Folate-Based Inhibitors of Thymidylate Synthase: Synthesis and Antitumor Activity of γ-Linked Sterically Hindered Dipeptide Analogues of 2-Desamino-2-methyl-N^{10}-propargyl-5,8-dideazafolic Acid (ICI 198583)†

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In an effort to synthesize inhibitors of thymidylate synthase (TS) that do not undergo polyglutamation, a series of γ-linked sterically hindered dipeptide analogues of 2-desamino-2-methyl-N^{10}-propargyl-5,8-dideazafolic acid (ICI 198583) was prepared. A methyl, ethyl, or propargyl group was incorporated into the γ-glutamyl amide bond of γ-linked L,L dipeptide derivatives of ICI 198583, such as ICI 198583-γ-L-Glu. In addition, steric bulk was introduced on either side of the γ-glutamyl bond of ICI 198583-γ-L-Glu or ICI 198583-γ-L-Ala. The resulting dipeptide analogues, *e.g.*, ICI 198583-γ-MeGlu and ICI 198583-γ-Aib, were apparently stable to *in vivo* hydrolysis but poorer inhibitors of TS and L1210 cell growth. However, introduction of 7-Me, 2′-F substitution into the quinazoline nucleus gave significant improvement in the inhibitory activity against thymidylate synthase. Compounds **28**−**30**, the 7-Me, 2′-F derivatives of ICI 198583-γ-MeGlu, ICI 198583-γ-EtGlu, and ICI 198583-γ-PgGlu, respectively, were potent inhibitors of TS (K_{iapp} = 0.21−1.1 nM) and L1210 cell growth (IC_{50} = 0.05−0.34 μM) and were similar to that seen with the most potent γ-linked L,D dipeptide derivatives of ICI 198583 previously synthesized. Furthermore, the low cross-resistance ratios for the L1210:R^{D1694}/L1210 cell line indicated that **28**−**30** do not undergo polyglutamation.

Introduction

In a recent paper from our laboratories the synthesis of γ-linked L,D dipeptide analogues of 2-desamino-2-methyl-N^{10}-propargyl-5,8-dideazafolic acid (ICI 198583), as inhibitors of thymidylate synthase (TS) that do not undergo polyglutamation, was described.[1] The design of this class of antifolates was based on γ-linked L,L dipeptide derivatives of ICI 198583, *e.g.*, ICI 198583-γ-L-Glu and ICI 198583-γ-L-Ala, because of their excellent TS inhibitory properties.[2] However, susceptibility of the γ-glutamyl bond to enzymatic degradation limited the utility of these dipeptide derivatives as nonpolyglutamatable inhibitors of TS in animal models since ICI 198583, a product of the enzymatic degradation, had been shown to undergo polyglutamation.[3] Replacement of the C-terminal residue of γ-linked L,L dipeptides by D-amino acids gave L,D dipeptide derivatives that were potent inhibitors of TS, nonsubstrates for folylpolyglutamyl synthetase (FPGS), and resistant to enzymatic degradation[1] (Figure 1). A second approach involved the replacement of the amidic hydrogen of dipeptide analogues of ICI 198583 by an alkyl group (*e.g.*, methyl) or the introduction of steric bulk on either side of the γ-glutamyl amide bond. We therefore prepared a variety of *N*-alkylated, and in particular *N*-methylated, analogues of active dipeptide derivatives with the view of determing whether incorporation of *N*-alkyl residues in quinazoline antifolate dipeptides would prevent enzymatic degradation and polyglutamation without compromising potency. Such agents, with activity independent of FPGS, were expected to display a different spectrum of activity to Tomudex,[4−6] particularly in tumors expressing low levels of FPGS. We now report here the synthesis of 14 sterically hindered γ-linked dipeptide analogues of ICI 198583.

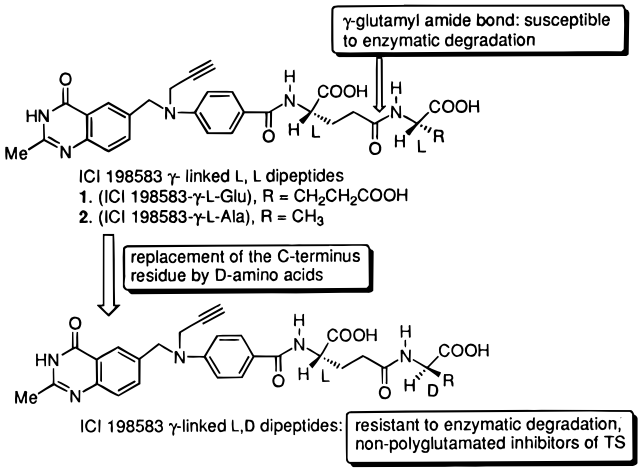

Figure 1.

Chemistry

The synthetic pathway to γ-linked sterically hindered dipeptides of ICI 198583 involved preparation of the

† Abbreviations: TS, thymidylate synthase; FPGS, folylpolyglutamyl synthetase; MTX, methotrexate: DEPC, diethyl cyanophosphoridate; Pg, propargyl; Glu, glutamic acid; Gly, glycine; Ala, alanine; Aib, α-aminoisobutyric acid; MeGlu, *N*-methylglutamic acid; EtGlu, *N*-ethylglutamic acid; PgGlu, *N*-propargylglutamic acid; 4-MeGlu, 4-methylglutamic acid; 4,4-diMeGlu, 4,4-dimethylglutamic acid; LDA, lithium diisopropylamide; LICA, lithium *N*-isopropylcyclohexylamide; NMM, *N*-methylmorpholine; DIEA, diisopropylethylamine; PyBOP, benzotriazolyloxytris(pyrrolidino)phosphonium hexafluorophosphate; Tr, trityl.

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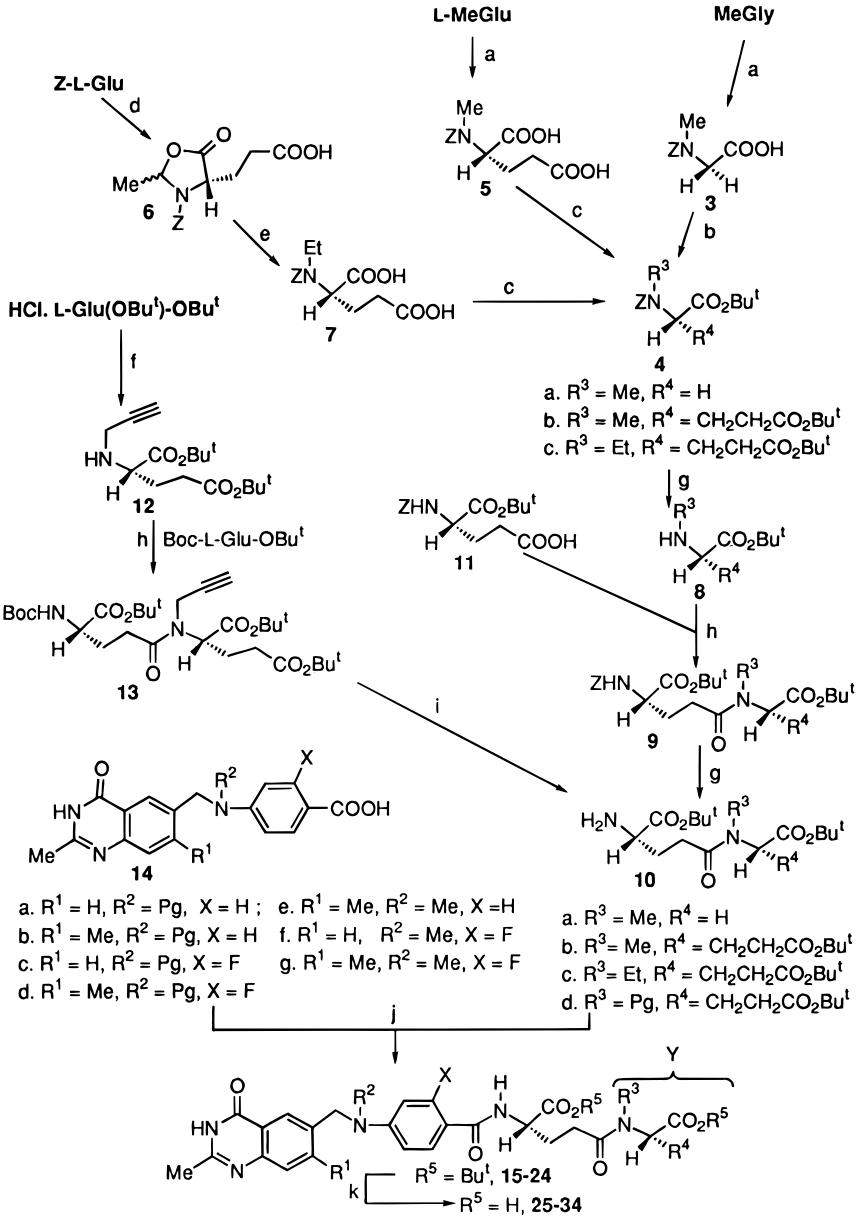
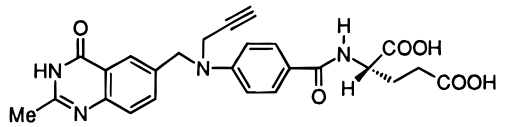
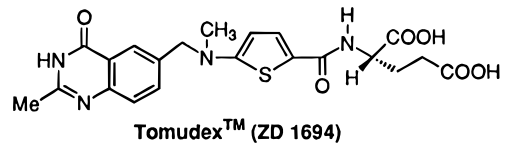
Scheme 1[a]



[a] Conditions: (a) aq $NaHCO_3$, $ClCO_2CH_2Ph$; (b) DCC, Bu^{t}OH, DMAP, $CHCl_3$; (c) $(CH_3)_2C{=}CH_2$, concd H_2SO_4, CH_2Cl_2; (d) paraldehyde, TsOH·H_2O, benzene, reflux; (e) Et_3SiH, TFA, $CHCl_3$, rt; (f) $CH{\equiv}CCH_2Br$, K_2CO_3, DMF, rt; (g) H_2, 10% Pd/C, EtOAc, rt; (h) $ClCO_2CH(CH_3)_2$, NMM, THF, −20 °C to rt; (i) TFA−CH_2Cl_2, rt; (j) DEPC, Et_3N; (k) TFA.



ICI 198583



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dipeptide derivatives by solution peptide synthesis followed by condensation with the appropriate pteroic acid analogue (Schemes 1−4). (The chemistry of peptide analogues of various antifolates has been described in a recent paper from our laboratories.[2])

The preparation of the dipeptides **10a**−**d**, key intermediates for the synthesis of *N*-alkyl derivatives **25**−**34**, is shown in Scheme 1. To prepare **8a,b**, the carboxyl function of MeGly or MeGlu was protected as a *tert*-butyl ester by first blocking the amino function with a benzyloxycarbonyl (Z) group and then esterifying the carboxyls using DCC / Bu^{t}OH[7] or isobutylene/H_2SO_4, respectively. Removal of the Z group by catalytic hydrogenolysis afforded **8a,b**.

The synthesis of Z-EtGlu (**7**) was accomplished by applying Freidinger's two-step procedure for Fmoc-protected *N*-alkyl amino acids.[8] In the first step Z-L-Glu was converted to the oxazolidinone **6** by treatment with paraldehyde under acid-catalyzed conditions (TsOH). Reduction of this intermediate with the triethylsilane−trifluoroacetic acid system afforded *N*-(benzyloxycarbonyl)-*N*-ethyl-L-glutamic acid (**7**, Z-Et-Glu). Di-*tert*-butyl *N*-ethyl-L-glutamate (**8c**) was then prepared by a sequence similar to that described for the *N*-Me derivative **8b** (Scheme 1). Subsequent condensation of **8a**−**c** with α-*tert*-butyl *N*-(benzyloxycarbonyl)-L-glutamate via isobutyl mixed anhydride activation[9]

Table 1. Quinazoline Antifolate Sterically Hindered Dipeptide Esters

compd	R^1	R^2	X	Y
15	H	Pg	H	MeGlu(OBu^t)-OBu^t
16	Me	Pg	H	MeGlu(OBu^t)-OBu^t
17	H	Pg	F	MeGlu(OBu^t)-OBu^t
18	Me	Pg	F	MeGlu(OBu^t)-OBu^t
19	Me	Pg	F	EtGlu(OBu^t)-OBu^t
20	Me	Pg	F	PgGlu(OBu^t)-OBu^t
21	H	Pg	H	MeGly-OBu^t
22	Me	Me	H	MeGlu(OBu^t)-OBu^t
23	H	Me	F	MeGlu(OBu^t)-OBu^t
24	Me	Me	F	MeGlu(OBu^t)-OBu^t
59a	H	Pg	H	Aib-OBu^t
59b	H	Pg	H	Pro-OBu^t

Table 2. Quinazoline Antifolate Sterically Hindered Dipeptides

compd	R^1	R^2	X	Y
25	H	Pg	H	MeGlu
26	Me	Pg	H	MeGlu
27	H	Pg	F	MeGlu
28	Me	Pg	F	MeGlu
29	Me	Pg	F	EtGlu
30	Me	Pg	F	PgGlu
31	H	Pg	H	MeGly
32	Me	Me	H	MeGlu
33	H	Me	F	MeGlu
34	Me	Me	F	MeGlu
60a	H	Pg	H	Aib
60b	H	Pg	H	Pro

afforded the Z-protected dipeptides **9a**−**c**, from which the key intermediates **10a**−**c** were obtained by catalytic hydrogenolysis (Scheme 1).

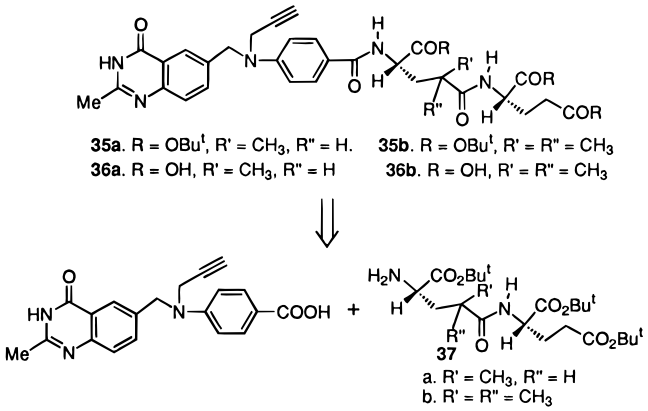
A different strategy was applied to prepare the *N*-propargyl derivative **10d**. Treatment of di-*tert*-butyl L-glutamate hydrochloride salt with propargyl bromide and K_2CO_3 as the base in DMF afforded di-*tert*-butyl *N*-propargyl glutamate (**12**) which was then coupled to Boc-L-Glu-OBu^t via isobutyl mixed anhydride activation to give the dipeptide **13** (Scheme 1). Selective removal of the Boc group was achieved with TFA−CH_2Cl_2 (v/v, 1:4) at room temperature over a 10 min period.

Dipeptides **10a**−**d** were then condensed with the pteroate analogues **14a**−**g**[1,2] using diethyl phosphorocyanidate (DEPC) carboxyl activation[10] to give the quinazoline antifolate dipeptide esters **15**−**24** (Scheme 1, Table 1), which were converted to the final products **25**−**34** by treatment with TFA (Scheme 1, Table 2).

The synthesis of quinazoline antifolate sterically hindered dipeptides **36a,b**, in which one or two methyl groups were introduced at the γ-center of the first glutamic residue of ICI 198583-γ-L-Glu (**1**), required the key intermediates tri-*tert*-butyl γ-4-methyl-L-glutamyl-L-glutamate (**37a**) and tri-*tert*-butyl γ-4,4-dimethyl-L-glutamyl-L-glutamate (**37b**), respectively (Scheme 2).

The monomethyl dipeptide derivative **37a** was prepared by the multistep sequence outlined in Scheme 3. We envisaged that introduction of the methyl group at the γ-center of a glutamate could be achieved through generation of the γ-enolate ester and quenching with MeI. Baldwin *et al.* applied this strategy to synthesize γ-hydroxyalkylated glutamates.[11] The γ-enolate of α-*tert*-butyl γ-methyl *N*-trityl-L-glutamate was generated using either LDA or LICA and then quenched with various carbonyl compounds to give γ-hydroxyalkylated glutamates. Rapoport *et al.* extended this and successfully prepared γ-alkylated glutamates from α-*tert*-butyl γ-

Scheme 2

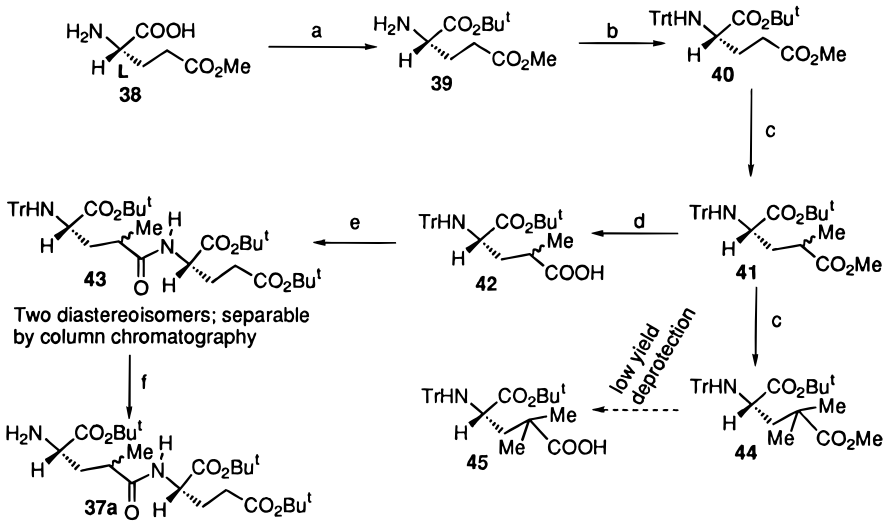


methyl *N*-[9-(9-phenylfluorenyl)]-L-glutamate using potassium bis(trimethylsilyl)amide ($(KN(SiMe_3)_2$) as the base to generate the γ-enolate ester instead of LDA or LICA.[12]

Our approach to the synthesis of **37a** involved differentiation of the two carboxyls of glutamic acid to enable chemoselectivity in later stages of the synthesis. The α-carboxyl was masked as its *tert*-butyl ester,[13,14] while a methyl ester was employed as a temporary protecting group for the γ-carboxy function of the glutamate (Scheme 3). Trityl protection of the amino group was used to prevent α-deprotonation and subsequent methylation or possible racemization. The γ-enolate ester of **40** was generated at −78 °C using KN-$(SiMe_3)_2$ as the base, and after quenching with MeI, the required product **41** was obtained as a mixture of two diastereoisomers, nonseparable by column chromatography. Methyl ester removal by treatment with aqueous LiOH in THF/H_2O gave **42** which was condensed with di-*tert*-butyl L-glutamate via isobutyl mixed anhydride activation to give the dipeptide derivative **43** as a mixture of two diastereoisomers, separable by column chromatography (R_f = 0.33, 0.28−20% EtOAc in hexanes). The stereochemistry at the γ-center of the MeGlu residue has not been determined. Only the diastereoisomer with the lower R_f (0.28) was taken forward to the next step since ^{1}H-NMR indicated the presence of an unidentified impurity in the diastereoisomer with the higher R_f. A pure sample of this isomer was later obtained by the γ-allyl ester route described in Scheme 4. We found that the allyl ester was superior to the methyl ester since it could be efficiently hydrolyzed under mild conditions (Scheme 4). In addition, the diastereoisomers of **43** were obtained in pure form. Finally, the trityl group was removed by catalytic hydrogenolysis to afford the key intermediate **37a**.

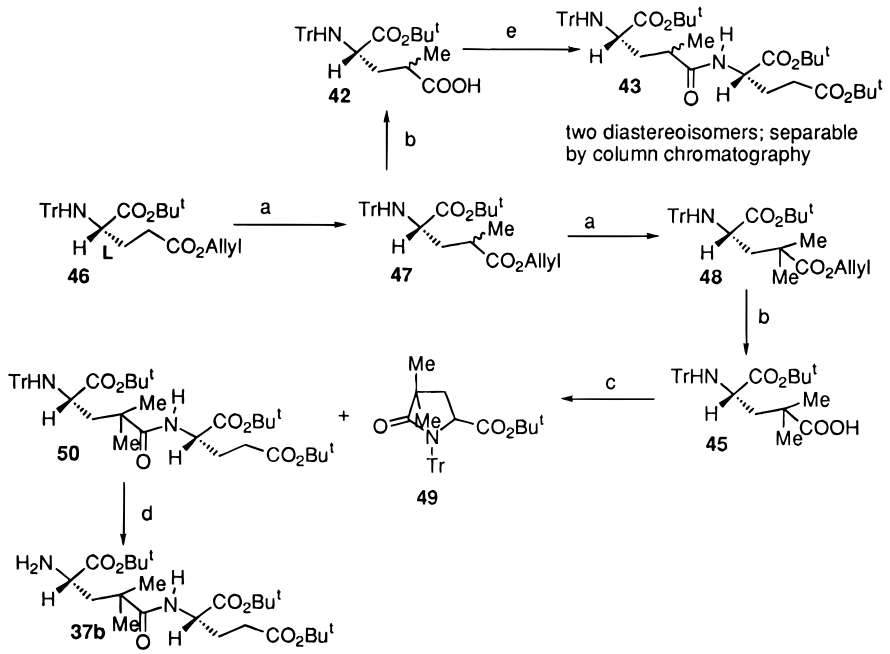
Initial attempts to prepare **44**, required for the preparation of dipeptide **37b**, directly from **40** using excess of base (3 equiv) and MeI (4 equiv) gave the γ-monomethylated glutamate derivative **41** and only about 3% of the desired product **44** (Scheme 3). The γ-dimethylated glutamate **44** was finally prepared from **41** by quenching its γ-enolate ester with 4 equiv of MeI. However, a workable route to **37b** from compound **44** was not possible since alkaline hydrolysis (NaOH, H_2O/THF) of α-*tert*-butyl γ-methyl *N*-trityl-4,4-dimethyl-L-glutamate (**44**) gave unacceptably low yields of **45** even when the reaction mixture was heated to reflux for 46 h.

Scheme 3[a]



[a] Conditions: (a) $CH_3CO_2C(CH_3)_3$, $HClO_4$, rt; (b) TrCl, Et_3N, $Pb(NO_3)_2$, $CHCl_3$, rt; (c) $KN(SiMe_3)_2$, MeI, THF, −78 °C; (d) $LiOH·H_2O$, H_2O/THF; (e) HCl·Glu(OBu^t)-OBu^t, $ClCO_2CH_2CH(CH_3)_2$, NMM, THF, −20 °C to rt; (f) H_2, 10% Pd/C, EtOAc, rt.

Scheme 4[a]

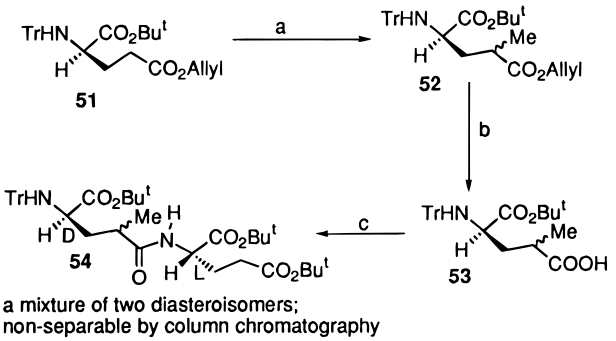


[a] Conditions: (a) $KN(SiMe_3)_2$, MeI, THF, −78 °C; (b) $Pd(PPh_3)_4$, pyrrolidine, CH_2Cl_2, rt; (c) HCl·Glu(OBu^t)-OBu^t, PyBOP, DMAP, DIEA, CH_2Cl_2, rt; (d) H_2, 10% Pd/C, EtOAc, rt; (e) HCl·Glu(OBu^t)-OBu^t, $ClCO_2CH_2CH(CH_3)_2$, NMM, THF, −20 °C to rt.

In view of this, allyl ester protection of the γ-carboxy function of the glutamate was utilized (Scheme 4). α-*tert*-Butyl γ-allyl *N*-trityl-L-glutamate (**46**) was synthesized as previously described.[15] Subsequently, the γ-dimethylated glutamate derivative **48** was prepared from **47** by generation of the γ-enolate ester at −78 °C with $KN(SiMe_3)_2$ and quenching with MeI (Scheme 4). The allyl ester was selectively removed by treatment with catalytic amounts of $Pd(PPh_3)_4$ and excess of pyrrolidine in CH_2Cl_2. Coupling of **45** to di-*tert*-butyl glutamate required activation of the γ-carboxyl with PyBOP[16] and catalytic amounts of DMAP in CH_2Cl_2 followed by addition of di-*tert*-butyl L-glutamate to give the desired dipeptide **50** in 45% yield and the cyclized byproduct **49** in 30% yield. It should be noted that attempts to prepare **50** using the isobutyl mixed anhydride method or azide method failed to give any product, whereas the acid chloride method resulted in the formation of the undesired pyroglutamic acid derivative **49**. This is not an unexpected finding since it is known that the steric bulk of an α,α-disubstituted carboxylic acid makes activation and amide bond formation more difficult.[17] Removal of the trityl group by catalytic hydrogenolysis finally afforded the key dipeptide intermediate **37b**.

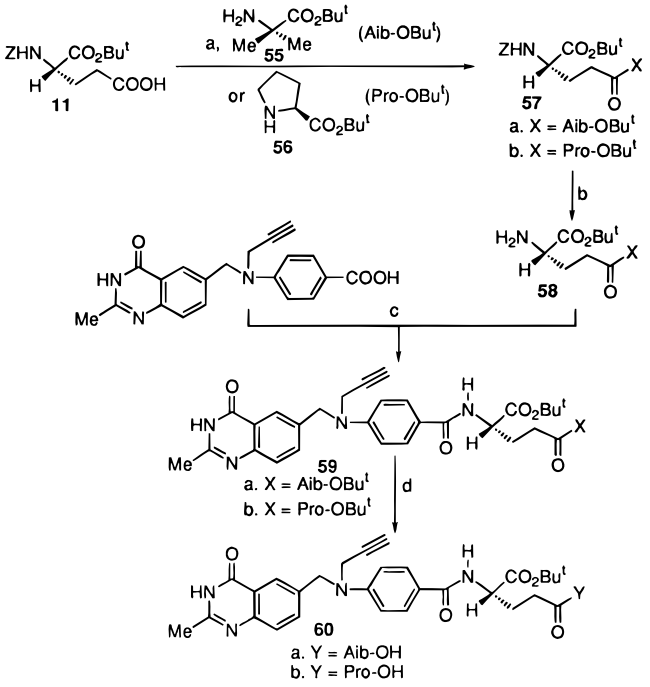
In order to check if the optical purity at the α-center of the glutamate was preserved during the synthetic sequence, the dipeptide **54** (Scheme 5) was synthesized by a route similar to the one employed for the synthesis of **43** except that allyl esters were used instead of methyl esters and the synthesis started with the D-glutamate derivative **51**. If racemization occurred at the α-center of the glutamate, it was expected that **43** and **54** would give the same ^{1}H-NMR spectra. However, **43** and **54**, each obtained as a mixture of two diastereoisomers, gave different ^{1}H-NMR spectra. For example, the ^{1}H-

Scheme 5[a]



[a] Conditions: (a) $KN(SiMe_3)_2$, MeI, THF, −78 °C; (b) $Pd(PPh_3)_4$, pyrrolidine, CH_2Cl_2; (c) HCl·Glu(OBu^t)-OBu^t, $ClCO_2CH_2CH(CH_3)_2$, NMM, THF, −20 °C to rt.

Scheme 6[a]



[a] Conditions: (a) $ClCO_2CH_2CH(CH_3)_2$, NMM, THF, −20 °C to rt; (b) H_2, 10% Pd/C, EtOAc, rt; (c) DEPC, Et_3N, DMF, 0 °C to rt; (d) TFA.

NMR spectrum of **43** showed one set of two doublets at 7.92 and 8.14 ppm for the amidic protons, whereas the ^{1}H-NMR spectrum of **54** showed one doublet at 7.99 ppm for the amidic protons. This suggests that the optical purity at the α-center of the glutamate had been preserved.

The quinazoline antifolate analogues **36a,b** were then prepared from the dipeptides **37a,b**, respectively, by the route employed for the synthesis of the *N*-alkyl derivatives, *i.e.*, DEPC coupling followed by TFA hydrolysis. Finally the synthesis of **60a,b** is shown in Scheme 6.

Conformational Equilibria of MeGlu, EtGlu, PgGlu, and MeGly Amide Derivatives

Normally an amide bond adopts the *trans* planar conformation. However, it has been reported that *N*-alkyl amide bonds tend to adopt both *cis* and *trans* conformations,[18,19] due to bulk of the alkyl substituent compared to that of the replaced amidic hydrogen. Both conformers normally interconvert, but if rotation around the *N*-alkylated amide bond is slowed, then the two conformers are distinguishable on the time scale of ^{1}H-NMR. This was the case with the MeGlu, EtGlu, PgGlu, and MeGly amide derivatives made in our study. Thus, for example, the ^{1}H-NMR of Z-MeGly (**3**) in DMSO at ambient temperature (25 °C) showed that the two conformers exist in similar populations. Raising the temperature increased the rate of rotation around the methylated amide bond resulting in a ^{1}H-NMR of **3** recorded at 345 K showing one peak for the *N*-Me, which represents the average environment between the two conformers. Similarly, the ^{1}H-NMR of Z-MeGlu (**5**) in DMSO at ambient temperature again showed that both *trans* and *cis* conformers are energetically comparable, since the ratio of the two singlets at 2.76 and 2.79 ppm corresponding to *N*-Me protons is about 3:2. After raising the temperature to 345 K, however, we observed one signal (singlet) for the *N*-Me group. The existence of the two conformers resulted in rather complicated ^{1}H-NMR spectra since a number of peaks are doubled even at high temperatures.

Biological Evaluation

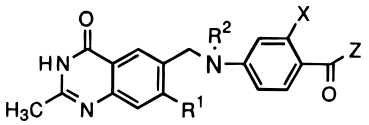
The antifolates listed in Table 2 were tested as inhibitors of partially purified TS from L1210 mouse leukemia cells that overproduce TS due to amplification of the TS gene.[20] The partial purification and assay method used in this study was as previously described, and it used (±)-5,10-methylenetetrahydrofolic acid at a concentration of 200 μM.[20] Inhibition of L1210 and L1210:1565 cell growth was also determined as previously described.[21] L1210:1565 is a L1210 mutant cell line with impaired reduced folate/MTX transport carrier. This cell line was made resistant to CI-920, a compound that uses the RFC transport system,[22] and hence is cross-resistant to MTX. The methodology for the *in vivo* stability was as previously described;[23] mice were administered with 100 mg/kg compound (ip) and killed 1 h later. Blood, liver, and kidneys were removed for quantitation by HPLC of the parent dipeptide derivative of ICI 198583 and any monoglutamate derivative of ICI 198583 present.

Results and Discussion

All compounds were tested as inhibitors of TS and L1210 cell growth. The results are shown in Table 3. Replacement of the γ-glutamyl amidic hydrogen of ICI 198583-γ-L-Glu (**1**) by methyl gave compound **25** which was approximately 5-fold less potent as an inhibitor of TS but stable to hydrolysis in mice. The same trend was observed with ICI 198583-γ-MeGly (**31**), again stable in mice but a considerably less potent inhibitor of TS (∼5-fold) than its parent dipeptide ICI 198583-γ-Gly (TS IC_{50} = 11 nM, L1210 IC_{50} = 0.11 μM).[2] Substitution of 7-Me into the quinazoline ring (compound **26**) or 2′-F into the benzene ring of **25** (compound **27**) enhanced TS inhibition by 2−4-fold. Combining both of these structural modifications to give compound **28** resulted in a 10-fold enhancement in TS inhibition but without any parallel improvement in the L1210 cell growth inhibition (IC_{50} = ∼0.24 μM).

Replacement of the N^{10}-propargyl substituent by a methyl in compounds **26**−**28** generally resulted in poorer inhibitors of TS and L1210 cell growth (compounds **32**−**34**, Table 3). Only the 7-Me, 2′-F derivative, compound **34**, had an inhibitory activity against TS

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



compd	R^1	R^2	X	Z	inhibition of L1210 TS, K_{iapp} (nM)	inhibition of cell growth, IC_{50} (μM)[a] L1210	W1L2	L1210:1565	L1210:R^{D1694}	stable to hydrolysis in mice?[b]
1	H	Pg	H	L-Glu-L-Glu	2.0	0.16 ± 0.069		4.6, 5.0	2.7, 2.9 (18)	no (ref 2)
25	H	Pg	H	L-Glu-L-MeGlu	11	0.24, 0.22	0.47	1.6, 2.3	2.2, 2.6 (10)	yes
26	Me	Pg	H	L-Glu-L-MeGlu	2.6	0.22, 0.24, 0.25	0.64	5	2.0, 2.1 (8)	
27	H	Pg	F	L-Glu-L-MeGlu	4.0	0.23, 0.27	0.44	1.8	2.6 (10)	
28	Me	Pg	F	L-Glu-L-MeGlu	1.1	0.34 ± 0.070	0.35, 0.70	4, 5.4	1.4 ± 0.32 (4)	yes
29	Me	Pg	F	L-Glu-L-EtGlu	0.35	0.07, 0.03	0.073	9.3, 5.4	0.22 (6)	
30	Me	Pg	F	L-Glu-L-PgGlu	0.21	0.11, 0.072	0.22, 0.21	4.2	0.57 (5)	
31	H	Pg	H	L-Glu-MeGly	50	1.1, 2.4	0.33	~70	7.4 (4)	yes
32	Me	Me	H	L-Glu-L-MeGlu	8.2	0.64, 0.56, 0.78	0.80	4.2, 9.8	4.0 (6)	yes
33	H	Me	F	L-Glu-L-MeGlu	23	2.4, 2.1, 1.5	2.3	5.1	9.2 (5)	
34	Me	Me	F	L-Glu-L-MeGlu	3.0	0.73, 0.60	0.63	5.6	2.6 (4)	
36a	H	Pg	H	L-4-MeGlu-L-Glu	8.6	0.41, 0.25	0.50	14	3.4 (10)	yes
36b	H	Pg	H	L-4,4-diMeGlu-L-Glu	18	1.4, 1.0	0.37	22	1.2, 3.5 (2)	yes
60a	H	Pg	H	L-Glu-Aib	42	2.5, 3.1	0.31	70	7.1 (2.5)	yes
60b	H	Pg	H	L-Glu-Pro	35	3.1, 4.2			10 (3)	yes

[a] Methodologies for the inhibition of mouse L1210 TS and L1210, L1210:1565 (impaired RFC), L1210:R^{D1694}, and human W1L2 cell growth are as described in refs 20, 21, and 24. [b] Methodology for the stability of these dipeptides in mice is described in ref 23.

comparable to that of the corresponding N^{10}-propargyl derivative **28**. These data support the previously reported[1] view that propargyl is a better N^{10}-substituent than methyl and 7-Me, 2′-F substitution pattern enhances binding to TS.

The impact of different *N*-substituents on the γ-glutamyl amide bond from the methyl was next explored. We chose, as the starting point for this study, compound **28**, a compound bearing the N^{10}-Pg, 7-Me, 2′-F substitution pattern in which the γ-glutamyl amide carried a methyl substituent. Replacement by ethyl or propargyl gave compound **29** or **30**, respectively. Relative to **28** these compounds were up to 5-fold more potent inhibitors of TS and L1210 cell growth (Table 3) supporting a degree of tolerance by the enzyme in the region of the *N*-substituent of the γ-glutamyl bond. Both analogues showed improvement in enzyme binding, but compound **29** was the most potent inhibitor of the L1210 and W1L2 cell growth (Table 3).

The consequences of introducing one or two methyl groups onto the γ-center of the first glutamic residue of ICI 198583-γ-L-Glu (**1**) were next studied. The monomethyl derivative **36a** was a 4-fold less potent inhibitor of TS and ~2-fold less growth inhibitory than ICI 198583-γ-L-Glu (Table 3). Introduction of two methyl groups, compound **36b**, resulted in a further drop in potency. The effect of introducing a methyl group on the other side of the γ-glutamyl amide bond was revealed by the synthesis of ICI 198583-γ-Aib (**60a**). The compound was a less potent inhibitor of TS and L1210 cell growth compared with its parent compound ICI 198583-γ-L-Ala (IC_{50} = 18 nM, L1210 IC_{50} = 0.56 μM).[2] When compounds **36a,b** and **60a** were administered to mice, no hydrolysis products were observed after 1 h, indicating that the introduction of steric bulk in close proximity to the γ-glutamyl amide bond significantly improved the enzymatic stability.

Further characterization of this interesting series of compounds revealed that the majority utilize the RFC/MTX transport carrier to enter cells. This is best illustrated by the poor activity in the L1210:1565 cell line, which exhibits an impaired RFC,[22] with cross-resistance ratios between 8 and 105, except compound **33** (Table 3).

These compounds were designed to overcome the antifolate-resistant phenotype due to a decreased level of polyglutamation. The ZD1694-resistant cell line (L1210:R^{D1694})[24] was used to address the question since in this cell line the predominant mechanism of resistance is a decreased ability to polyglutamate synthetic antifolates. A minor RFC transport defect has also been shown in this cell line to be a small but contributing mechanism of resistance. This cell line is >10000-fold resistant to ZD1694 but only 18-fold cross-resistant to ICI 198583[24] reflecting the decreased dependency of the latter compound on polyglutamation for activity. As expected, cross-resistance was observed with the γ-linked L,L dipeptide derivatives of ICI 198583 (compound **1**). Previous studies from our laboratory,[24] with a number of compounds that are structurally precluded from polyglutamation, suggested that compounds which use the RFC for cell entry but are not substrates for FPGS give cross-resistance values ranging from ~2 to 5. Compounds with a very low affinity of RFC gave values of ~1. Using these ratios as precedent we conclude that four compounds (**25**−**27**, **36a**) with high relative resistance ratios (8−10) are poor substrates for FPGS. We are not able to say clearly whether a low level of polyglutamation is occurring, and further work with a cell line expressing a low level of FPGS activity or radiolabeled studies would be required to establish this. The two most potent compounds in the L1210:R^{D1694} cell line were **29** and **30** (L-Glu-γ-EtGlu and L-Glu-γ-PgGlu, respectively), which is almost certainly due to their increased binding to TS.

Replacement of the γ-glutamyl amidic hydrogen by an alkyl group or introduction of steric hindrance to either side of this amide bond resulted in compounds which were stable *in vivo* but less potent than the parent γ-linked L,L dipeptide analogues of ICI 198583 *in vitro*. Reduced activity against the L1210:1565 cell line compared to the parental line indicated that these

dipeptides utilize the RFC transport system to enter cells. With regard to polyglutamation the picture is less clear. L1210:R^{D1694} cells were partially cross-resistant to analogues of ICI 198583-γ-L-Glu, such as **25**, which lack 7-Me substitution, and probably reflect a low level of FPGS substrate activity. However, incorporation of the 7-Me into these dipeptides was expected to prevent FPGS substrate activity as previously described when ICI 198583 was 7-methylated[25] and gave a low cross-resistance ratio.[24] Compounds **28**−**30** exhibited similar inhibitory activity against TS and L1210 cell growth as the most potent γ-linked L,D dipeptide analogues of ICI 198583, a class of compounds that do not undergo polyglutamation.

Experimental Section

Thin layer chromatography (TLC) was performed on precoated sheets of silica $60F_{254}$ (Merck Art 5735). Visualization was achieved by UV or chlorine−tolidine reagent. Merck silica 60 (Art 15111) was used in low-pressure column chromatography. Petrol refers to light petroleum (bp 60−80 °C). 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 70 eV, 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 determined 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 formed).

1. 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.

2. 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 *N*-Alkyl Amino Acids: *N*-(Benzyloxycarbonyl)-*N*-methylglycine (3). *N*-Methylglycine (7.0 g, 0.078 mol) was added to water (150 mL) and the pH of the solution adjusted to ∼9 with solid $NaHCO_3$. The solution was cooled to 0 °C and stirred vigorously (overhead mechanical stirring) while benzyl chloroformate (19 mL, 0.13 mol) was added dropwise over a 20 min period. The mixture was stirred for a further 2 h at 0 °C and an additional 2 h at room temperature, during which time the pH was periodically checked and adjusted to ∼9 with solid NaOH. The reaction mixture was then extracted with Et_2O (2 × 130 mL), and the aqueous solution was then acidified to pH 3 with 5 N HCl and the product isolated by extraction with EtOAc (2 × 130 mL). The combined organic extracts were dried (Na_2SO_4), and the solution was concentrated *in vacuo* to give *N*-(benzyloxycarbonyl)-*N*-methylglycine as a pale yellow oil (16.2 g, 92%): ^{1}H-NMR (DMSO-d_6, 72 °C) 2.91 (s, 3H, *N*-CH_3), 3.94 (s, 2H, CH_2CO$_2$H), 5.07 (s, 2H, PhCH_2), 7.33 (s, 5H, Ar*H*); MS (EI, *m*/*z*) 223 (M^+).

α-*tert*-Butyl *N*-(Benzyloxycarbonyl)-*N*-methylglycinate (4a). To a stirred solution of *N*-(benzyloxycarbonyl)-*N*-methylglycine (7.86 g, 0.035 mol) and *tert*-butyl alcohol (4.68 mL, 0.052 mmol) in $CHCl_3$ (30 mL) cooled in an ice−water bath was added a solution of DCC (8.0 g, 0.039 mol) in $CHCl_3$ (30 mL), and then DMAP (0.04 g) was added as catalyst. Stirring was continued at 0 °C for 2 h; then the mixture was kept at 4 °C overnight. Dicyclohexylurea was removed by filtration and the solvent evaporated *in vacuo*. The residue was dissolved in EtOAc (110 mL), AcOH (1.10 mL) was then added, and the solution was stored at 4 °C for 2 h. After filtration, the ethyl acetate solution was washed with 5% aqueous $NaHCO_3$ solution (2 × 70 mL) and H_2O (3 × 80 mL), dried ($MgSO_4$), and concentrated *in vacuo* to leave an oily residue. Purification by column chromatography on elution with 30% EtOAc in petrol gave the title compound **4a** (3.6 g, 37%) as an oil: ^{1}H-NMR (DMSO-d_6) 1.36, 1.41 (2 × s, 9H, C(CH_3)$_3$), 2.88, 2.91 (2 × s, 3H, *N*-CH_3), 3.91, 3.94 (2 × s, 2H, CH_2COOH), 5.05, 5.10 (2 × s, 2H, PhCH_2), 7.31, 7.36 (2 × m, 5H, Ar*H*); MS (EI, *m*/*z*) 279 (M^+). Anal. ($C_{15}H_{21}NO_4$) C, H, N.

***tert*-Butyl *N*-Methylglycinate (8a).** A solution of *tert*-butyl *N*-(benzyloxycarbonyl)-*N*-methylglycinate (1.60 g, 5.7 mmol) in EtOAc (120 mL) containing 10% Pd/C (0.23 g) was stirred under hydrogen for 2.5 h. The catalyst was removed by filtration and the filtrate concentrated *in vacuo*, yielding *tert*-butyl *N*-methylglycinate (0.71 g, 85%) as an oil: ^{1}H-NMR (DMSO-d_6) 1.41 (s, 9H, C(CH_3)$_3$), 2.25 (s, 3H, *N*-CH_3), 3.13 (s, 2H, CH_2); MS (EI, *m*/*z*) 145 (M^+).

***N*-(Benzyloxycarbonyl)-*N*-methyl-L-glutamic Acid (5).** *N*-Methyl-L-glutamic acid (5.0 g, 31.0 mmol) was dissolved in a saturated solution of $NaHCO_3$ (190 mL). The solution was cooled to 0 °C and stirred vigorously (overhead mechanical

stirring) while benzyl chloroformate (10.5 g, 62.0 mmol) was added dropwise over a 20 min period. The mixture was stirred for a further 2 h at 0 °C and then for a further 20 h at room temperature and then extracted with Et_2O (2 × 150 mL). The aqueous solution was acidified to pH 3 with 5 N HCl and the product isolated by extraction with EtOAc (2 × 150 mL). The pooled organic extracts were dried over Na_2SO_4, and the solvent was removed *in vacuo* to give a yellow oil which solidified on standing (6.9 g, 75%). Recrystallization from H_2O gave an analytically pure sample as a white solid: mp 98−101 °C (lit.[26] mp 79−80 °C); $[\alpha]^{25} = -35.0$ ($c = 1$, EtOH) (lit.[26] $[\alpha]^{27} = -25.7$ ($c = 1$, EtOH)); ^{1}H-NMR (DMSO-d_6, 67 °C) δ 1.94, 2.14 (2 × m, 2H, β-CH_2), 2.22 (t, $J = 6.3$ Hz, 2H, γ-CH_2), 2.79 (s, 3H, *N*-CH_3), 4.53 (dd, $J = 5.0$, 10.4 Hz, 1H, α-C*H*), 5.08 (s, 2H, ArCH_2), 7.33 (m, 5H, Ar*H*); MS (CI, *m*/*z*) 296 (M + H)$^+$. Anal. ($C_{14}H_{17}NO_6$) C, H, N.

Di-*tert*-butyl *N*-(Benzyloxycarbonyl)-*N*-methyl-L-glutamate (4b). To a suspension of *N*-(benzyloxycarbonyl)-*N*-methyl-L-glutamic acid (1.85 g, 6.3 mmol) in CH_2Cl_2 (52 mL) in a 500 mL pressure bottle was added concentrated H_2SO_4 (0.28 mL) followed by liquid isobutylene (25 mL) at −20 °C. The stoppered reaction vessel was shaken vigorously at room temperature for 28 h and then cooled, and a saturated solution of $NaHCO_3$ (100 mL) and EtOAc (200 mL) were added. The organic layer was separated and washed with a saturated $NaHCO_3$ solution (2 × 160 mL) and H_2O (100 mL), then dried (Na_2SO_4), and concentrated *in vacuo*. Purification by column chromatography using 10% EtOAc in CH_2Cl_2 as eluant gave the title compound **4b** (1.8 g, 70%) as an oil: ^{1}H-NMR (DMSO-d_6, 67 °C) δ 1.37, 1.38 (2 × s, 18H, 2 × C(CH_3)$_3$), 1.90, 2.07 (2 × m, 2H, β-CH_2), 2.20 (m, 2H, γ-CH_2), 2.79 (s, 3H, *N*-CH_3), 4.44 (dd, $J = 5.3$, 10.2 Hz, 1H, α-C*H*), 5.11 (m, 2H, ArCH_2), 7.33 (m, 5H, Ar*H*); MS (CI, *m*/*z*) 408 (M + H)$^+$. Anal. ($C_{22}H_{33}NO_6$) C, H, N.

Di-*tert*-butyl *N*-Methyl-L-glutamate (8b). The general procedure B was followed using di-*tert*-butyl *N*-(benzyloxycarbonyl)-*N*-methyl-L-glutamate (1.6 g, 3.9 mmol), EtOAc (120 mL), and 10% Pd/C (0.22 g). Workup as described gave the title compound **8b** (1.04 g, 98%) as an oil: ^{1}H-NMR (DMSO-d_6) δ 1.39, 1.42 (2 × s, 18H, C(CH_3)$_3$), 1.67 (m, 2H, β-CH_2), 2.19 (s, 3H, *N*-CH_3), 2.23 (m, 2H, γ-CH_2), 2.91 (dd, $J = 6.1$, 7.8 Hz, 1H, α-C*H*); MS (CI, *m*/*z*) 274 (M + H)$^+$.

***N*-(Benzyloxycarbonyl)-*N*-ethyl-L-glutamic Acid (7).** A stirred mixture of *N*-(benzyloxycarbonyl)-L-glutamic acid (10 g, 35.5 mmol), *p*-toluenesulfonic acid monohydrate (0.7 g, 3.7 mmol), and benzene (700 mL) was heated under reflux in a flask fitted with a Dean−Stark trap. After 80 min, paraldehyde (11 mL) was added to the mixture in portions during 20 min. Two further portions of paraldehyde (6.5 mL each) were added after total times of 2.5 and 5 h. After a further 30 min the mixture was cooled, decanted from separated tar, washed with water (200 mL followed by 3 × 100 mL), dried ($MgSO_4$), and evaporated. The residue was chromatographed on silica gel using increasingly polar mixtures of CH_2Cl_2 and EtOAc (100:0 to 60:40) as eluants to give a mixture of two diastereoisomeric products, **6**, as an oil (2.17 g). TFA (6 mL) and triethylsilane (3.3 mL, 20.5 mmol) were added to a solution of **6** (2.1 g, 6.8 mmol) in dry $CHCl_3$ (6 mL), and the solution was stirred under nitrogen at room temperature. Two further portions of triethylsilane (1.1 mL each) were added after total times of 16 and 40 h. After 64 h (total) the solution was concentrated and the residual gum chromatographed on silica gel with a gradient of MeOH−H_2O (95:5) in CH_2Cl_2 (0−10%). *N*-(Benzyloxycarbonyl)-*N*-ethyl-L-glutamic acid, **7**, was isolated as a gum (1.825 g, 17%): ^{1}H-NMR (DMSO-d_6−D_2O) δ 1.09 (t, $J = 6.8$ Hz, 3H, CH_2CH_3), 1.98, 2.14 (2 × m, 2H, β-CH_2), 2.28 (m, 2H, γ-CH_2), 3.13, 3.35 (2 × m, 2H, CH_2CH$_3$), 4.24 (m, 1H, α-C*H*), 5.11 (m, 2H, ArCH_2), 7.34 (m, 5H, Ar*H*); MS (EI, *m*/*z*) 309 (M)$^+$. Anal. ($C_{15}H_{19}NO_6$·0.25H_2O) C, H, N.

Di-*tert*-butyl *N*-(Benzyloxycarbonyl)-*N*-ethyl-L-glutamate (4c). A solution of **7** (2.878 g, 9.3 mmol) in CH_2Cl_2 (74 mL), contained in a pressure bottle, was cooled in acetone−dry ice, and concentrated H_2SO_4 (0.44 mL) and liquid isobutylene (39 mL) were added. The mixture was shaken at room temperature for 52 h, then cooled, and poured into saturated aqueous $NaHCO_3$ (150 mL). The products were extracted with EtOAc (150 mL, then 2 × 50 mL), and the combined organic solution was washed with brine (3 × 50 mL), dried (Na_2SO_4), and evaporated. The residue was chromatographed on silica gel using hexanes−CH_2Cl_2 (2:1, v/v), neat CH_2Cl_2, and CH_2Cl_2−EtOAc (19:1 and 9:1, v/v) in succession as eluants. Di-*tert*-butyl *N*-(benzyloxycarbonyl)-*N*-ethyl-L-glutamate, **4c**, was isolated as an oil (2.97 g, 76%): ^{1}H-NMR (DMSO-d_6) δ 1.08 (t, $J = 7.0$ Hz, 3H, CH_2CH_3), 1.32, 1.37, 1.39 (3 × s, 18H, C(CH_3)$_3$), 2.0 (m, 2H, β-CH_2), 2.23 (m, 2H, γ-CH_2), 3.05, 3.42 (2 × m, 2H, CH_2CH$_3$), 4.10 (m, 1H, α-C*H*), 5.13 (m, 2H, ArCH_2), 7.35 (m, 5H, Ar*H*); MS (EI, *m*/*z*) 422 (M + H)$^+$. Anal. ($C_{23}H_{35}NO_6$) C, H, N.

Di-*tert*-butyl *N*-Ethyl-L-glutamate (8c). The general procedure B was followed using **4c** (1.761 g, 4.18 mmol), EtOAc (125 mL), and 10% Pd/C (0.24 g). The reaction mixture was stirred under hydrogen at room temperature for 3 h, and the crude product was chromatographed with CH_2Cl_2−EtOH (100:0 to 97:3, v/v) as eluant. Di-*tert*-butyl *N*-ethyl-L-glutamate, **8c**, was isolated as a colorless oil (1.132 g, 94%): ^{1}H-NMR ($CDCl_3$) δ 1.08 (t, $J = 7.1$ Hz, 3H, CH_2CH_3), 1.44, 1.47 (2 × s, 18H, C(CH_3)$_3$), 1.83 (m, 2H, β-CH_2), 2.33 (m, 2H, γ-CH_2), 2.49, 2.63 (2 × m, 2H, CH_2CH$_3$), 3.10 (dd, $J = 7.4$, 6.4 Hz, 1H, α-C*H*); MS (EI, *m*/*z*) 287 (M)$^+$.

Di-*tert*-butyl *N*-Prop-2-ynyl-L-glutamate (12). Di-*tert*-butyl L-glutamate hydrochloride (3.0 g, 10.1 mmol), anhydrous K_2CO_3 (2.94 g, 21.3 mmol), DMF (15 mL), and propargyl bromide (80% solution in toluene, 1.2 mL, 10.6 mmol) were stirred together under argon at ambient temperature with protection from light. After 3 days the reaction mixture was concentrated and the residue partitioned between CH_2Cl_2 (100 mL) and H_2O (100 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL), and the combined CH_2Cl_2 solution was washed with H_2O (5 × 30 mL), dried ($MgSO_4$), and evaporated. Two portions of toluene were added and evaporated, and the residue was chromatographed on silica gel, using CH_2Cl_2−hexanes (1:2 to 3:1), neat CH_2Cl_2, and CH_2Cl_2−EtOAc (9:1) in succession as eluants. Di-*tert*-butyl *N*-prop-2-ynyl-L-glutamate, **12** (major, more polar product), was obtained as an oil (1.77 g, 59%): ^{1}H-NMR ($CDCl_3$) δ 1.44, 1.48 (2 × s, 18H, C(CH_3)$_3$), 1.84 (m, 2H, β-CH_2), 2.19 (t, $J = 2.5$ Hz, 1H, C≡C*H*), 2.35 (t, $J = 7.6$ Hz, 2H, γ-CH_2), 3.27 (dd, $J = 7.7$, 5.5 Hz, 1H, α-C*H*), 3.39 (m, 2H, CH_2-C≡C); MS (EI, *m*/*z*) 298 (M + H)$^+$.

Preparation of α-*tert*-Butyl *N*-Trityl-4-methyl-L-glutamate (42) and α-*tert*-Butyl *N*-Trityl-4,4-dimethyl-L-glutamate (45): α-*tert*-Butyl γ-Methyl L-Glutamate (39). γ-Methyl L-glutamate hydrochloride (12.88 g, 0.08 mol), *tert*-butyl acetate (500 mL), and 70% aqueous $HClO_4$ (12.6 g, 0.08 mol) were stirred at room temperature for 88 h. The mixture was then cooled in an ice bath and extracted with cooled 0.5 N HCl (3 × 130 mL). The combined aqueous extracts were immediately neutralized with solid $NaHCO_3$ and then extracted with Et_2O (3 × 200 mL). The ether extracts were combined, dried (Na_2SO_4), and filtered, and the solution was concentrated *in vacuo* to give α-*tert*-butyl γ-methyl L-glutamate (14.5 g) as a colorless oil. This was immediately used in the next experiment without further purification.

α-*tert*-Butyl γ-Methyl *N*-Trityl-L-glutamate (40). To a stirred solution of α-*tert*-butyl γ-methyl L-glutamate (13.71 g, 63.0 mmol) in dry $CHCl_3$ (50 mL) was added Et_3N (8.49 g, 84.0 mmol) followed by lead nitrate (8.34 g, 25 mmol) and then a solution of trityl chloride (11.67 g, 42.0 mmol) in dry $CHCl_3$ (20 mL). After stirring at room temperature for 28 h a white solid had formed. This was removed by filtration, and the filtrate was concentrated *in vacuo* to an orange residue. Purification by column chromatography, on gradient elution with CH_2Cl_2 in hexanes (10−100%), gave a semisolid product, a mixture of the desired product **40** and triphenylmethanol. This was treated with petrol; the white solid (triphenylmethanol, 3.9 g) was filtered off, and the filtrate was concentrated *in vacuo* to give the title compound **40** (9.46 g, 49%) as an oil, which solidified on standing at −20 °C for 4 weeks: mp 79−80 °C (hexanes); $[\alpha]^{25} = +23.22°$ ($c = 0.818$, $CHCl_3$) (lit.[11] $[\alpha]^{20} = +22.5°$ ($c = 0.75$, $CHCl_3$)); ^{1}H-NMR (DMSO-d_6) δ 1.13 (s, 9H, C(CH_3)$_3$), 1.80, 1.92 (2 × m, 2H, β-CH_2), 2.18, 2.44 (2 × m, 2H, γ-CH_2), 2.79 (d, $J = 9.0$ Hz, 1H, N*H*), 3.17 (m, 1H,

α-C*H*), 3.60 (s, 3H, OCH_3), 7.16−7.41 (m, 15H, Tr*H*); MS (CI, *m*/*z*) 460 $(M + H)^+$. Anal. ($C_{29}H_{33}NO_4$) C, H, N.

α-*tert*-Butyl γ-Methyl *N*-Trityl-4-methyl-L-glutamate (41). To a stirred solution of 0.6 M $KN(SiMe_3)_2$ in THF (13.85 mL, 8.25 mmol) at −78 °C was added a solution of α-*tert*-butyl γ-methyl *N*-trityl-L-glutamate (2.52 g, 5.5 mmol) in dry THF (25 mL) over a 5 min period under argon. The resulting yellowish solution was stirred at −78 °C for 50 min under argon, and then MeI (1.56 g, 0.68 mL, 11.0 mmol) was added under argon. Stirring was continued at −78 °C for 1 h and 15 min, and then the whitish slurry was poured into a saturated solution of NH_4Cl (200 mL). The mixture was extracted with Et_2O (2 × 200 mL), and the ether extracts were combined, dried (Na_2SO_4), and concentrated *in vacuo*. Purification by column chromatography on gradient elution with EtOAc in hexanes (3−9%) gave α-*tert*-butyl γ-methyl *N*-trityl-4-methyl-L-glutamate (1.62 g, 62%), a viscous oil, as a mixture of two diastereoisomers (2*S*,4*S* and 2*S*,4*R*): ^{1}H-NMR (DMSO-d_6) δ 0.98, 1.04 (2 × d, *J* = 7.0 Hz, 3H, γ-CH_3), 1.12, 1.14 (2 × s, 9H, C(CH_3)$_3$), 1.38, 1.54 (2 × m, 1H, β-C*H*), 1.93, 2.10 (2 × m, 1H, β-C*H*), 2.40 (m, 1H, γ-C*H*), 2.75, 2.80 (2 × d, *J* = 8.9, 9.4 Hz, 1H, N*H*), 3.10 (m, 1H, α-C*H*), 3.50, 3.66 (2 × s, 3H, CO_2CH_3), 7.16−7.39 (m, 15H, Tr*H*), a singlet at 3.55 ppm indicated that about 3% of **44** was present; MS (CI, *m*/*z*) 474 $(M + H)^+$. Anal. ($C_{30}H_{35}NO_4$) C, H, N.

α-*tert*-Butyl *N*-Trityl-4-methyl-L-glutamate (42). To a mixture of lithium hydroxide monohydrate (0.117 g, 2.79 mmol) and H_2O (1.86 mL) was added a solution of α-*tert*-butyl γ-methyl *N*-trityl-4-methyl-L-glutamate (1.1 g, 2.33 mmol) in THF (27 mL). The resulting mixture was refluxed for 3 h, then more H_2O (0.6 mL) was added, and refluxing was continued for 5 h. After that the organic solvent was removed by evaporation, the residue was treated with a half-saturated $NaHCO_3$ solution (100 mL), and the resulting mixture was acidified to pH 6 with 1 N HCl. The mixture was extracted with EtOAc (2 × 100 mL); the extracts were combined, dried (Na_2SO_4), and concentrated *in vacuo*. The residue was dried *in vacuo* over P_2O_5 to give α-*tert*-butyl *N*-trityl-4-methyl-L-glutamate (1.06 g) as a white foam contaminated with approximately 10% of the starting material. This was taken forward in the next step without further purification.

γ-Allyl ester protection provides a simpler and most efficient route to **42**: To a stirred solution of **47** (0.244 g, 0.49 mmol) in anhydrous CH_2Cl_2 (2.5 mL) under argon was added Pd(PPh_3)$_4$ (0.020 g) followed by pyrrolidine (0.09 mL). The resulting yellow solution was stirred at room temperature under argon for 4 h; then more catalyst (0.010 g) was added, and stirring was continued at room temperature overnight before the mixture was partitioned between Et_2O (50 mL) and 0.5 N HCl (40 mL). The aqueous layer was extracted with more Et_2O (2 × 50 mL), and the combined extracts were dried (Na_2SO_4) and concentrated *in vacuo*. The residue was treated with Et_2O (10 mL), the insoluble material was filtered off, and the filtrate was concentrated *in vacuo*. The product was further purified by column chromatography on elution with 3% MeOH in EtOAc and then dried *in vacuo* over P_2O_5. The title compound **42** was obtained as a white, solid foam (0.152 g, 68%): mp 62−65 °C (softens); ^{1}H-NMR (DMSO-d_6) δ 0.95, 1.04 (2 × d, *J* = 7.0 Hz, 3H, γ-CH_3), 1.11, 1.14 (2 × s, 9H, C(CH_3)$_3$), 1.30, 1.50 (2 × m, 1H, β-C*H*), 1.90−2.15, 2.30 (2 × m, 2H, β-C*H*, γ-C*H*), 2.76 (t, *J* = 9.2 Hz, 1H, N*H*), 3.10 (m, 1H, α-C*H*), 7.15−7.41 (m, 15H, Tr*H*); MS (FAB, *m*/*z*) 482 $(M + Na)^+$, 460 $(M + H)^+$.

α-*tert*-Butyl γ-Methyl *N*-Trityl-4,4-dimethyl-L-glutamate (44). To a stirred solution of 0.6 M $KN(SiMe_3)_2$ in THF (18.10 mL, 10.85 mmol) at −78 °C was added a solution of **40** (2.84 g, 6.19 mmol) in dry THF (28 mL) over a 5 min period under argon. The resulting solution was stirred at −78 °C for 50 min, then MeI (1.76 g, 12.38 mmol) was added, and stirring was continued at −78 °C for 1 h and 20 min. Workup and purification as described above afforded the γ-methyl glutamate derivative **41** as a colorless viscous oil (2.46 g, 84%). To a stirred solution of 0.6 M $KN(SiMe_3)_2$ in THF (16.0 mL, 9.60 mmol) at −78 °C was added a solution of **41** (2.33 g, 4.92 mmol) in dry THF (20 mL) over a 5 min period and under argon. The resultant yellow solution was stirred at −78 °C for 50 min; then MeI (2.79 g, 1.22 mL, 19.68 mmol) was added under argon. Stirring was continued at −78 °C for 3 h, then the white slurry was poured into a saturated solution of NH_4Cl (150 mL), and the mixture was extracted with Et_2O (2 × 200 mL). The organics were combined, dried (Na_2SO_4), and concentrated *in vacuo*. Purification by column chromatography, on gradient elution with EtOAc in hexanes (3−9%), gave the desired product **44** contaminated with the starting material **41** (NMR indicated a ratio of 2:5 monomethyl:dimethyl). It was possible, however, to isolated the desired product **44** by recrystallization of the crude product from MeOH/hexanes at −20 °C (the product was recrystallized three times from MeOH/hexanes (10:4, v/v) and twice from MeOH/hexanes (4:2, v/v)). The product was obtained as a white solid (0.850 g, 28% from **40**: mp 110−113 °C; ^{1}H-NMR (DMSO-d_6) δ 1.91, 1.93 (2 × s, 6H, 2 × γ-CH_3), 1.20 (s, 9H, C(CH_3)$_3$), 1.30 (dd, *J* = 3.8, 14.3 Hz, 1H, β-C*H*), 2.05 (dd, *J* = 8.7, 14.2 Hz, 1H, β-C*H*), 2.77 (d, *J* = 8.9 Hz, 1H, N*H*), 3.07 (m, 1H, α-C*H*), 3.56 (s, 3H, CO_2CH_3), 7.15−7.39 (m, 15H, Tr*H*); MS (CI, *m*/*z*) 386 $(M - CO_2Bu^t)^+$, 243 (Tr^+). Anal. ($C_{31}H_{37}NO_4$) C, H, N.

α-*tert*-Butyl γ-Allyl *N*-Trityl-4-methyl-L-glutamate (47). To a stirred solution of $KN(SiMe_3)_2$ (23.68 mL, 11.84 mmol, 0.5 M in toluene) in dry THF (22 mL) at −78 °C was added a solution of α-*tert*-butyl γ-allyl *N*-trityl-L-glutamate (**46**) (3.59 g, 7.4 mmol) in dry THF (26 mL) over a 5 min period under an argon atmosphere. The resulting pale yellow solution was stirred at −78 °C for 55 min, and then MeI (2.10 g, 0.92 mL, 14.8 mmol) was added. Stirring was continued at −78 °C for 30 min, then the white slurry was poured into a saturated solution of NH_4Cl (200 mL), and the resulting mixture was extracted with Et_2O (3 × 150 mL). The ether extracts were combined, dried (Na_2SO_4), and concentrated *in vacuo*. Purification by column chromatography, on gradient elution with EtOAc in petrol (6−8%), gave the title compound **47** (3.26 g, 88%), a colorless oil, as a mixture of two diastereoisomers (2*S*,4*S* and 2*S*,4*R*): ^{1}H-NMR (DMSO-d_6) δ 1.00, 1.07 (2 × d, *J* = 7.0 Hz, 3H, γ-CH_3), 1.12, 1.15 (2 × s, 9H, C(CH_3)$_3$), 1.38, 1.57 (2 × m, 1H, β-C*H*), 1.95, 2.15 (2 × m, 1H, β-C*H*), 2.45 (m, 1H, γ-C*H*), 2.80 (t, *J* = 9.4 Hz, 1H, N*H*), 3.12 (m, 1H, α-C*H*), 4.37, 4.48 [2 × dd, *J* = 13.7, 5.4 Hz, 4.60 (d, *J* = 5.3 Hz), 2H, $CH_2CH{=}CH_2$], 5.10−5.35 (m, 2H, CH=CH_2), 5.70−6.00 (m, 1H, C*H*=CH_2), 7.16−7.40 (m, 15H, Tr*H*); MS (CI, *m*/*z*) 500 $(M + H)^+$. ($C_{32}H_{37}NO_4 \cdot 0.5H_2O$) C, H, N.

α-*tert*-Butyl γ-Allyl *N*-Trityl-4,4-dimethyl-L-glutamate (48). To a stirred solution of $KN(SiMe_3)_2$ (16.2 mL, 0.5 M in toluene, 8.1 mmol) in dry THF (16 mL) at −78 °C was added a solution of α-*tert*-butyl γ-allyl *N*-trityl-4-methyl-L-glutamate (**47**) (2.24 g, 4.5 mmol) in dry THF (16 mL) over a 5 min period under argon. The resulting yellow solution was stirred at −78 °C for 55 min, and then MeI (1.12 mL, 2.56 g, 18 mmol) was added. Stirring was continued at −78 °C for 2 h, and then the yellowish slurry was poured into a saturated solution of NH_4Cl (200 mL). This was then extracted with Et_2O (3 × 150 mL); the ether extracts were combined, dried (Na_2SO_4), and concentrated *in vacuo*. Purification by column chromatography, on gradient elution with EtOAc in petrol (3−6%), gave the title compound **48** (2.08 g, 90%) as a colorless oil: ^{1}H-NMR (DMSO-d_6) δ 0.94, 0.96 (2 × s, 6H, 2 × γ-CH_3), 1.20 (s, 9H, C(CH_3)$_3$), 1.34 (dd, *J* = 14.2, 3.7 Hz, 1H, β-C*H*), 2.06 (dd, *J* = 14.3, 8.6 Hz, 1H, β-C*H*), 2.78 (d, *J* = 8.9 Hz, 1H, N*H*), 3.09 (m, 1H, α-C*H*), 4.41, 4.59 (2 × ddd, *J* = 15.0, 5.0, 1.2 Hz, 2H, $CH_2CH{=}CH_2$), 5.17 (dm, *J* = 11.6 Hz, 1H, CH=CH_2*cis*), 5.23 (dm, *J* = 17.3 Hz, 1H, CH=CH_2*trans*), 5.87 (m, 1H, C*H*=CH_2), 7.16−7.40 (m, 15H, Tr*H*), a doublet at 1.07 ppm indicated that about 4% of **47** was present; MS (CI, *m*/*z*) 514 $(M + H)^+$. Anal. ($C_{33}H_{39}NO_4 \cdot 0.5H_2O$) C, H, N.

α-*tert*-Butyl *N*-Trityl-4,4-dimethyl-L-glutamate (45). To a stirred solution of α-*tert*-butyl γ-allyl *N*-trityl-4,4-dimethyl-L-glutamate (1.4 g, 2.72 mmol) in dry dichloromethane (11 mL) under argon was added tetrakis(triphenylphosphine)palladium-(0) (0.091 g) followed by pyrrolidine (0.35 mL, excess). The resulting yellow solution was stirred at room temperature for 20 min, and then Et_2O (100 mL) and 1 N HCl (50 mL) were added. The two layers were separated, and the aqueous layer was washed with more Et_2O (100 mL). The ether extracts were combined, dried (Na_2SO_4), and concentrated *in vacuo*. The

residue was treated with diethyl ether (30 mL); the yellow precipitate was filtered off, and the filtrate was concentrated *in vacuo* yielding the title compound **45** (1.25 g, 97%) as a white solid foam: mp 66−68 °C; ^{1}H-NMR (DMSO-d_6) δ 0.88, 0.90 (2 × s, 6H, 2 × γ-CH_3), 1.20 (m, 10H, β-CH, C(CH_3)$_3$), 2.01 (dd, J = 14.2, 8.5 Hz, 1H, β-CH), 2.76 (d, J = 8.4 Hz, 1H, NH), 3.06 (m, 1H, α-CH), 7.17−7.48 (m, 15H, TrH); MS (CI, m/z) 474 (M + H)$^+$.

Preparation of Z-, Tr-, or Boc-Blocked Dipeptide *tert*-Butyl Esters: Di-*tert*-butyl *N*-[*N*-(Benzyloxycarbonyl)-L-γ-glutamyl]-*N*-methylglycinate (9a). To a stirred solution of α-*tert*-butyl *N*-(benzyloxycarbonyl)-L-glutamate (1.63 g, 4.8 mmol) and NMM (0.487 g, 4.83 mmol) in dry THF (8 mL) cooled to −20 °C was added isobutyl chloroformate (0.657 g, 4.83 mmol). After 10 min a solution of *tert*-butyl *N*-methylglycinate (0.70 g, 4.83 mmol) in THF (8 mL) was added. Stirring was continued for 10 min at −20 °C and then at room temperature for 4 h. *N*-Methylmorpholine hydrochloride was filtered off and the filtrate concentrated *in vacuo*. The residue was purified by column chromatography, on elution with a gradient of EtOAc in CH_2Cl_2 (5−25%) affording the title compound **9a** (1.73 g, 77%) as a yellow oil: ^{1}H-NMR (DMSO-d_6) δ 1.32, 1.38, 1.39, 1.41 (4 × s, 18H, 2 × C(CH_3)$_3$), 1.77, 1.87 (2 × m, 2H, β-CH_2), 2.24, 2.38 (2 × m, 2H, γ-CH_2), 2.79, 2.95 (2 × s, 3H, *N*-CH_3), 3.93(m) and 4.08(s) (3H, CH_2CO$_2$Bu^t, Glu α-CH), 5.03 (m, 2H, ArCH_2), 7.36 (m, 5H, ArH), 7.64 (t, J = 7.9 Hz, 1H, NH); MS (CI, m/z) 465 (M + H)$^+$. Anal. ($C_{24}H_{36}N_2O_7$·0.25H_2O) C, H, N.

Tri-*tert*-butyl *N*-[*N*-(Benzyloxycarbonyl)-L-glutamyl]-*N*-methyl-L-glutamate (9b). The general procedure A2 was followed using α-*tert*-butyl *N*-(benzyloxycarbonyl)-L-glutamate (1.31 g, 3.67 mmol), NMM (0.37 g, 3.67 mmol), dry THF (5 mL), isobutyl chloroformate (0.50 g, 3.67 mmol), and a solution of di-*tert*-butyl *N*-methyl-L-glutamate (1.00 g, 3.67 mmol) in dry THF (5 mL). The reaction mixture was stirred at −20 °C for 10 min and then at room temperature for 4 h. The crude product was purified by column chromatography using a gradient of EtOAc in CH_2Cl_2 (5−20%) as eluant. Trituration with Et_2O gave a white solid, the title compound **9b**. This was isolated by filtration, washed with petrol, and dried *in vacuo* (1.62 g, 74%): mp 92−93 °C; ^{1}H-NMR (DMSO-d_6) δ 1.32, 1.37, 1.38 (3 × s, 27H, 3 × C(CH_3)$_3$), 1.86, 2.03 (2 × m, 4H, 2 × β-CH_2), 2.12, 2.38 (2 × m, 4H, 2 × γ-CH_2), 2.60, 2.79 (2 × s, 3H, *N*-CH_3), 3.94 (m, 1H, Glu α-CH), 4.49, 4.76 (2 × dd, J = 4.8, 10.8 Hz, 1H, MeGlu α-CH), 5.03 (m, 2H, PhCH_2), 7.35 (s, 5H, ArH), 7.64 (t, J = 7.7 Hz, 1H, NH); MS (CI, m/z) 593 (M + H)$^+$. Anal. ($C_{31}H_{48}N_2O_9$) C, H, N.

Tri-*tert*-butyl *N*-[*N*-(Benzyloxycarbonyl)-L-γ-glutamyl]-*N*-ethyl-L-glutamate (9c). Isobutyl chloroformate (0.26 mL, 2 mmol) was added to a stirred solution of α-*tert*-butyl *N*-(benzyloxycarbonyl)-L-glutamate (0.675 g, 2.0 mmol) and NMM (0.22 mL, 2.0 mmol) in THF (2 mL) at −20 °C under argon. After 10 min a solution of di-*tert*-butyl *N*-ethyl-L-glutamate (0.575 g, 2 mmol) in THF (3 mL) was added. After a further 10 min at −20 °C the mixture was allowed to come to room temperature, and stirring was continued at this temperature for 20 h. The reaction mixture was then partitioned between CH_2Cl_2 (40 mL) and saturated aqueous $NaHCO_3$ (30 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL), and the combined organic solution was washed successively with saturated aqueous $NaHCO_3$ (20 mL) and H_2O (3 × 20 mL), dried ($MgSO_4$), and evaporated. The residue was chromatographed on silica gel using hexanes−CH_2Cl_2 (1:1, 1:2, and 1:3), neat CH_2Cl_2, and CH_2Cl_2−EtOAc (19:1, 9:1, 4:1, and 3:1) in succession as eluants. The title compound **9c** was isolated as a colorless gum (1.023 g, 84%): ^{1}H-NMR (DMSO-d_6, 383 K) δ 1.14 (t, J = 7.1 Hz, 3H, CH$_2$CH_3), 1.41, 1.42, 1.43 (3 × s, 27H, 3 × C(CH_3)$_3$), 2.01 (m, 4H, 2 × β-CH_2), 2.23, 2.41 (2 × m, 4H, 2 × γ-CH_2), 3.19, 3.40 (2 × m, 2H, CH_2-CH$_3$), 4.02 (m, 1H, α-CH), 4.21 (m, 1H, α-CH), 5.06 (s, 2H, PhCH_2), 6.98 (d, J = 7.7 Hz, 1H, NH), 7.34 (m, 5H, ArH); MS (FAB, m/z) 607 (M + H)$^+$. Anal. ($C_{32}H_{50}N_2O_9$) C, H, N.

Tri-*tert*-butyl *N*-[*N*-(*tert*-Butyloxycarbonyl)-L-γ-glutamyl]-*N*-prop-2-ynyl-L-glutamate (13). Isobutyl chloroformate (1.35 mL, 10.4 mmol) was added to a stirred solution of α-*tert*-butyl *N*-(*tert*-butyloxycarbonyl)-L-glutamate (3.2 g, 10.4 mmol) and NMM (1.15 mL, 10.4 mmol) in THF (13 mL) at −20 °C under argon. After 10 min a solution of di-*tert*-butyl *N*-prop-2-ynyl-L-glutamate (**12**) (3.089 g, 10.4 mmol) in THF (18 mL) was added. After a further 10 min at −20 °C the mixture was allowed to warm to ambient temperature. Stirring was continued at this temperature for 27 h, and then the reaction mixture was partitioned between CH_2Cl_2 (200 mL) and saturated aqueous $NaHCO_3$ (150 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL), and the combined CH_2Cl_2 solution was washed successively with saturated aqueous $NaHCO_3$ (100 mL) and H_2O (3 × 50 mL), dried ($MgSO_4$), and evaporated. The residue was chromatographed on silica gel using petrol−CH_2Cl_2 (1:1 and 1:2 in succession) followed by CH_2Cl_2−EtOAc (100:0 to 85:15) as eluants. The title compound **13** was isolated as a gum and crystallized from hexanes (4.104 g, 68%): mp 111−112 °C; ^{1}H-NMR (DMSO-d_6) δ 1.38, 1.39, 1.40 (3 × s, 36H, 4 × C(CH_3)$_3$), 1.93, 2.07, 2.23, 2.33 (4 × m, 8H, 2 × β-CH_2, 2 × γ-CH_2), 3.02 (m, 1H, C≡CH), 3.84 (m, 1H, α-CH), 4.12 (m, 2H, CH_2-C≡C), 4.52 (m, 1H, α-CH), 7.07 (m, 1H, NH); MS (FAB, m/z) 583 (M + H)$^+$. Anal. ($C_{30}H_{50}N_2O_9$) C, H, N.

***tert*-Butyl α-Methylalaninate (55).** α-Aminoisobutyric acid (6.18 g, 60.0 mmol), *tert*-butyl acetate (200 mL), and 70% aqueous $HClO_4$ (9.45 g, 66.0 mmol) were shaken at room temperature for 7 days in a 500 mL conical flask. The mixture was then cooled in an ice−water bath and extracted with cold 0.5 N HCl (4 × 50 mL). The combined extracts were washed with EtOAc (100 mL) and then immediately neutralized with solid $NaHCO_3$ while cooling in an ice bath. The neutralized solution was extracted with Et_2O (3 × 200 mL); the extracts were combined, dried (Na_2SO_4), and concentrated *in vacuo* (take care, the product is volatile). The product was obtained as a colorless oil 4.98 g (52%): ^{1}H-NMR (DMSO-d_6) δ 1.16 (s, 6H, C(CH_3)$_2$), 1.40 (s, 9H, C(CH_3)$_3$); MS (CI, m/z) 160 (M + H)$^+$.

Di-*tert*-butyl *N*-[*N*-(Benzyloxycarbonyl)-L-γ-glutamyl]-α-methylalaninate (57a). The general procedure A2 was followed using α-*tert*-butyl *N*-(benzyloxycarbonyl)-L-glutamate (3.033 g, 9.0 mmol), NMM (0.909 g, 9.0 mmol), dry THF (10 mL), isobutyl chloroformate (1.224 g, 9.0 mmol), and a solution of *tert*-butyl α-methylalaninate (**55**) (2.0 g, 12.5 mmol) in dry THF (5 mL). The reaction mixture was stirred at −20 °C for 10 min and then at room temperature for 1 h. The crude product was purified by column chromatography using 2% MeOH in CH_2Cl_2 as eluant. Reprecipitation from Et_2O/petrol gave a white solid, the title compound **57a**. This was isolated by filtration, washed with petrol, and dried *in vacuo* (3.86 g, 90%): mp 109−110 °C; ^{1}H-NMR (DMSO-d_6) δ 1.27 (s, 6H, C(CH_3)$_2$), 1.34, 1.39 (2 × s, 18H, 2 × C(CH_3)$_3$), 1.73, 1.90 (2 × m, 2H, β-CH_2), 2.13 (t, J = 7.3 Hz, 2H, 2 × γ-CH_2), 3.90 (m, 1H, Glu α-CH), 5.03 (m, 2H, PhCH_2), 7.36 (m, 5H, ArH), 7.64 (d, J = 7.5 Hz, 1H, Glu NH), 8.06 (s, 1H, α-methylalanine NH); MS (FAB, m/z) 501 (M + Na)$^+$. Anal. ($C_{25}H_{38}N_2O_7$) C, H, N.

Di-*tert*-butyl *N*-[*N*-(Benzyloxycarbonyl)-L-γ-glutamyl]-L-prolinate (57b). The general procedure A2 was followed using α-*tert*-butyl *N*-(benzyloxycarbonyl)-L-glutamate (3.15 g, 9.3 mmol), NMM (0.909 g, 9.0 mmol), dry THF (10 mL), isobutyl chloroformate (1.22 g, 9.0 mmol), and a solution of *tert*-butyl L-prolinate (1.54 g, 9.0 mmol) in dry THF (10 mL). The reaction mixture was stirred at −20 °C for 10 min and then at room temperature for 4 h. The crude product was purified by column chromatography using a gradient of EtOAc in CH_2Cl_2 (10−30%) as eluant. Trituration with petrol gave a white solid, the title compound **57b**. This was isolated by filtration, washed with petrol, and dried *in vacuo* (2.86 g, 65%): mp 106−108 °C; ^{1}H-NMR (DMSO-d_6) δ 1.37, 1.39 (2 × s, 18H, 2 × C(CH_3)$_3$), 1.62−2.10 (m, 6H, Glu β-CH_2, Pro 3- and 4-CH_2), 2.33 (m, 2H, Glu γ-CH_2), 3.42 (m, 2H, Pro 5-CH_2), 3.93 (m, 1H, Glu α-CH), 4.13, 4.40 (2 × dd, J = 8.8, 3.9 Hz, 1H, Pro 2-CH), 5.03 (m, 2H, PhCH_2), 7.35 (m, 5H, ArH), 7.59 (t, 1H, Glu NH); MS (CI, m/z) 491 (M + H)$^+$. Anal. ($C_{26}H_{38}N_2O_7$) C, H, N.

Tri-*tert*-butyl *N*-(*N*-Trityl-γ-4-methyl-L-glutamyl)-L-glutamate (43). To a stirred solution of α-*tert*-butyl *N*-trityl-4-methyl-L-glutamate (**42**) (0.760 g, purity 90%, approximately 1.50 mmol) and NMM (0.151 g, 1.50 mmol) in dry THF (3.5

mL) cooled to −20 °C was added isobutyl chloroformate (0.204 g, 1.50 mmol). After 10 min a suspension of di-*tert*-butyl L-glutamate hydrochloride (0.442 g, 1.50 mmol) in dry THF (4.5 mL) and NMM (0.151 g, 1.50 mmol) was added. Stirring was continued at −20 °C for 10 min and then for 1.5 h while the mixture was allowed to warm up to room temperature. The *N*-methylmorpholine hydrochloride was filtered off, and the filtrate was concentrated *in vacuo*. Purification by column chromatography on gradient elution with EtOAc in petrol (7−20%) gave in order of elution: (a) diastereoisomer with R_f = 0.33 (20% EtOAc in hexanes) as a gummy solid (0.100 g); ^{1}H-NMR (DMSO-d_6) δ 0.96 (d, J = 6.8 Hz, 3H, 4-MeGlu$_L$ γ-CH_3), 1.10 (s, 9H, 4-MeGlu$_L$ C(CH_3)$_3$), 1.34, 1.40 (2 × s, 18H, Glu$_L$ C(CH_3)$_3$), 1.60−2.15 (m, 4H, 4-MeGlu$_L$ β-CH_2, Glu$_L$ β-CH_2), 2.21 (t, J = 7.8 Hz, 2H, Glu$_L$ γ-CH_2), 2.53 (m, 1H, 4-MeGlu$_L$$\gamma$-C*H*), 2.77 (d, J = 9.6 Hz, 1H, 4-MeGlu$_L$ N*H*), 3.07 (m, 1H, 4-MeGlu$_L$ α-C*H*), 4.09 (m, 1H, Glu$_L$ α-C*H*), 7.14−7.40 (m, 15H, Tr*H*), 7.92 (d, J = 7.9 Hz, 1H, Glu$_L$ N*H*), peaks at 0.89 (d), 3.55 (t), and 3.79 (d) not assigned for, clean ^{1}H-NMR obtained when the reaction was repeated using a sample of **42** obtained through the γ-allyl route (Scheme 4); MS (CI, *m*/*z*) 701 (M + H)$^+$, 243 (Tr$^+$); and (b) diastereoisomer with R_f = 0.28 (20% EtOAc in hexanes) as a viscous colorless oil, which solidified on drying *in vacuo* over P_2O_5 (0.668, 64%), mp 58−66 °C (softens); ^{1}H-NMR (DMSO-d_6) δ 0.94 (d, J = 6.8 Hz, 3H, 4-MeGlu$_L$ γ-CH_3), 1.14 (s, 9H, 4-MeGlu$_L$ C(CH_3)$_3$), 1.39 (m, 19H, Glu$_L$ C(CH_3)$_3$, 4-MeGlu$_L$ β-C*H*), 1.85, 2.11 (2 × m, 3H, 4-MeGlu$_L$ β-C*H*, Glu$_L$ β-CH_2), 2.27 (t, J = 7.6 Hz, 2H, Glu$_L$ γ-CH_2), 2.38 (m, 1H, 4-MeGlu$_L$ γ-C*H*), 2.68 (d, J = 9.0 Hz, 1H, 4-MeGlu$_L$ N*H*), 3.04 (m, 1H, 4-MeGlu$_L$ α-C*H*), 4.13 (m, 1H, Glu$_L$ α-C*H*), 7.14−7.41 (m, 15H, Tr*H*), 8.14 (d, J = 7.4 Hz, 1H, Glu$_L$ N*H*); MS (CI, *m*/*z*) 701 (M + H)$^+$. Anal. ($C_{42}H_{56}N_2O_7$) C, H, N.

Tri-*tert*-butyl *N*-(*N*-Trityl-γ-4,4-dimethyl-L-glutamyl)-L-glutamate (50). To a stirred solution of α-*tert*-butyl *N*-trityl-4,4-dimethyl-L-glutamate (0.710 g, 1.50 mmol) and PyBOP (0.935 g, 1.80 mmol) in dry CH_2Cl_2 (1.9 mL) was added DIEA (0.464 g, 3.6 mmol) followed by DMAP (0.109 g, 0.9 mmol) and di-*tert*-butyl L-glutamate hydrochloride (0.531 g, 1.8 mmol). Stirring was continued at room temperature for 3 h, and then the solvent was removed by evaporation. Purification by column chromatography, on gradient elution with EtOAc in hexanes (5−20%), gave the following in order of elution.

Title compound 50: 0.445 g, 42%, viscous oil; ^{1}H-NMR (DMSO-d_6) δ 0.80, 0.95 (2 × s, 6H, 2 × γ-CH_3), 1.19 (s, 9H, 4-diMeGlu$_L$ C(CH_3)$_3$), 1.34 (s, 9H, Glu$_L$ C(CH_3)$_3$), 1.39 (m, 10H, Glu$_L$ C(CH_3)$_3$, 4-diMeGlu$_L$ β-C*H*), 1.78−1.98 (m, 3H, Glu$_L$ β-CH_2, 4-diMeGlu$_L$ β-C*H*), 2.22 (t, J = 7.2 Hz, 2H, Glu$_L$ γ-CH_2), 2.80 (d, J = 8.2 Hz, 1H, 4-diMeGlu$_L$ N*H*), 2.98 (m, 1H, 4-diMeGlu$_L$ α-C*H*), 4.04 (m, 1H, Glu$_L$ α-C*H*), 7.14−7.40 (m, 15H, Tr*H*); MS (CI, *m*/*z*) 715 (M + H)$^+$. Anal. ($C_{43}H_{58}N_2O_7$·0.3H_2O) C, H, N.

α-*tert*-Butyl *N*-tritylpyroglutamate (49): 0.205 g, 30%, white solid; mp 69−71 °C (softens); ^{1}H-NMR (DMSO-d_6) δ 0.96, 1.16 (2 × s, 6H, 2 × γ-CH_3), 1.27 (s, 9H, C(CH_3)$_3$), 1.86 (d, J = 13.5 Hz, 1H, β-C*H*), 2.50 (t, J = 12.9 Hz, 1H, β-C*H*), 4.06 (d, J = 10.7 Hz, 1H, α-C*H*), 7.15−7.31 (m, 15H, Tr*H*); MS (CI, *m*/*z*) 456 (M + H)$^+$. Anal. ($C_{30}H_{33}NO_3$) C, H, N.

Preparation of Free Base Dipeptide *tert*-Butyl Esters: Di-*tert*-butyl L-γ-Glutamyl-*N*-methylglycinate (10a). The general procedure B was followed using di-*tert*-butyl *N*-[*N*-(benzyloxycarbonyl)-L-γ-glutamyl]-*N*-methylglycinate (**9a**) (0.80 g, 1.72 mmol), EtOAc (90 mL), and 10% Pd/C (0.150 g). The reaction mixture was stirred at room temperature for 6 h. The catalyst was removed by filtration and the filtrate concentrated *in vacuo*, yielding di-*tert*-butyl L-γ-glutamyl-*N*-methylglycinate (0.53 g, 95%) as a yellow oil: ^{1}H-NMR (DMSO-d_6) δ 1.40, 1.41, 1.43 (3 × s, 18H, 2 × C(CH_3)$_3$), 1.59, 1.77 (2 × m, 2H, β-CH_2), 2.24, 2.40 (2 × m, 2H, γ-CH_2), 2.79, 2.99 (2 × s, 3H, N-CH_3), 3.17 (m, 1H, α-C*H*), 4.02(s) and 4.10(d) (2H, CH_2CO$_2$Bu^t); MS (EI, *m*/*z*) 331 (M + H)$^+$.

Tri-*tert*-butyl L-γ-Glutamyl-*N*-methyl-L-glutamate (10b). The general procedure B was followed using tri-*tert*-butyl *N*-[*N*-(benzyloxycarbonyl)-L-γ-glutamyl]-*N*-methyl-L-glutamate (**9b**) (0.80 g, 1.35 mmol), EtOAc (80 mL), and 10% Pd/C (0.11 g). The reaction mixture was stirred at room temperature for 3 h. Standard workup afforded the title compound **10b** (0.61 g, 99%) as an oil: ^{1}H-NMR (DMSO-d_6) δ 1.38, 1.39, 1.41, 1.42 (4 × s, 27H, C(CH_3)$_3$), 1.79, 2.05 (2 × m, 4H, 2 × β-CH_2), 2.12, 2.39 (2 × m, 4H, 2 × γ-CH_2), 2.60, 2.84 (2 × s, 3H, *N*-CH_3), 3.18 (dd, J = 8.3, 4.9 Hz, 1H, Glu α-C*H*), 4.53, 4.78 (2 × dd, J = 10.5, 4.6 Hz, 1H, MeGlu α-C*H*); MS (CI, *m*/*z*) 459 (M + H)$^+$.

Tri-*tert*-butyl L-γ-Glutamyl-*N*-ethyl-L-glutamate (10c). The general procedure B was followed using **9c** (0.826 g, 1.36 mmol), EtOAc (80 mL), and 10% Pd/C (0.12 g). The reaction mixture was stirred at room temperature for 4 h. After standard workup the product was chromatographed on silica gel using CH_2Cl_2−hexanes, neat CH_2Cl_2, and CH_2Cl_2−EtOAc (95:5 to 50:50) in succession as eluants. The title compound **10c** was isolated as an oil (0.600 g, 93%): ^{1}H-NMR (DMSO-d_6, 383 K) δ 1.16 (m, 3H, CH$_2$CH_3), 1.42, 1.43, 1.45 (3 × s, 27H, 3 × C(CH_3)$_3$), 1.64, 1.69, 1.91, 2.23, 2.40 (5 × m, 8H, 2 × β-CH_2, 2 × γ-CH_2), 2.84 (m, 1H, α-C*H*), 3.25, 3.39 (2 × m, CH_2-CH$_3$), 4.21 (m, 1H, α-C*H*); MS (FAB, *m*/*z*) 473 (M + H)$^+$.

Tri-*tert*-butyl L-γ-Glutamyl-*N*-prop-2-ynyl-L-glutamate (10d). TFA (17 mL) was added to a stirred solution of **13** (1.70 g, 2.9 mmol) in CH_2Cl_2 (68 mL) at 20 °C. After 10 min the reaction mixture was poured into vigorously stirred, ice-cooled saturated aqueous $NaHCO_3$ (500 mL). After shaking the CH_2Cl_2 layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 25 mL). The combined CH_2Cl_2 solution was washed with H_2O (2 × 100 mL), dried ($MgSO_4$), and evaporated. The residue was chromatographed using CH_2Cl_2−EtOAc (100:0 to 33:67) as eluant. The title compound **10d** was isolated as an oil (0.707 g, 50%): ^{1}H-NMR ($CDCl_3$−D_2O) δ 1.44, 1.46, 1.47 (3 × s, 27H, 3 × C(CH_3)$_3$), 1.85, 2.09, 2.33, 2.51, 2.66 (5 × m, 8H, 2 × β-CH_2, 2 × γ-CH_2), 3.33 (m, 1H, C≡C*H*), 3.71−4.44 (8 × d, 3H, α-C*H*, CH_2-C≡C) 4.59, 5.00 (2 × m, 1H, α-C*H*); MS (FAB, *m*/*z*) 483 (M + H)$^+$.

Di-*tert*-butyl L-γ-Glutamyl-α-methylalaninate (58a). The general procedure B was followed using **57a** (0.223 g, 0.47 mmol), EtOAc (30 mL), and 10% Pd/C (0.062 g). The reaction mixture was stirred at room temperature for 2.5 h. Standard workup afforded **58a** (0.158 g, 98%) as a colorless oil: ^{1}H-NMR (DMSO-d_6) δ 1.27 (s, 6H, C(CH_3)$_2$), 1.34, 1.40 (2 × s, 18H, 2 × C(CH_3)$_3$), 1.50, 1.80 (2 × m, 2H, β-CH_2), 2.12 (t, J = 7.8 Hz, 2H, γ-CH_2), 3.13 (dd, J = 4.9, 8.5 Hz, 1H, Glu α-C*H*); MS (LDI, *m*/*z*) 368 (M + Na)$^+$.

Di-*tert*-butyl L-γ-Glutamyl-L-prolinate (58b). The general procedure B was followed using **57b** (0.98 g, 2.0 mmol), EtOAc (50 mL), and 10% Pd/C (0.15 g). The reaction mixture was stirred at room temperature for 8 h. Standard workup afforded **58b** (0.70 g, 98%) as an oil: ^{1}H-NMR (DMSO-d_6) δ 1.37, 1.40 (2 × s, 18H, 2 × C(CH_3)$_3$), 1.55−2.10 (m, 6H, Glu β-CH_2, Pro 3- and 4-CH_2), 2.33 (t, J = 7.5 Hz, 2H, Glu γ-CH_2), 3.16 (m, 1H, Glu α-C*H*), 3.48 (t, J = 6.7 Hz, 2H, Pro 5-CH_2), 4.14, 4.40 (2 × dd, J = 8.8, 3.9 Hz, 1H, Pro 2-C*H*); MS (CI, *m*/*z*) 357 (M + H)$^+$.

Tri-*tert*-butyl γ-4-Methyl-L-glutamyl-L-glutamate (37a). To a solution of tri-*tert*-butyl *N*-(*N*-trityl-γ-4-methyl-L-glutamyl)-L-glutamate (0.608 g, 0.87 mmol) in EtOAc (60 mL) was added 10% Pd/C (0.160 g). The resulting mixture was degassed and then stirred at room temperature for 26 h under a hydrogen atmosphere. More catalyst (0.035 g) was then added, and stirring was continued under hydrogen for 20 h. The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo* to give a white semisolid, a mixture of triphenylmethane and tri-*tert*-butyl γ-4-methyl-L-glutamyl-L-glutamate (0.450 g). This was taken forward to the next step without further purification.

Tri-*tert*-butyl γ-4,4-Dimethyl-L-glutamyl-L-glutamate (37b). To a solution of **50** (0.430 g, 0.60 mmol) in EtOAc (50 mL) was added 10% Pd/C (0.065 g), and the resulting mixture was stirred under hydrogen for 26 h. Then more catalyst (0.075 g) was added, and stirring was continued under hydrogen for 16 h. The catalyst was removed by filtration, and the filtrate was concentrated *in vacuo* to give a white semisolid (0.400 g), a mixture of triphenylmethane and tri-*tert*-butyl γ-4,4-dimethyl-L-glutamyl-L-glutamate. This was taken forward to the next step without further purification.

Preparation of Tri-*tert*-butyl *N*-(*N*-Trityl-γ-4-methyl-D-glutamyl)-L-glutamate (54): α-*tert*-Butyl γ-Allyl *N*-Trityl-4-methyl-D-glutamate (52). The method followed that used to prepare **47** but used a solution of potassium hexamethyldisilazide (4.1 mL, 2.05 mmol, 0.5 M in toluene) in dry THF (3.0 mL), a solution of α-*tert*-butyl γ-allyl *N*-trityl-D-glutamate[15] (**51**) (0.62 g, 1.28 mmol) in dry THF (5.0 mL), and MeI (0.363 g, 0.16 mL, 2.56 mmol). Purification by column chromatography (twice), on gradient elution with ethyl acetate in hexanes (1−6%), gave the title compound **52** (0.340, 53%), a colorless oil, as a mixture of two diastereoisomers (2*R*,4*S* and 2*R*,4*R*): ^{1}H-NMR (DMSO-d_6) δ 1.00, 1.06 (2 × d, J = 7.0 Hz, 3H, γ-CH_3), 1.12, 1.15 (2 × s, 9H, C(CH_3)$_3$), 1.37, 1.56, 1.99, 2.14 (4 × m, 2H, β-CH_2), 2.44 (m, 1H, γ-CH), 2.80 (t, J = 9.4 Hz, 1H, NH), 3.12 (m, 1H, α-CH), 4.37, 4.47 [2 × ddd, J = 1.4, 5.4, 12.3 Hz, 4.60 (dd, J = 1.3, 5.3 Hz), 2H, CH_2CH=CH_2], 5.10−5.35 (m, 2H, CH=CH_2), 5.69−5.99 (m, 1H, CH=CH_2), 7.15−7.39 (m, 15H, TrH); MS (CI, m/z) 500 (M + H)$^+$. Anal. ($C_{32}H_{37}NO_4$) C, H, N.

α-*tert*-Butyl *N*-Trityl-4-methyl-D-glutamate (53). The method followed that used to prepare **45** but used **52** (0.240 g, 0.48 mmol), dry CH_2Cl_2 (2.5 mL), tetrakis(triphenylphosphine)palladium(0) (0.017 g), and pyrrolidine (0.06 mL, excess). Workup as described for **45** yielded the title compound **53** (0.190 g, 86%) as a white foam: mp 66−68 °C (softens); ^{1}H-NMR (DMSO-d_6) δ 0.94, 1.03 (2 × d, J = 6.7 Hz, 6H, 2 × γ-CH_3), 1.11, 1.14 (2 × s, 9H, C(CH_3)$_3$), 1.32, 1.50, 1.95, 2.10 (4 × m, 2H, β-CH_2), 2.30 (m, 1H, γ-CH), 2.80 (t, J = 8.6 Hz, 1H, NH), 3.12 (m, 1H, α-CH), 7.15−7.41 (m, 15H, TrH); MS (CI, m/z) 460 (M + H)$^+$. This was used in the next experiment without further purification.

Tri-*tert*-butyl *N*-(*N*-Trityl-γ-4-methyl-D-glutamyl)-L-glutamate (54). The method followed that used to prepare **43** but used α-*tert*-butyl *N*-trityl-4-methyl-D-glutamate (**53**) (0.180 g, 0.39 mmol), NMM (0.040 g, 0.39 mmol), dry THF (3.0 mL), isobutyl chloroformate (0.053 g, 0.39 mmol), and a suspension of di-*tert*-butyl L-glutamate hydrochloride salt (0.115 g, 0.39 mmol) in dry THF (2.5 mL) and *N*-methylmorpholine (0.040 g, 0.39 mmol). Purification by column chromatography, on gradient elution with ethyl acetate in petrol (5−20%), gave the title compound **54** as a viscous colorless oil, which solidified on drying *in vacuo* over P_2O_5 (0.205, 75%): mp 55−60 °C (softens); ^{1}H-NMR (DMSO-d_6) δ 0.91, 0.99 (2 × d, J = 6.2 Hz, 3H, 4-MeGlu$_D$ γ-CH_3), 1.10, 1.16 (2 × s, 9H, 4-MeGlu$_D$ C(CH_3)$_3$), 1.37, 1.38 (2 × s, 18H, 2 × Glu$_L$ C(CH_3)$_3$), 1.50−2.30 (m, 7H, 2 × β-CH_2, 4-MeGlu$_D$ γ-CH, Glu$_L$ γ-CH_2), 3.05 (m, 1H, 4-MeGlu$_D$ α-CH), 4.04, 4.18 (2 × m, 1H, Glu$_L$ α-CH), 7.14−7.41 (m, 15H, TrH), 7.99 (d, J = 7.8 Hz, 1H, Glu$_L$ NH); MS (CI, m/z) 701 (M + H)$^+$. Anal. ($C_{42}H_{56}N_2O_7$) C, H, N.

Preparation of Quinazoline Antifolate γ-Linked Dipeptide *tert*-Butyl Esters: Tri-*tert*-butyl *N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-γ-glutamyl]-*N*-methyl-L-glutamate (15). The general procedure C was followed using 4-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoic acid, trifluoroacetate salt (0.461 g, 1.0 mmol), tri-*tert*-butyl L-γ-glutamyl-*N*-methyl-L-glutamate (0.60 g, 1.3 mmol), dry 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 EtOAc as eluant. Reprecipitation from CH_2Cl_2−Et_2O (1:1, minimum amount)/petrol gave the title compound (0.56 g, 71%) as a white powder: mp 110−113 °C; ^{1}H-NMR (DMSO-d_6) δ 1.32, 1.33, 1.35, 1.36, 1.39 (5 × s, 27H, 3 × C(CH_3)$_3$), 1.86 (m, 4H, 2 × β-CH_2), 2.32 (s, 3H, quinazoline 2-CH_3), 2.15, 2.44 (2 × m, 4H, 2 × γ-CH_2), 2.61, 2.80 (2 × s, 3H, *N*-CH_3), 3.23 (s 1H, C≡CH), 4.29 (m, 3H, CH_2C≡C, Glu α-CH), 4.48, 4.77 (2 × m, 3H, quinazoline 6-CH_2, MeGlu α-CH), 6.82 (d, J = 8.7 Hz, 2H, 3′,5′-ArH), 7.54 (d, J = 8.4 Hz, 1H, quinazoline 8-H), 7.71 (t, J = 8.6 Hz, 3H, quinazoline 7-H, 2′,6′-ArH), 7.96 (s, 1H, quinazoline 5-H), 8.28 (t, 1H, Glu NH), 12.19 (s, 1H, lactam NH); MS (FAB, m/z) 810 (M + Na)$^+$, 788 (M + H)$^+$. Anal. ($C_{43}H_{57}N_5O_9$·0.5H_2O) C, H, N.

Tri-*tert*-butyl *N*-[*N*-[4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-γ-glutamyl]-*N*-methyl-L-glutamate (16). The general procedure C was followed using 4-[*N*-[(3,4-dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoic acid, trifluoroacetate salt (0.475 g, 1.0 mmol), tri-*tert*-butyl L-γ-glutamyl-*N*-methyl-L-glutamate (0.505 g, 1.1 mmol), dry DMF (15 mL), DEPC (0.359 g, 2.2 mmol), and Et_3N (0.222 g, 2.2 mmol). Purification by column chromatography, on gradient elution with MeOH in EtOAc (0−2%), afforded a pale yellow solid. Reprecipitation from CH_2Cl_2 (minimum amount)/petrol gave the title compound **16** (0.340 g, 42%) as a white solid: mp 114−116 °C; ^{1}H-NMR (DMSO-d_6) δ 1.33, 1.34, 1.36, 1.37, 1.40 (5 × s, 27H, 3 × C(CH_3)$_3$), 1.74−2.20 (m) and 2.42 (m obscured) (8H, 2 × β-CH_2, 2 × γ-CH_2), 2.31 (s, 3H, quinazoline 2-CH_3), 2.44 (s, 3H, quinazoline 7-CH_3), 2.62, 2.82 (2 × s, 3H, *N*-CH_3), 3.15 (s, 1H, C≡CH), 4.26 (s, 2H, CH_2C≡CH), 4.30 (m obscured, 1H, Glu α-CH), 4.44, 4.79 (2 × dd, J = 10.6, 4.9 Hz, 1H, MeGlu α-CH), 4.66 (s, 2H, quinazoline 6-CH_2), 6.81 (d, J = 8.9 Hz, 2H, 3′,5′-ArH), 7.43 (s, 1H, quinazoline 8-H), 7.74 (d, J = 7.74 Hz, 3H, quinazoline 5-H, 2′,6′-ArH), 8.22 (t, J = 7.44 Hz, 1H, Glu NH), 12.00 (s, 1H, lactam NH); MS (ESI, m/z) 802 (M + H)$^+$. Anal. ($C_{44}H_{59}N_5O_9$·0.25H_2O) C, H, N.

Tri-*tert*-butyl *N*-[*N*-[4-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]-L-γ-glutamyl]-*N*-methyl-L-glutamate (17). The general procedure C was followed using 4-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoic acid, trifluoroacetate salt (0.479 g, 1.0 mmol), tri-*tert*-butyl L-γ-glutamyl-*N*-methyl-L-glutamate (0.506 g, 1.1 mmol), dry 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 eluant. Reprecipitation from EtOAc−Et_2O (3:1, minimum amount)/petrol gave the title compound (0.430 g, 53%) as a white solid: mp 110−112 °C; ^{1}H-NMR (DMSO-d_6) δ 1.33, 1.34, 1.35, 1.36, 1.40 (5 × s, 27H, 3 × C(CH_3)$_3$), 1.80−2.20, 2.42 (2 × m, 8H, 2 × β-CH_2, 2 × γ-CH_2), 2.33 (s, 3H, quinazoline 2-CH_3), 2.60, 2.80 (2 × s, 3H, *N*-CH_3), 3.27 (s, 1H, C≡CH), 4.29 (m, 1H, Glu α-CH), 4.37 (s, 2H, CH_2C≡CH), 4.50 (dd) and 4.81 (dd obscured) (J = 10.1, 4.8 Hz, 1H, MeGlu α-CH), 4.79 (s, 2H, quinazoline 6-CH_2), 6.61 (d, J = 14.6 Hz, 1H, 3′-ArH), 6.66 (d, J = 8.6 Hz, 1H, 5′-ArH), 7.52 (m, 2H, 6′-ArH, quinazoline 8-H), 7.68 (dd, J = 8.4, 1.8 Hz, 1H, quinazoline 7-H), 7.95 (s, 1H, quinazoline 5-H), 8.02 (dd obscured) and 8.07 (dd) (J = 7.0, 4.2 Hz, 1H, Glu NH), 12.21 (s, 1H, lactam NH); MS (ESI, m/z) 806 (M + H)$^+$. Anal. ($C_{43}H_{56}FN_5O_9$·0.25H_2O) C, H, N, F.

Tri-*tert*-butyl *N*-[*N*-[4-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]-L-γ-glutamyl]-*N*-methyl-L-glutamate (18). The general procedure C was followed using 4-[*N*-[(3,4-dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoic acid, trifluoroacetate salt (1.94 g, 3.93 mmol), tri-*tert*-butyl L-γ-glutamyl-*N*-methyl-L-glutamate (1.8 g, 3.93 mmol), dry DMF (60 mL), DEPC (1.28 g, 7.86 mmol), and Et_3N (0.794 g, 7.86 mmol). The crude product was purified by column chromatography using a gradient of MeOH in EtOAc (0−3%) as eluant. Subsequent reprecipitation from CH_2Cl_2 (minimum amount)/petrol gave the title compound **18** (2.60 g, 81%) as a white solid: mp 122−124 °C; ^{1}H-NMR (DMSO-d_6) δ 1.33, 1.34, 1.35, 1.40 (4 × s, 27H, 3 × C(CH_3)$_3$), 1.70−2.20 (m) and 2.40 (m obscured) (8H, 2 × β-CH_2, 2 × γ-CH_2), 2.30 (s, 3H, quinazoline 2-CH_3), 2.43 (s, 3H, quinazoline 7-CH_3), 2.60, 2.80 (2 × s, 3H, *N*-CH_3), 3.25 (s, 1H, C≡CH), 4.30 (m, 3H, Glu α-CH, CH_2C≡CH), 4.50, 4.79 (2 × dd, J = 10.7, 4.6 Hz, 1H, MeGlu α-CH), 4.68 (s, 2H, quinazoline 6-CH_2), 6.61 (m, 2H, 3′,5′-ArH), 7.44 (s, 1H, quinazoline 8-H), 7.51 (t, J = 8.7 Hz, 1H, 6′-ArH), 7.68 (s, 1H, quinazoline 5-H), 8.00, 8.08 (2 × dd, J = 7.0, 4.3 Hz, 1H, Glu NH), 12.10 (s, 1H, lactam NH); MS (ESI, m/z) 820 (M + H)$^+$. ($C_{44}H_{58}FN_5O_9$) C, H, N, F.

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]-*N*-methylglycinate (21). The general procedure C was followed using 4-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoic acid, trifluoroacetate salt (0.553 g, 1.2 mmol), di-*tert*-butyl L-γ-glutamyl-*N*-methylglycinate (0.53 g, 1.6 mmol), dry DMF (15 mL), DEPC

(0.359 g, 2.2 mmol), and then Et_3N (0.222 g, 2.2 mmol). The crude product was purified by chromatography on a silica gel column using EtOAc as eluant. The product was reprecipitated from CH_2Cl_2/Et_2O at −20 °C yielding the title compound **21** as a white solid (0.22 g, 28%): mp 104−108 °C; ^{1}H-NMR (DMSO-d_6) δ 1.30, 1.39 (2 × s, 18H, 2 × C(CH_3)$_3$), 1.96 (m, 2H, Glu β-CH_2), 2.33 (s, 3H, quinazoline 2-CH_3), 2.26, 2.45 (2 × m, 2H, γ-CH_2), 2.79, 2.95 (2 × s, 3H, *N*-CH_3), 3.23 (s, 1H, C≡C*H*), 3.95 (d, J = 6.1 Hz) and 4.06 (s) (2H, MeGly α-CH_2), 4.25 (m, 1H, Glu α-C*H*), 4.33 (s, 2H, CH_2C≡C), 4.77 (s, 2H, quinazoline 6-CH_2), 6.81 (m, 2H, 3′,5′-Ar*H*), 7.54 (d, J = 8.3 Hz, 1H, quinazoline 8-*H*), 7.74 (m, 3H, quinazoline 7-*H*, 2′,6′-Ar*H*), 7.96 (s, 1H, quinazoline 5-*H*), 8.28 (t, J = 8.6 Hz, 1H, N*H*), 12.19 (s, 1H, lactam N*H*); MS (FAB, *m*/*z*) 682 (M + Na)$^+$. Anal. ($C_{36}H_{45}N_5O_7$·0.25H_2O) C, H, N.

Tri-*tert*-butyl *N*-[*N*-[4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-methylamino]benzoyl]-L-γ-glutamyl]-*N*-methyl-L-glutamate (22). The general procedure C was followed using 4-[*N*-[(3,4-dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-methylamino]benzoic acid, trifluoroacetate salt (0.450 g, 1.0 mmol), tri-*tert*-butyl L-γ-glutamyl-*N*-methyl-L-glutamate (0.430 g, 0.94 mmol), dry DMF (15 mL), DEPC (0.359 g, 2.2 mmol), and Et_3N (0.222 g, 2.2 mmol). Purification by column chromatography, on gradient elution with MeOH in EtOAc (0−4%), afforded a glass. Reprecipitation from CH_2Cl_2 (minimum amount)/petrol gave the title compound **22** (0.260 g, 36%) as a white solid: mp 139−140 °C; ^{1}H-NMR (DMSO-d_6) δ 1.33, 1.34, 1.36, 1.37, 1.40 (5 × s, 27H, 3 × C(CH_3)$_3$), 1.80−2.18 (m) and 2.40 (m obscured) (8H, 2 × β-CH_2, 2 × γ-CH_2), 2.30 (s, 3H, quinazoline 2-CH_3), 2.43 (s, 3H, quinazoline 7-CH_3), 2.61, 2.81 (2 × s, 3H, *N*-CH_3), 3.13 (s, 3H, N^{10}-CH_3), 4.25 (m, 1H, Glu α-C*H*), 4.48, 4.80 (2 × dd, J = 10.7, 4.7 Hz, 1H, MeGlu α-C*H*), 4.69 (s, 2H, quinazoline 6-CH_2), 6.70 (d, J = 8.9 Hz, 2H, 3′,5′-Ar*H*), 7.44, 7.53 (2 × s, 2H, quinazoline 5-*H* and 8-*H*), 7.71 (d, J = 8.8 Hz, 2H, 2′,6′-Ar*H*), 8.24 (t, J = 7.51 Hz, 1H, Glu N*H*), 12.10 (s, 1H, lactam N*H*); MS (ESI, *m*/*z*) 778 (M + H)$^+$. Anal. ($C_{42}H_{59}N_5O_9$·0.3H_2O) C, H, N.

Tri-*tert*-butyl *N*-[*N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-methylamino]-2-fluorobenzoyl]-L-γ-glutamyl]-*N*-methyl-L-glutamate (23). The general procedure C was followed using 4-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-methylamino]-2-fluorobenzoic acid, trifluoroacetate salt (0.410 g, 0.9 mmol), tri-*tert*-butyl L-γ-glutamyl-*N*-methyl-L-glutamate (0.410 g, 0.9 mmol), dry DMF (16 mL), DEPC (0.322 g, 1.98 mmol), and Et_3N (0.200 g, 1.98 mmol). The crude product was purified by column chromatography using a gradient of MeOH in EtOAc (0−2%) as eluant. Reprecipitation from CH_2Cl_2 (minimum amount)/petrol gave the title compound (0.465 g, 67%) as a white solid: mp 107−109 °C; ^{1}H-NMR (DMSO-d_6) δ 1.35 (m, 27H, 3 × C(CH_3)$_3$), 1.80−2.18, 2.42 (2 × m, 8H, 2 × β-CH_2, 2 × γ-CH_2), 2.32 (s, 3H, quinazoline 2-CH_3), 2.60, 2.80 (2 × s, 3H, *N*-CH_3), 3.13 (s, 3H, N^{10}-CH_3), 4.29 (m, 1H, Glu α-C*H*), 4.49 (m) and 4.79 (m obscured) (1H, MeGlu α-C*H*), 4.78 (s, 2H, quinazoline 6-CH_2), 6.54 (d, J = 16.1 Hz, 1H, 3′-Ar*H*), 6.61 (d, J = 9.3 Hz, 1H, 5′-Ar*H*), 7.56 (m, 3H, 6′-Ar*H*, quinazoline 7-*H* and 8-*H*), 7.84 (s, 1H, quinazoline 5-*H*), 7.87, 7.97 (2 × t, J = 6.20 Hz, 1H, Glu N*H*), 12.22 (s, 1H, lactam N*H*); MS (ESI, *m*/*z*) 782 (M + H)$^+$. Anal. ($C_{41}H_{56}FN_5O_9$) C, H, N, F.

Tri-*tert*-butyl *N*-[*N*-[4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-methylamino]-2-fluorobenzoyl]-L-γ-glutamyl]-*N*-methyl-L-glutamate (24). The general procedure C was followed using 4-[*N*-[(3,4-dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-methyl]-2-fluorobenzoic acid, trifluoroacetate salt (0.478 g, 1.0 mmol), tri-*tert*-butyl L-γ-glutamyl-*N*-methyl-L-glutamate (0.435 g, 0.95 mmol), dry 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 (0−5%) as eluant. Subsequent reprecipitation from CH_2Cl_2 (minimum amount)/petrol gave the title compound **24** (0.518 g, 69%) as a white solid: mp 162−164 °C; ^{1}H-NMR (DMSO-d_6) δ 1.34, 1.35, 1.36, 1.40 (4 × s, 27H, 3 × C(CH_3)$_3$), 1.73−2.18 (m) and 2.40 (m obscured) (8H, 2 × β-CH_2, 2 × γ-CH_2), 2.30 (s, 3H, quinazoline 2-CH_3), 2.42 (s, 3H, quinazoline 7-CH_3), 2.60, 2.80 (2 × s, 3H, *N*-CH_3), 3.11 (s, 3H, N^{10}-CH_3), 4.29 (m, 1H, Glu α-C*H*), 4.50, 4.79 (2 × dd, J = 10.7, 4.5 Hz, 1H, MeGlu α-C*H*), 4.69 (s, 2H, quinazoline 6-CH_2), 6.51 (d, J = 14.4 Hz, 1H, 3′-Ar*H*), 6.56 (d, J = 8.1 Hz, 1H, 5′-ArH), 7.48 (m, 3H, quinazoline 8-*H*, 6′-Ar*H*, quinazoline 5-*H*), 7.88, 7.98 (2 × t, J = 6.1 Hz, 1H, Glu N*H*), 12.12 (s, 1H, lactam N*H*); MS (ESI, *m*/*z*) 796 (M + H)$^+$. Anal. ($C_{42}H_{58}FN_5O_9$) C, H, N, F.

Tri-*tert*-butyl *N*-[*N*-[4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]-L-γ-glutamyl]-*N*-ethyl-L-glutamate (19). The general procedure C was followed using 4-[*N*-[(3,4-dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoic acid (0.341 g, 0.9 mmol), tri-*tert*-butyl L-γ-glutamyl-*N*-ethyl-L-glutamate (0.555 g, 1.17 mmol), dry DMF (5 mL), DEPC (0.30 g, 1.8 mmol), and Et_3N (0.21 g, 2.0 mmol). The crude product was chromatographed with CH_2Cl_2−EtOH (100:0 to 97:3) as eluant, and the glass obtained was triturated with hexanes to give the title compound **19** (0.519 g, 69%): mp 90−100 °C; ^{1}H-NMR (DMSO-d_6, 383 K) δ 1.13 (t, J = 7.1 Hz, 3H, CH_2CH_3), 1.39, 1.41, 1.45 (3 × s, 27H, 3 × C(CH_3)$_3$), 2.01, 2.22 (2 × m, 6H, β-CH_2, γ-CH_2), 2.34 (s, 3H, quinazoline CH_3), 2.45 (m, 5H, γ-CH_2, quinazoline CH_3), 2.96 (t, J = 2.3 Hz, 1H, C≡C*H*), 3.20, 3.40 (2 × m, 2H, CH_2CH_3), 4.22 (m, 3H, CH_2C≡C, α-C*H*), 4.42 (dd, J = 13.0, 7.0 Hz, 1H, α-C*H*), 4.69 (s, 2H, quinazoline 6-CH_2), 6.61, 6.67, 6.71, 6.75 (4 × d, J = 2.4 Hz, 2H, 3′,5′-Ar*H*), 7.42 (s, 1H, quinazoline 8-*H*), 7.60 (m, 2H, Glu N*H*, 6′-Ar*H*), 7.85 (s, 1H, quinazoline 5-*H*), 11.60 (br s, 1H, lactam N*H*); MS (ESI, *m*/*z*) 834 (M + H)$^+$. Anal. ($C_{45}H_{60}FN_5O_9$) C, H, N.

Tri-*tert*-butyl *N*-[*N*-[4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]-L-γ-glutamyl]-*N*-prop-2-ynyl-L-glutamate (20). The general procedure C was followed using 4-[*N*-[(3,4-dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoic acid (0.403 g, 1.06 mmol), tri-*tert*-butyl L-γ-glutamyl-*N*-prop-2-ynyl-L-glutamate (0.657 g, 1.36 mmol), dry DMF (6 mL), DEPC (0.39 g, 2.4 mmol), and Et_3N (0.24 g, 2.4 mmol). The crude product was chromatographed with CH_2Cl_2−EtOH (100:0 to 95:5) as eluant. The glass obtained was triturated with hexanes to give the title compound **20** (0.654 g, 73%): mp 90−97 °C; ^{1}H-NMR (DMSO-d_6, 383 K) δ 1.39, 1.40, 1.44 (3 × s, 27H, 3 × C(CH_3)$_3$), 2.01, 2.24, 2.53 (3 × m, 8H, β-CH_2, γ-CH_2), 2.32, 2.43 (2 × s, 6H, quinazoline CH_3 and CH_3), 2.91, 2.96 (2 × m, 2H, C≡C*H*), 4.08 (m) and 4.21 (d, J = 2.2 Hz) (4H, CH_2C≡C), 4.41 (dd, J = 13.6, 7.5 Hz, 1H, α-C*H*), 4.56 (dd, J = 9.0, 5.5 Hz, 1H, α-C*H*), 4.67 (s, 2H, quinazoline 6-CH_2), 6.59, 6.65, 6.69, 6.73 (4 × d, 2H, 3′,5′-Ar*H*), 7.40 (s, 1H, quinazoline 8-*H*), 7.60 (m, 2H, Glu N*H*, 6′-Ar*H*), 7.83 (s, 1H, quinazoline 5-*H*), 8.95 (br s, 1H, lactam N*H*); MS (ESI, *m*/*z*) 844 (M + H)$^+$. Anal. ($C_{46}H_{58}FN_5O_9$) C, H, N, F.

Tri-*tert*-butyl *N*-[*N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-γ-4-methyl-L-glutamyl]-L-glutamate (35a). The general procedure C was followed using tri-*tert*-butyl γ-4-methyl-L-glutamyl-L-glutamate (**37a**) (0.430 g, approximately 0.59 mmol), 4-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoic acid, trifluoroacetic acid salt (0.327 g, 0.71 mmol), dry DMF (6.5 mL), DEPC (0.231 g, 1.42 mmol), and Et_3N (0.143 g, 1.42 mmol). The crude product was purified by column chromatography using a gradient of MeOH in EtOAc (0−2%) as eluant. Reprecipitation from CH_2Cl_2−Et_2O (1:1, minimum amount)/petrol at −20 °C afforded the title compound **35a** as a white solid (0.170 g, 28%): mp 102−110 °C; ^{1}H-NMR (DMSO-d_6) δ 1.03 (d, J = 6.81 Hz, 3H, 4-MeGlu$_L$ γ-CH_3), 1.33, 1.36, 1.38 (3 × s, 27H, 3 × C(CH_3)$_3$), 1.65, 1.83, 2.05 (3 × m, 4H, 2 × β-CH_2), 2.20 (t, J = 7.7 Hz, 2H, Glu$_L$ γ-CH_2), 2.32 (s, 3H, quinazoline 2-CH_3), 2.50 (m, DMSO peak, 4-MeGlu$_L$ γ-C*H*), 3.22 (s, 1H, C≡C*H*), 4.07, 4.21 (2 × m, 2H, 4-MeGlu$_L$ α-C*H*, Glu$_L$ α-C*H*), 4.34 (s, 2H, CH_2C≡C), 4.78 (s, 2H, quinazoline 6-CH_2), 6.82 (d, J = 8.7 Hz, 2H, 3′,5′-Ar*H*), 7.54 (d, J = 8.4 Hz, 1H, quinazoline 8-*H*), 7.72 (t, J = 8.7 Hz, 3H, quinazoline 7-*H*, 2′,6′-Ar*H*), 7.96 (s, 1H, quinazoline 5-*H*), 8.06, 8.20 (2 × d, J = 7.6 Hz, 2H, Glu$_L$ N*H*, 4-MeGlu$_L$ N*H*), 12.18 (s, 1H, lactam N*H*); MS (ESI, *m*/*z*) 788 (M + H)$^+$. Anal. ($C_{43}H_{57}N_5O_9$·0.5H_2O) C, H, N.

Tri-*tert*-butyl *N*-[*N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-

6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-γ-4,4-dimethyl-L-glutamyl]-L-glutamate (35b). The general procedure C was followed using tri-*tert*-butyl γ-4,4-dimethyl-L-glutamyl-L-glutamate (**37b**) (0.360 g, approximately 0.43 mmol), 4-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoic acid, trifluoroacetate salt (0.200 g, 0.43 mmol), dry DMF (7.0 mL), DEPC (0.154 g, 0.95 mmol), and Et_3N (0.095 g, 0.95 mmol). The crude product was purified by column chromatography using a gradient of MeOH in EtOAc (0−2%) as eluant. Reprecipitation from Et_2O/petrol at −20 °C yielded the title compound **35b** (0.200 g, 58%) as a white powder: mp 101−104 °C; ^{1}H-NMR (DMSO-d_6) δ 1.11, 1.16 (2 × s, 6H, 2 × 4-diMeGlu$_L$ γ-CH_3), 1.30, 1.36 (2 × s, 27H, 3 × C(CH_3)$_3$), 1.95 (m, 4H, 2 × β-CH_2), 2.21 (t, J = 7.7 Hz, 2H, Glu$_L$ γ-CH_2), 2.32 (s, 3H, quinazoline 2-CH_3), 3.22 (s, 1H, C≡CH), 4.08, 4.23 (2 × m, 2H, Glu$_L$ α-CH, 4-diMeGlu$_L$ α-CH), 4.31 (s, 2H, CH_2C≡CH), 4.76 (s, 2H, quinazoline 6-CH_2), 6.81 (d, J = 8.7 Hz, 2H, 3′,5′-ArH), 7.52 (d, J = 8.4 Hz, 1H, quinazoline 8-H), 7.66 (m, 4H, quinazoline 7-H, 2′,6′-ArH, amidic NH), 7.95 (s, 1H, quinazoline 5-H), 8.19 (d, J = 7.3 Hz, 1H, amidic NH), 12.19 (s, 1H, lactam NH); MS (ESI, m/z) 802 (M + H)$^+$. Anal. ($C_{44}H_{59}N_5O_9$) C, H, N.

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]-α-methylalaninate (59a). The general procedure C was followed using 4-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoic acid, trifluoroacetate salt (0.461 g, 1.0 mmol), di-*tert*-butyl L-γ-glutamyl-α-methylalaninate (0.48 g, 1.4 mmol), dry DMF (15 mL), DEPC (0.359 g, 2.2 mmol), and then Et_3N (0.222 g, 2.2 mmol). The crude product was purified by chromatography on a silica gel column using EtOAc as eluant. The product was reprecipitated from CH_2Cl_2/Et_2O yielding the title compound **59a** as a white solid (0.486 g, 72%): mp 115−116 °C; ^{1}H-NMR (DMSO-d_6) δ 1.26 (s, 6H, C(CH_3)$_2$), 1.33, 1.39 (2 × s, 18H, 2 × C(CH_3)$_3$), 1.91, 1.99 (2 × m, 2H, Glu β-CH_2), 2.14 (m, 2H, γ-CH_2), 2.32 (s, 3H, quinazoline 2-CH_3), 3.27 (s, 1H, C≡CH), 4.25 (m, 1H, Glu α-CH), 4.34 (s, 2H, CH_2C≡C), 4.78 (s, 2H, quinazoline 6-CH_2), 6.83 (d, J = 8.9 Hz, 2H, 3′,5′-ArH), 7.54 (d, J = 8.4 Hz, 1H, quinazoline 8-H), 7.70 (dd, J = 2.0 Hz, 1H, quinazoline 7-H), 7.74 (d, J = 8.8 Hz, 2H, 2′,6′-ArH), 7.96 (d, J = 1.6 Hz, 1H, quinazoline 5-H), 8.07 (s, 1H, α-methylalanine NH), 8.34 (d, J = 7.4 Hz, 1H, Glu NH), 12.20 (s, 1H, lactam NH); MS (FAB, m/z) 674 (M + H)$^+$. Anal. ($C_{37}H_{47}N_5O_7$·0.5H_2O) C, H, N.

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]-L-prolinate (59b). The general procedure C was followed using 4-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoic acid, trifluoroacetate salt (0.461 g, 1.0 mmol), di-*tert*-butyl L-γ-glutamyl-L-prolinate (0.524 g, 0.15 mmol), dry DMF (15 mL), DEPC (0.359 g, 2.2 mmol), and then Et_3N (0.222 g, 2.2 mmol). The crude product was purified by chromatography on a silica gel column using EtOAc as eluant. The product was reprecipitated from cold Et_2O/petrol yielding the title compound **59b** as a white solid (0.192 g, 28%): mp 113−117 °C; ^{1}H-NMR (DMSO-d_6) δ 1.31, 1.35, 1.39 (3 × s, 18H, 2 × C(CH_3)$_3$), 1.70−2.10 (m, 6H, Glu β-CH_2, Pro 3- and 4-CH_2), 2.38 (t, J = 7.5 Hz, 2H, γ-CH_2), 2.32 (s, 3H, quinazoline 2-CH_3), 3.41 (m, 2H, Pro 5-CH_2), 3.19 (s, 1H, C≡CH), 4.15, 4.40 (2 × dd, J = 9.0, 3.8 Hz, 1H, Pro 2-CH), 4.32 (m, 3H, Glu α-CH, CH_2C≡C), 4.77 (s, 2H, quinazoline 6-CH_2), 6.83 (d, J = 8.9 Hz, 2H, 3′,5′-ArH), 7.53 (d, J = 8.4 Hz, 1H, quinazoline 8-H), 7.68 (dd, J = 8.4, 2.0 Hz, 1H, quinazoline 7-H), 7.73 (d, J = 9.0 Hz, 2H, 2′,6′-ArH), 7.97 (d, J = 2.0 Hz, 1H, quinazoline 5-H), 8.23 (t, 1H, NH), 12.20 (s, 1H, lactam NH); MS (FAB, m/z) 708 (M + Na)$^+$. Anal. ($C_{38}H_{47}N_5O_7$·0.5H_2O) C, H, N.

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]-*N*-methyl-L-glutamic Acid (25). The general procedure D was followed using **15** (0.167 g, 0.21 mmol) and TFA (10 mL). Compound **25** was obtained as a white powder (0.152 g, 90%): mp 205 °C dec; ^{1}H-NMR (DMSO-d_6) δ 1.98 (m, 4H, 2 × β-CH_2), 2.42 (s, 3H, quinazoline 2-CH_3), 2.14, 2.45 (2 × m, 4H, 2 × γ-CH_2), 2.65, 2.81 (2 × s, 3H, *N*-CH_3), 3.24 (s, 1H, C≡CH), 4.35 (br s, 3H, CH_2C≡C, Glu α-CH), 4.81 (s, 2H, quinazoline 6-CH_2), 4.58, 4.91 (dd, J = 11.0, 4.5 Hz, 1H, MeGlu α-CH), 6.83 (d, J = 8.7 Hz, 2H, 3′,5′-ArH), 7.59 (d, J = 8.4 Hz, 1H, quinazoline 8-H), 7.75 (m, 3H, 2′,6′-ArH, quinazoline 7-H), 8.00 (s, 1H, quinazoline 5-H), 8.32 (d, J = 7.3 Hz, 1H, Glu NH); MS (FAB, m/z) 642 (M + Na)$^+$. Anal. ($C_{31}H_{33}N_5O_9$·1.1TFA·0.5Et_2O·H_2O) C, H, N, F.

***N*-[*N*-[4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-γ-glutamyl]-*N*-methyl-L-glutamic Acid (26).** The general procedure D was followed using **16** (0.200 g, 0.25 mmol) and TFA (15 mL). Compound **26** was obtained as a white powder (0.180 g, 95%): mp 170 °C dec; ^{1}H-NMR (DMSO-d_6) δ 1.82−2.23 (m) and 2.46 (m obscured) (8H, 2 × β-CH_2, 2 × γ-CH_2), 2.31, 2.48 (2 × s, 6H, quinazoline 2-CH_3, quinazoline 7-CH_3), 2.65, 2.81 (2 × s, 3H, *N*-CH_3), 3.23 (s, 1H, C≡CH), 4.31 (m, 3H, CH_2C≡CH, Glu α-CH), 4.56, 4.91 (2 × dd, J = 10.9, 4.4 Hz, 1H, MeGlu α-CH), 4.71 (s, 2H, quinazoline 6-CH_2), 6.79 (d, J = 8.9 Hz, 2H, 3′,5′-ArH), 7.48 (s, 1H, quinazoline 8-H), 7.76 (m, 3H, quinazoline 5-H, 2′,6′-ArH), 8.33 (m, 1H, Glu NH); MS (ESI, m/z) 634 (M + H)$^+$. Anal. ($C_{32}H_{35}N_5O_9$·TFA·H_2O) C, H, N.

***N*-[*N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]-L-γ-glutamyl]-*N*-methyl-L-glutamic Acid (27).** The general procedure D was followed using **17** (0.220 g, 0.27 mmol) and TFA (15 mL). The title compound **27** was obtained as a white solid (0.213 g, 97%): mp 120 °C dec; ^{1}H-NMR (DMSO-d_6) δ 1.82−2.20 (m) and 2.41 (m obscured) (8H, 2 × β-CH_2, 2 × γ-CH_2), 2.43 (s, 3H, quinazoline 2-CH_3), 2.63, 2.80 (2 × s, 3H, *N*-CH_3), 3.27 (s, 1H, C≡CH), 4.37 (m, 3H, CH_2C≡CH, Glu α-CH), 4.54, 4.90 (2 × dd, J = 10.9, 4.4 Hz, 1H, MeGlu α-CH), 4.82 (s, 2H, quinazoline 6-CH_2), 6.61 (d, J = 15.0 Hz, 1H, 3′-ArH), 6.66 (d, J = 8.1 Hz, 1H, 5′-ArH), 7.52 (t, J = 8.7 Hz, 1H, 6′-ArH), 7.61 (d, J = 8.4 Hz, 1H, quinazoline 8-H), 7.77 (d, J = 8.5 Hz, 1H, quinazoline 7-H), 8.00 (s, 1H, quinazoline 5-H), 8.05 (m, 1H, Glu NH); MS (ESI, m/z) 638 (M + H)$^+$. Anal. ($C_{31}H_{32}FN_5O_9$·1.3TFA·H_2O·Et_2O) C, H, N, F.

***N*-[*N*-[4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]-L-γ-glutamyl]-*N*-methyl-L-glutamic Acid (28).** The general procedure D was followed using **18** (0.215 g, 0.26 mmol) and TFA (16 mL). The title compound **28** was obtained as a white solid (0.205 g, 98%): mp 150 °C dec; ^{1}H-NMR (DMSO-d_6) δ 1.82−2.20 (m) and 2.46 (m obscured) (8H, 2 × β-CH_2, 2 × γ-CH_2), 2.45, 2.48 (2 × s, 6H, quinazoline 2-CH_3 and 7-CH_3), 2.63, 2.81 (2 × s, 3H, *N*-CH_3), 3.25 (s, 1H, C≡CH), 4.33 (m, 3H, CH_2C≡CH, Glu α-CH), 4.58, 4.90 (2 × dd, J = 10.9, 4.4 Hz, 1H, MeGlu α-CH), 4.74 (s, 2H, quinazoline 6-CH_2), 6.61 (m, 2H, 3′,5′-ArH), 7.49 (s, 1H, quinazoline 8-H), 7.54 (t, J = 8.7 Hz, 1H, 6′-ArH), 7.73 (s, 1H, quinazoline 5-H), 8.08 (m, 1H, Glu NH); MS (ESI, m/z) 652 (M + H)$^+$. Anal. ($C_{32}H_{34}FN_5O_9$·1.3TFA·H_2O) C, H, N, F.

***N*-[*N*-[4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]-L-γ-glutamyl]-*N*-ethyl-L-glutamic Acid (29).** Compound **19** (0.461 g, 0.55 mmol) was stirred with TFA (25 mL) at room temperature under argon for 75 min. The solution was evaporated and the residue triturated with Et_2O to give a powder (0.470 g). A solution of this material (0.370 g) in 0.5 M aqueous $NaHCO_3$ (5 mL) was filtered and adjusted to pH 4 with 1 N HCl. The resulting suspension was centrifuged, and the precipitate was collected, washed with H_2O, and dried to give the title compound **29** (0.248 g, 84%): mp 159−162 °C; ^{1}H-NMR (DMSO-d_6, 383 K) δ 1.13 (t, J = 7.0 Hz, 3H, CH_2CH_3), 2.03, 2.26 (2 × m, 6H, β-CH_2, γ-CH_2), 2.34 (s, 3H, quinazoline CH_3), 2.45 (m, 5H, γ-CH_2, quinazoline CH_3), 2.96 (m, C≡CH), 3.24, 3.38 (2 × m, CH_2CH$_3$), 4.22 (d, J = 2.2 Hz, 2H, CH_2C≡C), 4.39, 4.48 (2 × m, 2H, α-CH), 4.69 (s, 2H, quinazoline 6-CH_2), 6.62, 6.68, 6.72, 6.75 (4 × d, J = 2.4 Hz, 2H, 3′,5′-ArH), 7.41 (s, 1H, quinazoline 8-H), 7.63 (m, 2H, Glu NH, 6′-ArH), 7.85 (s, 1H, quinazoline 5-H); MS (ESI, m/z) 666 (M + H)$^+$. Anal. ($C_{33}H_{36}N_5O_9F$·0.75H_2O) C, H, N.

***N*-[*N*-[4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluoroben-**

zoyl]-L-γ-glutamyl]-*N*-prop-2-ynyl-L-glutamic Acid (30). Compound **20** (0.544 g, 0.645 mmol) and TFA (29 mL) were stirred together at room temperature in the dark for 75 min. The solution was evaporated to a glass which was triturated with Et_2O and dissolved in 0.5 M aqueous $NaHCO_3$ (8 mL). The solution was filtered and adjusted to pH 4 with 1 N HCl. The resulting suspension was centrifuged and the precipitate washed three times with H_2O by resuspension−centrifugation−decantation, then collected by filtration, further washed, and dried to give the title compound **30** (0.387 g, 87%): mp 162−165 °C; ^{1}H-NMR (DMSO-d_6, 383 K) δ 2.07, 2.18, 2.34, 2.56 (4 × m, 11H, β-CH_2, γ-CH_2, quinazoline CH_3), 2.45 (s, 3H, quinazoline CH_3), 2.89, 2.97 (2 × m, 2H, C≡C*H*), 4.10 (m) and 4.22 (d, *J* = 2.3 Hz) (4H, CH_2C≡C), 4.47 (m, 1H, α-C*H*), 4.69 (s, 3H, α-C*H*, quinazoline 6-CH_2), 6.62, 6.68, 6.71, 6.75 (4 × d, *J* = 2.4 Hz, 2H, 3′,5′-Ar*H*), 7.41 (s, 1H, quinazoline 8-*H*), 7.64 (m, 2H, Glu N*H*, 6′-Ar*H*), 7.84 (s, 1H, quinazoline 5-*H*); MS (ESI, *m*/*z*) 676 (M + H)$^+$. Anal. ($C_{34}H_{34}FN_5O_9$·0.75H_2O) C, H, N, F.

***N*-[*N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-γ-glutamyl]-*N*-methylglycine (31).** The general procedure D was followed using **21** (0.075 g, 0.11 mmol) and TFA (5 mL). Compound **31** was obtained as a white solid (0.60 g, 72%): mp 140 °C dec; ^{1}H-NMR (DMSO-d_6) δ 2.01 (m, 2H, β-CH_2), 2.33, 2.42 (2 × m, 2H, γ-CH_2), 2.42 (s, 3H, quinazoline 2-CH_3), 2.81, 2.97 (2 × s, 3H, *N*-CH_3), 3.24 (s, 1H, C≡C*H*), 3.98 (d, *J* = 8.0 Hz) and 4.10 (s) (2H, MeGly α-CH_2), 4.36 (s, 3H, CH_2C≡C, Glu α-C*H*), 4.81 (s, 2H, quinazoline 6-CH_2), 6.84 (d, *J* = 8.3 Hz, 2H, 3′,5′-Ar*H*), 7.59 (d, *J* = 8.5 Hz, 1H, quinazoline 8-*H*), 7.76 (m, 3H, quinazoline 7-H, 2′,6′-Ar*H*), 8.01 (s, 1H, quinazoline 5-*H*), 8.33 (t, *J* = 7.0 Hz, 1H, N*H*); MS (FAB, *m*/*z*) 570 (M + Na)$^+$, 548 (M + H)$^+$. Anal. ($C_{28}H_{29}N_5O_7$·1.1TFA·0.8H_2O·0.8Et_2O) C, H, N, F.

***N*-[*N*-[4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-methylamino]benzoyl]-L-γ-glutamyl]-*N*-methyl-L-glutamic Acid (32).** The general procedure D was followed using **22** (0.135 g, 0.174 mmol) and TFA (12 mL). Compound **32** was obtained as a white powder (0.120 g, 100%): mp 155 °C dec; ^{1}H-NMR (DMSO-d_6) δ 1.80−2.16 (m) and 2.41 (m obscured) (8H, 2 × β-CH_2, 2 × γ-CH_2), 2.40, 2.47 (2 × s, 6H, quinazoline 2-CH_3 and 7-CH_3), 2.64, 2.80 (2 × s, 3H, *N*-CH_3), 3.13 (s, 3H, N^{10}-CH_3), 4.31 (m, 1H, Glu α-C*H*), 4.57, 4.91 (2 × dd, *J* = 11.0, 4.3 Hz, 1H, MeGlu α-C*H*), 4.72 (s, 2H, quinazoline 6-CH_2), 6.71 (d, *J* = 8.7 Hz, 2H, 3′,5′-Ar*H*), 7.47, 7.53 (2 × s, 2H, quinazoline 5-*H* and 8-*H*), 7.73 (d, *J* = 8.5 Hz, 2H, 2′,6′-Ar*H*), 8.28 (d, *J* = 7.2 Hz, 1H, Glu N*H*); MS (ESI, *m*/*z*) 610 (M + H)$^+$. Anal. ($C_{30}H_{35}N_5O_9$·TFA·1.5H_2O) C, H, N, F.

***N*-[*N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-methylamino]-2-fluorobenzoyl]-L-γ-glutamyl]-*N*-methyl-L-glutamic Acid (33).** The general procedure D was followed using **23** (0.295 g, 0.38 mmol) and TFA (23 mL). The title compound **33** was obtained as a white solid (0.270 g, 85%): mp 110 °C dec; ^{1}H-NMR (DMSO-d_6) δ 1.82−2.18 (m) and 2.40 (m obscured) (8H, 2 × β-CH_2, 2 × γ-CH_2), 2.45 (s, 3H, quinazoline 2-CH_3), 2.63, 2.81 (2 × s, 3H, *N*-CH_3), 3.13 (s, 3H, N^{10}-CH_3), 4.34 (m, 1H, Glu α-C*H*), 4.53, 4.90 (2 × dd, *J* = 10.9, 4.4 Hz, 1H, MeGlu α-C*H*), 4.83 (s, 2H, quinazoline 6-CH_2), 6.56 (d, *J* = 15.9 Hz, 1H, 3′-Ar*H*), 6.62 (d, *J* = 9.1 Hz, 1H, 5′-Ar*H*), 7.53 (t, *J* = 9.0 Hz, 1H, 6′-Ar*H*), 7.62 (d, *J* = 8.4 Hz, 1H, quinazoline 8-*H*), 7.70 (dd, *J* = 8.4, 1.8 Hz, 1H, quinazoline 7-*H*), 7.89 (d, *J* = 1.5 Hz, 1H, quinazoline 5-*H*), 7.97 (t, *J* = 5.5 Hz, 1H, Glu N*H*); MS (ESI, *m*/*z*) 614 (M + H)$^+$. Anal. ($C_{29}H_{32}FN_5O_9$·1.6TFA·H_2O·0.4Et_2O) C, H, N, F.

***N*-[*N*-[4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-methylamino]-2-fluorobenzoyl]-L-γ-glutamyl]-*N*-methyl-L-glutamic Acid (34).** The general procedure D was followed using **24** (0.296 g, 0.37 mmol) and TFA (23 mL). The title compound **34** was obtained as a white solid (0.285 g, 92%): mp 155 °C dec; ^{1}H-NMR (DMSO-d_6) δ 1.82−2.18 (m) and 2.40 (m obscured) (8H, 2 × β-CH_2, 2 × γ-CH_2), 2.43, 2.47 (2 × s, 6H, quinazoline 2-CH_3 and 7-CH_3), 2.63, 2.81 (2 × s, 3H, *N*-CH_3), 3.13 (s, 3H, N^{10}-CH_3), 4.34 (m, 1H, Glu α-C*H*), 4.54, 4.90 (2 × dd, *J* = 10.9, 4.4 Hz, 1H, MeGlu α-C*H*), 4.73 (s, 2H, quinazoline 6-CH_2), 6.53 (d, *J* = 13.1 Hz, 1H, 3′-Ar*H*), 6.57 (d, *J* = 6.9 Hz, 1H, 5′-Ar*H*), 7.48, 7.50 (2 × s, 2H, quinazoline 5-*H* and 8-*H*), 7.54 ((t obscured), *J* = 8.7 Hz, 1H, 6′-Ar*H*), 8.09 (m, 1H, Glu N*H*); MS (ESI, *m*/*z*) 628 (M + H)$^+$. Anal. ($C_{30}H_{34}FN_5O_9$·1.4TFA·0.5Et_2O·H_2O) C, H, N, F.

***N*-[*N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-γ-4-methyl-L-glutamyl]-L-glutamic Acid (36a).** The general procedure D was followed using **35a** (0.071 g, 0.09 mmol) and TFA (6 mL). Compound **36a** was obtained as a white powder (0.053 g, 76%): mp 160−165 °C dec; ^{1}H-NMR (DMSO-d_6) δ 1.02 (d, *J* = 6.8 Hz, 4-MeGlu$_L$ γ-CH_3), 1.65, 1.91, 2.09 (3 × m, 4H, 2 × β-CH_2), 2.25 (t, *J* = 7.6 Hz, 2H, Glu$_L$ γ-CH_2), 2.39 (s, 3H, quinazoline 2-CH_3), 2.50 (DMSO peak 4-MeGlu$_L$ γ-C*H*), 3.23 (s, 1H, C≡C*H*), 4.19 (m, 2H, Glu$_L$ α-C*H*, 4-MeGlu$_L$ α-C*H*), 4.35 (s, 2H, CH_2C≡CH), 4.80 (s, 2H, quinazoline 6-CH_2), 6.82 (d, *J* = 8.9 Hz, 2H, 3′,5′-Ar*H*), 7.58 (d, *J* = 8.4 Hz, 1H, quinazoline 8-*H*), 7.74 (d, *J* = 8.7 Hz, 3H, quinazoline 7-*H*, 2′,6′-Ar*H*), 7.99 (s, 1H, quinazoline 5-*H*), 8.10, 8.26 (2 × d, *J* = 7.7 Hz, 2H, Glu$_L$ N*H*, 4-MeGlu$_L$ N*H*); MS (ESI, *m*/*z*) 620 (M + H)$^+$. Anal. ($C_{31}H_{33}N_5O_9$·0.95TFA·0.4Et_2O·1.2H_2O) C, H, N, F.

***N*-[*N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-γ-4,4-dimethyl-L-glutamyl]-L-glutamic Acid (36b).** The general procedure D was followed using **35b** (0.068 g, 0.085 mmol) and TFA (6.5 mL). Compound **36b** was obtained as a white powder (0.050 g, 82%): mp >140 °C dec; ^{1}H-NMR (DMSO-d_6) δ 1.12, 1.17 (2 × s, 6H, 2 × 4-diMeGlu$_L$ γ-CH_3), 1.84−2.13 (m, 4H, β-CH_2), 2.25 (t, *J* = 7.4 Hz, 2H, Glu$_L$ γ-CH_2), 2.38 (s, 3H, quinazoline 2-CH_3), 3.23 (s, 1H, C≡C*H*), 4.20 (m, 1H, α-C*H*), 4.33 (m, 3H, CH_2C≡CH, α-C*H*), 4.79 (s, 2H, quinazoline 6-CH_2), 6.82 (d, *J* = 8.6 Hz, 2H, 3′,5′-Ar*H*), 7.57 (d, *J* = 8.4 Hz, 1H, quinazoline 8-*H*), 7.71 (m, 4H, quinazoline 7-*H*, 2′,6′-Ar*H*, amidic N*H*), 7.99 (s, 1H, quinazoline 5-*H*), 8.22 (d, *J* = 7.30 Hz, 1H, amidic N*H*); MS (ESI, *m*/*z*) 634 (M + H)$^+$. Anal. ($C_{32}H_{35}N_5O_9$·0.6TFA·0.8H_2O) C, H, N, F.

***N*-[*N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-γ-glutamyl]-α-methylalanine (60a).** The general procedure D was followed using **59a** (0.100 g, 0.15 mmol) and TFA (10 mL). Compound **60a** was obtained as a white solid (0.062 g, 63%): mp 155−158 °C; ^{1}H-NMR (DMSO-d_6) δ 1.29 (s, 6H, C(CH_3)$_3$), 1.89, 2.02 (2 × m, 2H, Glu β-CH_2), 2.18 (m, 2H, γ-CH_2), 2.38 (s, 3H, quinazoline 2-CH_3), 3.23 (s, 1H, C≡C*H*), 4.29 (m, 1H, Glu α-C*H*), 4.34 (s, 2H, 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* = 8.3 Hz, 1H, quinazoline 8-*H*), 7.75 (d, *J* = 8.1 Hz, 3H, 2′,6′-Ar*H*, quinazoline 7-*H*), 7.98 (s, 1H, quinazoline 5-*H*), 8.02 (s, 1H, α-methylalanine N*H*), 8.29 (d, *J* = 7.6 Hz, 1H, Glu N*H*); MS (FAB, *m*/*z*) 584 (M + Na)$^+$. Anal. ($C_{29}H_{31}N_5O_7$·0.7TFA·H_2O) C, H, N, F.

***N*-[*N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-γ-glutamyl]-L-proline (60b).** The general procedure D was followed using **59b** (0.067 g, 0.098 mmol) and TFA (5 mL). Compound **60b** was obtained as a white solid (0.054 g, 77%): mp 162 °C dec; ^{1}H-NMR (DMSO-d_6) δ 1.84, 2.08 (2 × m, 6H, Glu β-CH_2, Pro 3- and 4-CH_2), 2.39 (m, 5H, quinazoline 2-CH_3, γ-CH_2), 3.42 (m, 2H, Pro 5-CH_2), 3.23 (s, 1H, C≡C*H*), 4.23, 4.48 (2 × dd, *J* = 9.9, 3.5 Hz, 1H, Pro 2-C*H*), 4.34 (m, 3H, Glu α-C*H*, CH_2C≡C), 4.80 (s, 2H, quinazoline 6-CH_2), 6.83 (d, *J* = 8.8 Hz, 2H, 3′,5′-Ar*H*), 7.57 (d, *J* = 8.4 Hz, 1H, quinazoline 8-*H*), 7.74 (d, *J* = 8.7 Hz, 3H, 2′,6′-Ar*H*, quinazoline 7-*H*), 7.99 (s, 1H, quinazoline 5-*H*), 8.32 (t, 1H, *NH*); MS (FAB, *m*/*z*) 596 (M + Na)$^+$. Anal. ($C_{30}H_{31}N_5O_7$·1.1TFA·0.22Et_2O) C, H, N.

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