2H, m), 3.03 (4H, m), 3.75 (3H, s), 4.66 (1H, dd, J=9.0, 5.0 Hz), 6.46 (1H, s), 7.20—7.30 (3H, m), 7.38 (1H, m), 7.53 (1H, m), 7.73 (1H, br s), 7.75 (2H, d, J=8.2). SIMS m/z: 559 (MH^+).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(2-methoxycarbonylphenyl)-L-glutaminate (12Ba) IR (KBr): 3300, 3200, 2980, 2950, 2670, 2570, 1740, 1680, 1650, 1605, 1590, 1525, 1500, 1445, 1435, 1310, 1295, 1260, 1200, 1175, 1130, 1085, 830, 800, 760 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.38 (2H, m), 2.64 (2H, m), 2.90 (4H, m), 3.75 (3H, s), 3.88 (3H, s), 4.80 (1H, m), 6.38 (1H, s), 7.07 (2H, d, J=8.0), 7.07 (1H, t, J=8.0), 7.50 (1H, t, J=8.0), 7.65 (2H, d, J=8.0), 7.67 (1H, d, J=8.0), 7.97 (1H, dd, J=1.2, 8.0), 8.62 (1H, d, J=7.8), 11.14 (1H, br s). SIMS m/z: 574 (MH^+).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(3-ethoxycarbonylphenyl)-L-glutaminate (12Bb) IR (KBr): 3470, 3380, 3200, 2990, 2960, 2940, 2850, 1720, 1610, 1580, 1555, 1490, 1435, 1370, 1290, 1220, 1175, 1105, 1085, 1020, 760 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.32 (3H, t, J=7.2), 1.80—2.37 (2H, m), 2.46 (2H, m), 2.96 (4H, m), 3.66 (3H, s), 4.30 (2H, q, J=7.2), 4.49 (1H, m), 5.37 (2H, br s), 5.99 (2H, br s), 6.35 (1H, d, J=2.0), 7.33 (2H, d, J=8.2), 7.43 (1H, t, J=8.0), 7.62 (1H, dt, J=8.0, 1.4), 7.81 (2H, d, J=8.2), 7.83 (1H, dd, J=8.0, 1.4), 8.23 (1H, t, J=1.4), 8.71 (1H, d, J=7.2), 10.16 (1H, s), 10.35 (1H, d, J=2.0). SIMS m/z: 588 (MH^+).

Methyl N^2-[4-[3-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]benzoyl]-N^5-(3-ethoxycarbonylphenyl)-L-glutaminate (12Bc) IR (KBr): 3350, 2930, 1730, 1715, 1610, 1550, 1490, 1430, 1290 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 1.40 (3H, t, J=7.0), 1.90—2.10 (2H, m), 2.15—2.50 (2H, m), 2.58 (2H, m), 2.75 (2H, m), 3.78 (3H, s), 4.36 (2H, q, J=7.0), 4.41 (1H, m), 6.53 (1H, s), 7.25 (2H, d, J=8.2), 7.37 (1H, t, J=7.8), 7.70—7.85 (4H, m), 8.18 (1H, br s). SIMS m/z: 602 (MH^+).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(4-ethoxycarbonylphenyl)-L-glutaminate (12Bd) IR (KBr): 3375, 3180, 2975, 2920, 2850, 1735, 1700, 1605, 1575, 1535, 1500, 1420, 1405, 1365, 1305, 1275, 1250, 1215, 1175, 1105, 1020, 855, 770, 750 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.31 (3H, t, J=7.0), 1.80—2.35 (2H, m), 2.50 (2H, t, J=5.6), 2.97 (4H, m), 3.66 (3H, s), 4.28 (2H, q, J=7.0), 4.48 (1H, m), 5.37 (2H, br s), 5.99 (2H, br s), 6.36 (1H, s), 7.34 (2H, d, J=8.2), 7.71 (2H, d, J=8.8), 7.81 (2H, d, J=8.2), 7.90 (2H, d, J=8.8), 8.72 (1H, d, J=7.6), 10.28 (1H, s), 10.37 (1H, br s). SIMS m/z: 588 (MH^+).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(2-cyanophenyl)-L-glutaminate (12Ca) IR (KBr): 3420, 2930, 2850, 2330, 1740, 1680, 1610, 1575, 1545, 1500, 1470, 1430, 1330, 1315, 1250, 1210, 1155, 770 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.20—2.68 (2H, m), 2.77 (2H, m), 2.97 (4H, m), 3.65 (3H, s), 4.55 (1H, m), 5.37 (2H, br s), 5.99 (2H, br s), 6.36 (1H, s), 7.33 (2H, d, J=7.8), 7.46 (1H, t, J=7.6), 7.60 (1H, d, J=8.4), 7.78 (1H, t, J=8.4), 7.80 (2H, d, J=7.8), 8.08 (1H, d, J=7.6), 8.73 (1H, d, J=7.0), 10.36 (1H, br s). SIMS m/z: 541 (MH^+).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(3-cyanophenyl-L-glutaminate (12Cb) IR (KBr): 3380, 2920, 2850, 2230, 1735, 1605, 1545, 1480, 1425, 1320, 1305, 1285, 1255, 1210, 1165, 1090, 1015 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 2.05—2.45 (2H, m), 2.52 (2H, t, J=5.4), 2.99 (4H, s), 3.78 (3H, s), 4.79 (1H, m), 6.44 (2H, s), 7.18 (2H, d, J=8.4), 7.34 (2H, m), 7.69 (2H, d, J=8.4), 7.74 (1H, m), 7.91 (1H, s). SIMS m/z: 541 (MH^+).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(4-cyanophenyl)-L-glutaminate (12Cc) IR (KBr): 3390, 3200, 2930, 2850, 2230, 1740, 1610, 1580, 1535, 1505, 1330, 1315, 1260, 1220, 1180, 1090, 1020, 760 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.90—2.35 (2H, m), 2.56 (2H, m), 2.96 (4H, m), 3.65 (3H, s), 4.49 (1H, m), 5.36 (2H, s), 5.99 (2H, s), 6.35 (1H, d, J=1.8), 7.33 (2H, d, J=8.2), 7.75 (4H, s), 7.80 (2H, d, J=8.2), 8.70 (1H, d, J=7.6), 10.37 (2H, s). SIMS m/z: 541 (MH^+).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-[2-(1*H*-tetrazol-5-yl)phenyl]-L-glutaminate (12Da) IR (KBr): 3400, 3200, 2925, 2850, 1730, 1640, 1590, 1540, 1500, 1470, 1435, 1410, 1380, 1355, 1300, 1250, 1210, 1195, 1095, 1005 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.00—2.50 (2H, m), 2.64 (2H, t, J=7.0), 2.95 (4H, m), 3.66 (3H, s), 4.53 (1H, m), 6.59 (1H, s), 6.83 (2H, br s), 7.09 (1H, dt, J=8.2, 1.2), 7.32 (2H, d, J=8.2), 7.40 (2H, br s), 7.82 (2H, d, J=8.2), 8.18 (1H, dd, J=7.8, 1.6), 8.54 (1H, dd, J=8.4, 1.0), 8.76 (1H, d, J=7.4), 11.21 (1H, s). SIMS m/z: 584 (MH^+).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-[3-(1*H*-tetrazol-5-yl)phenyl]-L-glutaminate (12Db) IR (KBr): 3400, 3200, 2930, 2850, 1735, 1640, 1610, 1570, 1545, 1500, 1470, 1430, 1415, 1380, 1345, 1315, 1295, 1270, 1210, 1090, 1055, 1030 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.90—2.35 (2H, m), 2.50 (2H, m), 2.94 (4H, m), 3.65 (3H, s), 4.48 (1H, m), 5.82 (2H, br s), 6.43 (1H, s), 6.48 (2H, br s), 7.34 (2H, d, J=8.0), 7.36 (1H, t, J=8.0), 7.66 (1H, d, J=8.0), 7.83 (2H, d, J=8.0), 8.22 (1H, s), 8.74 (1H, d, J=7.4), 10.08 (1H, s), 10.65 (1H, s). SIMS m/z: 584 (MH^+).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-[4-(1*H*-tetrazol-5-yl)phenyl]-L-glutaminate (12Dc) IR (KBr): 3400, 3300, 2920, 2850, 1735, 1635, 1605, 1535, 1500, 1445, 1420, 1370, 1335, 1300, 1245, 1220, 1170, 1155, 1100, 1060, 1030, 1005 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.40—2.35 (2H, m), 2.50 (2H, m), 2.97 (4H, m), 3.66 (3H, s), 5.70 (2H, br s), 6.41 (1H, s), 7.34 (2H, d, J=8.0), 7.71 (2H, d, J=8.6), 7.82 (2H, d, J=8.0), 7.93 (2H, d, J=8.6), 8.73 (1H, d, J=8.0), 10.14 (1H, s), 10.58 (1H, s). SIMS m/z: 584 (MH^+).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(2-hydroxyphenyl)-L-glutaminate (12Ea) IR (KBr): 3380, 2940, 2860, 1735, 1630, 1605, 1575, 1530, 1495, 1450, 1430, 1360, 1310, 1215, 750 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.90—2.40 (2H, m), 2.96 (4H, m), 3.65 (3H, s), 4.46 (1H, m), 5.35 (2H, br s), 5.97 (2H, br s), 6.36 (1H, s), 6.70—7.00 (3H, m), 7.34 (2H, d, J=8.0), 7.69 (1H, d, J=7.4), 7.81 (2H, d, J=8.0), 8.70 (1H, d, J=7.4), 9.26 (1H, s), 9.70 (1H, s), 10.35 (1H, br s). SIMS m/z: 532 (MH^+).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(3-hydroxyphenyl)-L-glutaminate (12Eb) IR (KBr): 3380, 2950, 2850, 1735, 1640, 1610, 1580, 1535, 1495, 1450, 1410, 1360, 1310, 1235, 750 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.80—2.30 (2H, m), 2.46 (2H, t, J=7.4), 2.98 (4H, m), 3.66 (3H, s), 4.46 (1H, m), 6.42 (1H, d, J=6.8), 6.47 (2H, br s), 6.53 (1H, s), 6.93 (1H, d, J=6.8), 7.00 (1H, t, J=6.8), 7.10 (2H, br s), 7.17 (1H, s), 7.34 (2H, d, J=8.2), 7.83 (2H, d, J=8.2), 8.72 (1H, d, J=7.0), 9.35 (1H, s), 9.82 (1H, s), 11.04 (1H, br s). SIMS m/z: 532 (MH^+).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(4-hydroxyphenyl)-L-glutaminate (12Ec) IR (KBr): 3475, 3380, 3310, 2950, 2925, 2910, 2850, 1740, 1640, 1615, 1585, 1545, 1515, 1500, 1440, 1405, 1380, 1340, 1305, 1255, 1240, 1160, 1105, 1090 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.90—2.30 (2H, m), 2.41 (2H, t, J=7.4), 2.96 (4H, m), 3.65 (3H, s), 4.46 (1H, m), 5.38 (2H, br s), 6.00 (2H, br s), 6.40 (1H, d, J=1.0), 6.69 (2H, d, J=8.8), 7.34 (2H, d, J=8.8), 7.81 (2H, d, J=8.0), 8.72 (2H, d, J=8.0), 9.14 (1H, s), 9.67 (1H, s), 10.36 (1H, s). SIMS m/z: 532 (MH^+).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(3-methoxycarbonylmethylphenyl)-L-glutaminate (12F) IR (KBr): 3350, 3200, 2950, 2850, 1735, 1610, 1570, 1550, 1490, 1435, 1330, 1305, 1260, 1200, 1170, 1090, 1010 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.80—2.40 (2H, m), 2.47 (2H, t, J=7.2), 2.96 (4H, m), 3.61 (3H, s), 3.62 (2H, s), 3.65 (3H, s), 4.46 (1H, m), 5.64 (2H, br s), 6.27 (2H, s), 6.40 (1H, d, J=1.0), 6.91 (1H, d, J=7.6), 7.22 (1H, d, J=7.6), 7.33 (2H, d, J=8.0), 7.46 (1H, d, J=7.6), 7.49 (1H, s), 7.81 (2H, d, J=8.0), 8.69 (1H, d, J=7.2), 9.92 (1H, s), 10.51 (1H, d, J=1.0). SIMS m/z: 588 (MH^+).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(3-ethoxycarbonyl-4-hydroxyphenyl)-L-glutaminate (12G) IR (KBr): 3380, 3200, 1735, 1665, 1650, 1640, 1610, 1580, 1540, 1490, 1430, 1290, 1205 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.34 (3H, t, J=7.0), 1.95—2.70 (2H, m), 2.97 (4H, m), 3.65 (3H, s), 4.36 (2H, q, J=7.0), 4.45 (1H, m), 5.59 (2H, br s), 6.22 (2H, br s), 6.39 (1H, s), 6.92 (1H, d, J=9.0), 7.32 (2H, d, J=8.0), 7.66 (1H, dd, J=9.0, 2.6), 7.80 (2H, d, J=8.0), 8.08 (1H, d, J=2.6), 8.69 (1H, d, J=7.8), 9.92 (1H, s),

10.33 (1H, s), 10.50 (1H, br s). SIMS *m/z*: 604 (MH^+).

Diethyl *N*-[3-[N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-O^1-methyl-γ-L-glutamylamino]benzoyl]-L-glutamate (12H) IR (KBr): 3380, 2950, 2850, 1735, 1720, 1640, 1610, 1575, 1540, 1500, 1420, 1295, 1245, 1200, 1120 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 1.23 (3H, t, *J*=7.0), 1.30 (3H, t, *J*=7.0), 2.00—2.40 (4H, m), 2.40—2.60 (4H, m), 2.96 (4H, m), 3.78 (3H, s), 4.12 (2H, q, *J*=7.0), 4.23 (2H, q, *J*=7.0), 4.68—4.81 (2H, m), 6.39 (1H, s), 7.14 (2H, d, *J*=8.2), 7.27 (1H, t, *J*=8.0), 7.50 (1H, dt, *J*=7.9, 1.8), 7.70 (2H, d, *J*=8.2), 7.75 (1H, t, *J*=1.8), 7.83 (1H, dt, *J*=8.0, 1.8), 7.86 (1H, d, *J*=7.9), 7.95 (1H, d, *J*=8.6). SIMS *m/z*: 745 (MH^+).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(6-fluoro-3-methoxycarbonylphenyl)-L-glutaminate (12I) IR (KBr): 3370, 2950, 2850, 1720, 1610, 1575, 1540, 1490, 1440, 1420, 1295, 1245, 1200, 1110, 990, 795, 760 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.19 (2H, m), 2.58 (2H, t, *J*=5.4), 2.96 (4H, m), 3.66 (3H, s), 3.85 (3H, s), 4.51 (1H, m), 5.54 (2H, br s), 6.17 (2H, s), 6.38 (1H, s), 7.32 (2H, d, *J*=8.2), 7.37 (1H, dd, *J*=10.6, 8.6), 7.72 (1H, ddd, *J*=8.4, 5.0, 2.4), 7.80 (2H, d, *J*=8.2), 8.59 (1H, dd, *J*=7.6, 2.2), 8.67 (1H, d, *J*=7.6), 9.91 (1H, s), 10.46 (1H, d, *J*=1.8). SIMS *m/z*: 592 (MH^+).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(3,4-methylenedioxyphenyl)-L-glutaminate (12J) IR (KBr): 3390, 1740, 1640, 1610, 1580, 1550, 1500, 1490, 1430, 1240, 1205, 1190, 1140 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.90—2.25 (2H, m), 2.35—2.45 (2H, m), 2.96 (4H, m), 3.65 (3H, s), 4.47 (1H, m), 5.51 (2H, br s), 5.96 (2H, s), 6.13 (2H, br s), 6.38 (1H, s), 6.81 (1H, d, *J*=8.4), 6.93 (1H, dd, *J*=8.4, 2.0), 7.28 (1H, d, *J*=2.0), 7.33 (2H, d, *J*=8.2), 7.80 (2H, d, *J*=8.2), 8.69 (1H, d, *J*=7.0), 9.82 (1H, s), 10.44 (1H, br s). SIMS *m/z*: 560 (MH^+).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(4-benzylthiophenyl)-L-glutaminate (12K) IR (KBr): 3380, 3320, 2940, 2850, 1740, 1610, 1545, 1530, 1495, 1450, 1425, 1395, 1305, 1285, 1240, 1200, 1170, 1130, 1090, 825, 800 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.80—2.35 (2H, m), 2.46 (2H, t, *J*=7.2), 2.96 (4H, m), 3.65 (3H, s), 4.13 (2H, s), 4.48 (1H, m), 5.49 (2H, br s), 6.11 (2H, s), 6.38 (1H, s), 7.15—7.20 (9H, m), 7.50 (2H, d, *J*=8.6), 7.80 (2H, d, *J*=8.6), 8.69 (1H, d, *J*=7.2), 9.94 (1H, s), 10.43 (1H, s). SIMS *m/z*: 638 (MH^+).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(3-methoxycarbonyl-2-naphthyl)-L-glutaminate (12La) IR (KBr): 3370, 3320, 2950, 1735, 1690, 1610, 1575, 1545, 1485, 1445, 1430, 1360, 1340, 1295, 1210, 1150, 1130, 1075, 790, 750 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.00—2.45 (2H, m), 2.59 (2H, t, *J*=7.6), 2.95 (4H, m), 3.67 (3H, s), 3.88 (3H, s), 4.54 (1H, m), 5.34 (2H, s), 5.95 (2H, s), 6.35 (1H, d, *J*=2.0), 7.32 (2H, d, *J*=8.0), 7.49 (1H, t, *J*=7.8), 7.62 (1H, t, *J*=7.8), 7.80 (2H, d, *J*=8.0), 7.88 (1H, d, *J*=8.2), 8.02 (1H, d, *J*=7.8), 8.58 (1H, s), 8.63 (1H, s), 8.73 (1H, d, *J*=7.6), 10.34 (1H, d, *J*=2.0), 10.57 (1H, s). SIMS *m/z*: 624 (MH^+).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(4-methoxycarbonyl-2-naphthyl)-L-glutaminate (12Lb) IR (KBr): 3330, 3200, 2950, 2925, 2850, 1740, 1670, 1640, 1605, 1540, 1500, 1460, 1435, 1390, 1300, 1245, 1200, 1175, 1150, 1130, 1035, 1020, 830, 795, 750 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.00—2.50 (2H, m), 2.56 (2H, t, *J*=7.4), 2.99 (4H, m), 3.67 (3H, s), 3.95 (3H, s), 4.54 (1H, m), 6.36 (2H, br s), 6.51 (1H, s), 7.00 (2H, s), 7.32 (2H, d, *J*=8.4), 7.54 (2H, m), 7.83 (2H, d, *J*=8.4), 7.91 (1H, m), 8.29 (1H, d, *J*=2.2), 8.51 (1H, d, *J*=2.2), 8.61 (1H, m), 8.71 (1H, d, *J*=6.6), 10.33 (1H, s), 10.97 (1H, s). SIMS *m/z*: 624 (MH^+).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(5-methoxycarbonyl-2-naphthyl)-L-glutaminate (12Lc) IR (KBr): 3380, 2950, 2920, 2850, 1605, 1575, 1545, 1535, 1500, 1460, 1430, 1375, 1350, 1270, 1250, 1200, 1130, 1070, 1020, 830, 800, 750 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.85—2.45 (2H, m), 2.56 (2H, t, *J*=7.0), 2.96 (4H, m), 3.67 (3H, s), 3.93 (3H, s), 4.54 (1H, m), 5.67 (2H, br s), 6.29 (2H, s), 6.41 (1H, s), 7.33 (2H, d, *J*=8.2), 7.55 (1H, t, *J*=8.0), 7.66 (1H, dd, *J*=9.4, 2.2), 7.82 (2H, d, *J*=8.2), 8.01 (1H, d, *J*=7.2), 8.06 (1H, d, *J*=8.8), 8.42 (1H, d, *J*=1.6), 8.67 (1H, d, *J*=9.2), 9.03 (1H, d, *J*=8.8), 10.23 (1H, s), 10.54 (1H, s). SIMS *m/z*: 624 (MH^+).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(1*H*-benzotriazol-5-yl)-L-glutaminate (12M) IR (KBr): 3400, 2950, 1735, 1670, 1630, 1610, 1580, 1560, 1540, 1500, 1440, 1430, 1390, 1200, 1180, 1135 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.70—1.80 (2H, m), 2.05—2.35 (2H, m), 2.96 (4H, m), 3.66 (3H, s), 4.50 (1H, m), 5.42 (2H, br s), 6.03 (2H, br s), 6.37 (1H, s), 7.33 (2H, d, *J*=8.2), 7.36 (1H, dd, *J*=9.0, 1.8), 7.81 (2H, d, *J*=8.2), 7.87 (1H, d, *J*=9.0), 8.34 (1H, br s), 8.70 (1H, d, *J*=7.0), 10.23 (1H, s), 10.39 (1H, br s). SIMS *m/z*: 555 (MH^+).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(5-methoxycarbonyl-3-pyridyl)-L-glutaminate (12Na) IR (KBr): 3290, 2950, 2850, 1725, 1660, 1635, 1540, 1500, 1460, 1430, 1340, 1300, 1265, 1200, 1130, 1110, 800, 765 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.90—2.35 (2H, m), 2.54 (2H, t, *J*=7.6), 2.97 (4H, m), 3.70 (3H, s), 3.89 (3H, s), 4.52 (1H, m), 6.32 (2H, br s), 6.50 (1H, s), 6.99 (2H, s), 7.32 (2H, d, *J*=8.4), 7.81 (2H, d, *J*=8.4), 8.62 (1H, t, *J*=2.4), 8.71 (1H, d, *J*=7.6), 8.74 (1H, d, *J*=2.4), 8.88 (1H, d, *J*=2.4), 10.42 (1H, s), 10.96 (1H, s). SIMS *m/z*: 575 (MH^+).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(5-methoxycarbonyl-2-pyridyl)-L-glutaminate (12Nb) IR (KBr): 3400, 3200, 2950, 2850, 1690, 1650, 1610, 1580, 1530, 1500, 1435, 1385, 1300, 1275, 1200, 1180, 1120, 920, 830, 800, 780, 720 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.80—2.40 (2H, m), 2.60 (2H, t, *J*=7.2), 3.33 (4H, m), 3.65 (3H, s), 3.85 (3H, s), 4.45 (1H, m), 5.91 (2H, br s), 6.44 (1H, s), 6.54 (2H, br s), 7.33 (2H, d, *J*=8.4), 7.80 (2H, d, *J*=8.4), 8.20 (1H, dd, *J*=7.8, 1.0), 8.28 (1H, dd, *J*=7.8, 2.0), 8.67 (1H, d, *J*=7.2), 8.86 (1H, d, *J*=2.2), 10.69 (1H, br s), 10.91 (1H, s). SIMS *m/z*: 575 (MH^+).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(1*H*-tetrazol-5-yl)-L-glutaminate (12Oa) IR (KBr): 3370, 3200, 2920, 1715, 1640, 1610, 1590, 1540, 1500, 1430, 1200, 1180, 1130, 1035, 720 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.00—2.35 (2H, m), 2.61 (2H, m), 2.96 (4H, m), 3.64 (3H, s), 4.49 (1H, m), 5.60 (1H, s), 6.82 (2H, s), 7.33 (2H, d, *J*=7.8), 7.49 (2H, s), 7.80 (2H, d, *J*=7.8), 8.68 (1H, d, *J*=7.6), 11.26 (1H, s), 11.97 (1H, s). SIMS *m/z*: 508 (MH^+).

Methyl N^2-[4-[3-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]benzoyl]-N^5-(1*H*-tetrazol-5-yl)-L-glutaminate (12Ob) IR (KBr): 3300, 3220, 2930, 1735, 1690, 1660, 1640, 1610, 1540, 1500, 1440, 1400, 1340, 1240, 1220, 1040 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.75—1.90 (2H, m), 1.95—2.20 (2H, m), 2.50—2.80 (6H, m), 3.65 (3H, s), 4.49 (1H, m), 6.40 (2H, br s), 6.61 (1H, s), 7.07 (2H, br s), 7.32 (2H, d, *J*=8.0), 7.81 (2H, d, *J*=8.0), 8.70 (1H, d, *J*=7.0), 11.05 (1H, br s), 11.94 (1H, br s). SIMS *m/z*: 522 (MH^+).

Methyl N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-[4-(methoxycarbonylmethyl)thiazol-2-yl]-L-glutaminate (12Pa) IR (neat): 3380, 3200, 2950, 2850, 1735, 1605, 1570, 1545, 1535, 1500, 1430, 1340, 1270, 1200, 1160, 1050, 830, 795, 750 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.90—2.35 (2H, m), 2.57 (2H, t, *J*=7.6), 2.98 (4H, m), 3.61 (3H, s), 3.65 (3H, s), 3.69 (2H, s), 4.46 (1H, m), 5.53 (2H, br s), 6.15 (1H, s), 6.39 (1H, s), 6.96 (1H, s), 7.34 (2H, d, *J*=8.0), 7.80 (2H, d, *J*=8.0), 8.67 (1H, d, *J*=7.6), 10.46 (1H, br s). SIMS *m/z*: 595 (MH^+).

Methyl N^2-[4-[3-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]benzoyl]-N^5-[4-(methoxycarbonylmethyl)thiazol-2-yl]-L-glutaminate (12Pb) IR (KBr): 3370, 3200, 2930, 2850, 1740, 1610, 1575, 1550, 1495, 1430, 1370, 1330, 1270, 1210, 1160, 1095, 1020, 795, 760 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.85 (2H, m), 1.95—2.35 (2H, m), 2.57 (2H, t, *J*=7.2), 2.69 (4H, m), 3.60 (3H, s), 3.64 (3H, s), 3.68 (2H, s), 4.45 (1H, m), 5.31 (2H, s), 5.88 (2H, s), 6.42 (1H, s), 6.95 (1H, s), 7.31 (2H, d, *J*=8.2), 7.81 (2H, d, *J*=8.2), 8.66 (1H, d, *J*=7.4), 10.36 (1H, s). SIMS *m/z*: 609 (MH^+).

Ethyl *N*-[N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-O^1-methyl-γ-L-glutamyl]glycinate (12Q) IR (KBr): 3365, 2945, 1735, 1680, 1640, 1600, 1570, 1545, 1435, 1210, 1140 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.18 (3H, t, *J*=7.2), 1.80—2.20 (2H, m), 2.30 (2H, t, *J*=7.2), 2.96 (4H, m), 3.64 (3H, s), 3.80 (2H, d, *J*=6.0), 4.07 (2H, q, *J*=7.2), 4.41 (1H, m), 5.47 (2H, br s), 6.09 (2H, br s), 6.37 (1H, s), 7.33 (2H, d, *J*=9.5), 7.80 (2H, d, *J*=9.5), 8.32 (1H, t, *J*=6.0), 8.70 (1H, d, *J*=6.6), 10.42 (1H, br s). SIMS *m/z*: 526 (MH^+).

Methyl *N*-[N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-O^1-methyl-γ-L-glutamyl]-L-*tert*-leucinate (12R) IR (KBr): 3365, 2945, 1735, 1685, 1640, 1605, 1540, 1500, 1475, 1400, 1230, 1180, 1140 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.95 (9H, s), 1.80—2.20 (2H, m), 2.35 (2H, t, *J*=6.6), 2.97 (4H, m), 3.60 (3H, s), 3.75 (3H, s), 4.13 (1H, d, *J*=8.8), 4.36 (1H, m), 5.40 (2H, br s), 6.20 (2H, br s), 6.38 (1H, s), 7.33 (2H, d, *J*=8.2), 7.80 (2H, d, *J*=8.2), 7.98 (1H, d, *J*=8.8), 8.62 (1H, d, *J*=7.4), 10.50 (1H, br s). SIMS *m/z*: 568 (MH^+).

Methyl *N*-[N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-O^1-methyl-γ-L-glutamyl]-L-phenylalaninate (12Sa) IR (KBr): 3365, 2945, 1735, 1680, 1640, 1605, 1570, 1550, 1435, 1200,

1135 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.80—2.20 (2H, m), 2.24 (2H, t, J=8.8), 2.30 (6H, m), 3.58 (3H, s), 3.63 (3H, s), 4.30—4.60 (2H, m), 5.38 (2H, br s), 6.00 (2H, br s), 6.38 (1H, s), 7.22 (5H, m), 7.34 (2H, d, J=8.4), 7.80 (2H, d, J=8.4), 8.38 (1H, d, J=7.2), 8.67 (1H, d, J=7.4), 10.37 (1H, br s). SIMS m/z: 602 (MH$^+$).

Methyl *N*-[*N*^{2}-[4-[3-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]benzoyl]-*O*^{1}-methyl-γ-L-glutamyl]-L-phenylalaninate (12Sb) IR (KBr): 3365, 2945, 1735, 1680, 1640, 1610, 1570, 1545, 1435, 1200, 1135 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 1.90—2.25 (4H, m), 2.35 (2H, t, J=7.0), 2.68—2.83 (4H, m), 2.93 (1H, dd, J=13.6, 8.6), 3.13 (1H, dd, J=13.6, 5.6), 3.63 (3H, s), 3.72 (3H, s), 4.53 (1H, dd, J=9.2, 5.0), 4.67 (1H, d, J=8.6, 5.6), 6.55 (1H, s), 7.10—7.25 (5H, m), 7.31 (2H, d, J=8.2), 7.77 (2H, d, J=8.2). SIMS m/z: 616 (MH$^+$).

N^2-[4-[3-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]-benzoyl]-N^5-[3-(dihydroxyboryl)phenyl]-L-glutamine (4Ab). Typical Procedure A 1 N NaOH aqueous solution (6 ml) was added to a stirred solution of **12**Ab (1.0 g) in MeOH–THF (2 : 1, 15 ml) at room temperature. The reaction mixture was stirred for 5 h, then concentrated *in vacuo*, and the residue was dissolved in H_2O. A 1 N HCl solution (6 ml) was added to the mixture. The resulting precipitate was collected by filtration, washed with H_2O, and dried *in vacuo* to give **4**Ab (839 mg, 86%) as a colorless solid, mp >270 °C (dec). IR (KBr): 3320, 3200, 2930, 1660, 1640, 1545, 1490, 1425, 1380, 1340, 705 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.84 (2H, m), 1.90—2.30 (4H, m), 2.45 (2H, m), 2.71 (4H, m), 4.23 (1H, m), 5.71 (2H, br s), 6.29 (2H, br s), 6.49 (1H, s), 7.24 (1H, t, J=8.0), 7.31 (2H, d, J=8.0), 7.46 (1H, d, J=7.2), 7.70 (1H, br d, J=8.4), 7.82 (1H, br s), 7.84 (2H, d, J=8.0), 7.98 (2H, br s), 8.55 (1H, d, J=7.6), 9.85 (1H, s), 10.60 (1H, br s). SIMS m/z: 559 (MH$^+$). *Anal.* Calcd for $C_{27}H_{30}BN_7O_6 \cdot 2.5H_2O$: C, 53.65; H, 5.84; N, 16.22. Found: C, 53.62; H, 5.81; N, 16.41.

Compounds **4**A—S were prepared from the corresponding esters **12**A—S by the same method as that described for **4**Ab.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-[3-(dihydroxyboryl)phenyl]-L-glutamine (4Aa) mp >270 °C (dec). IR (KBr): 3320, 3200, 2930, 1655, 1640, 1550, 1495, 1425, 1370, 1340, 705 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.90—2.40 (2H, m), 2.45 (2H, m), 2.97 (4H, m), 4.25 (1H, m), 5.75 (2H, br s), 6.35 (2H, br s), 6.49 (1H, s), 7.25 (1H, t, J=8.0), 7.32 (2H, d, J=8.0), 7.52 (1H, d, J=7.2), 7.70 (1H, br d, J=8.4), 7.82 (1H, br s), 7.85 (2H, d, J=8.0), 7.88 (2H, br s), 8.53 (1H, d, J=7.4), 9.92 (1H, s), 10.67 (1H, br s). SIMS m/z: 545 (MH$^+$). *Anal.* Calcd for $C_{26}H_{28}BN_7O_6 \cdot 2.0H_2O$: C, 53.71; H, 5.55; N, 16.86. Found: C, 53.72; H, 5.71; N, 16.60.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(2-carboxyphenyl)-L-glutamine (4Ba) mp 192—195 °C. IR (KBr): 3320, 3200, 2910, 2830, 1655, 1640, 1580, 1520, 1495, 1440, 1370, 1285, 1240, 750 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.90—2.40 (2H, m), 2.50 (2H, m), 3.00 (4H, m), 4.40 (1H, m), 6.64 (1H, s), 6.67 (2H, br s), 7.07 (1H, dt, J=1.2, 7.8), 7.23 (2H, d, J=8.0), 7.29 (2H, s), 7.47 (1H, dt, J=1.6, 8.6), 7.83 (2H, d, J=8.0), 8.04 (1H, dd, J=1.6, 8.4), 8.52 (1H, d, J=8.4), 8.56 (1H, d, J=8.4), 11.36 (1H, s), 12.03 (1H, s). SIMS m/z: 546 (MH$^+$). *Anal.* Calcd for $C_{27}H_{27}N_7O_6 \cdot 2.9H_2O$: C, 54.25; H, 5.53; N, 16.40. Found: C, 54.51; H, 5.25; N, 16.09.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(3-carboxyphenyl)-L-glutamine (4Bb) mp 205—206 °C. IR (KBr): 3350, 3200, 2930, 2850, 1645, 1600, 1545, 1500, 1440, 1385, 1300, 1260, 1190, 1095, 1020, 905, 820, 760 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.90—2.40 (2H, m), 2.44 (2H, m), 2.96 (4H, m), 4.41 (1H, m), 5.66 (2H, br s), 6.32 (2H, br s), 6.40 (1H, s), 7.33 (2H, d, J=8.4), 7.39 (1H, t, J=7.8), 7.60 (1H, dt, J=7.8, 1.2), 7.78 (1H, dd, J=7.8, 1.2), 7.79 (2H, d, J=8.4), 8.22 (1H, t, J=1.2), 8.55 (1H, d, J=7.6), 10.12 (1H, s), 10.53 (1H, s). SIMS m/z: 546 (MH$^+$). *Anal.* Calcd for $C_{27}H_{27}N_7O_6 \cdot 3.1H_2O$: C, 53.92; H, 5.56; N, 16.30. Found: C, 53.73; H, 5.56; N, 16.46.

N^2-[4-[3-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]-benzoyl]-N^5-(3-carboxyphenyl)-L-glutamine (4Bc) mp 191—193 °C. IR (KBr): 3325, 3200, 2930, 1660, 1640, 1610, 1590, 1540, 1530, 1500, 1440, 1380, 1290, 1260, 760 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.85 (2H, m), 1.85—2.30 (2H, m), 2.46 (2H, m), 2.69 (4H, m), 4.44 (1H, m), 5.61 (2H, br s), 6.20 (2H, br s), 6.47 (1H, s), 7.31 (2H, d, J=8.2), 7.39 (1H, t, J=8.0), 7.60 (1H, d, J=7.2), 7.79 (1H, br d, J=8.0), 7.83 (2H, d, J=8.2), 8.22 (1H, s), 8.56 (1H, d, J=7.6), 10.11 (1H, s), 10.55 (1H, br s). SIMS m/z: 560 (MH$^+$). *Anal.* Calcd for $C_{28}H_{29}N_7O_6 \cdot 2.5H_2O$: C, 55.62; H, 5.67; N, 16.22. Found: C, 55.53; H, 5.66; N, 16.47.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(4-carboxyphenyl)-L-glutamine (4Bd) mp 208—210 °C. IR (KBr): 3375, 3200, 2920, 1640, 1600, 1455, 1405, 1380, 1305, 1255, 1100, 855, 770 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.80—2.35 (2H, m), 2.50 (2H, m), 2.96 (4H, m), 4.43 (1H, m), 5.70 (2H, br s), 6.36 (2H, br s), 6.41 (1H, s), 7.33 (2H, d, J=8.4), 7.73 (2H, d, J=8.8), 7.81 (2H, d, J=8.4), 7.87 (2H, d, J=8.8), 8.55 (1H, d, J=7.6), 10.25 (1H, s), 10.56 (1H, br s). SIMS m/z: 546 (MH$^+$). *Anal.* Calcd for $C_{27}H_{27}N_7O_6 \cdot 1.8H_2O$: C, 56.11; H, 5.34; N, 16.96. Found: C, 56.18; H, 5.52; N, 16.89.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(2-cyanophenyl)-L-glutamine (4Ca) mp 219—221 °C. IR (KBr): 3370, 3200, 2920, 2850, 1640, 1610, 1560, 1535, 1490, 1465, 1445, 1390, 1330, 1250, 770 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.10—2.50 (2H, m), 2.77 (2H, t, J=7.5), 2.97 (4H, m), 4.47 (1H, m), 6.21 (2H, br s), 6.48 (1H, s), 6.85 (2H, br s), 7.33 (2H, d, J=8.0), 7.46 (1H, dt, J=7.4, 1.0), 7.59 (1H, d, J=7.8), 7.78 (1H, dt, J=8.4, 1.4), 7.80 (2H, d, J=8.0), 8.07 (1H, dd, J=8.0, 1.0), 8.60 (1H, d, J=7.8), 10.86 (1H, s). SIMS m/z: 527 (MH$^+$). *Anal.* Calcd for $C_{27}H_{26}N_8O_4 \cdot 4.0H_2O$: C, 54.17; H, 5.72; N, 18.83. Found: C, 54.30; H, 5.50; N, 18.75.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(3-cyanophenyl)-L-glutamine (4Cb) mp 187—188 °C. IR (KBr): 3350, 3200, 2925, 2850, 2225, 1640, 1585, 1545, 1500, 1480, 1450, 1430, 1390, 1330, 1300, 1285, 1255, 1190, 1170, 1095, 1015, 790, 755 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.92—2.34 (2H, m), 2.50 (2H, m), 2.96 (4H, m), 4.44 (1H, m), 5.66 (2H, br s), 6.30 (2H, br s), 6.40 (1H, s), 7.33 (2H, d, J=8.0), 7.48 (2H, m), 7.81 (3H, d, J=8.0), 8.08 (1H, s), 8.57 (1H, d, J=7.0), 10.35 (1H, s), 10.54 (1H, s). SIMS m/z: 527 (MH$^+$). *Anal.* Calcd for $C_{27}H_{26}N_8O_4 \cdot 3.0H_2O$: C, 55.86; H, 5.56; N, 19.34. Found: C, 55.55; H, 5.24; N, 19.10.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(4-cyanophenyl)-L-glutamine (4Cc) mp 194—195 °C. IR (KBr): 3410, 2930, 2855, 2230, 1645, 1600, 1535, 1510, 1455, 1410, 1310, 1260, 1175, 1100, 840 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.50—2.45 (4H, m), 2.96 (4H, m), 4.40 (1H, m), 5.54 (2H, s), 6.19 (2H, br s), 6.38 (1H, s), 7.32 (2H, d, J=8.2), 7.75 (4H, s), 7.80 (2H, d, J=8.2), 8.54 (1H, d, J=8.0), 10.39 (1H, s), 10.46 (1H, s). SIMS m/z: 527 (MH$^+$). *Anal.* Calcd for $C_{27}H_{26}N_8O_4 \cdot 3.5H_2O$: C, 55.00; H, 5.64; N, 19.00. Found: C, 55.07; H, 5.42; N, 18.97.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-[2-(1*H*-tetrazol-5-yl)phenyl]-L-glutamine (4Da) mp 207—209 °C. IR (KBr): 3380, 3200, 2925, 2850, 1640, 1595, 1540, 1500, 1470, 1410, 1385, 1360, 1335, 1295, 1255, 1190, 1160, 1090, 750 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.00—2.40 (2H, m), 2.63 (2H, t, J=7.2), 2.97 (4H, m), 4.45 (1H, m), 6.61 (1H, s), 6.80 (2H, br s), 7.10 (1H, dt, J=7.8, 0.8), 7.29 (2H, dt, J=7.8, 0.8), 7.31 (2H, d, J=8.0), 7.53 (2H, s), 7.82 (2H, d, J=8.0), 8.17 (1H, dd, J=8.0, 0.8), 8.54 (1H, d, J=7.8), 8.62 (1H, d, J=7.8), 11.29 (1H, s). SIMS m/z: 570 (MH$^+$). *Anal.* Calcd for $C_{27}H_{27}N_{11}O_4 \cdot 1.85H_2O$: C, 53.79; H, 5.13; N, 25.55. Found: C, 53.97; H, 4.96; N, 25.26.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-[3-(1*H*-tetrazol-5-yl)phenyl]-L-glutamine (4Db) mp 187—189 °C. IR (KBr): 3340, 3200, 2925, 2850, 1645, 1570, 1545, 1500, 1455, 1400, 1300, 1280, 1255, 1190, 1090, 800, 760, 745 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.80—2.40 (2H, m), 2.50 (2H, m), 2.96 (4H, m), 4.42 (1H, m), 6.10 (2H, br s), 6.48 (1H, s), 6.77 (2H, br s), 7.33 (2H, d, J=8.0), 7.43 (1H, d, J=7.6), 7.67 (2H, m), 7.82 (2H, d, J=8.0), 8.30 (1H, s), 8.58 (1H, d, J=7.0), 10.13 (1H, s), 10.81 (1H, s). SIMS m/z: 570 (MH$^+$). HR-MS (FAB): Calcd for $C_{27}H_{28}N_{11}O_4$ [MH$^+$]: 570.2325. Found: 570.2354.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-[4-(1*H*-tetrazol-5-yl)phenyl]-L-glutamine (4Dc) mp 221—223 °C. IR (KBr): 3400, 3200, 2930, 2850, 1640, 1570, 1540, 1500, 1450, 1430, 1385, 1335 1310, 1255, 1185, 1155, 1095, 1075, 1020, 1000, 845, 750 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.80—2.35 (2H, m), 2.50 (2H, m), 2.97 (4H, m), 4.43 (1H, m), 6.15 (2H, br s), 6.47 (1H, s), 6.81 (2H, s), 7.33 (2H, d, J=8.2), 7.76 (2H, d, J=8.7), 7.82 (2H, d, J=8.2), 7.95 (2H, d, J=8.7), 8.58 (1H, d, J=8.0), 10.22 (1H, s), 10.84 (1H, s). SIMS m/z: 570 (MH$^+$). HR-MS (FAB): Calcd for $C_{27}H_{28}N_{11}O_4$ [MH$^+$]: 570.2325. Found: 570.2330.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(2-hydroxyphenyl)-L-glutamine (4Ea) mp 182—183 °C. IR (KBr): 3400, 3200, 2920, 2850, 1640, 1535, 1500, 1450, 1395, 1300, 1280, 1240, 1190, 1100, 750 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.80—2.30 (2H, m), 2.38 (2H, t, J=7.2), 2.97 (4H, m), 4.40 (1H, m), 5.82 (2H, br s), 6.43 (1H, s), 6.48 (2H, br s), 6.70—7.00 (3H, m), 7.34 (2H, d, J=8.0), 7.68 (1H, d, J=6.4), 7.82 (2H, d, J=8.0), 8.57 (1H, d, J=7.8), 9.28 (1H, s),

9.73 (1H, br s), 10.63 (1H, s). SIMS *m/z*: 518 (MH^+). *Anal.* Calcd for $C_{26}H_{27}N_7O_5 \cdot 2.6H_2O$: C, 55.33; H, 5.75; N, 17.37. Found: C, 54.98; H, 5.48; N, 17.49.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(3-hydroxyphenyl)-L-glutamine (6Eb) mp 187—189 °C. IR (KBr): 3330, 3200, 2920, 2850, 1640, 1610, 1545, 1495, 1445, 1385, 1340, 1225, 1185, 1155, 1090, 855, 770 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.80—2.30 (2H, m), 2.46 (2H, t, J=7.4), 2.99 (4H, m), 4.39 (1H, m), 5.97 (2H, br s), 6.42 (1H, d, J=8.0), 6.46 (1H, s), 6.62 (2H, br s), 6.92 (1H, d, J=8.0), 7.04 (1H, t, J=8.0), 7.17 (1H, s), 7.34 (2H, d, J=8.2), 7.83 (2H, d, J=8.2), 5.57 (1H, d, J=7.6), 9.34 (1H, s), 9.80 (1H, s), 10.73 (1H, br s). SIMS *m/z*: 518 (MH^+). *Anal.* Calcd for $C_{26}H_{27}N_7O_5 \cdot 2.0H_2O$: C, 56.41; H, 5.64; N, 17.71. Found: C, 56.28; H, 5.84; N, 17.81.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(4-hydroxyphenyl)-L-glutamine (4Ec) mp 196—198 °C. IR (KBr): 3400, 3340, 3220, 2930, 2855, 1650, 1545, 1515, 1450, 1400, 1340, 1305, 1240, 1170, 1100, 835, 770 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.85—2.30 (2H, m), 2.94 (2H, t, J=7.2), 2.96 (4H, m), 4.39 (1H, m), 5.58 (2H, br s), 6.21 (2H, br s), 6.38 (1H, s), 6.36 (2H, d, J=8.8), 7.33 (2H, d, J=8.2), 7.34 (2H, d, J=8.8), 7.81 (2H, d, J=8.2), 8.56 (1H, d, J=7.8), 9.14 (1H, s), 9.67 (1H, s), 10.48 (1H, br s). SIMS *m/z*: 518 (MH^+). *Anal.* Calcd for $C_{26}H_{27}N_7O_5 \cdot 2.7H_2O$: C, 55.16; H, 5.77; N, 17.32. Found: C, 55.05; H, 5.66; N, 17.39.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(3-carboxymethylphenyl)-L-glutamine (4F) mp 195—196 °C. IR (KBr): 3330, 3200, 2920, 1660, 1630, 1610, 1595, 1555, 1540, 1500, 1435, 1380, 1350, 1285, 1255, 1210, 1180, 1160, 1090, 1070, 770 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.90—2.40 (2H, m), 2.46 (2H, t, J=7.0), 2.96 (4H, m), 3.50 (2H, s), 4.41 (1H, m), 5.47 (1H, br s), 6.11 (2H, s), 6.37 (1H, s), 6.85 (1H, d, J=8.0), 7.21 (1H, t, J=8.0), 7.33 (2H, d, J=8.4), 7.47 (1H, d, J=8.0), 7.49 (1H, s), 7.81 (2H, d, J=8.4), 8.54 (1H, d, J=7.0), 9.93 (1H, s), 10.41 (1H, s). SIMS *m/z*: 560 (MH^+). *Anal.* Calcd for $C_{28}H_{29}N_7O_6 \cdot 4.2H_2O$: C, 52.94; H, 5.93; N, 15.43. Found: C, 52.85; H, 5.77; N, 15.45.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(3-carboxy-4-hydroxyphenyl)-L-glutamine (4G) mp 222—224 °C. IR (KBr): 3350, 3220, 1665, 1650, 1550, 1500, 1440, 1380 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.90—2.25 (2H, m), 2.35—2.45 (2H, m), 2.96 (4H, m), 4.39 (1H, m), 6.58 (1H, s), 6.70 (1H, d, J=8.6), 6.71 (2H, br s), 7.33 (2H, d, J=8.2), 7.50 (1H, dd, J=8.6, 2.6), 7.55 (2H, br s), 7.81 (2H, d, J=8.2), 7.95 (1H, d, J=2.6), 8.58 (1H, d, J=7.6), 9.73 (1H, s), 11.24 (1H, br s). SIMS *m/z*: 562 (MH^+). *Anal.* Calcd for $C_{27}H_{27}N_7O_7 \cdot 2.6H_2O$: C, 53.30; H, 5.33; N, 16.12. Found: C, 53.18; H, 5.25; N, 16.28.

***N*-[3-[N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-γ-L-glutamylamino]benzoyl]-L-glutamic Acid (4H)** mp 218—220 °C. IR (KBr): 3400, 3200, 2920, 1660, 1640, 1590, 1545, 1530, 1500, 1480, 1390 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.85—2.60 (8H, m), 2.95 (4H, m), 4.30—4.50 (2H, m), 5.62 (2H, br s), 6.28 (2H, br s), 6.38 (1H, s), 7.20—7.42 (5H, m), 7.53 (1H, d, J=7.2), 7.70—7.90 (4H, m), 7.96 (1H, br s), 8.45—8.60 (2H, m), 10.11 (1H, s), 10.53 (1H, br s). SIMS *m/z*: 675 (MH^+). *Anal.* Calcd for $C_{32}H_{34}N_8O_9 \cdot 3.0H_2O$: C, 52.74; H, 5.53; N, 15.38. Found: C, 52.65; H, 5.67; N, 15.39.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(3-carboxy-6-fluorophenyl)-L-glutamine (4I) mp 202—204 °C. IR (KBr): 3330, 3200, 2930, 2850, 1650, 1615, 1545, 1500, 1440, 1380, 1280, 1255, 1195, 1115, 1095, 770 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.90—2.45 (2H, m), 2.57 (2H, t, J=7.0), 2.97 (4H, m), 4.42 (1H, m), 5.85 (2H, br s), 6.43 (1H, d, J=1.8), 6.50 (2H, s), 7.32 (2H, d, J=8.4), 7.33 (1H, dd, J=10.6, 8.6), 7.70 (1H, ddd, J=8.4, 4.8, 2.2), 7.83 (2H, d, J=8.4), 8.53 (1H, d, J=7.8), 8.54 (1H, dd, J=7.8, 2.2), 9.87 (1H, s), 10.64 (1H, d, J=1.8). SIMS *m/z*: 564 (MH^+). *Anal.* Calcd for $C_{27}H_{26}FN_7O_6 \cdot 3.0H_2O$: C, 52.51; H, 5.22; F, 3.08; N, 15.88. Found: C, 52.43; H, 4.98; F, 3.00; N, 15.61.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(3,4-methylenedioxyphenyl)-L-glutamine (4J) mp 185—186 °C. IR (KBr): 3375, 3200, 1655, 1640, 1560, 1545, 1500, 1490, 1445, 1390, 1240, 1190, 1040 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.90—2.25 (2H, m), 2.35—2.45 (2H, m), 2.96 (4H, m), 4.41 (1H, m), 5.96 (2H, s), 6.00 (2H, br s), 6.46 (1H, s), 6.65 (2H, br s), 6.81 (1H, d, J=8.4), 6.93 (1H, dd, J=8.4, 2.0), 7.28 (1H, d, J=2.0), 7.32 (2H, d, J=8.2), 7.81 (2H, d, J=8.2), 8.55 (1H, d, J=7.8), 9.83 (1H, s), 10.74 (1H, br s). SIMS *m/z*: 546 (MH^+). *Anal.* Calcd for $C_{27}H_{27}N_7O_6 \cdot 1.8H_2O$: C, 56.11; H, 5.34; N, 16.96. Found: C, 56.11; H, 5.20; N, 17.23.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(4-benzylthiophenyl)-L-glutamine (4K) mp 167—168 °C. IR (KBr): 3300, 3180, 2920, 2850, 1650, 1590, 1530, 1495, 1450, 1395, 1335, 1305, 1280, 1245, 1200, 1160, 1090, 810, 720 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.94—2.35 (2H, m), 2.46 (2H, t, J=8.0), 2.97 (4H, m), 4.14 (2H, s), 4.42 (1H, m), 6.19 (2H, br s), 6.48 (1H, s), 6.83 (2H, s), 7.19—7.40 (9H, m), 7.51 (2H, d, J=8.6), 7.82 (2H, d, J=8.2), 8.55 (1H, d, J=8.0), 9.96 (1H, s), 10.85 (1H, s). SIMS *m/z*: 624 (MH^+). *Anal.* Calcd for $C_{33}H_{33}N_7O_4S \cdot 2.0H_2O$: C, 60.08; H, 5.65; N, 14.86; S, 4.86. Found: C, 59.75; H, 5.70; N, 14.70; S, 4.92.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(3-carboxy-2-naphthyl)-L-glutamine (4La) mp 235—237 °C. IR (KBr): 3330, 3200, 2930, 2850, 1640, 1580, 1540, 1500, 1480, 1450, 1390, 1365, 1280, 1255, 1210, 1150, 750 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.00—2.50 (2H, m), 2.80 (2H, m), 3.01 (4H, m), 4.43 (1H, m), 6.63 (1H, d, J=1.2), 7.18 (2H, d, J=8.2), 7.40 (1H, t, J=8.0), 7.52 (1H, t, J=7.0), 7.81 (1H, d, J=7.0), 7.83 (2H, d, J=8.2), 7.92 (1H, d, J=8.0), 8.55 (1H, d, J=8.4), 8.68 (1H, s), 8.95 (1H, s), 11.35 (1H, d, J=1.2). SIMS *m/z*: 596 (MH^+). *Anal.* Calcd for $C_{31}H_{29}N_7O_6 \cdot 1.6H_2O$: C, 59.63; H, 5.20; N, 15.70. Found: C, 59.65; H, 5.45; N, 15.65.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(4-carboxy-2-naphthyl)-L-glutamine (4Lb) mp 208—211 °C. IR (KBr): 3320, 3200, 2920, 2850, 1640, 1540, 1500, 1455, 1420, 1380, 1350, 1290, 1235, 1150, 750 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.80—2.50 (2H, m), 2.56 (2H, t, J=7.4), 2.98 (4H, m), 4.45 (1H, m), 6.27 (2H, br s), 6.49 (1H, s), 7.31 (2H, d, J=8.4), 7.51 (2H, m), 7.82 (1H, m), 7.83 (2H, d, J=8.4), 8.26 (1H, d, J=2.2), 8.49 (1H, d, J=1.6), 8.57 (1H, d, J=7.6), 8.77 (1H, m), 10.30 (1H, s), 10.90 (1H, s). SIMS *m/z*: 596 (MH^+). *Anal.* Calcd for $C_{31}H_{29}N_7O_6 \cdot 4.0H_2O$: C, 55.77; H, 5.59; N, 14.68. Found: C, 55.91; H, 5.17; N, 14.77.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(5-carboxy-2-naphthyl)-L-glutamine (4Lc) mp 211—212 °C. IR (KBr): 3400, 3300, 3200, 2920, 2850, 1640, 1545, 1535, 1500, 1460, 1430, 1380, 1340, 1250, 1220, 1200, 1155, 1140, 835 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.90—2.45 (2H, m), 2.56 (2H, t, J=7.4), 3.01 (4H, m), 4.45 (1H, m), 6.10 (2H, br s), 6.46 (1H, s), 6.75 (2H, s), 7.32 (2H, d, J=8.0), 7.52 (1H, t, J=7.6), 7.64 (1H, dd, J=9.4, 2.0), 7.83 (2H, d, J=8.0), 8.01 (2H, d, J=7.6), 8.41 (1H, d, J=2.0), 8.57 (1H, d, J=7.4), 8.79 (1H, d, J=9.6), 10.24 (1H, s), 10.80 (1H, s). SIMS *m/z*: 596 (MH^+). *Anal.* Calcd for $C_{31}H_{29}N_7O_6 \cdot 2.0H_2O$: C, 58.95; H, 5.27; N, 15.52. Found: C, 58.85; H, 5.45; N, 15.35.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(1*H*-benzotriazol-5-yl)-L-glutamine (4M) mp 212—214 °C. IR (KBr): 3330, 3200, 2920, 1640, 1560, 1540, 1500, 1445, 1390, 1305, 1250 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.95—2.40 (4H, m), 2.96 (4H, m), 4.43 (1H, m), 5.54 (2H, br s), 6.17 (2H, s), 6.39 (1H, s), 7.32 (2H, d, J=8.0), 7.35 (1H, dd, J=9.0, 1.8), 7.81 (2H, d, J=8.0), 7.86 (1H, d, J=9.0H), 8.34 (1H, br s), 8.55 (1H, d, J=8.0), 10.21 (1H, s), 10.46 (1H, br s). SIMS *m/z*: 541 (MH^+). *Anal.* Calcd for $C_{28}H_{28}N_8O_4 \cdot 2.0H_2O$: C, 58.32; H, 5.59; N, 19.43. Found: C, 58.07; H, 5.47; N, 19.61.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(5-carboxy-3-pyridyl)-L-glutamine (4Na) mp 214—216 °C. IR (KBr): 3330, 3200, 2900, 1850, 1640, 1560, 1543, 1517, 1500, 1440, 1380, 1200, 1090 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.95—2.45 (2H, m), 2.50 (2H, t, J=7.6), 2.98 (4H, m), 4.46 (1H, m), 6.50 (2H, br s), 6.53 (1H, s), 7.15 (2H, s), 7.32 (2H, d, J=7.2), 7.82 (2H, d, J=7.2), 8.58 (2H, s), 8.74 (1H, s), 8.87 (1H, s), 10.38 (1H, s), 11.03 (1H, s). SIMS *m/z*: 547 (MH^+). *Anal.* Calcd for $C_{26}H_{26}N_8O_6 \cdot 3.0H_2O$: C, 52.00; H, 5.37; N, 18.66. Found: C, 51.97; H, 5.47; N, 18.61.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(5-carboxy-2-pyridyl)-L-glutamine Ammonium Salt (4Nb) mp 260—262 °C. IR (KBr): 3380, 3200, 2930, 2850, 1650, 1610, 1585, 1540, 1530, 1395, 1365, 1290, 1150, 1120, 1090 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.80—2.70 (4H, m), 2.95 (4H, m), 4.26 (1H, m), 5.42 (2H, br s), 6.07 (2H, br s), 6.36 (1H, s), 7.30 (2H, d, J=7.8), 7.76 (2H, d, J=7.8), 8.13 (1H, d, J=6.8), 8.26 (1H, d, J=6.8), 8.74 (1H, s), 10.81 (1H, s). *Anal.* Calcd for $C_{26}H_{29}N_9O_6 \cdot 2.6H_2O$: C, 51.16; H, 5.65; N, 20.65. Found: C, 51.31; H, 6.01; N, 20.36.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-(1*H*-tetrazol-5-yl)-L-glutamine (4Oa) mp 207—209 °C. IR (KBr): 3380, 3270, 3200, 2910, 1680, 1630, 1605, 1540, 1490, 1450, 1380, 1330, 1240, 1155, 1040 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.95—2.50 (2H, m), 2.60 (2H, m), 2.96 (4H, m), 4.43 (1H, m), 5.88 (2H, br s), 6.43 (1H, s), 6.52 (2H, s), 7.32 (2H, d, J=8.4), 7.79 (2H, d, J=8.4), 8.54 (1H, d, J=8.0), 10.56 (1H, s), 11.88 (1H, br s). SIMS *m/z*: 494 (MH^+). HR-MS

(FAB): Calcd for $C_{21}H_{24}N_{11}O_4$ [MH^+]: 494.2012. Found: 494.2006.

N^2-[4-[3-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]-benzoyl]-N^5-(1*H*-tetrazol-5-yl)-L-glutamine (4Ob) mp 197—198 °C. IR (KBr): 3320, 3200, 2930, 1660, 1640, 1610, 1540, 1500, 1460, 1390, 1250, 1140, 1115 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.85 (2H, m), 1.90—2.65 (4H, m), 2.69 (4H, m), 4.40 (1H, m), 5.66 (2H, br s), 6.26 (2H, s), 6.48 (1H, s), 7.30 (2H, d, J=8.2), 7.81 (2H, d, J=8.2), 8.49 (1H, d, J=7.4), 10.57 (1H, s), 11.76 (1H, br s). SIMS m/z: 508 (MH^+). HR-MS (FAB): Calcd for $C_{22}H_{26}N_{11}O_4$ [MH^+]: 508.2168. Found: 508.2173.

N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-N^5-[4-(carboxymethyl)thiazol-2-yl]-L-glutamine (4Pa) mp 203—204 °C. IR (KBr): 3380, 3200, 2930, 1645, 1540, 1500, 1450, 1380, 1330, 1270, 1185, 1170 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.80—2.40 (2H, m), 2.57 (2H, t, J=6.6), 2.97 (4H, m), 3.57 (2H, s), 4.39 (1H, m), 5.73 (2H, br s), 6.37 (2H, s), 6.41 (1H, s), 6.92 (1H, s), 7.33 (2H, d, J=7.8), 7.80 (2H, d, J=7.8), 8.51 (1H, d, J=6.6), 10.57 (1H, br s), 12.12 (1H, s). SIMS m/z: 567 (MH^+). *Anal.* Calcd for $C_{25}H_{26}N_8O_6S \cdot 2.4H_2O$: C, 49.24; H, 5.09; N, 18.37. Found: C, 49.24; H, 4.92; N, 18.17.

N^2-[4-[3-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]-benzoyl]-N^5-[4-(carboxymethyl)thiazol-2-yl]-L-glutamine (4Pb) mp 189—191 °C. IR (KBr): 3330, 3200, 2930, 1640, 1540, 1500, 1450, 1380, 1330, 1270, 1185, 1160 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.85 (2H, m), 2.05 (2H, m), 2.22 (1H, m), 2.56 (2H, t, J=6.2), 2.69 (4H, m), 3.56 (2H, s), 4.38 (1H, m), 5.47 (2H, br s), 6.08 (2H, s), 6.44 (1H, d, J=1.6), 6.90 (1H, s), 7.30 (2H, d, J=8.4), 7.80 (2H, d, J=8.4), 8.49 (1H, d, J=8.0), 10.46 (1H, s). SIMS m/z: 581 (MH^+). *Anal.* Calcd for $C_{26}H_{28}N_8O_6S \cdot 2.0H_2O$: C, 50.64; H, 5.23; N, 18.17. Found: C, 50.73; H, 5.41; N, 18.25.

***N*-[N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]-benzoyl]-γ-L-glutamyl]glycine (4Q)** mp 188—190 °C. IR (KBr): 3400, 3170, 2920, 1635, 1540, 1495, 1455, 1385, 1290, 1250, 1230 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.80—2.15 (2H, m), 2.26 (2H, t, J=5.8), 2.96 (4H, s), 3.68 (1H, d, J=5.6), 3.87 (1H, d, J=5.6), 4.27 (1H, m), 5.48 (2H, br s), 6.12 (2H, br s), 6.38 (1H, s), 7.33 (2H, d, J=8.2), 7.78 (2H, d, J=8.2), 8.11 (1H, t, J=5.6), 8.48 (1H, d, J=7.2), 10.44 (1H, br s). SIMS m/z: 484 (MH^+). *Anal.* Calcd for $C_{22}H_{25}N_7O_6 \cdot 2.7H_2O$: C, 49.66; H, 5.76; N, 18.43. Found: C, 49.44; H, 5.43; N, 18.61.

***N*-[N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]-benzoyl]-γ-L-glutamyl]-L-*tert*-leucine (4R)** mp 200—203 °C. IR (KBr): 3325, 3200, 2950, 2920, 2860, 1640, 1540, 1500, 1475, 1455, 1400, 1370, 1295, 1230, 1180, 1110, 1090 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.93 (9H, s), 1.80—2.22 (2H, m), 2.35 (2H, t, J=6.6), 2.97 (4H, m), 4.12 (1H, d, J=8.8), 4.35 (1H, m), 5.62 (2H, br s), 6.28 (2H, br s), 6.40 (1H, s), 7.33 (2H, d, J=8.2), 7.80 (2H, d, J=8.2), 7.97 (1H, d, J=8.8), 8.56 (1H, d, J=7.4), 10.52 (1H, br s). SIMS m/z: 540 (MH^+). *Anal.* Calcd for $C_{26}H_{33}N_7O_6 \cdot 4.1H_2O$: C, 50.91; H, 6.77; N, 15.98. Found: C, 50.66; H, 6.31; N, 15.76.

***N*-[N^2-[4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]-benzoyl]-γ-L-glutamyl]-L-phenylalanine (4Sa)** mp 180—181 °C. IR (KBr): 3330, 3200, 2920, 1640, 1540, 1500, 1450, 1385, 1340, 1295, 1250, 1215, 1185, 1115, 1085 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.70—2.10 (2H, m), 2.21 (2H, m), 2.97 (6H, m), 4.32 (1H, m), 4.42 (1H, m), 5.92 (2H, br s), 6.45 (1H, s), 6.59 (2H, br s), 7.23 (5H, s), 7.33 (2H, d, J=8.2), 7.80 (2H, d, J=8.2), 8.20 (1H, d, J=7.8), 8.55 (1H, d, J=7.4), 10.71 (1H, br s). SIMS m/z: 574 (MH^+). *Anal.* Calcd for $C_{29}H_{31}N_7O_6 \cdot 2.5H_2O$: C, 56.30; H, 5.87; N, 15.85. Found: C, 56.56; H, 5.83; N, 15.72.

***N*-[N^2-[4-[3-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]benzoyl]-γ-L-glutamyl]-L-phenylalanine (4Sb)** mp 164—166 °C. IR (KBr): 3325, 3200, 2940, 1635, 1540, 1495, 1450, 1385, 1340, 1290, 1250, 1215, 1185, 1115, 1085 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.75—2.10 (4H, m), 2.21 (2H, m), 2.70 (4H, m), 2.84 (1H, dd, J=13.8, 9.8), 3.03 (1H, dd, J=13.8, 5.0), 4.20—4.50 (2H, m), 6.17 (2H, br s), 6.56 (1H, s), 6.77 (2H, br s), 7.21 (5H, s), 7.30 (2H, d, J=8.2), 7.80 (2H, d, J=8.2), 8.18 (1H, d, J=8.0), 8.54 (1H, d, J=7.4), 10.89 (1H, br s). SIMS m/z: 588 (MH^+). *Anal.* Calcd for $C_{30}H_{33}N_7O_6 \cdot 2.0H_2O$: C, 57.78; H, 5.98; N, 15.72. Found: C, 57.72; H, 5.93; N, 15.82.

Cell Lines The parent CCRF-CEM human lymphoblastic leukemia cell line and the MTX-resistant sublines CCRF-CEM R_1 characterized by increased DHFR activity, CCRF-CEM R_{BO} which has normal DHFR levels but impaired MTX transport and CCRF-CEM $R_{30/6}$ characterized by impaired polyglutamylation of MTX were routinely cultured in RPMI 1640 medium supplemented with 10% horse serum, penicillin (100 units/ml) and streptomycin (100 μg/ml) at 37 °C in a 5% CO_2 atmosphere.

Cell Growth Inhibition Assay Meth A cells were grown in MEM supplemented with 10% FBS in an atmosphere of 5% CO_2 at 37 °C. Logarithmically growing cells (4×10^4) in 2.0 ml of medium were seeded in 12-well plates. Test drugs were added at various concentrations prior to the cell seeding. Cells were incubated for 72 h, and the cell number was counted with a Coulter counter, Model ZM (Coulter Electronics Ltd., Luton, England). Exponentially growing CCRF-CEM cells were prepared at a density of $3—5 \times 10^4$ cells/ml and distributed in duplicate 5-ml portions into tissue culture tubes, to which 0.05 ml of drug solutions at various concentrations was added. The cells were incubated at 37 °C. After the indicated time, cell density was determined with a model B Coulter counter (Coulter Electronics, Hialeah, FL).

DHFR Inhibition Assay DHFR activity was measured by optical photometry, with a modification of the method of Bertino.[18] The reaction was carried out at 30 °C in flat-bottomed 96-well plates (Nunc-Immunoplate Maxisorp) pretreated with 1 mg/ml bovine serum albumin, utilizing 0.25 μg protein/ml (1.9 μU/ml) bovine liver DHFR, various concentrations of dihydrofolic acid and 300 μl of a reaction buffer containing 0.1 M Tris–HCl (pH 7.5), 150 mM KCl, 15 mM 2-mercaptoethanol and 125 mM NADPH. The reaction was started by adding a mixture of NADPH and dihydrofolic acid to the reaction buffer containing DHFR and drugs. Changes in the absorbance of NADPH and dihydrofolic acid were measured at 340 nm for 2 min at 2 s intervals using a Titertek Multiskan MCC/340 (Labsystems, Finland) controlled by a personal computer.

TS Inhibition Assay TS activity was measured by the method of Roberts[19] with a slight modification. In brief, 10 μl of crude TS fraction was added to 40 μl of reaction mixture in a flat-bottomed 96-well plate. Final concentrations of constituents in the reaction mixture were as follows; 800 μM tetrahydrofolic acid, 18 mM formaldehyde, 80 μM 5′-dUMP, 0.51 μM [5-^{3}H]5′-dUMP, 10.2 mM 2-mercapthoethanol, 100 mM sucrose, 68 mM NaF, 174 mM Tris–HCl (pH 7.5), 6.24 mg/ml bovine serum albumin and various concentrations of test drugs. The reaction mixture was incubated on a water bath at 37 °C for 1 h and then chilled on ice. Ice-cold 26.65 mg/ml trichloroacetic acid (20 μl), 10 μl of 3.33 mg/ml cold dUMP, and 220 μl of 114 mg/ml charcoal were added to the reaction mixture, and the whole was transferred to a centrifuge tube. After centrifugation, the amount of [^{3}H]H_2O released from [^{3}H]FdUMP in the supernatant of the reaction mixture was measured with a liquid scintillation counter.

***In Vivo* Antitumor Test** All tumors were transplanted subcutaneously on the side of the abdomen of mice on day 0. Meth A cells (1×10^6) or 0.1 ml of Colon 26 homogenate (1/5 dilution) were transplanted in BALB/c or CDF1 mice. P388 (1×10^6) cells were transplanted in BDF1 mice. Groups of 5 mice for each dosage regimen were treated intravenously with test drug solution (0.2 ml/20 g body weight) every other day for solid tumors (Meth A and Colon 26) or every day for leukemia (P388). Administration of the test drugs was started on day 7 for Colon 26, or at 24 h after tumor transplantation for the other tumors. Mice were killed and tumor weight was measured on day 14, day 21 and day 10 for Meth A, Colon 26 and P388, respectively. The results of treatment, expressed as a percentage of the control (T/C, %), calculated from tumor weights, are considered to reveal significant activity on the basis of the NCI protocols for antitumor agents.

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