Quinazoline Antifolate Thymidylate Synthase Inhibitors: γ-Linked L-D, D-D, and D-L Dipeptide Analogues of 2-Desamino-2-methyl-N^{10}-propargyl-5,8-dideazafolic Acid (ICI 198583)[†,‡]

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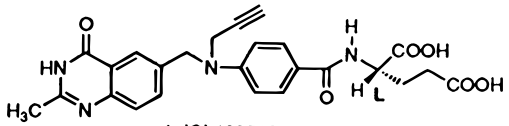
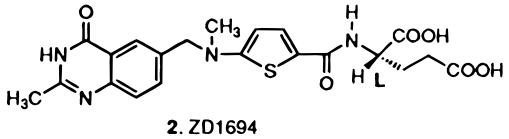
The syntheses of γ-linked L-D, D-D, and D-L dipeptide analogues of 2-desamino-2-methyl-N^{10}-propargyl-5,8-dideazafolic acid (ICI 198583) are described. The general methodology for the synthesis of these molecules involved the preparation of the dipeptide derivatives employing solution phase peptide synthesis followed by condensation of the dipeptide free bases with the appropriate pteroic acid analogue via diethyl cyanophosphoridate (DEPC) activation. In the final step, *tert*-butyl esters were removed by trifluoroacetic acid (TFA) hydrolysis. Z-L-Glu-OBu^t-γ-D-Ala-OBu^t, for example, was prepared from α-*tert*-butyl *N*-(benzyloxycarbonyl)-L-glutamate and *tert*-butyl D-alaninate via isobutyl-mixed anhydride coupling. The *Z*-group was removed by catalytic hydrogenolysis and the resulting dipeptide free base condensed with 2-desamino-2-methyl-N^{10}-propargyl-5,8-dideazapteroic acid via DEPC coupling. Finally, *tert*-butyl esters were removed by TFA hydrolysis to give ICI 198583-γ-D-Ala. The compounds were tested as inhibitors of thymidylate synthase and L1210 cell growth. Good enzyme and growth inhibitory activity were found with γ-linked L-D dipeptides, the best examples being the Glu-γ-D-Glu derivative **35** (K_i = 0.19 nM, L1210 IC_{50} = 0.20 ± 0.017 μM) and the Glu-γ-D-α-aminoadipate derivative **39** (K_i = 0.12 nM, L1210 IC_{50} = 0.13 ± 0.063 μM). In addition, ICI 198583 L-γ-D-linked dipeptides were resistant to enzymatic degradation in mice.

Introduction

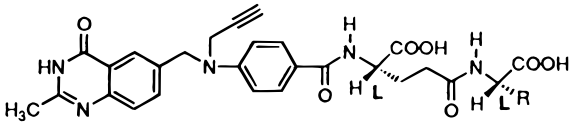
Polyglutamation of antifolates targeted toward thymidylate synthase (TS) can be considered as a desirable metabolic process since the polyglutamate metabolites are often more potent inhibitors of TS than the parent drug, and these molecules are retained within the cells for prolonged periods due to the polyionic character. In addition, it is believed that some degree of tumor selectivity may be achieved since in tumor cells in mice polyglutamation has been reported to occur more rapidly than in normal tissues.[1] Tomudex (ZD 1694)[2] is an example of a polyglutamatable inhibitor of TS which is currently in phase III clinical study for the treatment of colorectal cancer.[3] However, the FPGS substrate activity of antifolates such as ZD1694 may be of a disadvantage in tumors expressing low levels of, or an altered expression of, folylpolyglutamyl synthetase (FPGS)[4−6] or in tumors expressing high levels of γ-glutamyl hydrolases,[7,8] a group of enzymes that catalyze the cleavage of the γ-glutamyl amide bond of polyglutamates,[9] and may result in normal tissue toxicities due to polyglutamate retention. For these reasons, we were interested in designing and synthesizing potent inhibitors of TS which would not depend on polyglutamation for antitumor activity. However, as part of our requirement, compounds were designed to utilize the reduced folate/MTX carrier (RFC), a transport system commonly used by many classical antifolates such as MTX or ZD1694,[4] since use of the RFC transport system was found to increase the growth inhibitory potency of many water-soluble antifolates. In addition, utilization of the RFC transport system has been reported to offer some tumor selectivity.[10]



1. ICI 198583



2. ZD1694



ICI 198583 L-γ-L Linked Dipeptides

3, ICI 198583-γ-L-Glu, R = CH_2CH_2COOH

† Part of this work has been presented in preliminary form; see: Bavetsias, V.; Jackman, A. L.; Thornton, T. J.; Pawelczack, K.; Boyle, F. T.; Bisset, G. M. F. Quinazoline Antifolates Inhibiting Thymidylate Synthase: Synthesis of γ-Linked Peptide and Amide Analogues of 2-Desamino-2-Methyl-N^{10}-Propargyl-5,8-Dideazafolic Acid (ICI 198583). In *Advances in Experimental Medicine and Biology (Chemistry and Biology of Pteridines and Folates)*; Ayling, J. E., *et al.*, Eds.; Plenum Press: New York, 1993; Vol. 338, pp 593−596.

‡ Abbreviations: TS, thymidylate synthase; FPGS, folylpolyglutamyl synthetase; MTX, methotrexate; DEPC, diethyl cyanophosphoridate; Z, benzyloxycarbonyl; pg, propargyl; Glu, glutamic acid; Ala, alanine; Phe, phenylalanine; aad, α-aminoadipic acid; TFA, trifluoroacetic acid.

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ICI 198583 γ-linked L-L dipeptides (in which the second amino acid was other than glutamate) were the first class of dipeptides synthesized as inhibitors of TS that do not undergo polyglutamation.[11] Although these γ-linked L-L dipeptides were potent inhibitors of TS, their utility was limited because when they were injected into mice they were degraded to ICI 198583,[12] a compound known to be a substrate for FPGS. This degradation is believed to be the result of the enzymatic cleavage of the γ-glutamyl amide bond by γ-glutamyl hydrolases that have high activity in tissues such as liver. We therefore envisaged stabilizing the γ-glutamyl amide bond in γ-linked L-L dipeptide derivatives of ICI 198583 to enzymatic degradation by inverting the stereochemistry of the amino acids from L to D. It was also believed that the second amino acid could be glutamate since it had previously been reported that natural folates and folate analogues with a D-Glu instead of an L-Glu were not substrates for FPGS.[13]

With regard to TS inhibition, we believed that L-γ-D-dipeptide analogues of ICI 198583 could maintain the high affinity for TS shown by quinazoline antifolate L-γ-L dipeptides.[11] During the course of the study, a computer graphics model of the humanized *Escherichia coli* active site of TS was developed and indicated that the α-carboxyls of the diglutamate chain of ICI 198583-γ-L-Glu (**3**) play a crucial role for TS binding. The α-carboxyl of the first glutamate was shown to interact with a lysine residue (conserved Lys48) through a molecule of water, and the α-carboxyl of the second glutamate interacts electrostatically with an arginine residue in the active site of the enzyme. Further examination of the model indicated that the electrostatic interaction could take place regardless of the stereochemistry of the second (terminal) amino acid and therefore supported the view that L-D dipeptides may also maintain high affinity for TS possibly to a level shown by their L-L counterparts.[11] We report here the synthesis of 15 dipeptide analogues of ICI 198583, some of which include 2′-F and 7-Me substituents, which had been shown in other series to enhance further TS inhibition.[14,15]

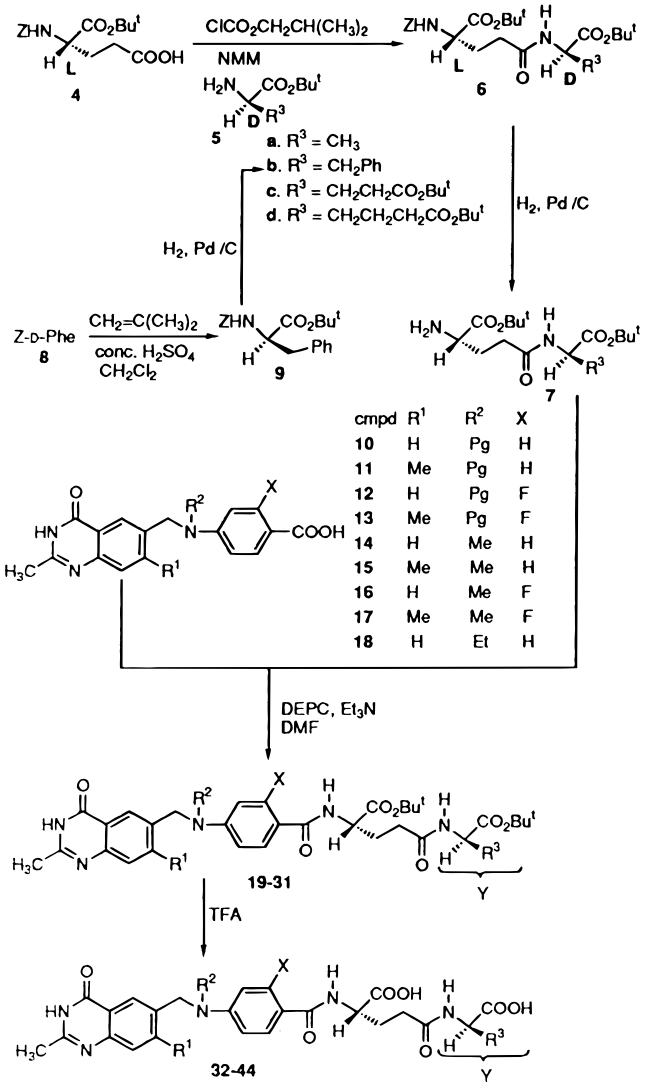
Chemistry

The chemistry of dipeptide analogues of various antifolates has been described in a recent paper from our laboratories.[11] Our route to γ-linked L-D dipeptide analogues of ICI 198583, summarized in Scheme 1, was identical with that used for the synthesis of L-γ-L dipeptide derivatives of ICI 198583 and is based on the strategy used in the synthesis of pteroyloligo-γ-glutamates[16] and the polyglutamates of ZD1694.[17]

For the synthesis of Z-blocked L-γ-D dipeptides **6** (Scheme 1), the mixed carbonic anhydride coupling method employing isobutyl chloroformate as the activating agent was used.[18] The carboxyl-protecting group *tert*-butyl was selected to avoid the possibility of $\gamma \rightarrow \alpha$ transpeptidation associated with alkaline carboxyl deprotection,[19] since the *tert*-butyl esters can be easily cleaved by treatment with trifluoroacetic acid under mild conditions in a racemization-free process.

D-Alanine *tert*-butyl ester (**5a**) was obtained commercially. Di-*tert*-butyl D-glutamate (**5c**) was prepared from the corresponding acid either by transesterification with *tert*-butyl acetate[20,21] and 70% perchloric acid in

Scheme 1



28% yield or by treatment with isobutylene/concentrated H_2SO_4 in CH_2Cl_2 in 45% yield. The preparation of **5b** is shown in Scheme 1. Z-D-Phe (**8**) was converted into the *tert*-butyl ester **9** by treatment with isobutylene/concentrated H_2SO_4 in CH_2Cl_2, and the Z-group was then removed by catalytic hydrogenolysis to afford **5b** in 69% overall yield. Finally, di-*tert*-butyl D-α-aminoadipate hydrochloride (**5d**) was prepared from D-α-aminoadipic acid by a method identical with that described for the L-enantiomer.[11]

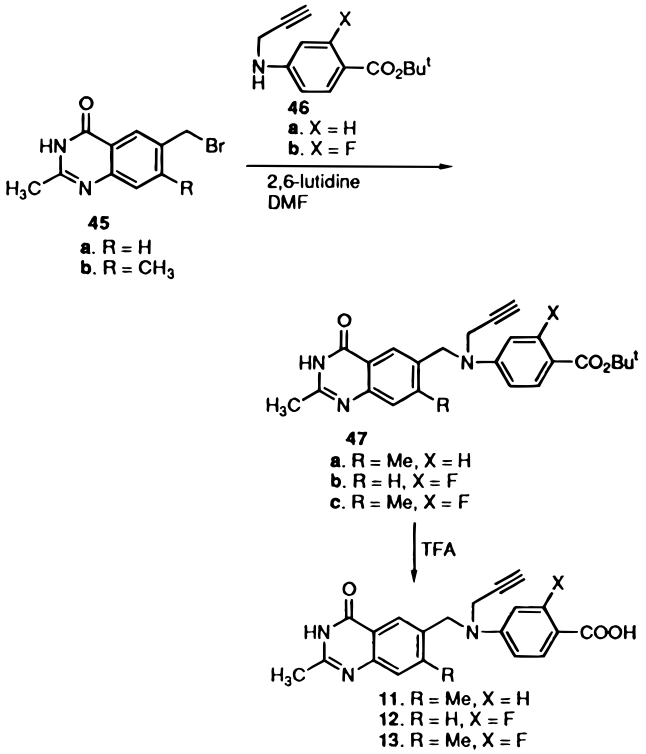
Quinazoline antifolate dipeptide *tert*-butyl esters **19**−**26** (Table 1) were prepared by condensing dipeptides **7** (obtained from **6** by catalytic hydrogenolysis, Scheme 1) with the appropriate pteroic acid analogues, *i.e.*, **10**[11] and **11**−**13**, via DEPC activation[22] (Scheme 1). The general route to pteroic acid analogues **11**−**13** is shown in Scheme 2. Compound **11**, for example, was prepared by coupling 6-(bromomethyl)-3,4-dihydro-2,7-dimethyl-4-oxoquinazoline (**45b**)[23] with *tert*-butyl 4-(prop-2-ynylamino)benzoate (**46a**)[24,25] followed by TFA hydrolysis. Quinazoline antifolate L-Glu-γ-D-Glu *tert*-butyl esters **27**−**31** (Table 1) were obtained by condensing **7c** with the appropriate pteroic acid analogue, *i.e.*, **14**,[11] **15**−**17**, and **18**,[11] again using DEPC (Scheme 1). 4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-methylamino]benzoic acid (**15**) was prepared from **48**[26]

Table 1. Preparation of Quinazoline Antifolate L-D Dipeptide Esters

compd	R^1	R^2	X	Y	yield (%)	mp (°C)	*m/z* $(M + H)^+$	formula	anal.
19	H	pg	H	Glu(OBu^t)-OBu^t	60	116−117	774	$C_{42}H_{55}O_9N_5 \cdot 0.5H_2O$	CHN
20	Me	pg	H	Glu(OBu^t)-OBu^t	58	159−161.5	810[a]	$C_{43}H_{57}N_5O_9 \cdot 0.5H_2O$	CHN
21	H	pg	F	Glu(OBu^t)-OBu^t	82	105−108	814[a]	$C_{42}H_{54}FN_5O_9 \cdot 0.5H_2O$	CHNF
22	Me	pg	F	Clu(OBu^t)-OBu^t	82	149.5−150.5	828[a]	$C_{43}H_{56}FN_5O_9 \cdot 0.5H_2O$	CHNF
23	H	pg	H	Ala-OBu^t	44	166−168	660	$C_{36}H_{45}N_5O_7$	CHN
24	H	pg	H	Phe-OBu^t	57	115−117.5	736	$C_{42}H_{49}N_5O_7 \cdot 1.5H_2O$	CHN
25	H	pg	H	aad(OBu^t)-OBu^t	74	108−109	788	$C_{43}H_{57}N_5O_9 \cdot 0.25H_2O$	CHN
26	Me	pg	F	aad(OBu^t)-OBu^t	63	149−150	820	$C_{44}H_{58}FN_5O_9$	CHNF
27	H	Me	H	Glu(OBu^t)-OBu^t	64	104.5−108.5	750	$C_{40}H_{55}N_5O_9$	CHN
28	Me	Me	H	Glu(OBu^t)-OBu^t	33	243.5−245	764	$C_{41}H_{57}N_5O_9 \cdot 0.6H_2O$	CHN
29	H	Me	F	Glu(OBu^t)-OBu^t	74	100−102	768	$C_{40}H_{54}FN_5O_9$	CHNF
30	Me	Me	F	Glu(OBu^t)-OBu^t	73	172−173	782	$C_{41}H_{56}FN_5O_9$	CHNF
31	H	Et	H	Glu(OBu^t)-OBu^t	51	110.5−113.5	763	$C_{41}H_{57}N_5O_9 \cdot 0.5H_2O$	CHN

[a] $(M + Na)^+$.

Scheme 2



by removing the POM-group under alkaline conditions (Scheme 3).

The synthesis of pteroic acid analogues **16** and **17** is shown in Scheme 4. Alkylation of *tert*-butyl 4-amino-2-fluorobenzoate (**51**)[24] with 6-(bromomethyl)-3,4-dihydro-2-methyl-4-oxo-3-[(pivaloyloxy)methyl]quinazoline (**49**)[27] or 6-(bromomethyl)-3,4-dihydro-2,7-dimethyl-4-oxo-3-[(pivaloyloxy)methyl]quinazoline (**50**)[24] in DMA, using 2,6-lutidine as base, afforded compounds **52** and **53** from which **54** and **55** were obtained by treatment with $NaBH_3(CN)$, HCHO, and AcOH. Removal of the *tert*-butyl and POM protecting groups was achieved upon treatment with TFA and then aqueous NaOH.

Quinazoline antifolate dipeptide *tert*-butyl esters **19**−**31** were deprotected with TFA to give quinazoline antifolates **32**−**44** (Table 2) as their TFA salts.

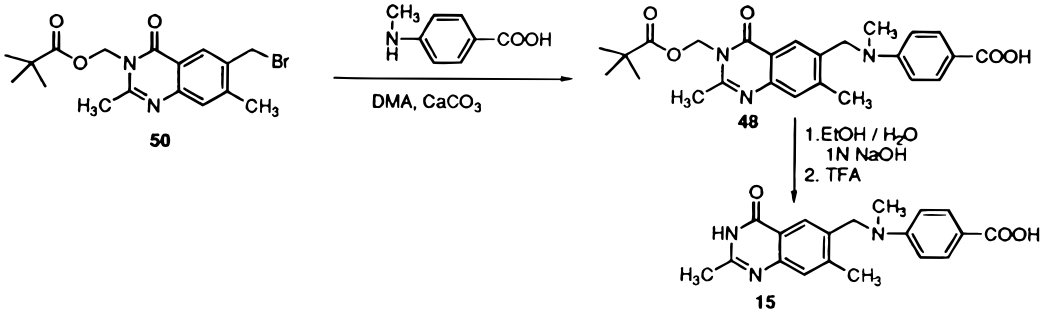
In an effort to determine whether stereochemical changes at the α-center of the first or both glutamic residues of ICI 198583-γ-L-Glu make the γ-glutamyl bond resistant to enzymatic degradation, antifolates **64a,b** were prepared. They were synthesized by a route identical with that described above for the synthesis of the L-D analogues **32**−**44**, except that α-*tert*-butyl *N*-(benzyloxycarbonyl)-D-glutamate (Z-D-Glu-OBu^t, **60**) was used in place of Z-L-Glu-OBu^t (**4**) (Scheme 5). α-*tert*-Butyl *N*-(benzyloxycarbonyl)-D-glutamate (**60**) was synthesized by a route similar to that employed for the synthesis of its L-enantiomer[28] (Scheme 5). D-Glutamic acid was converted to its corresponding γ-methyl ester hydrochloride **57** by treatment with $SOCl_2$/MeOH.[29] Benzyl chloroformate was then used for the introduction of the Z-group[30] and the α-carboxyl of **58** masked as its *tert*-butyl ester by treatment with Bu^{t}OH/$POCl_3$/pyridine.[28] In the last step, the γ-methyl ester was hydrolyzed under alkaline conditions to afford **60** in 33% overall yield.[31]

Stereochemical Integrity and NMR Analysis of Quinazoline Antifolate Dipeptides

Since there is no evidence to suggest that epimerization takes place in the mixed carbonic anhydride-coupling, hydrogenation, DEPC-coupling or acid hydrolysis steps, it was expected that the route employed for the preparation of **32**−**44** and **64a,b** would give isomerically pure antifolates. To confirm this, an HPLC chromatographic separation of **3** (ICI 198583-γ-L-Glu) and its three stereoisomers **32** (L-D), **64a** (D-L), and **64b** (D-D) using a β-cyclodextrin column was attempted. The chromatogram of the L-D stereoisomer, compound **32**, contained only one peak suggesting that this methodology provides isomerically pure products. However, the chromatogram of the D−D stereoisomer **64b** showed a 14% contamination with the L-D stereoisomer **32**, while the chromatogram of the D-L stereoisomer **64a** showed an 8% contamination with the L-L stereoisomer. These unexpected results led us to an investigation of the optical purity of the glutamate **60** which was used as one of the chiral building blocks for the preparation of both **64a,b**. Indeed, the specific optical rotation of **60** was found to be +20.0°, significantly lower than that reported for the L-enantiomer (−26.6°),[28] suggesting that this compound was enantiomerically impure. As a consequence, quinazoline antifolates **64a,b** were obtained as diastereoisomeric mixtures D-L/L-L and D-D/L-D, respectively. Compounds **64a,b** were not prepared in isomerically pure form since they were substantially less potent inhibitors of TS compared with ICI 198583-γ-D-Glu (**32**) (8- and 6-fold, respectively).

The proton NMR spectrum of compounds unsubstituted in the *p*-aminobenzoate residue, *e.g.*, **32**, shows the amidic hydrogen of the first glutamic residue as a doublet (J = 7.5 Hz) due to a vicinal coupling to GluL

Scheme 3



Scheme 4

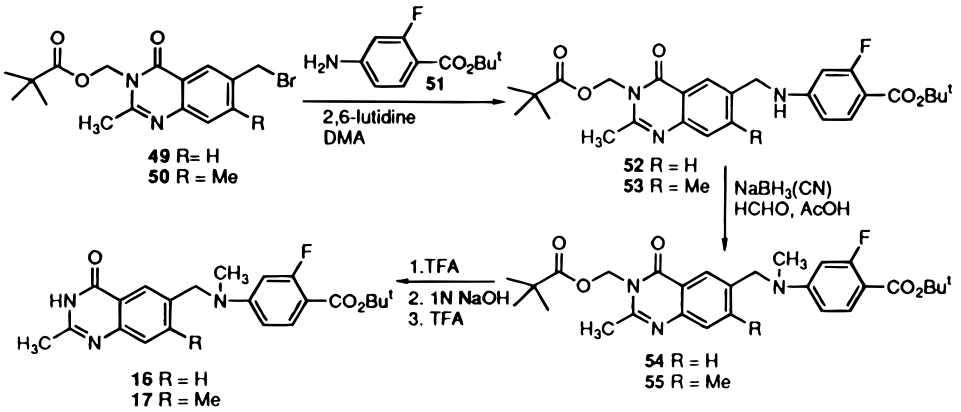


Table 2. Preparation of Quinazoline Antifolate L-D Dipeptides

compd	R^1	R^2	X	Y	yield (%)	mp (°C)	*m/z* $(M + H)^+$	formula	anal.
32	H	pg	H	Glu	100	146−148	606	$C_{30}H_{31}N_5O_9 \cdot 1.4TFA$	CHN
33	Me	pg	H	Glu	89	160 dec	642[a]	$C_{31}H_{33}N_5O_9 \cdot TFA \cdot 0.5Et_2O \cdot 1.3H_2O$	CHN
34	H	pg	F	Glu	81	115 dec	624[a]	$C_{30}H_{30}FN_5O_9 \cdot 1.5TFA \cdot 0.6Et_2O \cdot 0.5H_2O$	CHNF
35	Me	pg	F	Glu	92	155 dec	638	$C_{31}H_{32}FN_5O_9 \cdot TFA \cdot 0.5Et_2O \cdot 0.6H_2O$	CHNF
36	H	Pg	H	Ala	79	180 dec	570[a]	$C_{28}H_{29}N_5O_7 \cdot TFA \cdot 0.7Et_2O$	CHNF
37	H	pg	H	Phe	91	130 dec	624	$C_{34}H_{33}N_5O_7 \cdot TFA \cdot 0.7H_2O$	CHNF
38	H	pg	H	aad	97	145−146	620	$C_{31}H_{33}N_5O_9 \cdot 0.9TFA \cdot H_2O$	CHNF
39	Me	pg	F	aad	89	143−144 dec	652	$C_{32}H_{34}FN_5O_9 \cdot 0.8TFA \cdot 0.75H_2O$	CHNF
40	H	Me	H	Glu	59	105 dec	582	$C_{28}H_{31}N_5O_9 \cdot 1.5TFA \cdot 0.55Et_2O \cdot 1.1H_2O$	CHNF
41	Me	Me	H	Glu	90	150 dec	596	$C_{29}H_{33}N_5O_9 \cdot 1.1TFA \cdot 0.2Et_2O \cdot H_2O$	CHNF
42	H	Me	F	Glu	95	145−147	600	$C_{28}H_{30}FN_5O_9 \cdot 1.3TFA \cdot Et_2O \cdot 0.75H_2O$	CHNF
43	Me	Me	F	Glu	92	159−161	614	$C_{29}H_{32}FN_5O_9 \cdot TFA \cdot H_2O$	CHNF
44	H	Et	H	Glu	83	140 dec	596[a]	$C_{29}H_{33}N_5O_9 \cdot 0.95TFA \cdot 0.15Et_2O \cdot 1.3H_2O$	CHNF

[a] $(M+Na)^+$.

α-CH. However, in the 2′-F and 2′-F,7-Me derivatives, *e.g.*, compound **34**, **35**, **21** or **22**, the amidic hydrogen of the first glutamic residue appears as a double doublet or triplet. The same phenomenon was observed by Thornton *et al.*[15] with the 2′-F derivative of ICI 198583. This is due to an additional long range coupling to the 2′-F, and in the case of **22**, the existence of this coupling was demonstrated by a heteronuclear decoupling experiment. When the 2′-F signal was irradiated, the corresponding amidic hydrogen signal was collapsed to a doublet (Figure 1), revealing a coupling of 5.5 Hz between NH and 2′-F.

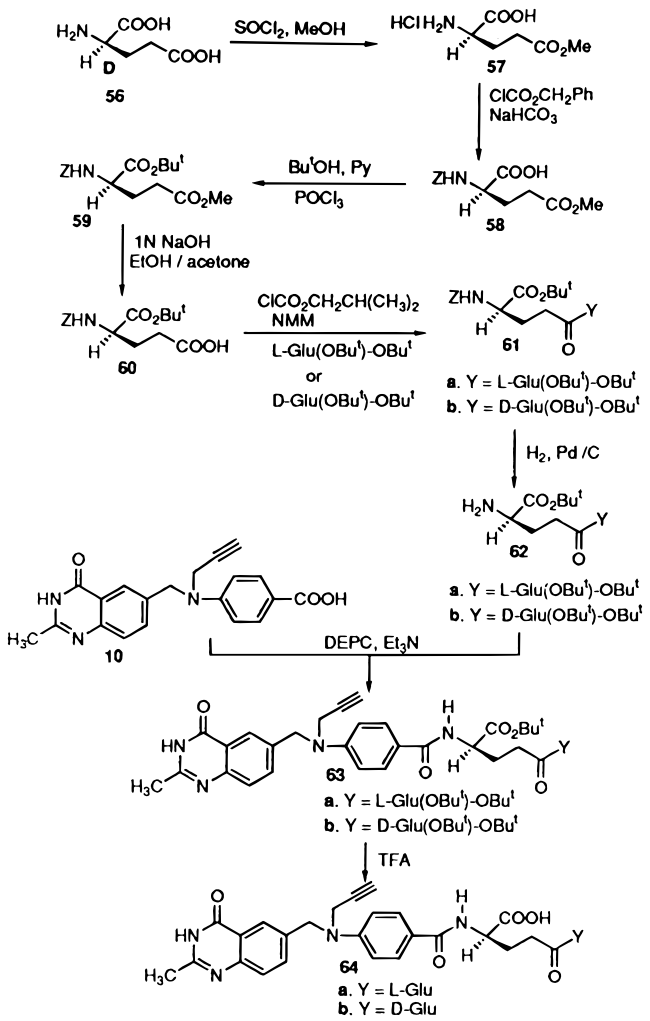
Biological Evaluation

The antifolates listed in Table 3 were tested as inhibitors of partially purified TS from L1210 mouse leukemia cells that overproduce TS due to amplification of the TS gene.[32] The partial purification and assay methods used in this study were as previously described, and they used (±)-5,10-methylenetetrahydrofolic acid at a concentration of 200 μM.[32] Inhibition of L1210 and L1210:1565 cell growth was also as previously described.[33] L1210:1565 is a L1210 mutant cell line with impaired reduced folate/MTX transport carrier. This cell line was made resistant to CI-90, a compound that uses the RFC transport system,[34] and hence is cross-resistant to MTX.

Results and Discussion

Replacement of the second glutamic residue of ICI 198583-γ-L-Glu by D-amino acids (Glu, Ala, Phe, aad) gave compounds **32** and **36**−**38** which were shown to be resistant to enzymatic degradation (ref 12; Table 4). ICI 198583-γ-D-Glu (**32**) was a potent inhibitor of TS (IC_{50} = 4.6 nM) and only 2-fold worse then ICI 198583-γ-L-Glu. However, replacing the propionate side chain of the D-glutamate of **32** by a methyl or benzyl group gave dicarboxylates **36** and **37** which were respectively 3- and 7-fold worse inhibitors of TS. On the other hand, lengthening the propionate side chain of the D-glutamate of **32** by one methylene gave ICI 198583-γ-aad (**38**) with inhibitory activity against TS similar to that of **32**. As indicated by an analysis of the computer graphics model

Scheme 5



of the humanized *E. coli* active site of TS (see Introduction), the L-D dipeptide derivatives **32** and **36**−**38** would be expected to exhibit similar TS inhibition to their L-L counterparts (Table 5).

As inhibitors of cell growth, **36** and **38** showed similar activity to that of **32**, but **37** was less active. Growth inhibition was generally similar to the L-L counterparts (Table 5). It appears that changing the stereochemistry of the second amino acid does not prevent uptake via the RFC since compounds **32**, **36**, and **38** were poorly active in the L1210/1565 cell line (Table 3).

Replacing the first glutamic residue of ICI 198583-γ-L-Glu (**3**) by its D-enantiomer gave compound **64a** and resulted in a substantial decrease (~20-fold) in TS inhibition (IC_{50} = 37 nM). The same trend was observed when both glutamic residues of **3** were replaced by their D-enantiomers (compound **64b**, TS IC_{50} = 27 nM). These results highlight the importance of the α-carboxyl for binding to TS and suggest that by changing the stereochemistry of the first glutamic residue of ICI 198583-γ-L-Glu from L to D, the α-carboxyl is no longer able to bind to the lysine residue in the active site of the enzyme. The lack of this interaction may account for the low inhibitory activity of **64a,b** for TS. The D-L dipeptide **64a** was unstable to hydrolysis when injected into mice, but the D-D analogue **64b** was apparently stable to enzymatic degradation.[12]

In an attempt to study how other structural modifications of the N^{10}-propargyl quinazoline L-Glu-γ-D-Glu derivative **32** affects TS and L1210 cell growth inhibition, compounds **33**−**35** were synthesized. Crystal structures of the ternary complex of *E. coli* TS with CB 3717 and 5-fluoro-2′-deoxyuridylate (FdUMP) indicate that CB 3717 binds in a partially folded conformation, with the *p*-aminobenzoyl ring inclined at 65° to the quinazoline ring.[35,36] The impact of 7-Me, 5-Me, or 7,9-di-Me substitution on this conformation was studied by Boyle *et al.* using molecular mechanics (SCANOPT) and semiempirical quantum mechanical energy calculations (AMPAC).[14] It was found that 7-Me substitution reinforces this conformation and, as predicted, TS inhibition was improved.[14] With this consideration in mind, a 7-methyl group was introduced into the quinazoline ring to give compound **33**. The substitution of a 2′-F in dipeptide **32** to give compound **34** originated from the observation that introduction of a 2′-F in ICI 198583 had a beneficial effect in binding to TS.[15] As expected, in our compounds substitution of a 7-Me into the quinazoline ring (compound **33**) or a 2′-F into the benzene ring (compound **34**) enhanced TS inhibition (each by ~2-fold compared with the parent **32**). Combining both of these modifications to give compound **35** resulted in 5-fold improvements in TS inhibition (K_i = 0.19 nM). However, compounds **32**−**35** all showed similar growth inhibitory activity against the L1210 cell line (IC_{50} ~ 0.2 μM), which may be a consequence of marginally slower cellular uptake.

Replacing the N^{10}-propargyl substituent in compound **32** by methyl (compound **40**) or ethyl (compound **44**) gave poorer inhibitors of TS and L1210 cell growth (Table 3). However, substitution of 7-Me or 2′-F into dipeptide **40** gave compounds **41** and **42** with respectively a 7- and 4-fold increase in TS inhibition. Substitution of a 2′-F into the benzene ring and a 7-Me into the quinazoline ring of dipeptide **40** gave compound **43**, which was a 17-fold better inhibitor of TS. Since the 2′-F,7-Me substitution pattern strengthened binding to TS in both the N^{10}-methyl and N^{10}-propargyl series, these substituents were introduced into ICI 198583-γ-D-aad (**38**). The resulting compound **39** showed similar inhibitory activity against TS and L1210 cell growth to that of **35** (TS IC_{50} = 0.56 ± 0.34 nM, L1210 IC_{50} = 0.13 ± 0.063 μM).

Some compounds were tested as inhibitors of the growth of L1210:R^{D1694} cell line which has a marked polyglutamation defect and is consequently cross-resistant (18-fold) to ICI 198583-γ-L-Glu.[37] Compound **32** had good activity against this cell line (IC_{50} = 0.68 ± 0.35, Table 5). This is in agreement with other findings which show that natural folate and folate analogues possessing a D-glutamate are not substrates for FPGS.[13] If the second amino acid is Ala, then the stereochemistry is unimportant as neither can be polyglutamated. This also indicates that although hydrolysis of the L−L bond occurs in mice, it does not occur in tissue culture to any significant extent (the breakdown product ICI 198583 has poor activity against this cell line because of its requirement for polyglutamation[37]). The cytotoxicity of ICI 198583-γ-L-aad (L1210:R^{D1694} IC_{50} = 2.9 ± 0.40, Table 5) was interesting in that the level of cross-resistance seen was quite high but was reduced when the terminal L-aad was replaced with the D-aad (compound **38**; Table 5). This may suggest that

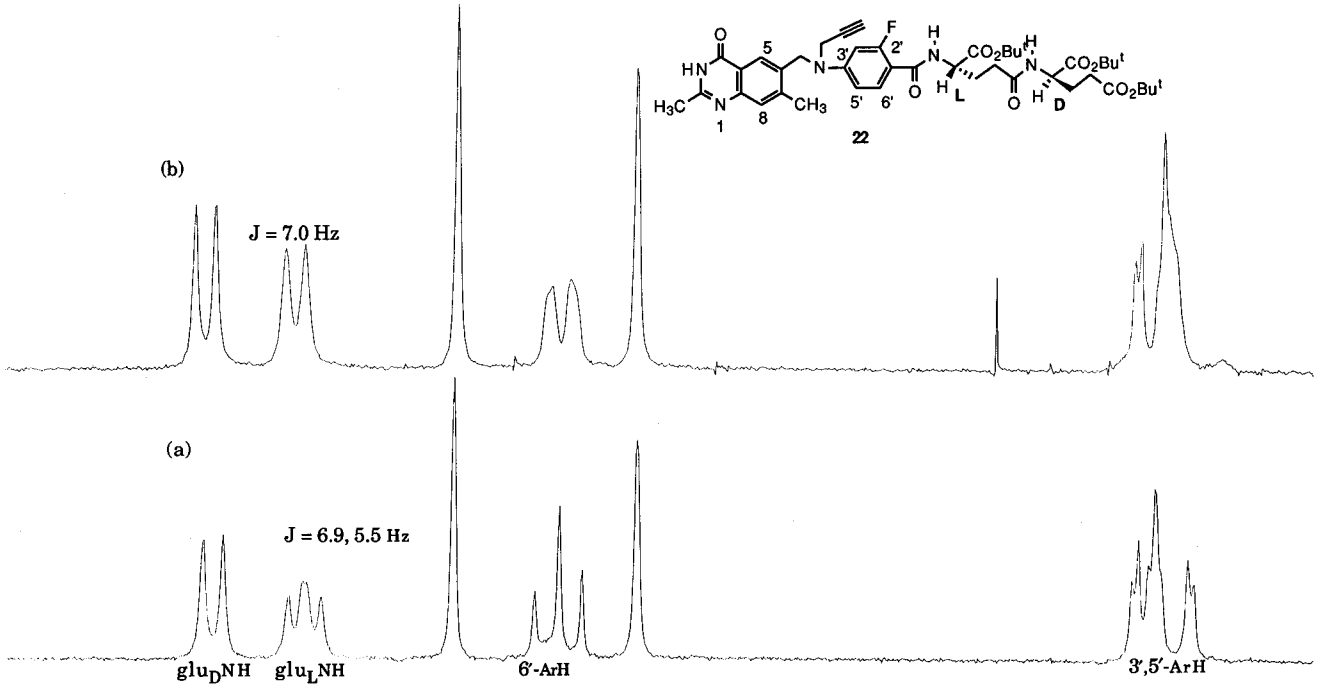
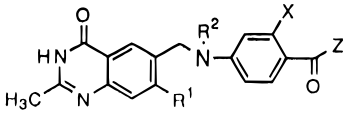


Figure 1. (a) ^{1}H NMR spectrum of **22** and (b) heteronuclear-decoupled spectrum of **22** obtained by irradiating the 2′-F signal and then recording the ^{1}H NMR spectrum.

Table 3. L1210 TS and Cell Growth Inhibition Data for Quinazoline Antifolate γ-Linked Dipeptides[a]



compd	R^1	R^2	X	Z	inhibition of L1210 TS, IC_{50} (nM)	inhibition of cell growth, IC_{50} (μM) L1210	W1L2	L1210:1565
3	H	pg	H	L-Glu-L-Glu	2.0	0.16 ± 0.069	0.09, 0.074	4.6, 5.0
32	H	pg	H	L-Clu-D-Glu	4.6	0.33 ± 0.25	0.15, 0.23	15, 16
33	Me	pg	H	L-Glu-D-Glu	1.9	0.21	0.23	7.8
34	H	pg	F	L-Glu-D-Glu	1.9	0.21	0.22	7.8
35	Me	pg	F	L-Glu-D-Glu	0.4, 0.92[b]	0.20 ± 0.017	0.17, 0.093	6.8, 8.7
36	H	pg	H	L-Glu-D-Ala	12	0.43 ± 0.13	0.15	16
37	H	pg	H	L-Glu-D-Phe	30	2.1 ± 0.36		>10
38	H	pg	H	L-Glu-D-aad	10, 4.2	0.08, 0.11	0.22	27
39	Me	pg	F	L-Glu-D-aad	0.56 ± 0.34[c]	0.13 ± 0.063	0.19, 0.3	8.1, 12
40	H	Me	H	L-Glu-D-Glu	34	3.2		
41	Me	Me	H	L-Glu-D-Glu	4.6	0.58	0.28	19
42	H	Me	F	L-Glu-D-Glu	8.8	0.65	0.9	21
43	Me	Me	F	L-Glu-D-Glu	2.0	0.26		
44	H	Et	H	L-Glu-D-Glu	17	3.6		
64a	H	pg	H	D-Glu-L-Glu	37	3.4	2.4	50
64b	H	pg	H	D-Glu-D-Glu	27	1.3	1.4	~100

[a] Methodologies for the inhibition of mouse L1210 TS and L1210, L1210:1565 (impaired RFC), and human W1L2 cell growth are as described in refs 32 and 33. [b] K_i = 0.19 nM. [c] K_i = 0.12 nM.

the L-Glu-γ-L-aad is a substrate for FPGS. Further work using isolated FPGS may clarify this.

Recent data demonstrate that cellular TS is only inhibited while the compounds, *e.g.*, **32** and **35**, are present in the medium. This is contrast to ICI 198583-γ-L-Glu that continues to inhibit the enzyme even after removal from the extracellular environment[37,38] which is consistent with the formation of slowly effluxable higher chain polyglutamates.

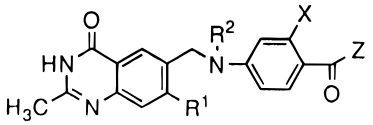
Compounds **35** and **39** were also tested as inhibitors of tumor growth in mice. Both compounds had activity against the L57789 $TK^{-/-}$ and $TK^{+/-}$ tumors and the HX62 human ovarian tumor xenograft.[39,40]

In conclusion, replacement of the second amino acid of γ-linked L-L dipeptides of ICI 198583 by their D-enantiomers produced dipeptide derivatives that were potent inhibitors of TS and also stable to hydrolysis when injected into mice. The best examples were the Glu-γ-D-Glu derivative **35** and the Glu-γ-D-aad derivative **39**. Reduced activity against the L1210:1565 cell line compared to the parental line indicated that L-D dipeptides utilize the RFC transport system to enter cells. Equivalent activity in the L1210:R^{D1694} line suggests that they were not substrates for FPGS. These compounds therefore represent an interesting group of potent, water-soluble, folate-based TS inhibitors that do not undergo polyglutamation but have antitumor activity.

Experimental Section

Thin layer chromatography (TLC) was performed on precoated sheets of silica gel $60F_{254}$ (Merck art. 5735). Visualiza-

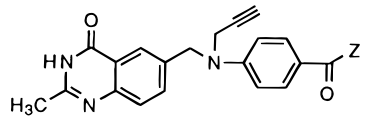
Table 4. Stability of Quinazoline Antifolate γ-Linked Dipeptides in Mice[a]



compd	R^1	R^2	X	Z	plasma concentration (μM): dipeptide (parent compound)	plasma concentration (μM): monoglutamate (hydrolysis product)
3	H	pg	H	L-Glu-L-Glu	27 ± 5	14 ± 2 (from ref 12)
32	H	pg	H	L-Glu-D-Glu	34 ± 6	ND[b] (from ref 12)
35	Me	pg	F	L-Glu-D-Glu	32 ± 11	ND
38	H	pg	H	L-Glu-D-aad	31 ± 4	ND
40	H	Me	H	L-Glu-D-Glu	17 ± 4	ND

[a] Liver only contained the dipeptide. Methodology is described in ref 12. [b] ND = not detected.

Table 5. L-D and L-D Dipeptides: Inhibition of L1210 TS and of L1210 and L1210:R^{D1694} Cell Growth



compd	Z	inhibition of L1210 TS, IC_{50} (nM)	inhibition of cell growth, IC_{50} (μM): L1210	inhibition of cell growth, IC_{50} (μM): L1210:R^{D1694}
3	L-Glu-L-Glu	2.0	0.16 ± 0.069	2.7, 2.9 (18)
32	L-Glu-D-Glu	4.6	0.33 ± 0.25	0.68 ± 0.35 (2)
	L-Glu-L-Ala	13	0.62, 0.79	2.1, 1.6 (2)
36	L-Glu-D-Ala	12	0.43 ± 0.13	0.88 (2)
	L-Glu-L-Phe	18	4.4	8.9 (2)
37	L-Glu-D-Phe	30	2.1 ± 0.36	
	L-Glu-L-aad	2	0.30 ± 0.031	2.9 ± 0.40 (10)
38	L-Glu-D-aad	10, 4.2	0.08, 0.11	0.44, 0.42 (4)

tion was achieved by UV or chlorine−tolidine reagent. Merck silica gel 60 (art. 15111) was used in low-pressure column chromatography. High-performance liquid chromatography (HPLC) analyses were performed using a Waters model 510 solvent delivery system, model 680 automated gradient controller, model U6K injector, and model 490 programmable wavelength detector set to monitor at 230 and 280 nm. Retention times were determined on a Trivector Trilab 3000 multichannel chromatography system. Separation of stereoisomers was performed on a 25 cm × 0.46 cm Astec cyclobond I (B) column (Technical Ltd., U.K.), and they were eluted isocratically with 82% 50 mM Na_2HPO_4/50 mM NaH_2PO_4 (1:1, v/v)−18% CH_3CN at a flow of 1 mL/min. Electron impact (EI) and chemical ionization (CI) mass spectra were determined with a VG 7070H spectrometer and a VG 2235 data system using the direct-insertion method, an ionizing voltage of 70eV, a trap current of 100 μA, and an ion-source temperature of 160 °C. Fast atom bombardment (FAB) mass spectra were determined with a VG ZAB-SE spectrometer. Electrospray ionization (ESI) mass spectra were recorded using a TSQ 700 triple-quadrupole mass spectrometer (Finnigan MAT) fitted with an electrospray ionization source (Analytica). Samples were dissolved in methanol:water (50:50, v/v) containing 1% acetic acid and infused into the mass spectrometer using a Harvard infusion pump (Cambridge) at 1 μL/min. Masses were scanned from 200 to 800 amu at a scanning speed of 3 s/scan. Proton NMR spectra were recorded using a Bruker AC250 spectrometer. Field strengths are expressed in units of δ (ppm) relative to tetramethylsilane, and peak multiplicities are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; dm, doublet of multiplets; t, triplet; q, quartet, br s, broad singlet; m, multiplet. Optical rotations were obtained using a Perkin-Elmer model 141 polarimeter. A sodium lamp was used as radiation source. Melting points were detemined on a Kofler block and are uncorrected. Elemental analyses were determined by C.H.N. Analysis Ltd., Leicester, U.K.

General Procedures. Procedure A: Preparation of Z-Blocked Dipeptide *tert*-Butyl Esters. To a stirred solution of α-*tert*-butyl *N*-(benzyloxycarbonyl)-L-glutamate (1.0 mmol) in dry THF (3.0 mL) and *N*-methylmorpholine (1.0 mmol) cooled to −20 °C was added isobutyl chloroformate (1.0 mmol) (a white precipitate had formed). In method A1 stirring was continued for 10 min at −20 °C, and then a suspension of the appropriate amino acid *tert*-butyl ester hydrochloride salt (1.0 mmol) in dry THF (3.0 mL) and *N*-methylmorpholine (1.0 mmol) was added to the reaction mixture. In method A2 stirring was continued for 10 min at −20 °C, and then a solution of the appropriate amino acid *tert*-butyl ester free base (1.0 mmol) in dry THF (3.0 mL) was added into the reaction mixture. Stirring was continued at −20 °C for 10 min and then for 1.5 h at room temperature. *N*-Methylmorpholine hydrochloride was filtered off, and the filtrate was concentrated *in vacuo* to give the crude product which was purified by column chromatography.

Procedure B: Hydrogenolysis of Z-Blocked Dipeptide *tert*-Butyl Esters. To a solution of the Z-protected dipeptide (1.0 mmol) in EtOAc (60 mL) was added 10% Pd/C (10−15% of the dipeptide's weight). The resulting black mixture was degassed and then stirred at room temperature for 3 h under a hydrogen atmosphere (balloon). The catalyst was filtered off and the filtrate evaporated to provide the dipeptide free base which was immediately taken forward into the next step used without further purification.

Procedure C: Preparation of Quinazoline Antifolate Dipeptide Esters. To a stirred solution of the dipeptide free base (1.2 mmol) in dry DMF (14 mL) cooled to 0 °C was added the appropriate pteroic acid analogue, trifluoroacetate salt (1.0 mmol) followed by DEPC (2.2 mmol) and Et_3N (2.2 mmol). Stirring was continued at 0 °C for 10 min and then for 2 h at room temperature under an argon atmosphere and in the dark. The solution was then diluted with EtOAc (100 mL) and H_2O (100 mL); the two layers were separated, and the aqueous layer was extracted with EtOAc (2 × 100 mL). The combined organic extracts were successively washed with 10% aqueous citric acid (2 × 100 mL), saturated aqueous $NaHCO_3$ (200 mL), dilute aqueous NaCl (150 mL), and H_2O (150 mL), dried (Na_2SO_4), and concentrated *in vacuo* to leave the crude product.

Procedure D: Acid Hydrolysis of the *tert*-Butyl Esters with Trifluoroacetic Acid. A solution of the appropriate antifolate dipeptide *tert*-butyl ester (1.0 mmol) in TFA (30 mL) was stirred at room temperature for 1.25 h with protection from the light. The solution was then concentrated *in vacuo*, and the oily residue was triturated with Et_2O. The solid was collected by filtration, washed with Et_2O (40 mL), and dried *in vacuo* over P_2O_5.

Preparation of Z-Blocked Dipeptide *tert*-Butyl Esters: Di-*tert*-butyl *N*-[*N*-(Benzyloxycarbonyl)-L-γ-glutamyl]-D-alaninate (6a). The general procedure A1 was followed using α-*tert*-butyl *N*-(benzyloxycarbonyl)-L-glutamate (2.02 g, 6.0 mmol), *N*-methylmorpholine (0.606 g, 6.0 mmol), dry THF (10 mL), isobutyl chloroformate (0.816 g, 6.0 mmol), and a suspension of D-alanine α-*tert*-butyl ester hydrochloride (1.09 g, 6.0 mmol) in dry THF (10 mL) and *N*-methylmorpholine (0.606 g, 6.0 mmol). Purification of the crude product by column chromatography, on elution with 2:1 (v/v) CH_2Cl_2/EtOAc, gave the title compound **6a** (2.30 g, 83%) as a white solid: mp 78−80 °C; NMR (Me_2SO-d_6) δ 1.21 (d, J = 7.3 Hz, 3H, Ala β-CH_3), 1.38, 1.39 (2 × s, 18H, 2 × C(CH_3)$_3$), 1.74, 1.90 (2 × m, 2H, β-CH_2), 2.18 (t, J = 7.5 Hz, 2H, γ-CH_2), 3.88 (m, 1H, Glu α-C*H*), 4.06 (m, 1H, Ala α-C*H*), 5.02 (ABq, J = 12.5 Hz, 2H, ArCH_2), 7.35 (m, 5H, Ar*H*), 7.64 (d, J = 7.7 Hz, 1H, Glu N*H*), 8.17 (d, J = 7.0 Hz, 1H, Ala N*H*); MS (FAB): m/z 465 $(M + H)^+$. Anal. ($C_{24}H_{36}N_2O_7$) C, H, N.

α-*tert*-Butyl *N*-(Benzyloxycarbonyl)-D-phenylalaninate (9). *N*-(Benzyloxycarbonyl)-D-phenylalanine (2.99 g, 10.0 mmol) was dissolved in CH_2Cl_2 (83 mL) in a 500 mL pressure bottle. To this solution was added concentrated H_2SO_4 (0.37 mL) followed by liquid isobutylene (41 mL) at −20 °C. The stoppered reaction vessel was shaken at room temperature for 28 h, and then the reactants were neutralized with a saturated solution of $NaHCO_3$. EtOAc (150 mL) was added; the two layers were separated, and the aqueous layer was washed with

more EtOAc (100 mL). The combined organic extracts were then washed with a saturated solution of $NaHCO_3$ (2 × 100 mL) and H_2O (2 × 100 mL), dried ($MgSO_4$), and concentrated *in vacuo* to give a white solid. Purification by column chromatography, on elution with 5% EtOAc in CH_2Cl_2, gave the title compound **9** (2.53 g, 71%) as a white solid: mp 80−81 °C; NMR (Me_2SO-d_6) δ 1.33 (s, 9H, C(CH_3)$_3$), 2.90 (m, 2H, β-CH_2), 4.13 (m, 1H, α-C*H*), 4.99 (m, 2H, ArCH_2OCO), 7.29 (m, 10H, 2 × Ar*H*), 7.72 (d, J = 7.7 Hz, 1H, N*H*); MS (CI) m/z 356 (M + H)$^+$. Anal. ($C_{21}H_{25}NO_4$) C, H, N.

***tert*-Butyl D-Phenylalaninate (5b).** The general procedure B was followed using **9** (2.45 g, 6.9 mmol), EtOAc (220 mL), and 10% Pd/C (0.260 g). The reaction mixture was stirred at room temperature for 15 h under a hydrogen atmosphere. Compound **5b** was obtained as a colorless oil (1.50 g, 98%): NMR (Me_2SO-d_6) δ 1.31 (s, 9H, C(CH_3)$_3$), 2.78 (m, 2H, β-CH_2), 3.43 (t, J = 6.8 Hz, 1H, α-C*H*), 7.21 (m, 5H, Ar*H*); MS (CI) m/z 222 (M + H)$^+$.

Di-*tert*-butyl *N*-[*N*-(Benzyloxycarbonyl)-L-γ-glutamyl]-D-phenylalaninate (6b). The general procedure A2 was followed using α-*tert*-butyl *N*-(benzyloxycarbonyl)-L-glutamate (1.051 g, 3.12 mmol), dry THF (5 mL), *N*-methylmorpholine (0.315 g, 3.12 mmol), isobutyl chloroformate (0.424 g, 3.12 mmol), and a solution of *tert*-butyl D-phenylalaninate (**5b**; 0.69 g, 3.12 mmol) in dry THF (5 mL). Purification by column chromatography, on gradient elution with 1:9−1:4 (v/v) EtOAc/CH_2Cl_2, gave the title compound **6b** as a colorless oil (1.45 g, 86%) which solidified on standing for a few weeks, giving a white solid: mp 79−80 °C; NMR (Me_2SO-d_6) δ 1.31, 1.38 (2 × s, 18H, 2 × C(CH_3)$_3$), 1.69, 1.85 (2 × m, 2H, Glu β-CH_2), 2.16 (t, J = 7.0 Hz, 2H, Glu γ-CH_2), 2.90 (m, 2H, Phe β-CH_2), 3.87 (m, 1H, Glu α-C*H*), 4.32 (m, 1H, Phe α-C*H*), 5.02 (m, 2H, PhCH_2OCO), 7.23 (m, 5H, Phe Ar*H*), 7.35 (m, 5H, *Ph*CH$_2$OCO), 7.62 (d, J = 7.7 Hz, 1H, Glu N*H*), 8.24 (d, J = 7.7 Hz, 1H, Phe N*H*); MS (CI) m/z 541 (M + H)$^+$. Anal. ($C_{30}H_{40}N_2O_7$) C, H, N.

Di-*tert*-butyl D-Glutamate (5c). a. Transesterification Method. D-Glutamic acid (1.3 g, 8.84 mmol), *tert*-butyl acetate (100 mL), and 70% aqueous $HClO_4$ (1.4 g, 9.7 mmol) were stirred at room temperature for 48 h. The solution was then cooled in an ice−water bath and extracted with 0.5 N HCl (2 × 60 mL). The combined aqueous extracts were immediately neutralized with solid $NaHCO_3$. The aqueous solution was extracted with Et_2O (2 × 140 mL); the ether extracts were pooled, dried (Na_2SO_4), and concentrated *in vacuo* to a colorless oily residue. This was diluted with Et_2O (25 mL), and then gaseous HCl was passed through the solution. The solution was left in a freezer overnight, and then the precipitated white solid was collected by filtration, washed with Et_2O (20 mL), and dried *in vacuo* over P_2O_5. The title compound **5c** was thus obtained as its hydrochloride salt (0.74 g, 28%): mp 121−122 °C; NMR (Me_2SO-d_6) δ 1.40, 1.45 (2 × s, 18H, 2 × C(CH_3)$_3$), 1.94, 2.27−2.50 (2 × m, 4H, β-CH_2, γ-CH_2), 3.89 (t, J = 6.6 Hz, 1H, α-C*H*), 8.38 (s, 3H, NH_3); MS (CI) m/z 260 (M + H)$^+$.

b. Isobutylene Method. D-Glutamic acid (1.90 g, 13.0 mmol) was suspended in CH_2Cl_2 (94 mL) in a 500 mL pressure bottle. To this suspension was added concentrated H_2SO_4 (0.58 mL) followed by liquid isobutylene (41 mL) at −20 °C, and the bottle was tightly stoppered. The reaction mixture was shaken at room temperature for 72 h and then neutralized with a saturated solution of $NaHCO_3$. EtOAc (100 mL) was added; the two layers were separated, and the aqueous layer was washed with more EtOAc (2 × 100 mL). The combined organic extracts were dried ($MgSO_4$) and then concentrated *in vacuo* to give the title compound **5c** as a pale yellow oil (1.50 g, 45%): NMR (Me_2SO-d_6) δ 1.40 (s, 18H, 2 × C(CH_3)$_3$), 1.75 (m, 2H, β-CH_2), 2.27 (m, 2H, γ-CH_2), 3.15 (dd, J = 8.1, 5.1 Hz, 1H, Glu α-C*H*); MS (CI) m/z 260 (M + H)$^+$.

Tri-*tert*-butyl *N*-[*N*-(Benzyloxycarbonyl)-L-γ-glutamyl]-D-glutamate (6c). The general procedure A2 was followed using α-*tert*-butyl *N*-(benzyloxycarbonyl)-L-glutamate (1.011 g, 3.0 mmol), *N*-methylmorpholine (0.303 g, 3.0 mmol), dry THF (10 mL), isobutyl chloroformate (0.408 g, 3.0 mmol), and a solution of di-*tert*-butyl D-glutamate (0.855 g, 3.3 mmol) in dry THF (10 mL). The crude product was purified by column chromatography using 2% MeOH in CH_2Cl_2 as eluent. Trituration with hexanes afforded the title compound **6c** as a white solid. This was collected by filtration, washed with hexanes, and dried *in vacuo* (1.36 g, 78%): mp 110 °C; NMR (Me_2SO-d_6) δ 1.39 (s, 27H, C(CH_3)$_3$), 1.73, 1.89 (2 × m, 4H, β-CH_2), 2.23 (m, 4H, γ-CH_2), 3.89 (m, 1H, GluL α-C*H*), 4.10 (m, 1H, GluD α-C*H*), 5.03 (ABq, J = 14.0 Hz, 2H, PhCH_2), 7.36 (m, 5H, Ar*H*), 7.63 (d, J = 7.7 Hz, 1H, GluL N*H*), 8.13 (d, J = 7.7 Hz, 1H, GluD N*H*); MS (FAB) m/z 579 (M + H)$^+$. Anal. ($C_{30}H_{46}N_2O_9$) C, H, N.

Di-*tert*-butyl D-α-Aminoadipate Hydrochloride (5d). D-α-Aminoadipic acid (1.0 g, 6.2 mmol), *tert*-butyl acetate (85 mL), and 70% aqueous $HClO_4$ (0.99 g) were stirred at room temperature for 24 h. The mixture was then cooled in an ice−water bath and extracted with 0.5 N HCl (2 × 50 mL). The combined aqueous extracts were immediately neutralized with solid $NaHCO_3$. The aqueous solution was extracted with Et_2O (2 × 150 mL); the ether extracts were pooled and dried ($MgSO_4$), and the solvent was reduced in volume to *ca*. 50 mL. This solution was acidified by addition of a few drops of dry ethereal HCl and then cooled in an ice−water bath. The resulting white solid was isolated by filtration, washed with ether (2 × 10 mL), and dried *in vacuo*: 0.93 g (48%); mp 133−134 °C; NMR (Me_2SO-d_6) δ 1.40, 1.47 (2 × s, 18H, C(CH_3)$_3$), 1.54, 1.64 (2 × m, 2H, β-CH_2), 1.73 (m, 2H, γ-CH_2), 2.24 (t, J = 7.2 Hz, 2H, δ-CH_2), 3.88 (t, J = 5.9 Hz, 1H, α-C*H*), 8.19 (s, 3H, NH_3).

Tri-*tert*-butyl *N*-[*N*-(Benzyloxycarbonyl)-L-γ-glutamyl]-D-2-aminoadipate (6d). The general procedure A1 was followed using α-*tert*-butyl *N*-(benzyloxycarbonyl)-L-glutamate (4.65 g, 14.0 mmol), *N*-methylmorpholine (1.39 g, 14.0 mmol), THF (60 mL), isobutyl chloroformate (1.88 g, 14.0 mmol), and a suspension of **5d** (4.27 g, 14.0 mmol) in dry THF (30 mL) and *N*-methylmorpholine (1.39 g, 14.0 mmol). Purification of the crude product by column chromatography, on elution with 1% MeOH in CH_2Cl_2, gave the required product which was recrystallized from petroleum ether (100 mL), collected by filtration, and washed with petroleum ether (3 × 50 mL): 7.46 g, (90%); mp 87−88 °C; NMR (Me_2SO-d_6) δ 1.39 (s, 27H, C(CH_3)$_3$), 1.53, 1.64, 1.78, 1.91 (4 × m, 6H, Glu β-CH_2, aad β-CH_2, aad γ-CH_2), 2.20 (m, 4H, Glu γ-CH_2, aad δ-CH_2), 3.89 (m, 1H, Glu α-C*H*), 4.06 (m, 1H, aad α-C*H*), 5.04 (ABq, J = 12.4 Hz, 2H, ArCH_2), 7.35 (m, 5H, Ar*H*), 7.57 (d, J = 7.7 Hz, 1H, Glu N*H*), 8.08 (d, J = 7.6 Hz, 1H, aad N*H*); MS (ESI) m/z 615 (M + Na)$^+$. Anal. ($C_{31}H_{48}N_2O_9$·0.25H_2O) C, H, N.

Hydrogenolysis of Z-Blocked Dipeptide *tert*-Butyl Esters: Di-*tert*-butyl L-γ-Glutamyl-D-alaninate (7a). The general procedure B was followed using **6a** (0.696 g, 1.5 mmol), EtOAc (40 mL), and 10% Pd/C (0.125 g). Compound **7a** was obtained as a white sticky solid (0.480 g, 97%) and used immediately in the next experiment without further purification: NMR (Me_2SO-d_6) δ 1.21 (d, J = 7.3 Hz, 3H, Ala CH_3), 1.38, 1.41 (2 × s, 18H, 2 × C(CH_3)$_3$), 1.57, 1.77 (2 × m, 2H, Glu β-CH_2), 2.18 (t, J = 7.9 Hz, 2H, Glu γ-CH_2), 3.17 (dd, J = 5.2, 8.2 Hz, 1H, Glu α-C*H*), 4.07 (m, 1H, Ala α-C*H*), 8.16 (d, J = 6.9 Hz, 1H, Ala N*H*); MS (FAB) m/z 331 (M + H)$^+$.

Di-*tert*-butyl L-γ-Glutamyl-D-phenylalaninate (7b). The general procedure B was followed using **6b** (0.700 g, 1.30 mmol), EtOAc (90 mL), and 10% Pd/C (0.096 g). The title compound **7b** was obtained as a colorless oil (0.514 g, 98%): NMR (Me_2SO-d_6) δ 1.32, 1.40 (2 × s, 18H, 2 × C(CH_3)$_3$), 1.70 (m, 2H, Glu β-CH_2), 2.15 (m, 2H, Glu γ-CH_2), 2.90 (m, 2H, Phe β-CH_2), 3.11 (dd, J = 8.0, 5.1 Hz, 1H, Glu α-C*H*), 4.33 (m, 1H, Phe α-C*H*), 7.23 (m, 5H, CH$_2$*Ph*), 8.24 (d, J = 7.7 Hz, 1H, Phe N*H*); MS (CI) m/z 407 (M + H)$^+$.

Tri-*tert*-butyl L-γ-Glutamyl-D-glutamate (7c). The general procedure B was followed using *N*-[*N*-(benzyloxycarbonyl)-L-γ-glutamyl]-D-glutamate (**6c**; 2.4 g, 4.15 mmol), EtOAc (240 mL), and 10% Pd/C (0.330 g). The product **7c** was obtained as a colorless oil (1.84 g, 100%) and used immediately without further purification: NMR (Me_2SO-d_6) δ 1.39, 1.41 (2 × s, 27H, 3 × C(CH_3)$_3$), 1.56−1.87 (m, 4H, 2 × β-CH_2), 2.22 (m, 4H, 2 × γ-CH_2), 3.14 (dd, J = 5.1, 8.1 Hz, 1H, GluL α-C*H*), 4.11 (m, 1H, GluD α-C*H*), 8.13 (d, J = 7.6 Hz, 1H, GluD N*H*); MS (CI) m/z 445 (M + H)$^+$.

Tri-*tert*-butyl L-γ-Glutamyl-D-2-aminoadipate (7d). The general procedure B was followed using **6d** (5.38 g, 9.08 mmol),

EtOAc (300 mL), and 10% Pd/C (0.82 g). Standard workup afforded **7d** as a colorless oil (4.2 g, 100%): NMR (Me_2SO-d_6) δ 1.39, 1.40 (2 × s, 27H, 3 × C(CH_3)$_3$), 1.50−1.80 (m, 6H, Glu β-CH_2, aad β-CH_2 and γ-CH_2), 2.20 (m, 4H, Glu γ-CH_2, aad δ-CH_2), 3.13 (dd, J = 5.1, 8.0 Hz, 1H, Glu α-C*H*), 4.05 (m, 1H, aad α-C*H*), 8.13 (d, J = 7.5 Hz, 1H, aad, N*H*); MS (FAB) m/e 459 $(M + H)^+$.

Preparation of Pteroic Acid Analogues: *tert*-Butyl 4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoate (47b). To a suspension of 6-(bromomethyl)-3,4-dihydro-2-methyl-4-oxoquinazoline[41] (**45a**; 5.0 g, 19.8 mmol) and *tert*-butyl 2-fluoro-4-(prop-2-ynylamino)benzoate[24] (**46b**; 4.43 g, 17.8 mmol) in anhydrous DMF (60 mL) was added 2,6-lutidine (5.14 mL, 44.2 mmol). The reaction mixture was stirred under argon at 80 °C for 19 h, then it was allowed to cool to ∼45 °C and concentrated *in vacuo*. The residue was partitioned between EtOAc (700 mL) and H_2O (250 mL); the two layers were separated, and the aqueous layer was extracted with more EtOAc (200 mL). The combined organic extracts were successively washed with aqueous NH_4OH (H_2O/NH_3, v/v 10:1, 2 × 200 mL), a dilute aqueous solution of NaCl (200 mL), and H_2O (200 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (250 mL), and to this solution was added Merck silica gel (art. 7734; 50 g). The solvent was removed *in vacuo*, and the resulting free running powder was placed on a silica gel column made up in EtOAc. Elution of the column with EtOAc afforded the required product as a white solid: 2.9 g (39%); mp 226−229 °C; NMR (Me_2SO-d_6) δ 1.48 (s, 9H, C(CH_3)$_3$), 2.33 (s, 3H, quinazoline 2-CH_3), 3.28 (s, 1H, C≡C*H*), 4.37 (s, 2H, CH_2C≡C), 4.81 (s, 2H, quinazoline 6-CH_2), 6.62 (m, 2H, 3′,5′-Ar*H*), 7.56 (m, 2H, 6′-Ar*H*, quinazoline 8-*H*), 7.67 (dd, J = 2.1, 7.9 Hz, 1H, quinazoline 7-*H*), 7.93 (s, 1H, quinazoline 5-*H*), 12.21 (s, 1H, lactam N*H*); MS (CI) m/z 421 (M^+). Anal. ($C_{24}H_{24}FN_3O_3$) C, H, N, F.

4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoic Acid, Trifluoroacetate Salt (12). A solution of **47b** (2.2 g, 5.2 mmol) in TFA (21 mL) was stirred at room temperature for 45 min with protection from the light. The TFA was then removed *in vacuo*, and the residue was triturated with EtOAc. The product, a white solid, was collected by filtration, washed with petroleum ether, and dried *in vacuo* over P_2O_5: 2.4 g (96%); mp >300 °C; NMR (Me_2SO-d_6) δ 2.43 (s, 3H, quinazoline 2-CH_3), 3.30 (s, 1H, C≡C*H*), 4.40 (s, 2H, CH_2C≡C), 4.85 (s, 2H, quinazoline 6-CH_2), 6.63 (m, 2H, 3′,5′-Ar*H*), 7.63 (m, 2H, 6′-Ar*H*, quinazoline 8-*H*), 7.76 (d, J = 8.5 Hz, 1H, quinazoline 7-*H*), 7.99 (s, 1H, quinazoline 5-*H*); MS (ESI) m/z 366 $(M + H)^+$. Anal. ($C_{20}H_{16}FN_3O_3$·TFA·0.25H_2O), C, H, N. F.

***tert*-Butyl 4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoate (47c).** To a suspension of 6-(bromomethyl)-3,4-dihydro-2,7-dimethyl-4-oxoquinazoline[23] (**45b**; 4.0 g, 15.0 mmol) and *tert*-butyl 2-fluoro-4-(prop-2-ynylamino)benzoate[24] (**46b**; 3.73 g, 15.0 mmol) in anhydrous DMF (110 mL) was added 2,6-lutidine (3.6 mL, 29.9 mmol). The reaction mixture was stirred under argon at 70 °C for 16 h; then more 2,6-lutidine (1.3 mL, 11.17 mmol) was added, and stirring was continued at 80 °C for 8 h. Workup and purification as described for **47b** afforded the required compound as a white solid: 2.3 g (35%); mp >310 °C; NMR (Me_2SO-d_6) δ 1.49 (s, 9H, C(CH_3)$_3$), 2.32, 2.43 (2 × s, 6H, quinazoline 2-CH_3 and 7-CH_3), 3.26 (s, 1H, C≡C*H*), 4.32 (s, 2H, CH_2C≡C), 4.71 (s, 2H, quinazoline 6-CH_2), 6.60 (m, 2H, 3′,5′-Ar*H*), 7.44 (s, 1H, quinazoline 8-*H*), 7.65 (m, 2H, 6′-Ar*H*, quinazoline 5-*H*), 12.09 (s, 1H, lactam N*H*); MS (EI) m/z 435 (M^+). Anal. ($C_{25}H_{26}FN_3O_3$) C, H, N, F.

4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoic Acid, Trifluoroacetate Salt (13). The method followed that used to prepare **12** but used *tert*-butyl 4-[*N*-[(3,4-dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoate (**47c**; 2.1 g, 4.8 mmol) in TFA (21 mL). The required compound was obtained as a white solid: 2.55 g; mp >310 °C; NMR (Me_2SO-d_6) δ 2.39, 2.45 (2 × s, 6H, quinazoline 2-CH_3 and 7-CH_3), 3.26 (s, 1H, C≡C*H*), 4.33 (s, 2H, CH_2C≡C), 4.74 (s, 2H, quinazoline 6-CH_2), 6.59 (m, 2H, 3′,5′-Ar*H*), 7.46 (s, 1H, quinazoline 8-*H*), 7.68 (m, 2H, quinazoline 5-*H*, 6′-Ar*H*); MS (ESI) m/z 380 $(M + H)^+$; HRMS (FAB) found 380.1410, calcd for $C_{21}H_{18}FN_3O_3$ 380.1414.

***tert*-Butyl 4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoate (47a).** The method followed that used to prepare **47b** but used 6-(bromomethyl)-3,4-dihydro-2,7-dimethyl-4-oxoquinazoline[23] (**45b**; 5.0 g, 18.7 mmol), *tert*-butyl 4-(prop-2-ynylamino)-benzoate[24,25] (**46a**; 4.87 g, 21.1 mmol), anhydrous DMF (95 mL), and 2,6-lutidine (6.10 mL, 52.45 mmol). The reaction mixture was stirred under argon at 85 °C for 20 h. The desired product was obtained as a white solid: 4.32 g (55%); mp 232−233 °C; NMR (Me_2SO-d_6) δ 1.49 (s, 9H, C(CH_3)$_3$), 2.43, 2.50 (2 × s, 6H, quinazoline 2-CH_3 and 7-CH_3), 3.23 (s, 1H, C≡C*H*), 4.31 (s, 2H, CH_2C≡C), 4.69 (s, 2H, quinazoline 6-CH_2), 6.78 (d, J = 8.9 Hz, 2H, 3′,5′-Ar*H*), 7.43 (s, 1H, quinazoline 8-*H*), 7.67 (s, 1H, quinazoline 5-*H*), 7.71 (d, J = 8.7 Hz, 2H, 2′,6′-Ar*H*), 12.09 (s, 1H, lactam N*H*); MS (CI) m/z 417 (M^+). Anal. ($C_{25}H_{27}N_3O_3$·0.25H_2O) C, H, N.

4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoic Acid, Trifluoroacetate Salt (11). The method followed that used to prepare **12** but used *tert*-butyl 4-[*N*-[(3,4-dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoate (**47a**; 2.31 g, 5.55 mmol) in TFA (21 mL). The required product was obtained as a pale yellow solid: 2.9 g (97%); mp >310 °C; NMR (Me_2SO-d_6) δ 2.46, 2.50 (2 × s, 6H, quinazoline 2-CH_3 and 7-CH_3), 3.25 (s, 1H, C≡C*H*), 4.34 (s, 2H, CH_2C≡C), 4.74 (s, 2H, quinazoline 6-CH_2), 6.79 (d, J = 9.0 Hz, 2H, 3′,5′-Ar*H*), 7.50 (s, 1H, quinazoline 8-*H*), 7.73 (s, 1H, quinazoline 5-*H*), 7.77 (d, J = 8.8 Hz, 2H, 2′,6′-Ar*H*); MS (ESI) m/z 362 $(M + H)^+$. Anal. ($C_{21}H_{19}N_3O_3$·1.5TFA·0.5H_2O) C, H, N, F.

***tert*-Butyl 4-[*N*-[[3,4-Dihydro-2-methyl-4-oxo-3-[(pivaloyloxy)methyl]-6-quinazolinyl]methyl]amino]-2-fluorobenzoate (52).** A mixture of 6-(bromomethyl)-3,4-dihydro-2-methyl-4-oxo-3-[(pivaloyloxy)methyl]quinazoline[27] (**49**; 9.15 g, 25.0 mmol), *tert*-butyl 4-amino-2-fluorobenzoate[24] (**51**; 5.80 g, 27.5 mmol), 2,6-lutidine (2.94 g, 0.0275 mol), and a few crystals of NaI in dry DMA (30 mL) was stirred under argon at 95 °C for 10 h. The reaction mixture was then allowed to cool to room temperature; the DMA was removed *in vacuo*, and the residue was partitioned between EtOAc (300 mL) and H_2O (200 mL). The two layers were separated, and the organic layer was washed with H_2O (200 mL), dried ($MgSO_4$), and concentrated *in vacuo* to a yellow oil which crystallized overnight. This was dissolved in CH_2Cl_2, and to the resulting solution was added Merck silica gel (art. 7734; 100 g). The solvent was then removed under reduced pressure to give a free-running pale yellow powder which was placed on a silica gel column made up in 1:2 (v/v) EtOAc/hexanes. Elution of the column with a gradient of EtOAc in hexanes (50−100%) afforded the required product as a pale yellow solid: 10.3 g (83%); mp 149−150 °C; NMR (Me_2SO-d_6) δ 1.14 (s, 9H, POM C(CH_3)$_3$), 1.46 (s, 9H, CO_2C(CH_3)$_3$), 2.60 (s, 3H, quinazoline 2-CH_3), 4.50 (d, J = 5.9 Hz, 2H, CH_2NH), 6.05 (s, 2H, POM CH_2), 6.33 (d, J = 14.4 Hz, 1H, 3′-Ar*H*), 6.47 (d, J = 8.6 Hz, 1H, 5′-Ar*H*), 7.40−7.61 (m, 3H, CH_2N*H*, 6′-Ar*H*, quinazoline 8-*H*), 7.80 (d, J = 8.5 Hz, 1H, quinazoline 7-*H*), 8.06 (s, 1H, quinazoline 5-*H*); MS (ESI) m/z 498 $(M + H)^+$.

***tert*-Butyl 4-[*N*-[[3,4-Dihydro-2-methyl-4-oxo-3-[(pivaloyloxy)methyl]-6-quinazolinyl]methyl]-*N*-methylamino]-2-fluorobenzoate (54).** To a stirred solution of **52** (10.3 g, 0.021 mol) in glacial AcOH (150 mL) was added 37% aqueous formaldehyde (17 mL). Stirring was continued at room temperature for 10 min, and then sodium cyanoborohydride (2.83 g, 0.046 mol) was added over a period of 5 min (caution: evolution of HCN takes place). The AcOH was removed *in vacuo*, and the resulting residue was partitioned between EtOAc (300 mL) and H_2O (200 mL). The two layers were separated, and the organic layer was extracted with EtOAc (150 mL). The combined EtOAc extracts were washed with saturated aqueous $NaHCO_3$ (300 mL) and H_2O (300 mL), dried ($MgSO_4$), and concentrated *in vacuo* to a yellow oil. Purification by column chromatography, on gradient elution with EtOAc in CH_2Cl_2 (0−30%), gave the required product as a yellow oil: 8.4 g (80%). A small amount of the product (0.320

g) was rechromatographed on a silica gel column using 35% EtOAc in hexanes as eluent to yield a yellow gum that turned into a pale yellow solid on drying *in vacuo* over P_2O_5: mp 74−77 °C (softens); NMR (Me_2SO-d_6) δ 1.13 (s, 9H, POM C(CH_3)$_3$), 1.48 (s, 9H, C(CH_3)$_3$), 2.60 (s, 3H, quinazoline 2-CH_3), 3.13 (s, 3H, N^{10}-CH_3), 4.82 (s, 2H, CH_2NCH_3), 6.04 (s, 2H, POM CH_2), 6.53 (dd, J = 15.2, 2.3 Hz, 1H, 3′-Ar*H*), 6.60 (dd, J = 9.0, 2.4 Hz, 1H, 5′-Ar*H*), 7.62 (m, 3H, quinazoline 7-*H* and 8-H, 6′-Ar*H*), 7.88 (d, J = 1.3 Hz, 1H, quinazoline 5-*H*); MS (ESI) m/z 534 $(M + Na)^+$, 512 $(M + H)^+$. Anal. ($C_{28}H_{34}FN_3O_5$) C, H, F; N: calcd, 8.21; found, 7.66.

4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)-methyl]-*N*-methylamino]-2-fluorobenzoic Acid, Trifluoroacetate Salt (16). A solution of **54** (6.58 g, 0.0192 mol) in TFA (35 mL) was stirred at room temperature for 1 h with protection from the light and then concentrated *in vacuo* to a brown oil which was treated with EtOH (30 mL) and Et_2O (30 mL). The precipitated white solid was collected by filtration, washed with Et_2O, and dried *in vacuo* over P_2O_5: 4.54 g. This solid (4.34 g) was treated with EtOH (200 mL), H_2O (60 mL), and aqueous NaOH (1 N, 34 mL, 34 mmol), and the resulting solution was stirred at room temperature for 4 h and then acidified to pH 4 with 2 N HCl. The precipitated pale yellow solid was collected by filtration, washed with H_2O, and dried *in vacuo* over P_2O_5: 3.20 g (84% from **54**). To obtain the trifluoroacetate salt, part of this solid (1 g, 2.93 mmol) was dissolved in TFA (40 mL). The solution was allowed to stand at room temperature for 10 min and then concentrated *in vacuo*, and the resulting residue was triturated with Et_2O (50 mL). The required product was collected by filtration as a pale yellow solid and dried *in vacuo* over P_2O_5: 1.35 g; mp 297−298 °C; NMR (Me_2SO-d_6) δ 2.39 (s, 3H, quinazoline 2-CH_3), 3.13 (s, 3H, N^{10}-CH_3), 4.80 (s, 2H, CH_2NCH_3), 6.52 (d, J = 15.4 Hz, 1H, 3′-Ar*H*), 6.60 (d, J = 9.0 Hz, 5′-Ar*H*), 7.58 (d, J = 8.5 Hz, 1H, quinazoline 8-*H*), 7.66 (m, 2H, quinazoline 7-*H*, 6′-Ar*H*), 7.87 (s, 1H, quinazoline 5-*H*); MS (ESI) m/z 342 $(M + H)^+$. Anal. ($C_{18}H_{16}FN_3O_3$·TFA) C, H, N; F; calcd, 16.69; found, 16.28.

***tert*-Butyl 4-[*N*-[[3,4-Dihydro-2,7-dimethyl-4-oxo-3-[(pivaloyloxy)methyl]-6-quinazolinyl]methyl]amino]-2-fluorobenzoate (53).** The method followed that used to prepare **52** but used 6-(bromomethyl)-3,4-dihydro-2,7-dimethyl-4-oxo-3-[(pivaloyloxy)methyl]quinazoline[24] (**50**; 5.7 g, 15.0 mmol), *tert*-butyl 4-amino-2-fluorobenzoate[24] (**51**; 3.48 g, 16.5 mmol), 2,6-lutidine (1.76 g, 15.0 mmol), and a few crystals of NaI in dry DMA (30 mL). The crude product was dissolved in CH_2Cl_2 (200 mL), and to the resulting solution was added Merck silica gel (art. 7734; 60 g). The mixture was concentrated *in vacuo* to a fine powder which was placed on a silica gel column made up in 1:2 (v/v) EtOAc/hexanes. Elution with a gradient of EtOAc in hexanes (30−50%) afforded the required product as a yellow solid: 5.75 g (75%); mp 131−132 °C; NMR (Me_2SO-d_6) δ 1.12 (s, 9H, POM C(CH_3)$_3$), 1.48 (s, 9H, $CO_2C(CH_3)_3$), 2.47 (s, 3H, quinazoline 7-CH_3), 2.58 (s, 3H, quinazoline 2-CH_3), 4.44 (d, J = 5.3 Hz, 2H, CH_2NH), 6.02 (s, 2H, POM CH_2), 6.34 (d, J = 14.4 Hz, 1H, 3′-Ar*H*), 6.49 (d, J = 8.8 Hz, 1H, 5′-Ar*H*), 7.35 (t, 1H, CH_2NH), 7.48 (s, 1H, quinzaoline 8-*H*), 7.55 (t, J = 8.9 Hz, 1H, 6′-Ar*H*), 7.90 (s, 1H, quinazoline 5-*H*); MS (ESI) m/z 512 (M^+).

***tert*-Butyl 4-[*N*-[[3,4-Dihydro-2,7-dimethyl-4-oxo-3-[(pivaloyloxy)methyl]-6-quinazolinyl]methyl]-*N*-methylamino]-2-fluorobenzoate (55).** The method followed that used to prepare **54** but used **53** (9.15 g, 17.9 mmol), glacial AcOH (150 mL), 37% aqueous formaldehyde (15 mL), and sodium cyanoborohydride (2.44 g, 39.4 mol). Workup as described for **54** afforded a yellow foam which was triturated with Et_2O. The required product was then collected by filtration as a white solid, washed with Et_2O and hexanes, and dried *in vacuo* over P_2O_5: 6.21 g (66%); mp 174−175 °C; NMR (Me_2SO-d_6) δ 1.12 (s, 9H, POM C(CH_3)$_3$), 1.48 (s, 9H, $CO_2C(CH_3)_3$), 2.44, 2.57 (2 × s, 2H, quinazoline 7-CH_3 and 2-CH_3), 3.12 (s, 3H, N^{10}-CH_3), 4.73 (s, 2H, CH_2NH), 5.99 (s, 2H, POM, CH_2), 6.50 (d, J = 14.6 Hz, 1H, 3′-Ar*H*), 6.55 (dd, J = 2.3, 8.5 Hz, 1H, 5′-Ar*H*), 7.48, 7.50 (2 × s, 2H, quinazoline 8-*H* and 5-*H*), 7.61 (t, J = 8.8 Hz, 1H, 6′-Ar*H*); MS (ESI) m/z 526 $(M + H)^+$. Anal. ($C_{29}H_{36}FN_3O_5$) C, H, N, F.

4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)-methyl]-*N*-methylamino]-2-fluorobenzoic Acid, Trifluoroacetate Salt (17). A solution of **55** (3.0 g, 5.7 mmol) in TFA (30 mL) was stirred at room temperature for 1 h with protection from the light and then concentrated *in vacuo* to a dark green oil. Trituration with Et_2O (50 mL) afforded a buff solid which was dried *in vacuo* over P_2O_5 (2.5 g) and then treated with EtOH (100 mL), H_2O (30 mL), and aqueous NaOH (1 N, 17 mL). The resulting solution was stirred at room temperature for 3 h and then acidified to pH 4 with 2 N HCl. The precipitated solid was collected by filtration, washed with H_2O (50 mL), dried *in vacuo* over P_2O_5, and then dissolved in TFA (30 mL). The purple solution was allowed to stand at room temperature for 10 min and then concentrated *in vacuo* to an oily residue. Trituration with Et_2O (50 mL) gave a light purple solid which was collected by filtration, washed with Et_2O, and dried *in vacuo* over P_2O_5: 1.73 g (69% from **55**); mp 312−314 °C dec; NMR (Me_2SO-d_6) δ 2.37, 2.45 (2 × s, 6H, quinazoline 2-CH_3 and 7-CH_3), 3.13 (s, 3H, N^{10}-CH_3), 4.73 (s, 2H, CH_2NCH_3), 6.51 (d, J = 15.1 Hz, 1H, 3′-Ar*H*), 6.55 (d, J = 8.9 Hz, 1H, 5′-Ar*H*), 7.47, 7.48 (2 × s, 2H, quinazoline 5-*H* and 8-*H*), 7.68 (t, J = 8.7 Hz, 1H, 6′-Ar*H*); MS (ESI) m/z 356 $(M + H)^+$. Anal. ($C_{19}H_{18}FN_3O_3$·0.7TFA·0.4H_2O) C, H, N, F.

4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)-methyl]-*N*-methylamino]benzoic Acid, Trifluoroacetate Salt (15). A mixture of 6-(bromomethyl)-3,4-dihydro-2,7-dimethyl-4-oxo-3-[(pivaloyloxy)methyl]quinazoline[24] (**50**; 3.0 g, 7.89 mmol), 4-(methylamino)benzoic acid (2.38 g, 15.78 mmol), and $CaCO_3$ (1.18 g, 11.83 mmol) in dry DMA (25 mL) was heated at 60 °C for 21 h; then it was allowed to cool to room temperature, filtered, and washed with DMA (30 mL). The filtrate was concentrated *in vacuo* to a brown residue which was triturated with EtOAc. The precipitated brown solid was collected by filtration and treated with EtOH (60 mL), H_2O (50 mL), and aqueous NaOH (1 N, 30 mL, 30 mmol). The resulting solution was stirred at room temperature for 1.5 h and acidified to pH 4, and the precipitated solid was collected by filtration and dried *in vacuo* over P_2O_5. Then it was suspended in TFA (30 mL), and the resulting cloudy solution was concentrated *in vacuo* to a brown residue which was triturated with Et_2O. The precipitated brown solid was collected by filtration and then dried *in vacuo* over P_2O_5: 2.86 (80%); mp >270 °C; NMR (Me_2SO-d_6) δ 2.43, 2.47 (2 × s, 6H, quinazoline 2-CH_3 and 7-CH_3), 3.15 (s, 3H, N^{10}-CH_3), 4.74 (s, 2H, quinazoline 6-CH_2), 6.72 (d, J = 9.0 Hz, 2H, 3′,5′-Ar*H*), 7.51 (s, 2H, quinazoline 5-*H* and 8-*H*), 7.74 (d, J = 8.9 Hz, 2H, 2′,6′-Ar*H*); MS (ESI) m/z 338 $(M + H)^+$; HRMS (FAB) found 338.1509, calcd for $C_{19}H_{19}N_3O_3$ 338.1505.

Preparation of Quinazoline Antifolate γ-Linked Dipeptide *tert*-Butyl Esters: Di-*tert*-butyl *N*-[*N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-γ-glutamyl]-D-alaninate (23). The general procedure C was followed using **7a** (0.480 g, 1.45 mmol), 4-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoic acid, trifluoroacetate salt (**10**; 0.461 g, 1.0 mmol), DMF (20 mL), DEPC (0.326 g, 2.0 mmol), and Et_3N (0.202 g, 2.0 mmol). Purification by column chromatography eluting with EtOAc gave a pale yellow solid. Subsequent reprecipitation from CH_2Cl_2 (minimum amount)/petroleum ether yielded the title compound **23** (0.290 g, 44%) as a white solid: mp 166−168 °C; NMR (Me_2SO-d_6) δ 1.19 (d, J = 7.3 Hz, 3H, Ala β-CH_3), 1.37, 1.40 (2 × s, 18H, 2 × C(CH_3)$_3$), 1.96 (m, 2H, β-CH_2), 2.23 (m, 2H, γ-CH_2), 2.32 (s, 3H, quinazoline 2-CH_3), 3.23 (s, 1H, C≡C*H*), 4.07 (quintet, J = 7.3 Hz, 1H, Ala α-C*H*), 4.24 (m, 1H, Glu α-C*H*), 4.34 (s, 2H, $CH_2C{\equiv}C$), 4.78 (s, 2H, quinazoline 6-CH_2), 6.83 (d, J = 8.8 Hz, 2H, 3′,5′-Ar*H*), 7.54 (d, J = 8.4 Hz, 1H, quinazoline 8-*H*), 7.69 (d, J = 9.6 Hz, 1H, quinazoline 7-*H*), 7.73 (d, J = 8.9 Hz, 2H, 2′,6′-Ar*H*), 7.96 (s, 1H, quinazoline 5-*H*), 8.19 (d, J = 7.0 Hz, 1H, Ala N*H*), 8.31 (d, J = 7.2 Hz, 1H, Glu N*H*), 12.19 (s, 1H, lactam N*H*); MS (FAB) m/z 660 $(M + H)^+$. Anal. ($C_{36}H_{45}N_5O_7$) C, H, N.

The procedure C was repeated with the appropriate dipeptide *tert*-butyl ester free bases **7b**−**d** and the appropriate pteroic acid analogues **10**−**18** to give the coupled quinazoline γ-linked dipeptide *tert*-butyl esters **19**−**22** and **24**−**31**. Yields

and mass spectral and analytical data of these products are given in Table 1. The ^{1}H NMR spectra of these compounds were consistent with the assigned structures.

Preparation of Quinazoline Antifolate γ-Linked Dipeptides: *N*-[*N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-γ-glutamyl]-D-alanine (36). The general procedure D was followed using **23** (0.100 g, 0.15 mmol) and TFA (8 mL). The title compound **36** was obtained as a white solid (0.084 g, 79%): mp 180 °C dec; NMR (Me_2SO-d_6) δ 1.21 (d, J = 7.3 Hz, 3H, Ala β-CH_3), 1.94, 2.03 (2 × m, 2H, β-CH_2), 2.22 (m, 2H, γ-CH_2), 2.38 (s, 3H, quinazoline 2-CH_3), 3.23 (s, 1H, C≡C*H*), 4.16 (m, 1H, Ala α-C*H*), 4.35 (m, 3H, Glu α-C*H*, CH_2C≡C), 4.80 (s, 2H, quinazoline 6-CH_2), 6.83 (d, J = 8.3 Hz, 2H, 3′,5′-Ar*H*), 7.57 (d, J = 8.4 Hz, 1H, quinazoline 8-*H*), 7.74 (d, J = 8.4 Hz, 3H, 2′,6′-Ar*H*), 7.99 (s, 1H, quinazoline 5-*H*), 8.17 (d, J = 7.3 Hz, 1H, Ala N*H*), 8.30 (d, J = 7.1 Hz, 1H, Glu N*H*); MS (FAB) m/z 570 (M + Na)$^+$, 548 (M + H)$^+$. Anal. ($C_{28}H_{29}N_5O_7$·TFA·0.7Et_2O) C, H, N.

The procedure D was repeated with the appropriate quinazoline γ-linked dipeptide *tert*-butyl esters **19**−**22** and **24**−**31** to yield the quinazoline γ-linked dipeptides **32**−**35** and **37**−**44**, all of which had ^{1}H NMR spectra consistent with the assigned structures. Yields and analytical data are gathered in Table 2.

Preparation D-Glu-L-Glu and D-Glu-D-Glu Derivatives of ICI 198583: γ-Methyl-D-Glutamate Hydrochloride (57). To a flask containing MeOH (116 mL) and cooled to −10 °C was slowly added $SOCl_2$ (17 mL, 23.0 mmol) followed by D-glutamic acid (25 g, 0.17 mmol). The flask was fitted with a condenser; the acetone−dry ice bath was removed, and the solution was stirred for 30 min and then poured into Et_2O (330 mL). The precipitate was filtered off and washed well with Et_2O (150 mL) to give the title compound **57** (20.50 g, 61%) as a white solid: mp 171−175 °C (lit.[29] mp 161 °C for the L-enantiomer); NMR (Me_2SO-d_6) δ 2.07 (m, 2H, β-CH_2), 2.51 (m, 2H, γ-CH_2), 3.61 (s, 3H, CO_2CH_3), 3.94 (m, 1H, α-C*H*), 8.44 (br s, 3H, NH_3); MS (CI) m/z 162 (M − Cl)$^+$.

γ-Methyl *N*-(Benzyloxycarbonyl)-D-glutamate (58). To a vigorously stirred solution (overhead stirring) of $NaHCO_3$ (19.0 g, 0.22 mol) in H_2O (240 mL) cooled to 5 °C was added γ-methyl D-glutamate hydrochloride (**57**; 22.0 g, 0.11 mol) followed by addition of benzyl chloroformate (30 mL, 0.21 mol) over a 15 min period. Stirring was continued at 5 °C for 1.5 h and then at room temperature for 2 h. After extracting with Et_2O (3 × 30 mL), the reaction mixture was acidified to pH 2 with 1 N HCl. EtOAc (120 mL) was then added into the mixture; the two layers were separated, and the aqueous layer was washed with more EtOAc (2 × 120 mL). The organic extracts were combined and dried (Na_2SO_4), and the solvent was removed *in vacuo* to give a pale yellow oil which solidified on standing. Recrystallization from CCl_4 gave the title compound **58** (12.0 g). Because of the low yield, the pH of the acidic solution, obtained during the workup, was adjusted to 9 with NaOH. Benzyl chloroformate (30 mL, 0.21 mol) was added, and the preparation was repeated as described above to give an additional 14.4 g of the product (81% overall yield) as a white solid: mp 64−66 °C (lit.[30] mp 72−73 °C for the L-enantiomer); NMR (Me_2SO-d_6) δ 1.80, 1.98 (2 × m, 2H, β-CH_2), 2.39 (m, 2H, γ-CH_2), 3.57 (s, 3H, CO_2CH_3), 3.98 (m, 1H, α-C*H*), 5.02 (s, 2H, PhCH_2), 7.35 (s, 5H, Ar*H*), 7.62 (d, J = 8.1 Hz, 1H, CON*H*); MS (CI) m/z 296 (M + H)$^+$. Anal. ($C_{14}H_{17}NO_6$) C, H, N.

α-*tert*-Butyl γ-Methyl *N*-(Benzyloxycarbonyl)-D-glutamate (59). To a stirred solution of γ-methyl *N*-(benzyloxycarbonyl)-D-glutamate (11 g, 0.037 mol) in dry Bu^{t}OH (70 mL, 0.74 mol) and dry pyridine (30 mL, 0.37 mol) cooled to −5 °C was added $POCl_3$ (3.45 mL, 0.037 mol) over a 30 min period. Stirring was continued at −5 °C for 15 min and then at room temperature for 50 min. Evaporation of the solvents left a residue which was partitioned between H_2O (40 mL) and Et_2O (40 mL). The two layers were separated, and the aqueous layer was washed with more Et_2O (40 mL). The organic extracts were combined, washed with aqueous saturated $NaHCO_3$ (2 × 40 mL), 10% aqueous citric acid (2 × 80 mL), brine (2 × 40 mL), and finally H_2O (40 mL), and dried (Na_2SO_4), and the solvent was removed *in vacuo* to give the title compound **59** (10.5 g, 82%) as a pale yellow oil: NMR (Me_2SO-d_6) δ 1.39 (s, 9H, $CO_2C(CH_3)_3$), 1.80, 1.94 (2 × m, 2H, β-CH_2), 2.40 (m, 2H, γ-CH_2), 3.58 (s, 3H, CO_2CH_3), 3.93 (m, 1H, α-C*H*), 5.04 (s, 2H, PhCH_2), 7.36 (s, 5H, Ar*H*), 7.67 (d, J = 7.8 Hz, 1H, N*H*); MS (CI) m/z 352 (M + H)$^+$. Anal. ($C_{18}H_{25}NO_6$·0.25H_2O) C, H, N.

α-*tert*-Butyl *N*-(Benzyloxycarbonyl)-D-glutamate (60). To a stirred solution of **59** (9.7 g, 27.6 mmol) in acetone (15 mL), EtOH (15 mL), and H_2O (15 mL) was added aqueous NaOH (1 N, 35 mL, 35.0 mmol). After stirring at room temperature for 24 h, the organic solvents were removed *in vacuo*, and the remainder was diluted with H_2O (80 mL). The alkaline solution was acidified to pH 4.5 with 1 N HCl and then extracted with EtOAc (3 × 100 mL). The organic extracts were combined and dried ($MgSO_4$), and the solvent was evaporated to give a viscous oil (7.6 g, 82%), which solidified on standing to give a white solid. An analytically pure sample was obtained after purification by column chromatography using 5% MeOH in $CHCl_3$ as eluent: mp 80−83 °C (lit.[28] mp 82−83.5 °C for the L-enantiomer); NMR (DMSO-d_6) δ 1.39 (s, 9H, C(CH_3)$_3$), 1.75, 1.90 (2 × m, 2H, β-CH_2), 2.29 (m, 2H, γ-CH_2), 3.93 (m, 1H, α-C*H*), 5.03 (s, 2H, PhCH_2), 7.35 (s, 5H, Ar*H*), 7.64 (d, J = 7.2 Hz, 1H, N*H*); MS (CI) m/z 338 (M + H)$^+$; [α]^{20} = +20.0° (c = 1.2, MeOH) (lit.[28] [α]^{20} = −26.6° (c = 1.2, MeOH) for the L-enantiomer). Anal. ($C_{17}H_{23}NO_6$) C, H, N.

Tri-*tert*-butyl *N*-[*N*-(Benzyloxycarbonyl)-D-γ-glutamyl]-L-glutamate (61a). The general procedure A1 was followed using α-*tert*-butyl *N*-(benzyloxycarbonyl)-D-glutamate (2.022 g, 6.0 mmol), *N*-methylmorpholine (0.606 g, 6.0 mmol), THF (7 mL), isobutyl chloroformate (0.816 g, 6.0 mmol), and a suspension of di-*tert*-butyl L-glutamate hydrochloride (1.77 g, 6.0 mmol) in THF (8 mL) and *N*-methylmorpholine (0.606 g, 6.0 mmol). The crude product was purified by column chromatography using a gradient of EtOAc in CH_2Cl_2 (8−20%) as eluent. Trituration with petroleum ether gave the title compound (2.7 g, 78%) as a white solid: mp 108−111 °C; NMR (Me_2SO-d_6) δ 1.38 (s, 27H, 3 × C(CH_3)$_3$), 1.72, 1.90 (2 × m, 4H, 2 × β-CH_2), 2.25 (m, 4H, 2 × γ-CH_2), 3.89 (m, 1H, Glu$_D$ α-C*H*), 4.10 (m, 1H, Glu$_L$ α-C*H*), 5.04 (m, 2H, ArCH_2), 7.35 (m, 5H, Ar*H*), 7.63 (d, J = 7.7 Hz, 1H, Glu$_D$ N*H*), 8.13 (d, J = 7.4 Hz, 1H, Glu$_L$ N*H*); MS (CI) m/z 579 (M + H)$^+$. Anal. ($C_{30}H_{46}N_2O_9$) C, H, N.

Tri-*tert*-butyl D-γ-Glutamyl-L-glutamate (62a). The general procedure B was followed using tri-*tert*-butyl *N*-[*N*-(benzyloxycarbonyl)-D-γ-glutamyl]-L-glutamate (0.754 g, 1.3 mmol), EtOAc (90 mL), and 10% Pd/C (0.108 g). Compound **62a** was obtained as a colorless oil (0.570 g, 98%) and used immediately without further purification.

Tri-*tert*-butyl *N*-[*N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-D-γ-glutamyl-L-glutamate (63a). The general procedure C was followed using tri-*tert*-butyl D-γ-glutamyl-L-glutamate (0.500 g, 1.1 mmol), 4-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoic acid, trifluoroacetate salt (**10**; 0.461 g, 1.0 mmol), DMF (15 mL), DEPC (0.359 g, 2.2 mmol), and Et_3N (0.222 g, 2.2 mmol). The crude product was purified by chromatography using a gradient of MeOH in EtOAc (0−2%) as eluent. Reprecipitation from CH_2Cl_2 (minimum amount)/petroleum ether gave the title compound **63a** (0.470 g, 61%) as a white solid: mp 112−116 °C; NMR (Me_2SO-d_6) δ 1.37, 1.39 (2 × s, 27H, 3 × C(CH_3)$_3$), 1.71, 1.88 (2 × m, 4H, 2 × β-CH_2), 2.24 (m, 4H, 2 × γ-CH_2), 2.33 (s, 3H, quinazoline 2-CH_3), 3.23 (s, 1H, C≡C*H*), 4.10 (m, 1H, Glu$_L$ α-C*H*), 4.24 (m, 1H, Glu$_D$ α-C*H*), 4.34 (s, 2H, CH_2C≡C), 4.78 (s, 2H, quinazoline 6-CH_2), 6.83 (d, J = 8.8 Hz, 2H, 3′,5′-Ar*H*), 7.54 (d, J = 8.4 Hz, 1H, quinazoline 8-*H*), 7.72 (m, 3H, quinazoline 7-*H*, 2′,6′-Ar*H*), 7.96 (s, 1H, quinazoline 5-*H*), 8.16 (d, J = 7.5 Hz, 1H, Glu$_L$ N*H*), 8.32 (d, J = 7.3 Hz, 1H, Glu$_D$ N*H*), 12.19 (s, 1H, lactam N*H*); MS (FAB) m/z 774 (M + H)$^+$. Anal. ($C_{42}H_{55}N_5O_9$) C, H, N.

***N*-[*N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)-methyl]-*N*-prop-2-ynylamino]benzoyl]-D-γ-glutamyl]-L-glutamaic Acid (64a).** The general procedure D was followed using **63a** (0.218 g, 0.28 mmol) and TFA (15 mL). The title

compound **64a** was obtained as a yellow solid (0.204 g, 88%): mp 90 °C dec; NMR (Me_2SO-d_6) δ 1.74, 1.92 (2 × m, 4H, 2 × β-CH_2), 2.23 (m, 4H, 2 × γ-CH_2), 2.45 (s, 3H, quinazoline 2-CH_3), 3.24 (s, 1H, C≡C*H*), 4.18 (m, 1H, Glu$_L$ α-C*H*), 4.36 (m, 3H, CH_2C≡C, Glu$_D$ α-C*H*), 4.82 (s, 2H, quinazoline 6-CH_2), 6.83 (d, J = 8.8 Hz, 2H, 3′,5′-Ar*H*), 7.61 (d, J = 8.4 Hz, 1H, quinazoline 8-*H*), 7.77 (m, 3H, quinazoline 7-*H*, 2′,6′-Ar*H*), 8.02 (s, 1H, quinazoline 5-*H*), 8.16 (d, J = 7.7 Hz, 1H, Glu$_L$ N*H*), 8.33 (d, J = 7.4 Hz, 1H, Glu$_D$ N*H*); MS (FAB) m/z 606 (M + H)$^+$. Anal. ($C_{30}H_{31}N_5O_9$·1.7TFA·0.25Et_2O·0.5H_2O) C, H, N.

Tri-*tert*-butyl *N*-[*N*-(Benzyloxycarbonyl)-D-γ-glutamyl]-D-glutamate (61b). The general procedure A1 was followed using α-*tert*-butyl *N*-(benzyloxycarbonyl)-D-glutamate (1.43 g, 4.24 mmol), THF (6 mL), *N*-methylmorpholine (0.428 g, 4.24 mmol), isobutyl chloroformate (0.577 g, 4.24 mmol), and a solution of di-*tert*-butyl D-glutamate (1.25 g, 4.24 mmol) in THF (6 mL). The crude product was purified by column chromatography using a gradient of EtOAc in CH_2Cl_2 (10−20%) as eluent. Trituration with petroleum ether afforded the title compound **61b** (1.50 g, 61%) as a white solid: mp 84−86 °C; NMR (Me_2SO-d_6) δ 1.38, (s, 27H, 3 × C(CH_3)$_3$), 1.72, 1.89 (2 × m, 4H, 2 × β-CH_2), 2.26 (m, 4H, 2 × γ-CH_2), 3.89, 4.10 (2 × m, 2H, 2 × Glu$_D$ α-C*H*), 5.03 (m, 2H, ArCH_2), 7.35 (m, 5H, Ar*H*), 7.64, 8.12 (2 × d, J = 7.7 Hz, 2H, 2 × Glu$_D$ N*H*); MS (FAB) m/z 579 (M + H)$^+$. Anal. ($C_{30}H_{46}N_2O_9$) C, H, N.

Tri-*tert*-butyl D-γ-Glutamyl-D-glutamate (62b). The general procedure B was followed using tri-*tert*-butyl *N*-[*N*-(benzyloxycarbonyl)-D-γ-glutamyl]-D-glutamate (0.718 g, 1.24 mmol), EtOAc (90 mL), and 10% Pd/C (0.100 g). The title compound **62b** was obtained as a colorless oil (0.460 g, 84%) and immediately used without further purification.

Tri-*tert*-butyl *N*-[*N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-D-γ-glutamyl]-D-glutamate (63b). The general procedure C was followed using tri-*tert*-butyl D-γ-glutamyl-D-glutamate (0.444 g, 1.0 mmol), 4-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoic acid, trifluoroacetate salt (**10**; 0.461 g, 1.0 mmol), DMF (15 mL), DEPC (0.359 g, 2.2 mmol), and Et_3N (0.222 g, 2.2 mmol). The crude product was purified by column chromatography using a gradient of MeOH in EtOAc (0−2%) as eluent. Reprecipitation from CH_2Cl_2 (minimum amount)/petroleum ether afforded the title compound **63b** (0.400 g, 52%) as a white solid: mp 110−116 °C; NMR (Me_2SO-d_6) δ 1.37, 1.39 (2 × s, 27H, 3 × C(CH_3)$_3$), 1.71, 1.89 (2 × m, 4H, 2 × β-CH_2), 2.24 (m, 4H, 2 × γ-CH_2), 2.33 (s, 3H, quinazoline 2-CH_3), 3.23 (s, 1H, C≡C*H*), 4.10, 4.24 (2 × m, 2H, 2 × Glu$_D$ α-C*H*), 4.34 (s, 2H, CH_2C≡C), 4.78 (s, 2H, quinazoline 6-CH_2), 6.83 (d, J = 8.7 Hz, 2H, 3′,5′-Ar*H*), 7.54 (d, J = 8.4 Hz, 1H, quinazoline 8-*H*), 7.72 (m, 3H, quinazoline 7-*H*, 2′,6′-Ar*H*), 7.96 (s, 1H, quinazoline 5-*H*), 8.13, 8.32 (2 × d, J = 7.5 Hz, 2H, 2 × Glu$_D$ N*H*), 12.19 (s, 1H, lactam N*H*); MS (FAB) m/z 797 (M + Na)$^+$. Anal. ($C_{42}H_{55}N_5O_9$) C, H, N.

***N*-[*N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-D-γ-glutamyl]-D-glutamic Acid (64b).** The general procedure D was followed using **63b** (0.208 g, 0.27 mmol) and TFA (15 mL). Compound **64b** was obtained as a white solid (0.180 g, 89%): mp 95 °C dec; NMR (Me_2SO-d_6) δ 1.74, 1.72 (2 × m, 4H, 2 × β-CH_2), 2.23 (m, 4H, 2 × γ-CH_2), 2.42 (s, 3H, quinazoline 2-CH_3), 3.24 (s, 1H, C≡C*H*), 4.18, 4.36 (2 × m, 2H, 2 × Glu$_D$ α-C*H*), 4.30 (s, 2H, CH_2C≡C), 4.81 (s, 2H, quinazoline 6-CH_2), 6.83 (d, J = 8.8 Hz, 2H, 3′,5′-Ar*H*), 7.60 (d, J = 8.4 Hz, 1H, quinazoline 8-*H*), 7.77 (m, 3H, quinazoline 7-*H*, 2′,6′-Ar*H*), 8.00 (s, 1H, quinazoline 5-*H*), 8.13, 8.33 (2 × d, J = 7.5 Hz, 2H, 2 × Glu$_D$ N*H*); MS (FAB) m/z 628 (M + Na)$^+$, 606 (M + H)$^+$. Anal. ($C_{30}H_{31}N_5O_9$·1.15TFA·H_2O) C, H, N.

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Supporting Information Available: ^{1}H NMR spectral data of quinazoline γ-linked dipeptide *tert*-butyl esters **19**−**22** and **24**−**31** and quinazoline γ-linked dipeptides **32**−**35** and **37**−**44** (2 pages). Ordering information is given on any current masthead page.

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