Novel 6–5 Fused Ring Heterocycle Antifolates with Potent Antitumor Activity: Bridge Modifications and Heterocyclic Benzoyl Isosters of 2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine Antifolate

Yoshihiko KOTAKE, Tatsuo OKAUCHI, Atsumi IIJIMA, Kentaro YOSHIMATSU, and Hiroaki NOMURA*

Research and Development Division, Eisai Company, Ltd., 5–1–3 Tokodai, Tsukuba-shi, Ibaraki 300–26, Japan.
Received October 31, 1994; accepted January 5, 1995

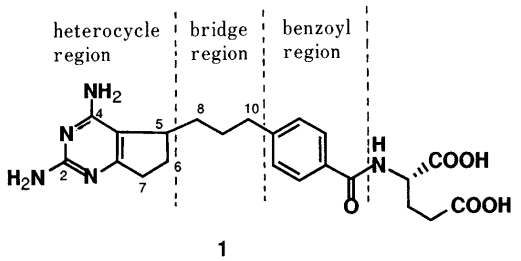
Structural modifications of an extremely potent inhibitor of dihydrofolate reductase (DHFR) activity and tumor cell growth, *N*-[4-[3-(2,4-diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)propyl]benzoyl]-L-glutamic acid (1), have led to the synthesis of new cyclopenta[*d*]pyrimidine-based antifolates, including those with low alkyl substituted trimethylene bridges (2a, b) and isosterically modified bridges (ethyleneoxa, 2c; ethyleneamino, 2d; the *N*-methyl- and *N*-ethyl derivatives of 2d, 2e, f) and those in which the benzene ring of 1 has been replaced by heterocyclic isosters (indole, 2g; indoline, 2h; thiophene, 2i). These new analogs are highly potent as DHFR and cell growth inhibitors, and most of them are more potent than methotrexate (MTX) and 10-ethyl-10-deazapterin (10-EDAM) in inhibiting tumor cell growth (P388 MTX-sensitive and MTX-resistant, colon 26 and KB) on 72 h drug exposure. Among them, 2a (the 10-methyl derivative of 1) and 2i were most potent, being 2- to 3-fold more potent than 10-EDAM. On 4 h drug exposure, the growth-inhibitory activity of these analogs was radically influenced by even minor structural changes. Compounds 1, 2a—e, g—i were much more cytotoxic in colon 26 cell line than were MTX and 10-EDAM, with 2d and 2i being most potent, followed by 2a. Structure–activity relationships and their possible significance are discussed.

Key words 6–5 fused ring heterocyclic antifolate; structure–activity relationship; cell growth inhibition; dihydrofolate reductase; 6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine; methotrexate

Structural modifications in the folate molecule have been extensively studied by many researchers[1] for about four decades. The majority of the modified structures of antifolates have been those containing a 6–6 fused; heterocyclic ring, *e.g.*, the pteridine (methotrexate, MTX; 10-ethyl-10-deazaaminopterin, 10-EDAM[2]), the 5,10-dideaza-5,6,7,8-tetrahydropteridine (DDATHF[3]), and the quinazoline ring (D1694[4]). Recently a series of antifolates with a 6–5 fused heterocyclic ring (pyrrolo[2,3-d]pyrimidine antifolates) have been reported, as exemplified by TNP351[5] and LY231514.[6]

The search for new cancer chemotherapy agents in our laboratory has led to the discovery of cyclopenta[*d*]pyrimidine-based antifolates,[7,8] a new class of 6–5 fused ring antifolates. 2,4-Diamino-6,7-dihydrocyclopenta[*d*]pyrimidine antifolate containing the trimethylene bridge (**1**),[7] proved to be an extremely potent inhibitor of dihydrofolate reductase (DHFR) and was shown to be more growth-inhibitory to a number of tumor cell lines than were MTX and 10-EDAM. It was therapeutically effective against several experimental tumors in mice with a potency higher than that of MTX and comparable to that of 10-EDAM. A shorter bridged analog of this series which contained the ethylene bridge[9] showed potent enzyme inhibition and highly potent cell growth inhibition, but was slightly less potent than **1** on direct comparison *in vitro*. The structure of the bridge region controls the conformational flexibility of the molecule, thereby determining whether it can interact suitably with the DHFR active site.[10] Therefore modification in the bridge region of antifolate structures has been one of the major determinants modulating the antifolate activity at both enzyme and cellular levels.[1] Thus, the trimethylene series, which are probably better inhibitors, as exemplified by **1**,[7] than the ethylene series,[9] should be further studied. Heterocyclic replacement of the benzene ring of **1** has also been another site of interest from the structure–activity relationship (SAR) point of view.[11] Certain analogs with thiophene[11,12] and/or indole[13] rings have been known to possess higher cytotoxic activities than the corresponding benzene counterparts. For this reason, compounds with the thiophene (**2i**), indole (**2g**) or indoline ring (**2h**) were added to our synthetic targets. Substitutions of other amino acids for L-glutamate in classical folate analogs has had only limited success, and compounds containing L-glutamate have been shown to be generally the most potent[14] as folate-relating enzyme inhibitors. These findings stimulated us to explore SAR in the present series of antifolates, in which the structures of the bridge region and/or the benzoate region are further modified. Work on these modifications was pursued under the restrictions of a three-atom chain for the bridge length in the molecule, and L-glutamyl as the amino acid moiety.

In the present paper we describe the synthesis and inhibitory activity of a series of cyclopenta[*d*]pyrimidine-based antifolates containing a variety of bridge structures,



1

* To whom correspondence should be addressed.

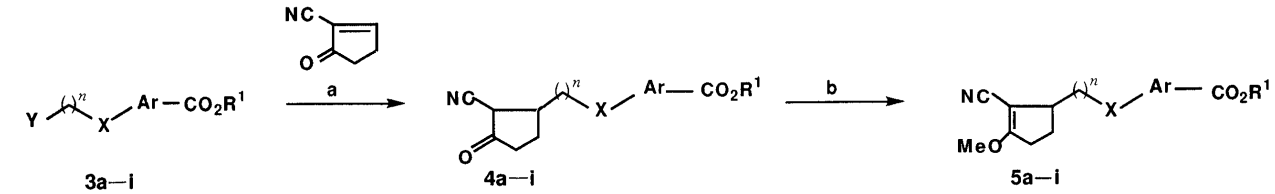
e.g., trimethylene with a low alkyl group ($-CH_2CH_2CHR-$), ethyleneamino ($-CH_2CH_2N^{10}R-$) and ethyleneoxa ($-CH_2CH_2O-$) units, and/or containing an alternative aromatic ring in the benzoyl region (indole, indoline or thiophene ring).

Chemistry

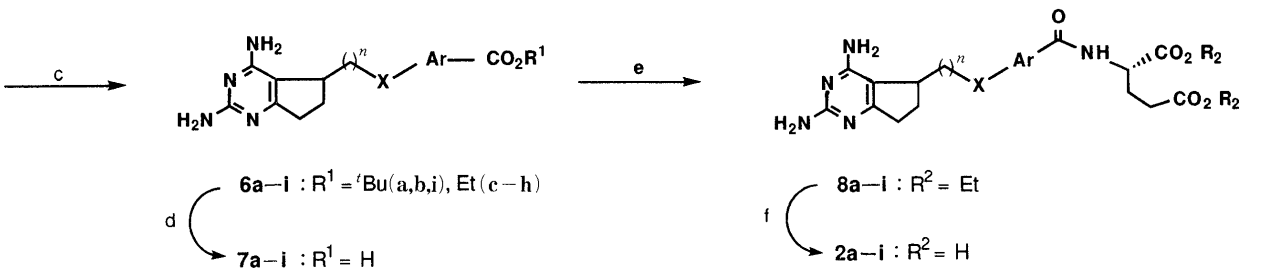
The general approach to the synthesis of these new antifolates with a variety of bridge structures (**2a—i**) involves 1) carbon–carbon radical coupling[15] of appropriately functionalized alkyl halides (**3a—i**) with 2-cyano-2-cyclopenten-1-one[7] in the presence of tributyltin hydride followed by *O*-methylation to yield ω-(2-cyano-3-methoxy-2-cyclopentenyl)alkylbenzoates (**5a, b**), their isosteric hetero atom analogs (**5c—f**) and their heterocyclic isosters of the benzene ring (**5g—i**), 2) cyclization of **5a—i** with guanidine to give 2,4-diamino-6,7-dihydrocyclopenta[*d*]pyrimidines with the corresponding aralkyl moiety at position 5 and 10-hetero atom isosteric side chains (**6a—i**), 3) deprotection to the corresponding carboxylic acid **7a—i**, and 4) amidation with diethyl glutamate and deesterification. The sequence of the reactions is summarized in Chart 1. All of the alkyl halides (**3a—i**) used as coupling partners are novel, and their synthetic routes are outlined in Charts 2 and 3.

An efficient approach to the appropriate propyl halides with the γ-methyl- (**3a**) and γ-ethyl (**3b**) substituents began with a Horner–Emmons reaction[16] using *tert*-butyl 4-acylbenzoate[17] (**9a, b**) and ethyl diethylphosphonoacetate (Chart 2). The resulting ethyl cinnamates obtained as (*EZ*) mixture were subjected to catalytic hydrogenation, followed by hydrolysis with 1 N NaOH to afford the propionic acids (**12a, b**) in good yields. The acids were reduced with excess BH_3–tetrahydrofuran (THF) (freshly prepared from $NaBH_4$ and BF_3–Et_2O) to the corresponding phenylpropanols (**13a, b**), which, on mesylation followed by halogenation using metal halides, gave the desired alkyl halides (**3a, b**) in excellent yields. Alkyl halides containing a hetero atom (**3c**, X = oxygen; **3d—f**, X = nitrogen) at position 10 of **1** were prepared smoothly from ethyl 4-hydroxy- or 4-aminobenzoate (**10c, d**) as shown in Chart 2.

O-Alkylation of the sodium salt of **10c** with ethyl bromoacetate yielded the diester **11c**. *N*-Alkylation of ethyl 4-aminobenzoate (**10d**) with ethyl bromoacetate in the presence of *N,N*-diisopropylethylamine gave compound **11d** in high yield. The *N*-methyl (**11e**) and *N*-ethyl derivatives (**11f**) were obtained from **11d** by treatment with dimethyl sulfate or diethyl sulfate, respectively, in the presence of $NaHCO_3$. On alkaline hydrolysis (1 N NaOH) of the diesters (**11c—f**), saponification occurred selectively at the aliphatic ester group to give the monoacids (**12c—f**). Reduction of **12c—f** with BH_3–THF followed by mesylation and halogenation gave the alkyl halides containing a hetero atom (**3c—f**). In the latter case, no aziridine was formed during the course of

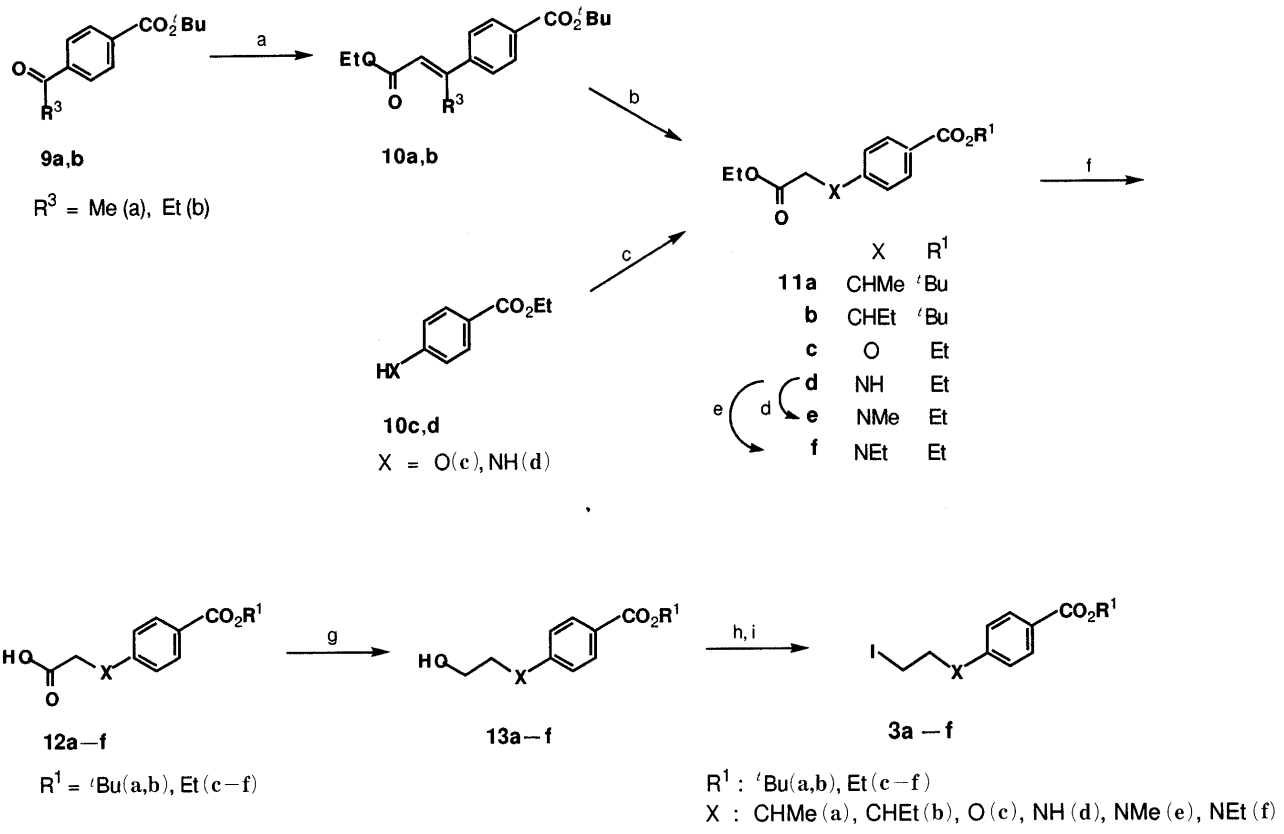


R1: tBu(a,b,i), Et(c–h)
Ar : benzene(a–f), indole(g), indoline(h), thiophene(i)
X : CHMe(a), CHEt(b), O(c), NH(d), NMe(e), NEt(f), CH_2(g–i)
n : 2(a–f,i), 1(g,h)
Y : I(a–h), Br(i)



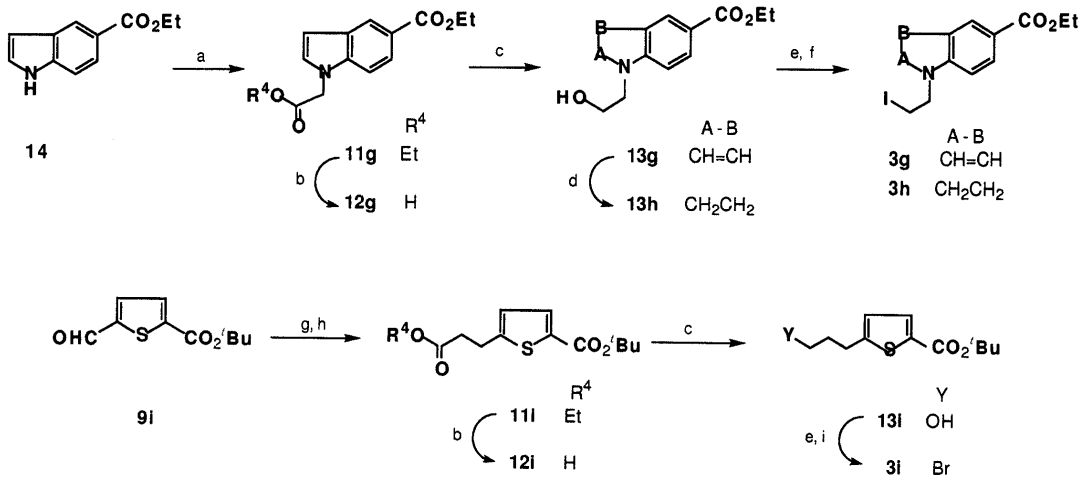
a: Bu_3SnH, AIBN; b: $TMSCHN_2$, iso-Pr_2EtN, MeOH; c: guanidine carbonate; d: 1N HCl-AcOH or 1N NaOH
e: DPPA (or CDI), diethyl L-glutamate HCl, Et_3N; f: 1N NaOH

Chart 1



a: $(EtO)_2POCH_2CO_2Et$, NaH; b: Pd/C, H_2,; c: NaH or iso-Pr_2EtN, $BrCH_2CO_2Et$;
d: $(MeO)_2SO_4$, $NaHCO_3$; e: $(EtO)_2SO_4$, $NaHCO_3$; f: 1N NaOH; g: BH_3 -THF;
h: MsCl, Et_3N i: NaI

Chart 2



a: NaH, $BrCH_2CO_2Et$; b: 1N NaOH; c: BH_3-THF; d: $NaBH_3CN$; e: MsCl, Et_3N;
f: NaI; g: $(EtO)_2POCH_2CO_2Et$, NaH; h: Pd-C, H_2; i: LiBr

Chart 3

halogenation of the intermediary amino alcohols (**13d—f**).

Indole (or indoline) isosters[13] of the folate analogs (**2g, h**), in which the nitrogen atom at position 10 is incorporated into an indole (or indoline) ring, were prepared by the synthetic methods shown in Charts 1 and 3. Alkyl halides containing the indole-5-carboxylate (**3g**) or indoline-5-carboxylate moiety (**3h**) were prepared from ethyl indole-5-carboxylate (**14**) as shown in Chart 3. *N*-Alkylation of **14** with ethyl bromoacetate, followed by alkaline hydrolysis, gave the acetic acid (**12g**). Reduction of **12g** with BH_3–THF gave the indol-1-ylethanol **13g**, and further reduction by the method of Gribble[18] led to the indolin-1-ylethanol **13h**. These alcohols (**13g, h**) were converted to the corresponding ethyl iodides (**3g, h**) in a similar way to that described above. The synthesis of the thiophene analog **3i** is outlined in Charts 1 and 3. *tert*-Butyl 5-formylthiophen-2-carboxylate (**9i**)[19] was converted to the propionic acid (**12i**). Borane reduction of **12i** and mesylation of the resulting alcohol **13i**, followed by bromination, gave the bromide (**3i**).

Preparation of the key intermediates (**4a—i**) began with the radical coupling reaction[15] of 2-cyano-2-cyclopenten-1-one with the coupling partner (**3a—i**) in the presence of tributyltin hydride, which yielded the corresponding 2,3-disubstituted cyclopentanones **4a—i**. Because of poor stability during the subsequent annulation reaction, these cyclopentanones (**4a—i**) were converted, by reaction with trimethylsilyl)diazomethane ($TMSCHN_2$), to the methyl enol ethers **5a—i**, which, on cyclization with guanidine carbonate, afforded 2,4-diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidines **6a—i**. Saponification of the ester group of **6a—i** proceeded with 1 N HCl–AcOH or 1 N NaOH to yield the acids **7a—i**. Condensation of **7a—c, g—i** with diethyl L-glutamate in the presence of DPPA progressed smoothly and yielded the diesters of **8a—c, g—i**. In contrast, the acids **7d—f** failed to undergo amidation under similar conditions, but when *N,N*-carbonyldiimidazole was used instead of DPPA, compounds **7d—f** underwent amidation successfully to yield **8d—f**. Finally, upon deesterification, these antifolate diesters (**8a—i**) led to the corresponding antifolate diacids **2a—i**. This series of antifolates has a chiral center at position 5 besides the α-carbon of the L-glutamate moiety. Compounds **2a, b** are also chiral additionally at position 10. Therefore, synthesis according to Chart 1 gave compounds as a mixture of either two (**2c—i**) or four (**2a, b**) diastereomers which could not be separated by conventional means.[20]

Results and Discussion

Modification in the bridge region involved 1) introduction of a lower alkyl group at position 10 of **1** and 2) isosteric replacement of C^{10}-methylene by a hetero atom, resulting in the new cyclopenta[*d*]pyrimidine antifolates which possessed bridge structures consisting of either $-CH_2CH_2CHR-$ (**2a, b**), $-CH_2CH_2NR-$ (**2d—f**) or $-CH_2CH_2O-$ (**2c**). These novel 6–5 fused heterocyclic antifolates were examined for their inhibition of DHFR[21] purified from bovine liver (Sigma, D-6385) and compared with MTX and 10-EDAM[22] as positive controls. Compounds with the benzoyl ring (**2a—f**) were all found to be highly inhibitory and approximately similar in potency (Table 1).

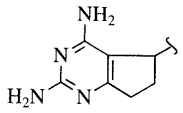
The C^{10}-methyl analog **2a** showed DHFR-inhibitory activity 1.7- and 2.5-fold more potent than those of **1** and 10-EDAM, respectively, and comparable to that of MTX. The C^{10}-ethyl analog **2b** was slightly less potent than **1** and MTX but comparable to 10-EDAM. The (N^{10}-methyl)- and (N^{10}-ethyl)ethyleneamine analogs (**2e, f**) were found to resemble each other very closely in their levels of activity, comparable to that of **1**. The other compound, the N^{10}-hydrogen compound (**2d**), which was 2.4-fold less potent than **1**, was the least active enzyme inhibitor among these ethyleneamino-bridge compounds. The ethyleneoxa-bridge compound (**2c**) showed activity 4-fold less than that of **1**. Thus, replacement of the C^{10}-methylene by a hetero atom (N or O) was an unfavorable modification as regards DHFR inhibition.

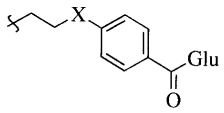
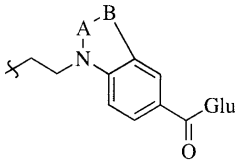
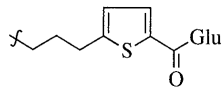
Heterocyclic analogs of **1** (**2g—i**) were found to retain a high level of DHFR-inhibitory activity. The thiophene isoster (**2i**), whose ring size is smaller than that of the phenyl group, proved to be a little more potent than the parent benzene compound **1** as an inhibitor of DHFR. Potencies of the indole analogs (**2g, h**) were approximately comparable to those of **1** and 10-EDAM. These results indicate that structural modification of the central region in this series, including changes in the size of 10-substituents and annular rings, has generally little effect on target DHFR inhibition, suggesting that the DHFR cavity[10] has an open region able to accommodate bulky groups (10-substituents or bicyclic rings) in the central region and to hold the molecule strictly in a favorable way required for binding to the enzyme active site.

The present series of antifolates were examined for growth inhibition[23] of four tumor cell lines, *i.e.*, P388 mouse leukemia cells (MTX-sensitive and MTX-resistant sublines), colon 26 mouse colorectal carcinoma and KB human epidermoid carcinoma cells, on prolonged exposure (72 h), in comparison with MTX and 10-EDAM. The results are shown in Table 1 as the concentration required to inhibit cell growth by 50% (IC_{50}). Most of the compounds were highly inhibitory to the growth of cells, and their potencies—except that of the ethyleneoxa compound (**2c**)—were as much as 1 log order greater than that of MTX. The IC_{50} values of these compounds were found to be at a similar level, with a range of less than 5 nM (0.9—3.4 nM), against P388 (MTX-sensitive), colon 26 and KB cells, as compared with 19—31 nM for MTX and 3—4.8 nM for 10-EDAM, respectively. Compounds which potently inhibited the growth of P388 cells (MTX-sensitive) tended to inhibit the growth of other cell lines (MTX-resistant P388, colon 26 and KB cells) potently as well.

The potencies of compounds in this series were considered to be mediated by DHFR inhibition as the sole locus of action.[8] In general terms, the difference in potency as DHFR inhibitors (except for **2d**) was similar to the difference in potency as cell growth inhibitors on 72 h drug exposure, suggesting a possible cause-and-effect relationship. Previous reports have shown that variable potency of antifolates as cell growth inhibitors is determined by differences in biochemical parameters.[24]

Table 1. DHFR-Inhibitory and Tumor Cell Growth-Inhibitory Activities



Compound	IC_{50} (nM[a])				
	DHFR	P388	P388 : MTXr (E-2)	Colon 26	KB
MTX	1.3	23.0	223	31.0	19.0
10-EDAM	3.8	4.3	44	4.8	3.0
					
X					
1 : CH_2	2.5	2.5	19	3.0	1.7
2a : HCMe	1.5	2.6	27	1.8	1.2
2b : HCEt	3.9	2.2	26	2.7	2.3
2c : O	10.0	6.9	56	5.7	5.3
2d : NH	5.9	1.7	18	2.0	1.7
2e : NMe	3.1	2.2	22	3.8	1.9
2f : NEt	3.0	3.4	29	4.4	1.6
					
A–B					
2g : CH=CH	3.8	2.6	29	3.0	1.7
2h : CH_2–CH_2	2.5	3.2	25	4.2	3.4
					
2i :	1.9	2.3	19	1.8	0.96

a) The method of measurement is described in Experimental.

Elevated potencies of some of these compounds were shown to result from their highly efficient uptake into the cells and greater substrate activity for folylpolyglutamate synthase (FPGS),[8,25] in addition to their elevated potency against DHFR.

Based on Table 1, the C^{10}-methyl (**2a**) and C^{10}-ethyl derivatives (**2b**) were approximately equipotent to **1**, though the magnitude of the cytotoxic activity was cell-dependent, and favorable for cell growth-inhibition which caused a 8- to 17-fold and 1.3- to 2.7-fold increase in potency over MTX and 10-EDAM, respectively. Compared with low alkyl introduction onto the trimethylene bridge, isosteric replacement of the C^{10}-methylene by a –NH– or low alkyl-introduced amine moiety (–NR–), which gave **2d—f**, had only a slight effect, as far as cell growth inhibition on prolonged exposure was concerned. The N^{10}-hydrogen (**2d**) and N^{10}-methyl derivative (**2e**) showed a potency close to that of **1**, and the replacement by NEt (**2f**) resulted in the same level or a slight decrease in potency, depending on the cell lines. Of interest is the observation that **2d** was highly cell growth-inhibitory in spite of its modest DHFR-inhibitory potency. The 4-fold decrease in the enzyme inhibition of **2d** when compared with that of **2a** was not reflected in its cell growth inhibition, which was equipotent to **2a**. A similar trend was observed upon comparison of **2d** with **2f** and/or others. The ethyleneoxa compound, **2c**, was less cytotoxic than others in this series, but nevertheless the IC_{50} values were less than 0.1 μM.

Another interesting modification is the heterocyclic replacement of the benzene ring of **1** by either thiophene, indole or indoline.[11–13] As shown in Table 1, heterocyclic isosters, **2g—i**, were found to be highly cytotoxic with a level of potency approximately similar to those of the parent benzene compound **1** and 10-EDAM. These results suggest that C^{10}-alkyl substitution, isosteric replacement of the C^{10} atom by the NH or *N*-alkyl group and/or replacement by heterocycles in the central aromatic region do not cause a great variation in potency on 72 h exposure. The maximum difference in IC_{50} values was 5.5-fold, observed on comparison between **2i** and **2c** against KB cells.

Against MTX-resistant P388 cells (E-2 subline), which were shown to be resistant as a result of impaired polyglutamation,[25] compounds **1** and **2a—i** inhibited to a much greater extent (4- to 13-fold) than MTX

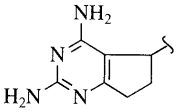
(IC_{50} = 223 nM), but the resistance could not be said to have been overcome (IC_{50} for **1** and **2a**—**i** = 18—56 nM; the degree of cross-resistance is 7- to 12-fold). Cross-resistance of the P388 subline E-2 suggests that substrate activity for FPGS is critical for the elevation of cytotoxic activity. A striking result observed with **1** was markedly low cross-resistance with MTX (14-fold)[8] in assays against CCRF-CEM/R2 cell line (a subline with a severe defect in MTX uptake), which is resistant to MTX (190-fold).

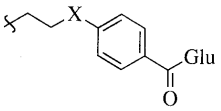
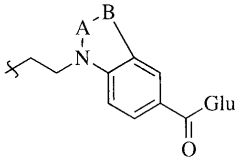
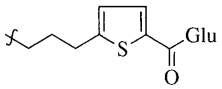
As mentioned above, large differences in potency could not be demonstrated among these analogs during prolonged exposure. However, differences in potency due to structural changes were found to vary depending on drug exposure time and to increase markedly when determined on short-term exposure.[24,26] When cells (colon 26) were exposed for 4 h to a range of concentrations of each drug and allowed to grow in a drug-free medium, the IC_{50} values increased and the differences in potency among the compounds were amplified. The ratios of the values for 4 h exposure to those for 72 h exposure were 20- to 600-fold for the present series of compounds (except **2f**), over 1000-fold for MTX and over 6000-fold for 10-EDAM, indicating that the activities of the 5,6-dihydrocyclopenta[*d*]pyrimidine antifolates (**1** and **2a**—**e**, **g**, **i**) are less dependent on exposure time than are those of the reference compounds. Among the members in the present series, relatively larger time dependence was observed with the N^{10}-alkyl introduced ethyleneamine compounds (**2f** > **2e** > **2d**), where the dependence tended to increase with increasing size of the alkyl group. The smaller time dependence of the potency may result from their increase in FPGS substrate activity and cellular uptake.[8,27] The compounds (except for **2f** and **2h**) inhibited the cell growth with IC_{50} values in the range of 0.052—2.5 μM, being much more potent than were MTX and 10-EDAM (IC_{50} values > 40 μM). Thus, the difference in potency between the two classes of antifolates, the present series (**1** and **2a**—**e**, **g**, **i**) and the pteridine series (MTX and 10-EDAM), was enlarged to 16- to over 800-fold.[28]

The relative activity as cell growth inhibitors on short-term exposure was in the order **2d** > **2i** > **2a** > **2b** > **2e** > **2c** > **1** > **2g** > **2h** ≫ **2f**, 10-EDAM, MTX (32 : 20 : 9.3 : 6.4 : 4.3 : 3.8 : 1 : 0.68 : 0.16 : < 0.04 : < 0.04 : < 0.04). The differences of activity among these compounds were markedly greater as compared to the corresponding differences on prolonged exposure. The ethyleneamino compound (**2d**) was most potent, followed by the thiophene isoster (**2i**), and the methyl- and ethyl-substituted trimethylene compounds (**2a**, **b**). The N^{10}-methyl derivative **2e** retained a high level of potency, while the N^{10}-ethyl derivative (**2f**) showed a marked drop in potency (IC_{50} = > 40 μM). Thus, there was a difference of over 800-fold in potency between **2d** and **2f** as cell growth inhibitors.

Viewed differently, Table 2 shows that alkylation at N^{10}, but not at C^{10}, decreases cell growth inhibition with increasing the steric bulk of the substituent. Such an inverse relation could not be observed with the C^{10}-alkyl compounds because even the C^{10}-ethyl compound (**2b**) was more potent than **1**. Thus, for reasons which are not clear, the C^{10}-alkyl compounds are superior to **1** and the N^{10}-alkyl-introduced ethyleneamines (**2e**, **f**) as far as cell growth inhibition on short-term exposure is concerned.

Table 2. Growth Inhibition of Colon 26 Mouse Colorectal Carcinoma Cells on 4 h Drug Exposure



Compound	Growth inhibition IC_{50} (μM[a]) 72 h	4 h
MTX	0.047	> 40
10-EDAM	0.0064	> 40
 X		
1 : CH_2	0.0027	1.67
2a : HCMe	0.0024	0.18
2b : HCEt	0.0023	0.26
2c : O	0.0085	0.44
2d : NH	0.0026	0.052
2e : NMe	0.0029	0.39
2f : NEt	0.0038	> 40
 A–B		
2g : CH = CH	0.0042	2.5
2h : CH_2–CH_2	0.0040	10.5
		
2i :	0.0013	0.082

a) The method of measurement is described in Experimental. The conditions were slightly different from those for Table 1.

Based on a series of our recent biochemical and cell growth inhibition studies, the large difference in potency between **2d** and **2f** is likely to be due not to a difference in DHFR-inhibitory activity but to differences in transport ability and FPGS substrate activity in cells.[8,29] The highly enhanced cytotoxicity of **2d** appeared to result from increased cell membrane transport and very good substrate activity for FPGS.[29] Greater antifolate uptake and retention function of polyglutamates are generally important for potency on short-term exposure.[24] Our earlier finding that **2d** is most potent in this series against colon 26 cells and CCRF–CEM human leukemia cells[8] is of interest in connection with the bridge structure, which is the most similar in the present series to that of the folic acid molecule. Although aminopterin (AMT) has been well known for its extremely high level[1] of both cellular uptake and substrate activity for FPGS, the N^{10}-hydrogen derivative **2d** was found to be superior to AMT in these biochemical criteria.[8]

The ring size of the central region of the molecule appears to be an important factor in cell growth inhibition

on short-term exposure. When the benzene ring of **1** was replaced by thiophene, 20-fold enhancement in potency over **1** was observed. Replacement of the benzene ring by a heterobicyclic ring (indole or indoline) caused a decrease (1.5- and 6.3-fold) in activity on short-term exposure. The indoline compound **2h** with its bulkier attendant with the partial loss of planarity was 4-fold less potent than the indole compound (**2g**), but still showed at least 4-fold enhancement in potency over **2f**. Compound **2f** can be regarded as a pyrroline ring-opened analog of the indoline compound **2h**. Earlier reports have indicated that the substrate activity for CCRF–CEM FPGS was in the order AMT > MTX > naphthoyl analog of MTX (K_m = 4.1, 42 and 82 μM)[30] and **2d** > **2e** > AMT > MTX (K_m = 1.5, 3.3, 5.0, 63 μM, respectively),[8] indicating that the effect on the intracellular metabolism to form γ-polyglutamate derivatives varies inversely with the size of the 10-substituent and/or with the size of the central ring system in the molecule. Thus, introduction of a bulky substituent onto the ethyleneamino nitrogen (*e.g.*, **2f**) and/or isosteric replacement by a larger ring (**2g, h**) in the central region does not appear to be of great advantage in terms of cell growth inhibition on short-term drug exposure.

The sensitivity of the cells to short-term drug exposure might provide more information about the efficacy of antifolates *in vivo*, and should be a reliable indicator for predicting responsiveness in mice bearing the same tumor.[26] Recently, we reported good *in vivo* effectiveness of **1** against colon 26 carcinoma, which responded poorly to MTX and 10-EDAM.[7] This correlated with the *in vitro* anti-colon 26 cell activities of these three antifolates on short-term exposure—that is, the fact that colon 26 colorectal carcinoma cells are highly sensitive to **1**, and less sensitive to MTX and 10-EDAM. Based on the extension of this relationship, compounds **2a—e, g, i**, which possess potencies comparable to or higher than that of **1** on short-term exposure, can be predicted to be effective against colon 26 in mice, while **2f** should be inactive. The *in vivo* efficacies of these compounds will be reported elsewhere.

Experimental

Column chromatography was performed on silica gel (Merck, particle size 0.063—0.200 mm for normal chromatography and 6.3—40 μ for flash chromatography). All melting points were determined on a Yanagimoto micromelting point apparatus without correction. IR spectra were obtained on a Nicolet 205 FT-IR spectrometer. ^{1}H-NMR spectra were measured on a Varian Unity 400 (400 MHz) spectrometer, and chemical shifts are expressed in δ units from tetramethylsilane (TMS) as an internal standard; coupling constants (J) are reported in hertz. Abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad peak; Hz, hertz. Mass spectra (MS) were obtained on a JEOL HX100 mass spectrometer. Elemental analysis were carried out at the Analytical Chemistry Section of Eisai Tsukuba Research Laboratories.

Ethyl 3-[4-(*tert*-Butoxycarbonyl)phenyl]-3-methylacrylate (10a) A solution of ethyl (diethylphosphono)acetate (18 g, 80 mmol) in THF (50 ml) was added dropwise to a stirred suspension of NaH (60% oil dispersion in mineral oil, 3.2 g, 80 mmol, washed with hexane 10 ml × 2) in THF (50 ml) at 0 °C over a period of 30 min. After stirring for 30 min at room temperature (r.t.) the mixed suspension became clear. Then a solution of *tert*-butyl 4-acetylbenzoate (**9a**, 16 g, 73 mmol) in THF (150 ml) was added dropwise at r.t. over a 30-min period and the mixed solution was warmed to 60 °C and stirred continuously at this temperature for 3 h. The reaction mixture was poured into an aqueous $NaHCO_3$ solution (150 ml). The organic layer was washed with brine (150 ml × 2), and dried over $MgSO_4$. This was evaporated *in vacuo* to afford **10a** as a pale yellow oil (20 g, quant.).

***tert*-Butyl 4-(2-Ethoxycarbonyl-1-methylethyl)benzoate (11a)** An ethanol solution (100 ml) of **10a** (20 g, 69 mmol) was subjected to hydrogenation in the presence of 10% Pd on charcoal under a H_2 atmosphere. After filtration, the solution was evaporated to give **11a** as a colorless oil (20 g, quant.). ^{1}H-NMR ($CDCl_3$) δ: 1.18 (3H, d, J = 7.2 Hz), 1.30 (3H, d, J = 6.8 Hz), 1.58 (9H, s), 2.55 (1H, dd, J = 15.2, 7.2 Hz), 2.61 (1H, dd, J = 15.2, 7.2 Hz), 3.27—3.38 (1H, m), 4.00—4.12 (2H, m), 7.26 (2H, d, J = 8.0 Hz), 7.91 (2H, d, J = 8.0 Hz).

3-[4-(*tert*-Butoxycarbonyl)phenyl]-3-methylpropionic Acid (12a) A solution of **11a** (20 g, 69 mmol) in EtOH (200 ml) was treated with 1 N NaOH (80 ml). The mixture was stirred for 3 h at r.t. and evaporated to dryness. The residue was dissolved in water, and to this solution 1 N HCl (80 ml) was added. The white precipitate that formed was collected by filtration, washed with water, and dried to give **12a** (18 g, 98%). Colorless prisms (hexane), mp 68—70 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.34 (3H, d, J = 6.8 Hz), 1.60 (9H, s), 2.62 (1H, dd, J = 11.6, 3.6 Hz), 2.69 (1H, dd, J = 11.6, 3.6 Hz), 3.34 (1H, dt, $J_d = J_t$ = 7.2 Hz), 7.27 (2H, d, J = 8.4 Hz), 7.94 (2H, d, J = 8.4 Hz).

***tert*-Butyl 4-(3-Hydroxy-1-methylpropyl)benzoate (13a)** A stirred suspension of $NaBH_4$ (11.6 g, 306 mmol) in THF (150 ml) was treated dropwise with $BF_3 \cdot Et_2O$ (58 g, 408 mmol) over 30 min at −78 °C under N_2. The mixture was stirred for 1 h at 0 °C and then cooled to −78 °C, and a solution of **12a** (18 g, 68 mmol) in THF (200 ml) was added dropwise over 1 h. The dry ice bath was removed and the reaction mixture was allowed to equilibrate to ambient temperature. The reaction was quenched by adding MeOH dropwise (200 ml), then the mixture was filtered and the filtrate evaporated *in vacuo*. The residue was dissolved in Et_2O (200 ml), washed with brine (50 ml × 4), and dried over $MgSO_4$. The solution was evaporated to give **13a** as a slightly colored oil (16 g, quant.). ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, d, J = 7.2 Hz), 1.58 (9H, s), 1.61 (1H, br s), 1.79—1.93 (2H, m), 2.90—3.01 (1H, m), 3.47—3.63 (2H, m), 7.25 (2H, d, J = 8.0 Hz), 7.92 (2H, d, J = 8.0 Hz).

***tert*-Butyl 4-(3-Iodo-1-methylpropyl)benzoate (3a)** The hydroxy compound **13a** (16 g, 68 mmol) was dissolved in CH_2Cl_2 (100 ml) and treated with methanesulfonyl chloride (10 g, 67 mmol) in the presence of triethylamine (10.1 g, 100 mmol) at −78 °C for 1 h. The reaction mixture was poured into 0.5 M $NaHSO_3$ (100 ml). After partition between CH_2Cl_2 and H_2O, the organic layer was dried over $MgSO_4$ and evaporated to give the mesylate of **13a** as a colorless oil (22 g). ^{1}H-NMR ($CDCl_3$) δ: 1.31 (3H, d, J = 6.8 Hz), 1.59 (9H, s), 1.95—2.14 (2H, m), 2.93 (3H, s), 2.92—3.03 (1H, m), 3.98—4.05 (1H, m), 4.10—4.18 (1H, m), 7.24 (2H, d, J = 8.0 Hz), 7.94 (2H, d, J = 8.0 Hz). The mesylate was dissolved in acetone (300 ml), and NaI (15 g, 0.1 mol) was added. The mixture was refluxed for 12 h and cooled. After filtration, the solution was evaporated to give a residue, which was purified by chromatography on a silica gel column using hexane–AcOEt (4 : 1) as the eluent to give the title compound **3a** (20 g, 94%) as a pale yellow oil. IR (neat): 2974, 2931, 1713, 1610, 1368, 1311, 1293, 1168, 1118, 1018, 850, 775 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.29 (3H, d, J = 6.8 Hz), 1.59 (9H, s), 2.02—2.16 (2H, m), 2.86—3.0 (2H, m), 3.09 (1H, dt, J_d = 10.0 Hz, J_t = 6.4 Hz), 7.26 (2H, d, J = 8.0 Hz), 7.94 (2H, d, J = 8.0 Hz).

***tert*-Butyl 4-(3-Bromo-1-methylpropyl)benzoate (3a′)** This compound was prepared in a manner similar to that described for the iodo compound using NaBr instead of NaI. A pale yellow oil. IR (neat): 2973, 2932, 1713, 1610, 1311, 1293, 1168, 1118, 1018 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.29 (3H, d, J = 6.8 Hz), 1.59 (9H, s), 2.12 (1H, dt, $J_d = J_t$ = 6.8 Hz), 3.03 (1H, dt, $J_d = J_t$ = 6.8 Hz), 3.15 (1H, dt, J_d = 10.0 Hz, J_t = 6.8 Hz), 3.31 (1H, dt, J_d = 10.0 Hz, J_t = 6.8 Hz), 7.25 (2H, d, J = 8.4 Hz), 7.93 (2H, d, J = 8.4 Hz).

***tert*-Butyl 4-[3-(2-Cyano-3-oxocyclopentanyl)-1-methylpropyl]benzoate (4a)** A solution of the methylpropyl iodide (**3a**, 7.9 g, 25 mmol) in benzene (250 ml) was kept at refluxing temperature with constant stirring under N_2. To this, two solutions were added dropwise and simultaneously over a period of 1 h: 2-cyano-2-cyclopenten-1-one (5.4 g, 51 mmol) in benzene (50 ml), and tributyltin hydride (11 g, 37.5 mmol) and a catalytic amount of azobisisobutyronitrile (AIBN) in benzene (50 ml). After addition was complete, the mixed solution was evaporated to dryness. The residue was dissolved in Et_2O (300 ml), washed with saturated KF (150 ml × 3), and filtered. The filtrate was dried over $MgSO_4$ and evaporated to dryness. The residue was purified by chromatography on a silica gel column, with a linear gradient of hexane–AcOEt (4 : 1 to 2 : 1) as the eluent to give **4a** (1.6 g, 18.8%) as a colorless oil. ^{1}H-NMR ($CDCl_3$)

δ: 1.290 (3H × 1/2, d, J=6.8 Hz), 1.294 (3H × 1/2, d, J=6.8 Hz), 1.30—1.84 (5H, m), 1.59 (9H, s), 2.16—2.40 (3H, m), 2.71—2.84 (1H, m), 2.75 (1H, d, J=12 Hz), 7.23 (2H, d, J=8.4 Hz), 7.93 (2H, d, J=8.4 Hz). MS (DI-EI) m/z: 341 (M^+).

***tert*-Butyl 4-[3-(2-Cyano-3-methoxy-2-cyclopentenyl)-1-methylpropyl]-benzoate (5a)** *N*,*N*-Diisopropylethylamine (0.65 g, 5.1 mmol) and a 10% solution of $TMSCH_2N_2$ in hexane (11 g) were added to a solution of **4a** (1.6 g, 4.7 mmol) in MeOH–CH_3CN (1 : 1, 150 ml) and the mixture was stirred for 5 h at r.t. The reaction was quenched with a small amount of AcOH and the mixture was evaporated to dryness. The residue was dissolved in Et_2O, washed with brine, dried over $MgSO_4$, and evaporated to give an oil. This was purified by chromatography on a silica gel column using hexane–AcOEt (4 : 1) as the eluent to give the methyl enol ether **5a** (1.6 g, 96%) as a colorless oil. IR (neat): 2978, 2935, 2203, 1712, 1632, 1612 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.10—1.76 (5H, m), 1.26 (3H, d, 6.8 Hz), 1.58 (9H, s), 1.97—2.10 (1H, m), 2.36—2.46 (2H, m), 2.68—2.85 (2H, m), 3.99 (3H × 1/2, s), 4.01 (3H × 1/2, s), 7.218 (2H × 1/2, d, J=8.4 Hz), 7.221 (2H × 1/2, d, J=8.4 Hz), 7.91 (2H, d, J=8.4 Hz). FAB-MS m/z: 356 (MH^+).

***tert*-Butyl 4-[3-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)-1-methylpropyl]benzoate (6a)** The methyl enol ether **5a** (1.6 g, 4.5 mmol) and guanidine carbonate (2.4 g, 13.5 mmol) were dissolved in *tert*-BuOH (70 ml). The mixture was placed in an autoclave and allowed to react at 160 °C for 12 h. After cooling, the reaction mixture was filtered to remove a precipitate and the filtrate was evaporated to dryness. The residue was chromatographed on a silica gel column using $CHCl_3$–MeOH (10 : 1) as the eluent to give **6a** (0.88 g, 51%) as a colorless powder, mp 104—106 °C. IR (KBr): 3368, 3329, 3184, 2961, 2931, 1711, 1610, 1582, 1445, 1294, 1167, 1119 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.11—1.80 (6H, m), 1.24 (3H × 1/2, d, J=6.8 Hz), 1.25 (3H × 1/2, d, J=6.8 Hz), 1.59 (9H, s), 2.04—2.21 (1H, m), 2.60 (1H, ddt, J=17.2, 9.6, 4.0 Hz), 2.66—2.85 (2H, m), 2.86—3.01 (1H, m), 4.31 (1H, br s), 4.41 (1H, br s), 4.65 (1H, br s), 4.66 (1H, br s), 7.21 (2H, d, J=8.0 Hz), 7.92 (2H, d, J=8.0 Hz). FAB-MS m/z: 383 (MH^+). *Anal.* Calcd for $C_{22}H_{30}N_4O_2 \cdot 1/10H_2O$: C, 68.76; H, 7.92; N, 14.58. Found: C, 67.55; H, 7.86; N, 14.98.

4-[3-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)-1-methylpropyl]benzoic Acid (7a) A mixed solution containing the ester **6a** (0.8 g, 2.1 mmol) and 1 N HCl–AcOH (30 ml) was stirred at r.t. over 2 h and evaporated to dryness to give the corresponding acid **7a** as a colorless powder (0.69 g, quant.). IR (KBr): 3368, 3196, 2966, 2933, 2857, 1656, 1588, 1539, 1455, 1380, 787, 774 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 0.90—1.04 (1/2H, m), 1.19 (3H, t, J=6.8 Hz), 1.20—1.42 (2H, m), 1.44—1.68 (3H × 1/2, m), 2.44—2.65 (1H, m), 2.66—2.78 (1H, m), 2.87—2.96 (1H, m), 5.72 (2H, br s), 5.98 (2H, br s), 7.28 (2H × 1/2, d, J=8.4 Hz), 7.30 (2H × 1/2, d, J=8.4 Hz), 7.84 (2H × 1/2, d, J=8.4 Hz), 7.85 (2H × 1/2, d, J=8.4 Hz). FAB-MS m/z: 327 (MH^+).

***N*-[4-[3-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)-1-methylpropyl]benzoyl]-L-glutamic Acid (2a)** i) DPPA (0.67 g, 2.4 mmol) and Et_3N (0.7 g, 7 mmol) were added to a mixed solution of **7a** (0.38 g, 1.2 mmol) and diethyl L-glutamate hydrochloride (0.56 g, 2.3 mmol) in *N*,*N*-dimethylformamide (DMF) (60 ml) under cooling in an ice bath. The mixture was stirred for 30 min at 0 °C and for an additional 2 h at r.t. The reaction mixture was filtered, and the filtrate was evaporated to dryness. The residue was purified by chromatography on a silica gel column using $CHCl_3$–MeOH (10 : 1) as the eluent to give diethyl *N*-[4-[3-(2,4-diamino-6,7-dihydro-5*H*-cyclopenta-[*d*]pyrimidin-5-yl)-1-methylpropyl]benzoyl]-L-glutamate (**8a**) as a pale yellow oil.

ii) A 1 N NaOH solution (6.5 ml) was added to a solution of **8a** in EtOH (40 ml) and stirred at r.t. for 5 h. The reaction mixture was neutralized by adding 1 N HCl and evaporated to give a residue. This was purified by chromatography on a silica gel column using $CHCl_3$–MeOH–AcOH (10 : 10 : 1) as the eluent. The fractions containing **2a** were collected and evaporated to dryness to give a colorless powder, which was further purified by conversion to the sodium salt in diluted alkali and acidification with diluted HCl. The precipitate that formed was collected and dried over P_2O_5 to give **2a** as a colorless powder (0.21 g, 38%), mp 175—177 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.90—1.39 (5H, m), 1.42—1.68 (3H, m), 1.85—2.09 (3H, m), 2.22—2.44 (3H, m), 2.45—2.74 (2H, m), 2.85—2.95 (1H, m), 4.27—4.39 (1H, m), 5.93 (2H, br s), 6.13 (2H, br s), 7.20—7.30 (2H, m), 7.70—7.80 (2H, m), 8.34—8.44 (1H, m). FAB-MS m/z: 456 (MH^+).

***tert*-Butyl 4-(2-Ethoxycarbonyl-1-ethylethyl)benzoate (11b)** In a manner similar to that described for **10a**, the ketone **9b**[17] (16.5 g, 70 mmol) was converted to 3-aryl-3-ethylacrylate (**10b**) by reaction with ethyl (diethylphosphono)acetate. Catalytic hydrogenation of **10b** yielded the title compound as a colorless oil (20.8 g, 96%). ^{1}H-NMR ($CDCl_3$) δ: 0.77 (3H, t, J=7.2 Hz), 1.16 (3H, t, J=7.2 Hz), 1.52—1.78 (2H, m), 1.58 (9H, s), 2.55 (2H, dd, J=15.2, 7.2 Hz), 2.65 (2H, dd, J=15.2, 7.2 Hz), 2.99—3.13 (1H, m), 3.96—4.07 (2H, m), 7—24 (2H, d, J=8.0 Hz), 7.90 (2H, d, J=8.0 Hz).

3-[4-(*tert*-Butoxycarbonyl)phenyl]-3-ethylpropionic Acid (12b) In a manner similar to that described for **12a**, **11b** (20.5 g, 67 mmol) was hydrolyzed to the acid **12b** (18 g, 97%) as colorless prisms (AcOEt–hexane), mp 87—89 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.78 (3H, t, J=7.2 Hz), 1.52—1.81 (2H, m), 1.58 (9H, s), 2.61 (1H, dd, J=16.0, 7.2 Hz), 2.69 (1H, dd, J=16.0, 7.2 Hz), 3.00—3.10 (1H, m), 7.23 (2H, d, J=8.4 Hz), 7.93 (2H, d, J=8.4 Hz).

***tert*-Butyl 4-(3-Hydroxy-1-ethylpropyl)benzoate (13b)** In a manner similar to that described for **13a**, **12b** (18 g, 65 mmol) was reduced to the alkanol **13b** as a colorless oil (15 g, 87%). ^{1}H-NMR ($CDCl_3$) δ: 0.76 (3H, t, J=7.2 Hz), 1.16 (1H, t, J=5.6 Hz), 1.55—1.85 (3H, m), 1.59 (9H, s), 1.92—2.02 (1H, m), 2.63—2.73 (1H, m), 3.38—3.57 (2H, m), 7.21 (2H, d, J=8.0 Hz), 7.92 (2H, d, J=8.0 Hz).

***tert*-Butyl 4-(1-Ethyl-3-iodopropyl)benzoate (3b)** In a manner similar to that described for **3a**, mesylation of **13b** (15 g, 57 mmol), followed by iodination gave **3b**. A pale yellow oil (19 g, 89%). ^{1}H-NMR ($CDCl_3$) δ: 0.77 (3H, t, J=7.2 Hz), 1.59—1.78 (2H, m), 1.59 (9H, s), 1.98—2.10 (1H, m), 2.13—2.25 (1H, m), 2.67—2.72 (1H, m), 2.76—2.86 (1H, m), 3.02—3.10 (1H, m), 7.22 (2H, d, J=8.0 Hz), 7.93 (2H, d, J=8.0 Hz).

***tert*-Butyl 4-[3-(2-Cyano-3-methoxy-2-cyclopentenyl)-1-ethylpropyl]-benzoate (5b)** In a manner similar to that described for **4a**, the reaction of **3b** (19 g, 51 mmol) with 2-cyano-2-cyclopenten-1-one gave the 3-oxo compound **4b**, which on *O*-methylation yielded **5b** as a colorless oil (11 g, 58%). IR (neat): 2966, 2931, 2873, 2204, 1712, 1633, 1610, 1457, 1353, 1293, 1167, 1118, 850 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.74 (3H × 1/2, t, J=7.2 Hz), 0.75 (3H × 1/2, t, J=7.2 Hz), 0.92—1.06 (1H × 1/2, m), 1.22—1.78 (7H + 1H × 1/2, m), 1.59 (9H, s), 1.96—2.09 (1H, m), 2.35—2.52 (3H, m), 2.72—2.82 (lH, m), 4.00 (3H × 1/2, s), 4.01 (3H × 1/2, s), 7.18 (2H × 1/2, d, J=8.0 Hz), 7.18 (2H × 1/2, d, J=8.0 Hz), 7.91 (2H, d, J=8.0 Hz).

***tert*-Butyl 4-[3-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)-1-ethylpropyl]benzoate (6b)** In a manner similar to that described for **6a**, **6b** was prepared by the reaction of **5b** (11 g, 30 mmol) with guanidine carbonate as colorless crystals (4.7 g, 40%), mp 157—159 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.73 (3H × 1/2, t, J=7.2 Hz), 0.75 (3H × 1/2, t, J=7.2 Hz), 1.06—1.78 (7H, m), 1.59 (9H, s), 2.04—2.19 (1H, m), 2.37—2.64 (2H, m), 2.66—3.00 (2H, m), 4.25 (1H, br s), 4.36 (1H, br s), 4.61 (1H, br s), 4.63 (1H, br s), 7.17 (2H, d, J=8.0 Hz), 7.91 (2H, d, J=8.0 Hz). FAB-MS m/z: 397 (MH^+). *Anal.* Calcd for $C_{23}H_{32}N_4O_2 \cdot 0.75H_2O$: C, 67.37; H, 8.23; N, 13.66. Found: C, 67.50; H, 8.03; N, 13.37.

4-[3-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)-1-ethylpropyl]benzoic Acid (7b) Saponification of the ester **6b** (4.4 g, 11 mmol) gave **7b** as a colorless powder (3.7 g, 99%). ^{1}H-NMR (DMSO-d_6) δ: 0.65 (3H × 1/2, t, J=7.1 Hz), 0.68 (3H × 1/2, t, J=7.1 Hz), 0.80—0.92 (1H × 1/2, m), 1.12—1.32 (1H, m), 1.38—1.90 (6H + 1H × 1/2, m), 1.91—2.04 (1H, m), 2.30—2.72 (2H, m), 2.86—2.94 (1H, m), 6.30 (2H, br s), 6.63 (2H, br s), 7.24 (2H × 1/2, d, J=8.0 Hz), 7.26 (2H × 1/2, d, J=8.0 Hz),7.83 (2H × 1/2, d, J=8.0 Hz), 7.84 (2H × 1/2, d, J=8.0 Hz). FAB-MS m/z: 341 (MH^+).

***N*-[4-[3-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)-1-ethylpropyl]benzoyl]-L-glutamic Acid (2b)** Amidation of **7b** (3.5 g, 10 mmol) gave **8b**, which on saponification gave **2b** as a colorless powder (1.9 g, 40%), mp 177—179 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.65 (3H × 1/2, t, J=7.1 Hz), 0.68 (3H × 1/2, t, J=7.1 Hz), 0.83—0.94 (1H × 1/2, m), 1.12—1.31 (1H × 1/2, m), 1.38—1.69 (5H, m), 1.86—2.06 (3H, m), 2.28—2.66 (9H, m), 2.85—2.93 (1H, m), 4.29—4.35 (1H, m), 6.09 (2H, br s), 6.25 (2H, br s), 7.18—7.26 (2H, m), 7.72—7.80 (2H, m), 8.34—8.42 (1H, m). FAB-MS m/z: 470 (MH^+).

Ethyl 4-(Ethoxycarbonyl)phenoxyacetate (11c) A solution of ethyl 4-hydroxybenzoate (**10c**, 11 g, 67 mmol) in THF (50 ml) was added dropwise to a stirred suspension of NaH (60% suspension in mineral oil, 3.0 g, 74 mmol, washed with hexane 10 ml × 2) in THF (50 ml) at 0 °C. Stirring was continued for 30 min at r.t., and the mixture became clear. A solution of ethyl bromoacetate (13 g, 77 mmol) in THF was added at 0 °C, and the mixture was stirred at r.t. for 1 h, then poured into an aqueous solution of Na_2CO_3. The combined organic layers were washed with brine, dried over $MgSO_4$ and evaporated to give **11c** (17 g, 91%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.29 (3H, t, J=7.2 Hz),

1.37 (3H, t, J=7.2 Hz), 4.28 (2H, q, J=7.2 Hz), 4.34 (2H, q, J=7.2 Hz), 6.92 (2H, d, J=8.8 Hz), 8.00 (2H, d, J=8.8 Hz).

4-(Ethoxycarbonyl)phenoxyacetic Acid (12c) The ester **11c** (17 g, 67 mmol) was hydrolyzed to **12c** as colorless needles (14 g, 93%), mp 130—131 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.38 (3H, t, J=7.2 Hz), 4.35 (2H, q, J=7.2 Hz), 4.74 (2H, s), 6.94 (2H, d, J=8.8 Hz), 8.02 (2H, d, J=8.8 Hz).

Ethyl 4-(2-Hydroxyethoxy)benzoate (13c) This was prepared from **12c** (14 g, 62 mmol) as colorless prisms (13 g, quant.), mp 61—63 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.38 (3H, t, J=7.2 Hz), 4.00 (2H, t, J=4.4 Hz), 4.14 (2H, t, J=4.4 Hz), 4.35 (2H, q, J=7.2 Hz), 6.94 (2H, d, J=9.2 Hz), 8.00 (2H, d, J= 9.2 Hz).

Ethyl 4-(2-Iodoethoxy)benzoate (3c) Mesylation of **13c** (13 g, 62 mmol), followed by iodination gave **3c** as colorless needles (16 g, 89%), mp 58.5—59 °C. IR (neat): 2975, 1709, 1608, 1509, 1315, 1278, 1266, 1249, 1172, 1164, 1112, 993, 769 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.38 (3H, t, J=7.2 Hz), 3.44 (2H, t, J=6.8 Hz), 4.31 (2H, t, J=6.8 Hz), 4.35 (2H, q, J=7.2 Hz), 6.92 (2H, d, J=8.8 Hz), 8.00 (2H, d, J=8.8 Hz).

Ethyl 4-(2-Bromoethoxy)benzoate (3c') This was prepared in a manner similar to that described for **3a'**. Colorless needles, mp 75—76 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.38 (3H, t, J=7.2 Hz), 3.66 (2H, t, J=6.0 Hz), 4.35 (2H, t, J=6.0 Hz), 4.35 (2H, q, J=7.2 Hz), 6.93 (2H, d, J=8.8 Hz), 8.01 (2H, d, J=8.8 Hz). FAB-MS m/z: 275 (MH^+). *Anal.* Calcd for $C_{11}H_{13}BrO_3$: C, 48.37; H, 4.80. Found: C, 48.13; H, 4.65.

Ethyl 4-[2-(2-Cyano-3-methoxy-2-cyclopentenyl)ethoxy]benzoate (5c) The reaction of **3c** (7.4 g, 27 mmol) with 2-cyano-2-cyclopentene-1-one gave the 3-oxo compound **4c**, which, on *O*-methylation with $TMSCHN_2$, yielded **5c** as colorless plates (1.25 g, 15%), mp 69—71 °C. IR (KBr): 2981, 2945, 2199, 1702, 1628, 1609, 1510, 1359, 1285, 1258, 1175, 1103, 1051, 975, 846 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.38 (3H, t, J=7.2 Hz), 1.61—1.74 (1H, m), 1.78—1.90 (1H, m), 2.13—2.32 (2H, m), 2.47—2.54 (2H, m), 3.06—3.17 (1H, m), 4.06 (3H, s), 4.12 (2H, t, J=6.4 Hz), 4.34 (2H, t, J=7.2 Hz), 6.90 (2H, d, J=8.8 Hz), 7.99 (2H, d, J=8.8 Hz). FAB-MS m/z: 316 (MH^+). *Anal.* Calcd for $C_{18}H_{21}NO_4$: C, 68.55; H, 6.71; N, 4.44. Found: C, 68.36; H, 6.72; N, 4.33.

Ethyl 4-[2-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)-ethoxy]benzoate (6c) This was prepared by the reaction of **5c** (1 g, 3.2 mmol) with guanidine carbonate (1.7 g, 9.5 mmol) as colorless needles (0.75 g, 69%), mp 218—220 °C. IR (KBr): 3444, 3321, 3158, 2941, 1796, 1687, 1627, 1605, 1574, 1510, 1450, 1257, 1169, 1108, 773 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.39 (3H, t, J=7.2 Hz), 1.80—2.08 (3H, m), 2.20—2.34 (1H, m), 2.64 (1H, ddd, J=17.2, 9.6, 2.4 Hz), 2.83—2.95 (2H, m), 3.26—3.38 (2H, m), 4.03—4.15 (2H, m), 4.36 (2H, q, J=7.2 Hz), 4.69 (2H, br s), 4.71 (2H, br s), 6.93 (2H, d, J=8.8 Hz), 8.01 (2H, d, J=8.8 Hz). FAB-MS m/z: 343 (MH^+).

4-[2-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)eth-oxy]benzoic Acid (7c) Saponification of the ester **6c** (0.62 g, 1.8 mmol) gave **7c** (0.48 g) as a colorless powder. IR (KBr): 3382, 3191, 3093, 2966, 2927, 1667, 1604, 1591, 1549, 1534, 1507, 1467, 1379, 1245, 784 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.64—1.85 (2H, m), 1.94—2.08 (2H, m), 2.32—2.48 (1H, m), 2.58—2.72 (1H, m), 3.00—3.12 (1H, m), 4.04 (2H, t, J=7.2 Hz), 5.63 (2H, br s), 6.01 (2H, br s), 6.94 (2H, d, J=8.8 Hz), 7.84 (2H, d, J=8.8 Hz). FAB-MS m/z: 315 (MH^+).

Diethyl *N*-[4-[2-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)ethoxy]benzoyl]-L-glutamate (8c) Amidation of **7c** (0.48 g) with diethyl L-glutamate hydrochloride (0.73 g, 3 mmol) gave **8c** as a pale yellow oil.

***N*-[4-[2-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)-ethoxy]benzoyl]-L-glutamic Acid (2c)** Saponification of the diester **8c** gave **2c** (0.16 g, 20% from **7c**) as a colorless powder, mp 167—169 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.66—2.10 (6H, m), 2.27—2.35 (2H, m), 2.36—2.48 (1H, m), 2.60—2.74 (1H, m), 3.01—3.07 (1H, m), 4.05 (2H, t, J=6.8 Hz), 4.29—4.38 (1H, m), 5.82—5.98 (2H, br), 6.18—6.30 (2H, br), 6.97 (2H, d, J=8.8 Hz), 7.82 (2H, d, J=8.8 Hz), 8.34 (1H, d, J=7.2 Hz). FAB-MS m/z: 444 (MH^+).

Ethyl 4-[*N*-(Ethoxycarbonylmethyl)amino]benzoate (11d) A mixture containing ethyl 4-aminobenzoate (**10d**, 33 g, 0.2 mol), ethyl bromoacetate (36.7 g, 0.22 mol), and *N*,*N*-diisopropylethylamine (28.4 g, 0.22 mol) and DMF (300 ml) was heated at 70 °C for 24 h under N_2. The reaction mixture was evaporated and the residue was dissolved in Et_2O (600 ml), washed with brine (150 ml × 4), dried over $MgSO_4$, and evaporated to dryness. The residue was purified by chromatography on a silica gel column using hexane–AcOEt (3 : 1) as the eluent to give **11d** (44 g, 87%) as pale yellow prisms, mp 62—64 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.31 (3H, t, J=7.2 Hz), 1.36 (3H, t, J=7.2 Hz), 3.95 (2H, d, J=4.4 Hz), 4.27 (2H, q, J=7.2 Hz), 4.37 (2H, q, J=7.2 Hz), 6.57 (2H, d, J=8.8 Hz), 7.90 (2H, d, J=8.8 Hz).

***N*-(4-Ethoxycarbonylphenyl)aminoacetic Acid (12d)** Saponification of diester **11d** (18.8 g, 75 mmol) gave **12d** (16 g, 96%) as colorless needles (hexane–AcOEt), mp 159—161 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.37 (3H, t, J=7.2 Hz), 4.05 (2H, s), 4.33 (2H, q, J=7.2 Hz), 6.60 (2H, d, J=8.8 Hz), 7.92 (2H, d, J=8.8 Hz).

Ethyl 4-[*N*-(2-Hydroxyethyl)amino]benzoate (13d) This was prepared from **12d** (15.8 g, 71 mmol) as colorless prisms (9 g, 61%), mp 61—63 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.36 (3H, t, J=7.2 Hz), 1.55—1.70 (lH, br), 3.37 (2H, t, J=5.2 Hz), 3.87 (2H, t, J=5.2 Hz), 4.32 (2H, q, J=7.2 Hz), 4.40—4.55 (lH, br), 6.60 (2H, d, J=8.8 Hz), 7.88 (2H, d, J=8.8 Hz).

Ethyl 4-[*N*-(2-Iodoethyl)amino]benzoate (3d) Mesylation of **13d** (9 g, 43 mmol), followed by iodination gave **3d** as colorless needles (9.5 g, 69%), mp 83—84 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.37 (3H, t, J=7.2 Hz), 3.34 (2H, t, J=6.8 Hz), 3.60 (2H, t, J=6.8 Hz), 4.32 (2H, q, J=7.2 Hz), 4.36—4.56 (1H, br), 6.59 (2H, d, J=8.8 Hz), 7.89 (2H, d, J=8.8 Hz).

Ethyl 4-[2-[(2-Cyano-3-oxocyclopentan-1-yl)ethyl]amino]benzoate (4d) The reaction of **3d** (9.5 g, 30 mmol) with 2-cyano-2-cyclopenten-1-one gave **4d** as slightly yellow prisms (2.4 g, 27%). ^{1}H-NMR ($CDCl_3$) δ: 1.36 (3H, t, J=7.2 Hz), 1.50—1.70 (1H, m), 1.88—2.10 (2H, m), 2.30—2.46 (2H, m), 2.50—2.64 (2H, m), 2.92 (1H, d, J=12.0 Hz), 3.42 (2H, t, J=7.2 Hz), 4.32 (2H, q, J=7.2 Hz), 6.58 (2H, d, J=8.8 Hz), 7.89 (2H, d, J=8.8 Hz). *Anal.* Calcd for $C_{17}H_{20}N_2O_3 \cdot 0.25H_2O$: C, 66.98; H, 6.78; N, 9.19. Found: C, 67.28; H, 6.67; N, 8.79.

Ethyl 4-[*N*-[2-(2-Cyano-3-methoxy-2-cyclopentenyl)ethyl]amino]ben-zoate (5d) *O*-Methylation of **4d** (2.4 g, 8 mmol) gave **5d** as colorless plates (2.2 g, 88%), mp 95—97 °C. IR (KBr): 3373, 2855, 2204, 1672, 1633, 1601, 1534, 1351, 1284, 1276, 1174, 1128 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.36 (3H, t, J=7.2 Hz), 1.52—1.78 (2H, m), 1.99—2.09 (1H, m), 2.12—2.22 (1H, m), 2.48—2.55 (2H, m), 2.96—3.06 (1H, m), 3.22—3.32 (2H, br), 4.04—4.16 (1H, br), 4.06 (3H, s), 4.31 (2H, q, J=7.2 Hz), 6.56 (2H, d, J=18.8 Hz), 7.87 (2H, d, J=18.8 Hz). FAB-MS m/z: 315 (MH^+). *Anal.* Calcd for $C_{18}H_{22}N_2O_3$: C, 68.77; H, 7.05; N, 8.82. Found: C, 68.59; H, 7.05; N, 8.82.

Ethyl 4-[*N*-[2-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)ethyl]amino]benzoate (6d) The reaction of **5d** (2.2 g, 6.8 mmol) with guanidine carbonate gave **6d** as a colorless crystalline powder (4 g, 60%), mp 228—230 °C. IR (KBr): 3374, 3308, 3190, 2982, 2956, 2925, 2876, 2854, 1674, 1638, 1602, 1574, 1287, 1268, 1175, 1126, 771 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.37 (3H, t, J=7.2 Hz), 1.74—2.00 (3H, m), 2.20—2.33 (1H, m), 2.65 (1H, ddd, J=17.2, 9.2, 2.4 Hz), 2.82—2.95 (1H, m), 3.10—3.32 (3H, m), 4.02—4.12 (1H, br), 4.32 (2H, q, J=7.2 Hz), 4.68 (2H, br s), 4.72 (2H, br s), 6.61 (2H, d, J=8.8 Hz), 7.89 (2H, d, J=8.8 Hz). FAB-MS m/z: 342 (MH^+). *Anal.* Calcd for $C_{18}H_{23}N_5O_2 \cdot 1/5H_2O$: C, 62.66; H, 6.84; N, 20.30. Found: C, 62.65; H, 6.45; N, 20.37.

4-[2-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)ethyl-amino]benzoic Acid (7d) Saponification of ester **6d** (0.61 g, 1.8 mmol) gave **7d** as a colorless powder (0.5 g, 89%). ^{1}H-NMR (DMSO-d_6) δ: 1.38—1.52 (1H, m), 1.72—1.92 (2H, m), 1.94—2.08 (1H, m), 2.40—2.50 (1H, m), 2.62—2.74 (1H, m), 2.98—3.15 (3H, m), 5.95 (2H, br s), 6.37 (1H, t, J=4.8 Hz), 6.43 (2H, br s), 6.54 (2H, d, J=8.8 Hz), 7.64 (2H, d, J=8.8 Hz). FAB-MS m/z: 314 (M^+).

Diethyl *N*-[4-[*N'*-[2-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyri-midin-5-yl)ethyl]amino]benzoyl]-L-glutamate(8d) *N*,*N'*-Carbonyldi-imidazole (CDI, 0.65 g, 4 mmol) was added in small portions to a stirred suspension of **7d** (0.5 g, 1.6 mmol) in anhydrous DMF (40 ml) at 0 °C under N_2, and the mixture was stirred for 1 h at r.t. To this mixed solution, diethyl L-glutamate hydrochloride (1.15 g, 4.8 mmol) and Et_3N (0.49 g, 4.8 mmol) were added. The whole was heated at 70 °C for 24 h under N_2, cooled and filtered, and the filtrate was evaporated to dryness. The residue was purified by chromatography on a silica gel column using $CHCl_3$–MeOH (5 : 1) as the eluent to give **8d** as a pale yellow oil (0.25 g, 31.3%). FAB-MS m/z: 499 (MH^+).

***N*-[4-[N^{10}-[2-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)ethyl]amino]benzoyl]-L-glutamic Acid (2d)** Saponification of **8d** (0.25 g, 0.5 mmol) gave **2d** as a colorless powder (0.10 g, 45%), mp 179—182 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.36—1.51 (1H, m), 1.69—2.07 (1H, m), 2.21—2.52 (3H, m), 2.59—2.72 (1H, m), 3.00—3.16 (3H, m), 4.26—4.35 (1H, m), 5.83 (2H, br s), 6.15 (1H, t, J=4.8 Hz), 6.54 (2H, d, J=8.4 Hz), 7.64 (2H, d, J=8.4 Hz), 8.02 (1H, d, J=7.2 Hz). FAB-MS m/z: 443 (MH^+).

Ethyl *N*-(4-Ethoxycarbonylphenyl)-*N*-methylaminoacetate (11e) A mixture containing the diester **11d** (27.2 g, 108 mmol), $NaHCO_3$ (18.1 g,

216 mmol) and dimethyl sulfate (68.3 g, 540 mmol) was stirred at 90 °C for 1 h under N_2, then evaporated to dryness, and the residue was partitioned between AcOEt and H_2O. The combined organic layers were washed with brine, dried over $MgSO_4$ and evaporated. The residue was purified by chromatography on a silica gel column using hexane–AcOEt (1 : 3) as the eluent to give **11e** (18 g, 63%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, t, J=7.2 Hz), 1.36 (3H, t, J=7.2 Hz), 3.13 (3H, s), 4.11 (2H, s), 4.19 (2H, q, J=7.2 Hz), 4.32 (2H, q, J=7.2 Hz), 6.64 (2H, d, J=8.8 Hz), 7.92 (2H, d, J=8.8 Hz).

***N*-(4-Ethoxycarbonylphenyl)-*N*-methylaminoacetic Acid (12e)** Saponification of the diester amine **11e** (18 g, 68 mmol) in alkaline ethanol gave **12e** as a colorless powder (14.5 g, 55%), mp 110—112 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.36 (3H, t, J=7.2 Hz), 3.13 (3H, s), 4.17 (2H, s), 4.32 (2H, q, J=7.2 Hz), 6.66 (2H, d, J=9.2 Hz), 7.93 (2H, d, J=9.2 Hz).

Ethyl 4-[*N*-(2-Hydroxyethyl)-*N*-methylamino]benzoate (13e) Reduction of **12e** (14.2 g, 60 mmol) with $NaBH_4$ gave **13e** as a colorless oil (12.8 g, 95%). ^{1}H-NMR ($CDCl_3$) δ: 1.37 (3H, t, J=7.2 Hz), 1.58—1.68 (1H, br), 3.07 (3H, s), 3.58 (2H, t, J=5.6 Hz), 3.82—3.89 (2H, m), 4.32 (2H, q, J=7.2 Hz), 6.71 (2H, d, J=9.2 Hz), 7.90 (2H, d, J=9.2 Hz).

Ethyl 4-[*N*-(2-Iodoethyl)-*N*-methylamino]benzoate (3e) Mesylation of **13e** (12.8 g, 57.3 mmol), followed by iodination gave **3e** as a pale yellow oil (17.7 g, 93%). ^{1}H-NMR ($CDCl_3$) δ: 1.37 (3H, t, J=7.2 Hz), 3.08 (3H, s), 3.23 (2H, t, J=7.6 Hz), 3.79 (2H, t, J=7.6 Hz), 4.33 (2H, q, J=7.2 Hz), 6.65 (2H, d, J=9.2 Hz), 7.93 (2H, d, J=9.2 Hz).

Ethyl 4-[*N*-[2-(2-Cyano-3-methoxy-2-cyclopentenyl)ethyl]-*N*-methylamino]benzoate (5e) This was prepared from **3e** (16.7 g, 50 mmol) *via* **4e**. A pale yellow oil (3.1 g, 19%). IR (neat): 2982, 2203, 1699, 1633, 1606 1525, 1463, 1386, 1359, 1279, 1184, 1109 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.36 (3H, t, J=7.2 Hz), 1.54—1.70 (2H, m), 1.96—2.08 (1H, m), 2.10—2.22 (1H, m), 2.48—2.55 (2H, m), 2.83—2.95 (1H, m), 3.02 (3H, s), 3.48 (2H, t, J=7.6 Hz), 4.05 (3H, s), 4.32 (2H, q, J=7.2 Hz), 6.64 (2H, d, J=8.8 Hz), 7.91 (2H, d, J=8.8 Hz). FAB-MS m/z: 329 (MH^+).

Ethyl 4-[*N*-[2-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)ethyl]-*N*-methylamino]benzoate (6e) Compound **6e** was prepared from **5e** (2.2 g, 6.8 mmol) as a colorless crystalline powder (1.9 g, 59%), mp 244—248 °C (dec). IR (KBr): 3372, 3295, 3156, 2981, 2949, 2908, 1679, 1665, 1627, 1598, 1525, 1448, 1278, 1178, 1108, 771 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.37 (3H, t, J=7.2 Hz), 1.50—1.74 (1H, m), 1.82—2.06 (1H, m), 2.20—2.34 (1H, m), 2.69 (1H, ddd, J=17.6, 9.6, 4.0 Hz), 2.81—2.93 (1H, m), 2.99—3.14 (1H, m), 3.02 (3H, s), 3.35—3.55 (2H, m), 4.32 (2H, q, J=7.2 Hz), 4.45 (2H, br s), 4.69 (2H, br s), 6.64 (2H, d, J=8.8 Hz), 7.91 (2H, d, J=8.8 Hz). FAB-MS m/z: 356 (MH^+). *Anal.* Calcd for $C_{19}H_{25}N_5O_2 \cdot 1/5H_2O$: C, 63.56; H, 7.13; N, 19.51. Found: C, 63.56; H, 6.99; N, 19.75.

4-[*N*-[2-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)ethyl]-*N*-methylamino]benzoic Acid (7e) Saponification of the ester **6e** (1.4 g, 4 mmol) gave **7e** (1.7 g, 96%) as a colorless powder. IR (KBr): 3335, 3198, 2947, 1652, 1603, 1527, 1455, 1380, 1319, 1280, 1187, 788 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.32—1.47 (1H, m), 1.50—1.84 (2H, m), 1.94—2.08 (1H, m), 2.35—2.45 (1H, m), 2.58—2.70 (1H, m), 2.90—3.02 (1H, m), 5.63 (2H, br s), 6.09 (2H, br s), 6.69 (2H, d, J=8.8 Hz), 7.69 (2H, d, J=8.8 Hz). FAB-MS m/z: 328 (MH^+).

***N*-[4-[*N'*^{10}-[2-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)ethyl]-*N'*^{10}-methylamino]benzoyl]-L-glutamic Acid (2e)** Compound **2e** was prepared from **7e** (1.2 g, 3.7 mmol) *via* **8e** as a colorless powder (0.4 g, 24%), mp 178—181 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.36—1.48 (1H, m), 1.71—1.83 (2H, m), 1.84—2.10 (3H, m), 2.30 (2H, t, J=7.2 Hz), 2.37—2.50 (1H, m), 2.60—2.74 (1H, m), 2.91 (3H, s), 2.94—3.02 (1H, m), 3.24—3.48 (2H, m), 4.26—4.36 (1H, m), 5.92 (2H, br s), 6.32 (2H, br s), 6.68 (2H, d, J=8.8 Hz), 7.69 (2H, d, J=8.8 Hz), 8.10 (1H, d, J=7.2 Hz). FAB-MS m/z: 457 (MH^+). *Anal.* Calcd for $C_{22}H_{28}N_6O_5 \cdot 1/4H_2O$: C, 55.69; H, 6.37; N, 17.71. Found: C, 55.94; H, 6.39; N, 17.78.

Ethyl *N*-(4-Ethoxycarbonylphenyl)-*N*-ethylaminoacetate (11f) Reaction of **11d** (25 g, 0.1 mmol) with diethyl sulfate (77 g, 0.5 mol) in the presence of $NaHCO_3$ (42 g, 0.5 mol) gave the diester amine **11f** (23 g, 82%).

***N*-(4-Ethoxycarbonylphenyl)-*N*-ethylaminoacetic Acid (12f)** Saponification of **11f** (23 g, 82 mmol) gave **12f** as colorless needles (20 g, 97%), mp 135—137 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.24 (3H, t, J=7.2 Hz), 1.36 (3H, t, J=7.2 Hz), 3.52 (2H, q, J=7.2 Hz), 4.12 (2H, s), 4.32 (2H, q, J=7.2 Hz), 6.62 (2H, d, J=9.2 Hz), 7.92 (2H, d, J=9.2 Hz).

Ethyl 4-[*N*-Ethyl-*N*-(2-hydroxyethyl)amino]benzoate (13f) This was synthesized from **12f** (20 g, 80 mmol) as colorless prisms (11 g, 58%), mp 73—75 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.19 (3H, t, J=7.2 Hz), 1.36 (3H, t, J=7.2 Hz), 1.68 (1H, t, J=5.2 Hz), 3.49 (2H, q, J=7.2 Hz), 3.55 (2H, t, J=6.0 Hz), 3.80—3.88 (2H, m), 4.32 (2H, q, J=7.2 Hz), 6.69 (2H, d, J=9.2 Hz), 7.89 (2H, d, J=9.2 Hz).

Ethyl 4-[*N*-Ethyl-*N*-(2-iodoethyl)amino]benzoate (3f) This was synthesized from **13f** (10.8 g, 45.5 mmol) *via* the mesylate as a slightly yellow oil (11 g, 70%). ^{1}H-NMR ($CDCl_3$) δ: 1.21 (3H, t, J=7.2 Hz), 1.36 (3H, t, J=7.2 Hz), 3.21 (2H, t, J=8.0 Hz), 3.47 (2H, q, J=7.2 Hz), 3.72 (2H, t, J=8.0 Hz), 4.32 (2H, q, J=7.2 Hz), 6.62 (2H, d, J=9.2 Hz), 7.91 (2H, d, J=9.2 Hz).

Ethyl 4-[*N*-[2-(2-Cyano-3-methoxy-2-cyclopentenyl)ethyl]-*N*-ethylamino]benzoate (5f) This was synthesized from **3f** (11 g, 32 mmol) *via* **4f**. Colorless prisms (2.5 g, 23%), mp 73—74 °C. IR (KBr): 2954, 2201, 1691, 1639, 1601, 1525, 1405, 1368, 1356, 1291, 1274, 1183, 769 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.19 (3H, t, J=7.2 Hz), 1.35 (3H, t, J=7.2 Hz), 1.54—1.74 (2H, m), 1.96—2.08 (1H, m), 2.12—2.24 (1H, m), 2.48—2.56 (2H, m), 2.86—3.06 (1H, m), 3.42 (4H, q, J=7.2 Hz), 4.05 (3H, s), 4.31 (2H, q, J=7.2 Hz), 6.61 (2H, d, J=8.8 Hz), 7.88 (2H, d, J=8.8 Hz). FAB-MS m/z: 343 (MH^+). *Anal.* Calcd for $C_{20}H_{26}N_2O_3$: C, 70.15; H, 7.65; N, 8.18. Found: C, 69.89; H, 7.64; N, 8.12.

Ethyl 4-[*N*-[2-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)ethyl]-*N*-ethylamino]benzoate (6f) This was prepared from **5f** (2.5 g, 7.3 mmol) as a colorless crystalline powder (1.8 g, 67%), mp 301—303 °C. IR (KBr): 3481, 3360, 3336, 2983, 2879, 1679, 1656, 1626, 1590, 1525, 1446, 1408, 1369, 1282, 1266, 1183, 1158, 1118, 1107, 771 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.18 (3H, t, J=7.2 Hz), 1.36 (3H, t, J=7.2 Hz), 1.52—1.76 (1H, m), 1.84—2.04 (2H, m), 2.20—2.34 (1H, m), 2.70 (1H, ddd, J=17.7, 9.6, 4.0 Hz), 2.82—2.92 (1H, m), 3.05—3.14 (1H, m), 3.30—3.52 (4H, m), 4.32 (2H, q, J=7.2 Hz), 4.47 (2H, br s), 4.68 (2H, br s), 6.62 (2H, d, J=9.2 Hz), 7.89 (2H, d, J=9.2 Hz). FAB-MS m/z: 370 (MH^+). *Anal.* Calcd for $C_{20}H_{27}N_5O_2$: C, 65.02; H, 7.37; N, 18.96. Found: C, 64.18; H, 7.15; N, 18.94.

4-[*N*-[2-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)ethyl]-*N*-ethylamino]benzoic Acid (7f) Saponification of **6f** (1.5 g, 4.1 mmol) gave **7f** as a colorless powder (1.35 g, 96%). ^{1}H-NMR (DMSO-d_6) δ: 1.10 (3H, t, J=7.2 Hz), 1.40—1.53 (1H, m), 1.76—1.88 (2H, m), 2.00—2.13 (1H, m), 2.47 (1H, ddd, J=17.2, 9.6, 1.6 Hz), 2.66—2.78 (1H, m), 2.97—3.07 (1H, m), 3.24—3.48 (4H, m), 5.90 (2H, br s), 6.39 (2H, br s), 6.69 (2H, d, J=8.8 Hz), 7.70 (2H, d, J=8.8 Hz). FAB-MS m/z: 342 (MH^+).

***N*-[4-[*N'*^{10}-[2-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)ethyl]-*N'*^{10}-ethylamino]benzoyl]-L-glutamic Acid (2f)** This was prepared from **7f** (1.2 g, 3.5 mmol) *via* **8f** as a colorless powder (0.43 g, 26%), mp 176—178 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.07 (3H, t, J=6.8 Hz), 1.37—1.52 (1H, m), 1.75—2.12 (5H, m), 2.29 (2H, t, J=7.2 Hz), 2.45 (1H, ddd, J=16.8, 9.6, 2.4 Hz), 2.63—2.752 (1H, m), 2.96—3.04 (1H, m), 3.22—3.44 (2H, m), 4.26—4.36 (1H, m), 5.97 (2H, br s), 6.38 (2H, br s), 6.65 (2H, d, J=8.8 Hz), 7.67 (2H, d, J=8.8 Hz), 8.05 (1H, d, J=7.2 Hz). FAB-MS m/z: 471 (MH^+). *Anal.* Calcd for $C_{23}H_{30}N_6O_5 \cdot H_2O$: C, 56.55; H, 6.60; N, 17.20. Found: C, 56.62; H, 6.49; N, 17.24.

(5-Ethoxycarbonyl-1*H*-indol-1-yl)acetic Acid (12g) Ethyl indole-5-carboxylate (29 g, 0.153 mol) was converted to **12g** by reaction with ethyl bromoacetate, followed by saponification in alkali. A colorless powder (33 g, 87%), mp 171—173 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.41 (3H, t, J=7.2 Hz), 4.39 (2H, q, J=7.2 Hz), 4.92 (2H, s), 6.67 (1H, d, J=3.2 Hz), 7.14 (1H, d, J=3.2 Hz), 7.24 (1H, d, J=8.8 Hz), 7.95 (1H, dd, J=8.8, 1.6 Hz), 8.41 (1H, d, J=1.6 Hz).

Ethyl 1-(2-Hydroxyethyl)-1*H*-indole-5-carboxylate (13g) Reduction of indole-acetic acid **12g** (33 g, 0.133 mol) with $NaBH_4$ gave **13g** as a colorless powder (30 g, 97%). ^{1}H-NMR ($CDCl_3$) δ: 1.42 (3H, t, J=7.2 Hz), 1.50—1.70 (1H, br), 3.95—4.05 (2H, m), 4.32 (2H, t, J=5.2 Hz), 4.39 (2H, q, J=7.2 Hz), 6.62 (1H, dd, J=3.2, 0.8 Hz), 7.23 (1H, d, J=3.2 Hz), 7.38 (1H, d, J=8.8 Hz), 7.92 (1H, dd, J=8.8, 1.6 Hz), 8.41 (1H, J=1.6 Hz).

Ethyl 1-(2-Iodoethyl)-1*H*-indole-5-carboxylate (3g) This was prepared from **13g** (29 g, 0.124 mol) *via* the mesylate. Slightly colored needles (15 g, 33%), mp 79—80 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (3H, t, J=7.2 Hz), 3.46 (2H, t, J=7.6 Hz), 4.40 (2H, q, J=7.2 Hz), 4.54 (2H, t, J=7.6 Hz), 6.63 (1H, dd, J= 3.2, 0.8 Hz), 7.19 (1H, d, J=3.2 Hz), 7.34 (1H, d, J=8.8 Hz), 7.95 (1H, dd, J=8.8, 1.6 Hz), 8.41 (1H, J=1.6 Hz).

Ethyl 1-[2-(2-Cyano-3-methoxy-2-cyclopentenyl)ethyl-1*H*-indole-5-carboxylate (5g) This was prepared from **3g** (7.3 g, 21 mmol) *via* **4g** as a pale yellow oil (0.7 g, 9.5%). IR (neat): 2958, 2203, 1699, 1633, 1614 1455, 1360, 1308, 1285, 1259, 1190, 1084, 755 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (3H, t, J=7.2 Hz), 1.54—1.70 (1H, m), 1.86—1.98 (1H, m),

1.99—2.10 (1H, m), 2.23—2.35 (1H, m), 2.42—2.49 (2H, m), 2.80—2.92 (1H, m), 4.04 (3H, s), 4.27 (2H, q, J=7.2 Hz), 4.40 (2H, q, J=7.2 Hz), 6.61 (1H, dd, J=3.2, 0.8 Hz), 7.19 (1H, d, J=3.2 Hz), 7.36 (1H, d, J=8.4 Hz), 7.93 (1H, dd, J=8.4, 1.6 Hz), 8.40 (1H, J=1.6 Hz). FAB-MS m/z: 339 (MH^+).

Ethyl 1-[2-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)-ethyl]-1*H*-indole-5-carboxylate (6g) This was prepared from **5g** (0.7 g, 2.1 mmol) as a colorless powder (0.5 g, 66%), mp 192—194 °C. IR (KBr): 3481, 3369, 3330, 3183, 2946, 1702, 1683, 1654, 1622, 1477, 1446, 1306, 1257, 1190 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (3H, t, J=7.2 Hz), 1.79—1.93 (2H, m), 2.16—2.28 (2H, m), 2.67 (1H, ddd, J=17.6, 5.6, 3.6 Hz), 2.78—2.91 (2H, m), 4.13—4.35 (2H, m), 4.32 (2H, br s), 4.40 (2H, q, J=7.2 Hz), 4.72 (2H, br s), 6.62 (1H, dd, J=2.8, 0.4 Hz), 7.16 (1H, d, J=2.8 Hz), 7.34 (1H, d, J=8.8 Hz), 7.93 (1H, dd, J=8.8, 1.6 Hz), 8.41 (1H, dd, J=1.6, 0.4 Hz). FAB-MS m/z: 366 (MH^+). *Anal.* Calcd for $C_{20}H_{23}N_5O_2$: C, 65.74; H, 6.34; N, 19.16. Found: C, 65.59; H, 6.34; N, 19.07.

1-[2-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)ethyl]-1*H*-indole-5-carboxylic Acid (7g) Saponification of **6g** (0.5 g, 1.4 mmol) gave **7g** as a colorless powder (0.41 g). ^{1}H-NMR (DMSO-d_6) δ: 1.58—1.73 (2H, m), 1.88—2.10 (2H, m), 2.33—2.43 (1H, m), 2.56—2.68 (1H, m), 2.78—3.91 (1H, m), 2.98—3.08 (1H, m), 4.20 (2H, q, J=7.2 Hz), 5.63 (2H, br s), 5.64 (1H, br s), 6.11 (2H, br s), 6.54 (1H, d, J=3.2 Hz), 7.46 (1H, d, J=3.2 Hz), 7.56 (1H, d, J=8.8 Hz), 7.72 (1H, dd, J=8.8, 3.2 Hz), 8.18 (1H, d, J=3.2 Hz). FAB-MS m/z: 338 (MH^+).

***N*-[1-[2-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)ethyl]-1*H*-indole-5-carbonyl]-L-glutamic Acid (2g)** This was prepared from **7g** (0.4 g, 1.2 mmol) as a colorless powder (0.2 g, 36%), mp 188—190 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.60—1.75 (2H, m), 1.90—2.12 (4H, m), 2.36 (2H, t, J=7.6 Hz, m), 2.30—2.47 (1H, m), 2.52—2.70 (1H, m), 3.02—3.12 (1H, m), 4.23 (H, t, J=7.6 Hz), 4.35—4.44 (1H, m), 5.83—5.98 (2H, br), 6.24—6.40 (2H, br), 6.54 (1H, d, J=2.4 Hz), 7.47 (1H, d, J=2.4 Hz), 7.53—7.60 (1H, m), 7.65—7.71 (1H, m), 8.15 (1H, d, J=1.6 Hz), 8.38 (2H, d, J=7.2 Hz). FAB-MS m/z: 467 (MH^+). *Anal.* Calcd for $C_{23}H_{26}N_6O_5 \cdot 3/4H_2O$: C, 57.55; H, 5.77; N, 17.51. Found: C, 57.53; H, 5.62; N, 17.45.

Ethyl 1-(2-Hydroxyethyl)indoline-5-carboxylate (13h) Sodium cyanoborohydride (37.5 g, 0.6 mol) was added to a stirred solution of the indole-alcohol **13g** (24 g, 0.1 mol) in AcOH (500 ml). The mixture was stirred at r.t. for 3 h and evaporated. To this residue, AcOEt (500 ml) and 28% NH_4OH (300 ml) were added, and the mixture was vigorously stirred. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by chromatography on a silica gel column eluted with hexane–AcOEt (2 : 3—1 : 2) to give **13h** as a pale yellow oil (22 g, 93%). ^{1}H-NMR ($CDCl_3$) δ: 1.36 (3H, t, J=7.2 Hz), 1.80—1.90 (1H, br), 3.04 (2H, t, J=8.4 Hz), 3.34 (2H, t, J=5.4 Hz), 3.57 (2H, t, J=8.4 Hz), 3.80—3.87 (2H, br), 4.31 (2H, q, J=7.2 Hz), 6.45 (1H, d, J=8.4 Hz), 7.72 (1H, d, J=1.2 Hz), 7.82 (1H, dd, J=8.4, 1.2 Hz).

Ethyl 1-(2-Iodoethyl)indoline-5-carboxylate (3h) This was prepared from **13h** (22 g, 93 mmol) *via* the mesylate as slightly colored prisms (31 g, 97%), mp 54—56 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.36 (3H, t, J=7.2 Hz), 3.06 (2H, t, J=8.4 Hz), 3.27 (2H, t, J=5.4 Hz), 3.54—3.62 (4H, m), 4.31 (2H, q, J=7.2 Hz), 6.38 (1H, d, J=8.4 Hz), 7.72 (1H, d, J=1.2 Hz), 7.82 (1H, dd, J=8.4, 1.2 Hz).

Ethyl 1-[2-(2-Cyano-3-methoxy-2-cyclopentenyl)ethyl]indoline-5-carboxylate (5h) This was prepared from **3h** (24 g, 70 mmol) *via* **4h**. A pale yellow oil (6.8 g, 29%). IR (neat): 2933, 2854, 2202, 1698, 1629, 1611, 1506, 1447, 1354, 1270, 1180, 1106, 769 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.36 (3H, t, J=7.2 Hz), 1.54—1.68 (2H, m), 2.02—2.23 (2H, m), 2.48—2.54 (2H, m), 2.92—3.04 (1H, m), 3.02 (2H, t, J=8.8 Hz), 3.17—3.33 (2H, m), 3.44—3.60 (2H, m), 4.05 (3H, s), 4.30 (2H, q, J=7.2 Hz), 6.36 (1H, d, J=8.4 Hz), 7.70 (1H, d, J=1.2 Hz), 7.81 (1H, dd, J=8.4, 1.2 Hz). FAB-MS m/z: 341 (MH^+).

Ethyl 1-[2-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)-ethyl]indoline-5-carboxylate (6h) This was prepared from **5h** (6.8 g, 20 mmol) as a colorless powder (5.3 g, 72%), mp 220—222 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.36 (3H, t, J=7.2 Hz), 1.75—1.93 (3H, m), 2.20—2.33 (1H, m), 2.64 (1H, ddd, J=17.2, 9.6, 2.4 Hz), 2.84—2.95 (1H, m), 2.95—3.08 (1H, m), 3.03 (2H, t, J=8.4 Hz), 3.12—3.22 (1H, m), 3.25—3.39 (2H, m), 3.58—3.66 (1H, m), 4.32 (2H, q, J=7.2 Hz), 4.71 (2H, br s), 4.80 (2H, q, J=7.2 Hz), 6.45 (1H, d, J=8.0 Hz), 7.74 (1H, d, J=1.2 Hz), 7.82 (1H, dd, J=8.0, 1.2 Hz). FAB-MS m/z: 368 (MH^+). *Anal.* Calcd for $C_{20}H_{25}N_5O_2 \cdot 1/2H_2O$: C, 63.81; H, 6.96; N, 18.60. Found: C, 63.92; H, 6.78; N, 18.73.

1-[2-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)ethyl]-indoline-5-carboxylic Acid (7h) Saponification of **6h** (4.3 g, 12 mmol) gave **7h** as a colorless powder (3.35 g, 84%). ^{1}H-NMR (DMSO-d_6) δ: 1.37—1.50 (1H, m), 1.70—1.86 (2H, m), 1.94—2.06 (1H, m), 2.39 (1H, ddd, J=16.8, 9.6, 2.8 Hz), 2.58—3.70 (1H, m), 2.94—3.02 (1H, m), 3.06—3.23 (2H, m), 3.36—3.50 (2H, m), 5.62 (1H, br s), 5.64 (1H, br s), 6.43 (1H, d, J=8.4 Hz), 7.49 (1H, d, J=1.2 Hz), 7.60 (1H, dd, J=8.4, 1.2 Hz). FAB-MS m/z: 340 (MH^+).

***N*-[1-[2-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)ethyl]indoline-5-carbonyl]-L-glutamic Acid (2h)** This was prepared from **7h** (1.5 g, 4.4 mmol) as a colorless powder (0.86 g, 38%), mp 184—187 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.38—1.52 (1H, m), 1.73—2.10 (5H, m), 2.30 (2H, t, J=7.2 Hz), 2.37—2.48 (1H, m), 2.61—2.74 (1H, m), 2.90 (2H, t, J=7.6 Hz), 2.95—3.05 (1H, m), 3.05—3.24 (2H, m), 3.35—3.50 (2H, m), 4.26—4.34 (1H, m), 5.89 (2H, br s), 6.29 (2H, br s), 6.45 (1H, dd, J=8.4, 2.0 Hz), 7.53 (1H, s), 7.57 (1H, d, J=8.4 Hz), 8.06 (2H, d, J=6.8 Hz). FAB-MS m/z: 469 (MH^+). *Anal.* Calcd for $C_{23}H_{28}N_6O_5 \cdot 0.85H_2O$: C, 57.20; H, 6.18; N, 17.40. Found: C, 57.16; H, 6.24; N, 17.39.

***tert*-Butyl 5-(2-Ethoxycarbonylethyl)thiophene-2-carboxylate (11i)** In a manner similar to those described for **10a** and **11a**, *tert*-butyl 5-formylthiophene-2-carboxylate[19] (6.65 g, 31.3 mmol) gave **11i** as a colorless oil (7.6 g, 81.6%). ^{1}H-NMR ($CDCl_3$) δ: 1.26 (3H, t, J=7.2 Hz), 1.56 (9H, s), 2.68 (2H, t, J=7.6 Hz), 3.15 (2H, d, J=7.6 Hz), 4.15 (2H, q, J=7.2 Hz), 6.79 (1H, d, J=3.6 Hz), 7.54 (1H, d, J=3.6 Hz).

3-[5-(*tert*-Butoxycarbonyl)thiophen-2-yl]propionic Acid (12i) The diester **11i** (7.6 g, 26.8 mmol) was partially hydrolyzed to give monoacid **12i** as colorless prisms (6.1 g, 84.4%). ^{1}H-NMR ($CDCl_3$) δ: 1.58 (9H, s), 2.75 (2H, t, J=7.6 Hz), 3.16 (2H, d, J=7.6 Hz), 6.81 (1H, d, J=4.4 Hz), 7.53 (1H, d, J=4.4 Hz).

***tert*-Butyl 5-(3-Hydroxypropyl)thiophene-2-carboxylate (13i)** Compound **12i** (6.1 g, 26.8 mmol) was subjected to borane reduction to give the thiophene alcohol **13i** as a pale-colored oil (6 g, 92.4%). ^{1}H-NMR ($CDCl_3$) δ: 1.10 (lH, br s), 1.56 (9H, s), 1.94 (2H, tt, J=7.6, 6.4 Hz), 2.93 (2H, d, J=7.6 Hz), 3.70 (2H, d, J=6.4 Hz), 6.78 (1H, d, J=3.6 Hz), 7.54 (1H, d, J=3.6 Hz).

***tert*-Butyl 5-(3-Bromopropyl)thiophene-2-carboxylate (3i)** This was prepared from **13i** (6 g, 23.9 mmol) as a pale yellow oil (6.2 g, 85.1%). ^{1}H-NMR ($CDCl_3$) δ: 1.56 (9H, s), 2.21 (2H, tt, J=7.2, 6.4 Hz), 3.00 (2H, d, J=7.2 Hz), 3.42 (2H, d, J=6.4 Hz), 6.81 (1H, d, J=3.6 Hz), 7.55 (1H, d, J=3.6 Hz).

***tert*-Butyl 5-[3-(2-Cyano-3-oxo-1-cyclopentanyl)propyl]thiophene-2-carboxylate (4i)** On reaction with 2-cyano-2-cyclopenten-1-one (**3i**, 4.25 g, 14 mmol) gave **4i** as a colorless oil (0.83 g, 17.8%). IR (neat): 2979, 2935, 2246, 1760, 1704, 1695, 1463, 1369, 1302, 1282, 1258, 1169, 1097, 825, 754 cm^{-1}. FAB-MS m/z: 334 (MH^+).

***tert*-Butyl 5-[3-(2-Cyano-3-methoxy-2-cyclopenten-1-yl)propyl]thiophene-2-carboxylate (5i)** *O*-Methylation of **4i** (0.83 g, 2.5 mmol) gave **5i** as a colorless oil (0.82 g, 94.3%). IR (neat): 2978, 2936, 2204, 1704, 1632, 1462, 1368, 1353, 1300, 1283, 1258, 1169, 1097, 824, 753 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.44—1.84 (2H, m), 1.56 (9H, s), 2.03—2.14 (1H, m), 2.43—2.50 (2H, m), 2.76—2.92 (3H, m), 4.04 (3H, s), 6.76 (1H, d, J=3.6 Hz), 7.54 (lH, d, J=3.6 Hz). FAB-MS m/z: 348 (MH^+).

5-[3-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)propyl]thiophene-2-carboxylic Acid (7i) Reaction of **5i** (0.82 g, 2.35 mmol) with guanidine gave the 2,4-diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidine ester (**6i**), which on saponification gave **7i** as a colorless powder (0.33 g, 44.2%). IR (KBr): 3291, 3117, 1666, 1637, 1568, 1530, 1462, 1367, 1081, 1051, 1013, 770 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.28—1.33 (1H, m), 1.54—1.73 (4H, m), 1.90—2.08 (1H, m), 2.35—2.46 (1H, m), 2.52—2.68 (1H, m), 2.72—2.84 (2H, m), 2.90—3.00 (lH, m), 5.96 (2H, br s), 6.23 (2H, br s), 6.84 (2H, d, J=3.6 Hz), 7.45 (2H, d, J=3.6 Hz). FAB-MS m/z 319 (MH^+). *Anal.* Calcd for $C_{15}H_{18}N_4O_2S \cdot 0.5H_2O$: C, 55.03; H, 5.85; N, 17.11. Found: C, 54.67; H, 5.61; N, 17.04.

Diethyl *N*-[5-[3-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)propyl]thiophene-2-carbonyl]-L-glutamate (8i) Amidation of **7i** (0.30 g, 0.94 mmol) with diethyl L-glutamate gave **8i** as a slightly colored oil. FAB-MS m/z: 504 (MH^+).

***N*-[5-[3-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)-propyl]thiophene-2-carbonyl]-L-glutamic Acid (2i)** Saponification of the diester **8i** gave **2i** as a colorless powder (0.15 g), mp 191—194 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.20—1.34 (1H, m), 1.55—1.73 (4H, m), 1.83—2.08 (3H, m), 2.24—2.48 (3H, m), 2.56—2.69 (lH, m), 2.72—2.84 (2H, m), 2.92—3.02 (1H, m), 4.24—4.36 (1H, m), 5.87 (2H, br s), 6.20

(2H, br s), 6.85 (2H, d, $J=3.6$ Hz), 7.65 (2H, d, $J=3.6$ Hz), 8.38 (1H, d, $J=7.2$ Hz). FAB-MS m/z: 448 (MH^+). *Anal.* Calcd for $C_{20}H_{25}N_5O_5S \cdot 1H_2O$: C, 51.60; H, 5.85; N, 14.88. Found: C, 51.28; H, 5.54; N, 14.88.

Enzyme Inhibition Assay Enzyme-inhibitory activity was determined by spectrophotometric assay, based on the change in molar absorbance at 340 nm due to conversion of dihydrofolate to tetrahydrofolate, essentially according to the method of Misra *et al.*[21] For the measurement, purified DHFR enzyme from bovine liver cells (Sigma, D-6385) was used. The IC_{50} values (molar concentration required for 50% inhibition of enzyme activity) for DHFR inhibitors were obtained from plots of activity versus drug concentration.

Cell Growth Inhibition Assay a) By Long-Term Treatment (72 h): P388 leukemia cells (MTX-sensitive and MTX-resistant sublines), colon 26 colorectal carcinoma cells and colon 38 colorectal carcinoma cells, and KB human epidermoid carcinoma cells were used in this test. Cells were seeded at 2.5×10^3 cells per well in 96-well plates, and incubated at 37 °C under a 5% CO_2 atmosphere for 24 h in RPMI 1640 medium (which normally contains 2 mM folic acid) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY), penicillin (100 unit), streptomycin (100 μg/ml), 0.05 mM 2-mercaptoethanol, and 1 mM sodium pyruvate. To this culture was added a solution of drug in the RPMI 1640 medium at a final concentration of 0.1 nM—1 μM at time zero. The culture was continued at 37 °C under the 5% CO_2 atmosphere for 72 h. At the end of this period a cell count was made by the MTT colorimetric method[23] to determine the drug concentration required for 50% cell growth inhibition (IC_{50}). All experiments were repeated at least once. The values reported in Table 1 are averages from all experiments.

b) By Short-Term Treatment: The method used was a modification of those described in earlier reports.[24,26] Colon 26 cells were grown in suspension culture (initial density, 2.5×10^3 cells/0.1 ml) in folate-free RPMI 1640 medium supplemented with 10% dialyzed fetal calf serum (Gibco) and 10 nM leucovorin at 37 °C under a 5% CO_2 atmosphere for 24 h, and exposed to a range of drug concentrations (0.156—40 μM) at time zero. Following 4 h of drug exposure, the cells were harvested by removal of the supernatant, and then washed with drug-free complete medium an appropriate number of times to insure removal of drugs to levels that would not cause detectable inhibition over the time course of the experiment. Drug-free control samples were washed an equal number of times. The cells were resuspended to the initial volume in the drug-free complete medium and incubated for the additional period required to reach 72 h total time. At the same time, determinations on long-term drug exposure experiments were carried out for comparative purpose. In this case, colon 26 cells were incubated for the entire period (72 h) in the presence of the drug; other conditions were as described above. In all experiments, cells in each sample were counted at 72 h total period from seeding, and IC_{50} values were determined as described above.

References and Notes

1) For a review of antifolates: Rosowsky A., *Progress in Medicinal Chemistry*, **26**, 1—251 (1989).
2) *a*) Piper J. R., Johnson C. A., Otter G. M., Sirotnak F. M., *J. Med. Chem.*, **35**, 3002—3006 (1992); *b*) DeGraw J. I., Brown V., Tagawa H., Kisliuk R. L., Gaumont Y., Sirotnak F. M., *ibid.*, **25**, 1227—1230 (1982); *c*) Sirotnak F. M., DeGraw J. I., Schmid F. A., Goutas L. J., Moccio D. M., *Cancer Chemother. Pharmacol.*, **12**, 26—30 (1984).
3) Taylor E. C., Harrington P. J., Fletcher S. R., Beardsley G. P., Moran R. G., *J. Med. Chem.*, **28**, 914—921 (1985); Beardsley G. P., Moroson B. A., Taylor E. C., Moran R. G., *J. Biol. Chem.*, **264**, 328—333 (1989); Beardsley G. P., Taylor E. C., Shih C., Poore G. A., Grindey G. B., Moroson B. A., Moran R. G., *Proc. Am. Assoc. Cancer Res.*, **27**, 259 (1986).
4) Jackman A. L., Taylor G. A., Gibson W., Kimbell R., Brown M., Calvert A. H., Judson I. R., Hughes L. R., *Cancer Res.*, **51**, 5579—5586 (1991).
5) Miwa T., Hitaka T., Akimoto H., Nomura H., *J. Med. Chem.*, **34**, 556—560 (1991); Akimoto H., Hitaka T., Miwa T., Yukishige K., Kusanagi T., Ootsu K., *Proc. Am. Assoc. Cancer Res.*, **32**, 327 (1991).
6) Shih C., Grindey G. B., Barnett G. B., Pearce H. L., Engelhardt J. A., Todd G. C., Rinzel S. M., Worzalla J. F., Gossett L. S., Everson T. P., Wilson T. M., Kobierski M. E., Winter M. A., Kuhnt D., Taylor E. C., Moran R. G., *Proc. Am. Assoc. Cancer Res.*, **33**, 411 (1992); Grindey G. B., Shih C., Barnett C. J., Pearce H. L., Engelhardt J. A., Todd G. C., Rinzel S. M., Worzalla J. F., Gossett L. S., Everson T. P., Wilson T. M., Kobierski M. E., Winter M. A., Kuhnt D., Taylor E. C., Moran R. G., *ibid.*, **33**, 411 (1992); Taylor E. C., Kuhnt D., Shih C., Rinzel S. M., Grindey G. B., Barredo J., Jannatipour M., Moran R. G., *J. Med. Chem.*, **35**, 4450—4454 (1992).
7) Kotake Y., Iijima A., Yoshimatsu K., Tamai N., Ozawa Y., Koyanagi N., Kitoh K., Nomura H., *J. Med. Chem.*, **37**, 1616—1624 (1994).
8) McGuire J. J., Bergoltz V. V., Heitzman K. J., Haile W. H., Russel C. A., Bolanowska W. E., Kotake Y., Haneda T., Nomura H., *Cancer Res.*, **54**, 2673—2679 (1994); McGuire J. J., Bergoltz V. V., Heitzman K. J., Russel C. A., Bolanowska W. E., Haile W. H., Kotake Y., Haneda T., Nomura H., *Proc. Am. Assoc. Cancer Res.*, **35**, 303 (1994).
9) *N*-[4-(2-(2,4-Diamino-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamic acid.[7]
10) Bolin J. T., Filman D. J., Matthews D. A., Hamlin R. C., Kraut J., *J. Biol. Chem.*, **257**, 13650—13662 (1982).
11) Roberts E. C., Shealy Y. F., *J. Med. Chem.*, **14**, 125 (1971); *idem*, *ibid.*, **15**, 1310 (1972); *idem*, *J. Heterocycl. Chem.*, **11**, 547 (1974).
12) Marsham P. R., Hughes L. R., Jackman A. L., Hayter A. J., Oldfield, J., Wardleworth J. M., Bishop J. A. M., O'Connor B. M., Calvert A. H., *J. Med. Chem.*, **34**, 1594—1605 (1991).
13) Marsham P. R., Jackman A. L., Hayter A. J., Daw M. R., Snowden J. L., O'Connor B. M., Bishop J. A. M., Calvert A. H., Hughes L. R., *J. Med. Chem.*, **34**, 2209—2218 (1991).
14) Wright W. E., Jr., Cosulich D. B., Fahrenbach M. J., Waller C. W., Smith J. M., Jr., Hultquist M. E., *J. Am. Chem. Soc.*, **71**, 3014 (1949).
15) Giese B., *Angew. Chem. Int. Ed. Engl.*, **24**, 553—565 (1985); Giese B., GonzalesGomez J. A., Witzel T., *ibid.*, **23**, 69—70 (1984).
16) Nagaoka H., Kishi Y., *Tetrahedron*, **37**, 3873—3888 (1981).
17) Ritchie P. D., Wright A. E., *J. Chem. Soc.*, **1960**, 902—903; Sergeev P. G., Sladkov A. M., *Zhur. Obshchei Khim.*, **27**, 817—819 (1957).
18) Gribble G. W., Lord P. D., Skotnicki J., Dietz S. E., Eaton J. T., Johnson J., *J. Am. Chem. Soc.*, **96**, 7812—7814 (1974).
19) Carpenter A. J., Chadwick D. J., *Tetrahedron*, **41**, 3803—3812 (1985).
20) For the sake of brevity, we have chosen to omit stereochemical designations in the text and experimental section.
21) Misra D. K., Humphreys S. R., Friedkin M., Goldin A., Crawford E. J., *Nature* (London), **189**, 39—42 (1961); Delcamp T. J., Susten S. S., Blankenship D. T., Freishem J. H., *Biochemistry*, **22**, 633—639 (1983).
22) 10-EDAM used as a reference drug was synthesized according to the method previously described.[2a,b] Based on spectral data and chromatographic analysis the product was pure and unequivocally identical with an authentic sample.[8]
23) Alley M. C., Scudiero D. A., Monks A., Hursey M. L., Czerwinski M. J., Fine D. L., Abott B. J., Mayo J. G., Shoemaker R. H., Boyd M. R., *Cancer Res.*, **48**, 589—601 (1988); Mosmann T., *J. Immunol. Methods*, **65**, 55—63 (1983).
24) Sirotnak F. M., DeGraw J. I., Moccio D. M., Goutas L. J., *Cancer Chemother. Pharmacol.*, **12**, 18 (1984); Samuels L. L., Moccio D. M., Sirotnak F. M., *Cancer Res.*, **45**, 1488—1495 (1985); Balinska M., Galivan J., Coward J. K., *Cancer Res.*, **45**, 1489—1495 (1981).
25) FPGS is the enzyme responsible for biosynthesis of polyglutamyl derivatives by polyglutamation of folate and its analogs. Antifolate polyglutamates (*e.g.*, MTX polyglutamates) are as potent inhibitors of DHFR as the parent drug[31] and are selectively retained by cells.[24] Polyglutamation is a critical factor in the effectiveness of antifolates as chemotherapeutic agents.
26) McCloskey D. E., McGuire J. J., Russell C. A., Rowan B. G., Bertino J. R., Pizzorno G., Mini E., *J. Biol. Chem.*, **266**, 6181—6187 (1991); Li W. W., Lin J. T., Chang Y. M., Schweitzer B., Bertino J. R., *Int. J. Cancer*, **49**, 234—238 (1991).
27) Rosowsky A., Bader H., Freisheim J. H., *J. Med. Chem.*, **34**, 203—208 (1991).
28) The ratio of [IC_{50} for EDAM]/[IC_{50} for **2d**] = > 800.
29) McGuire *et al.*[8] have recently reported that **2d** retains extremely high substrate activity for CCRF-CEM FPGS. It is among the most active substrates known and exhibits greater uptake into the cells, based on inhibitory activity of ^{3}H-MTX uptake. Substrate

activity for CCRF-CEM FPGS was in the order **2d** > **1** > **2e** (V_{rel}/K_m = 0.54, 0.32 and 0.16, respectively; where $V_{rel} = V_{max}$ relative to AMT), while the corresponding values for 10-EDAM, AMT and MTX were 0.24, 0.2 and 0.013, respectively. The level of membrane transport into CCRF-CEM cells was in the order **1** > **2e** > **2d** > AMT (IC_{50} as a competitive inhibitor of ^{3}H-MTX uptake = 0.3, 0.6, 0.7 and 2.6 μM, respectively).

30) Piper J. R., Johnson C. A., Maddry J. A., Malik N. D., McGuire J. J., Otter J. M., Sirotnak F. M., *J. Med. Chem.*, **36**, 4161—4171 (1993).

31) McGuire J. J., Mini E., Hseih P., Bertino J. R., *Cancer Res.*, **45**, 6395—6400 (1985).