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Synthesis of Novel Quinazoline-Based Antifolates with Modified Glutamate Side Chains as Potential Inhibitors of Thymidylate Synthase and Antitumour Agents

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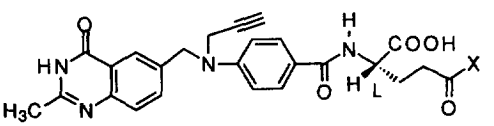
Abstract: Several novel antifolates, derivatives of 2-desamino-2-methyl-N^{10}-propargyl-5,8-dideazafolic acid, were synthesised as inhibitors of thymidylate synthase (TS) and antitumour agents. This was accomplished by first developing routes to the key intermediates Glu-OMe-γ-ψ[CSNH]Glu(OEt)-OEt (**8**), Glu-OBu^t-γ-ψ[CH_2NH]Glu(OBu^t)-OBu^t (**16**), Glu-OMe-γ-ψ[CN_4]Gly-OMe (**23**) and its 2,5-disubstituted regioisomer (**22**), followed by DEPC coupling to 4-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)-methyl]-*N*-prop-2-ynylamino]benzoic acid (**9**) or 4-[*N*-[(3,4-dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)-methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoic acid (**24**), and finally removal of the protecting groups. The resulting quinazoline-based antifolates with modified glutamate side chains, and in particular, the tetrazole derivatives **26** and **29** displayed potent TS and L1210 cell growth inhibitory activities (*e.g.*, for **26**: TS IC_{50} = 2.4 nM, L1210 IC_{50} = 1.3 μM).

Introduction

Over the last two decades there has been extensive interest in the thymidylate synthase (TS) enzyme as a target to cancer chemotherapy because of its critical role in DNA synthesis. Thymidylate synthase catalyses the conversion of 2′-deoxyuridine 5′-monophosphate (dUMP) to 2′-deoxythymidine 5′-monophosphate (dTMP), a reaction in which N^5, N^{10}-methylene-5,6,7,8-tetrahydrofolate (5,10-CH_2FH_4) serves as a one-carbon unit donor.[1]



1 (ICI 198583), X = OH
2 (ICI 198583-γ-L-Glu), X = L-Glu
3 (ICI 198583-γ-L-Ala), X = L-Ala
4 (ICI 198583-γ-Gly), X = Gly

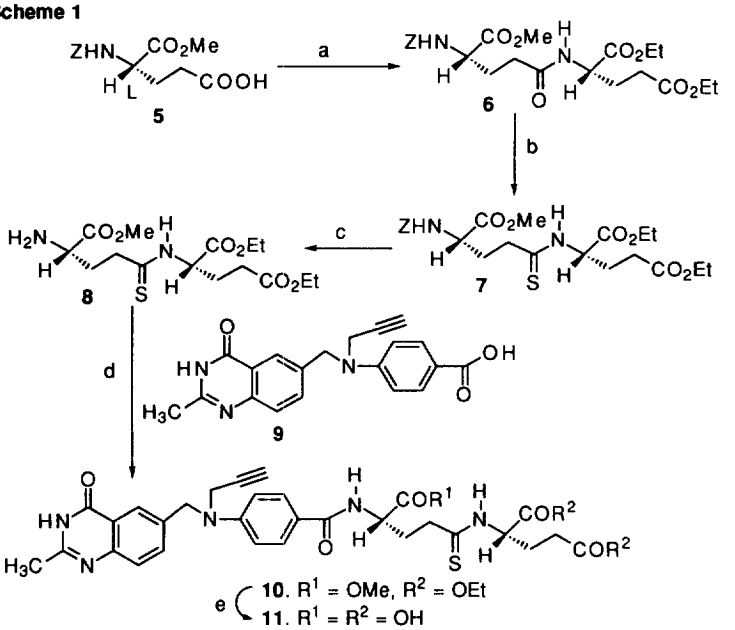
As part of our search for non-polyglutamatable, quinazoline-based inhibitors of TS we have recently

reported the synthesis and antitumour activity of a series of γ-linked dipeptide analogues of 2-desamino-2-methyl-N^{10}-propargyl-5,8-dideazafolic acid (ICI 198583).[2-4] This work stems from the synthesis of γ-linked L,L dipeptide derivatives of ICI 198583, which were a class of compounds that exhibited excellent TS inhibitory properties,[2] but in animal models were metabolically unstable because of susceptibility of the γ-glutamyl amide bond to enzymatic hydrolysis.[2,5] In an attempt to stabilise these γ-linked L,L dipeptides while retaining or even enhancing their biological activity, the γ-glutamyl bond was replaced by a series of isosteric groups,[6] thioamide (CSNH), methyleneamino (CH_2NH), and 1,5-disubstituted tetrazole (CN_4).

Results and Discussion

Thioamide isosteres are one of the most common modifications in biologically active peptides since their stereochemical resemblance to the parent amide is very close, and both adopt the *trans* planar conformation.[6,7]

Scheme 1

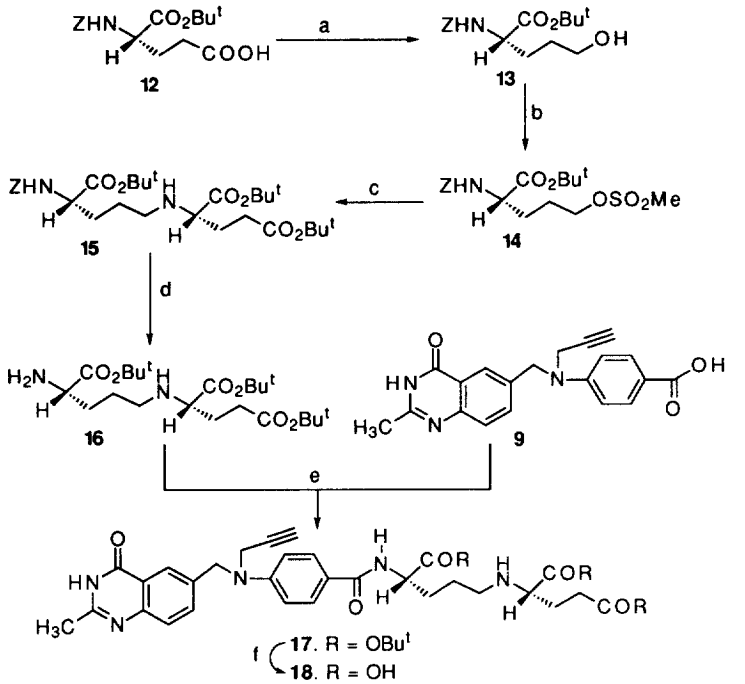


Conditions: (a) i) NMM (1 eq), $ClCO_2CH_2CH(CH_3)_2$ (1 eq), THF, -20°C, 10 min, ii) HCl·Glu(OEt)-OEt (1eq), NMM (1eq), THF, -20°C to R.T; (b) Lawesson's reagent (0.5 eq),THF; c) i) 30% HBr in AcOH, ii) sat. aq. $NaHCO_3$; d) Et_3N (2.2 eq), DEPC (2.2 eq), DMF; e) 1N aq. NaOH, MeOH/H_2O.

The synthetic route to the thioamide analogue of ICI 198583-γ-L-Glu (**2**), compound **11**, is shown in Scheme 1. Firstly, Z-L-Glu-OMe-γ-L-Glu(OEt)-OEt (**6**) was obtained in 84% yield by condensing diethyl glutamate hydrochloride with α-methyl *N*-(benzyloxycarbonyl)-L-glutamate via mixed isobutyl anhydride activation.[8] This dipeptide was then converted to the thioamide **7** in 90% yield with 0.5 equivalents of Lawesson's reagent[9] in tetrahydrofuran. Treatment of **7** with a 30% solution of HBr in acetic acid gave L-Glu-OMe-γ-ψ[CSNH]-L-Glu(OEt)-OEt[10] as its hydrobromide salt which was dissolved in aqueous saturated sodium bicarbonate and then the free base **8** was extracted with ethyl acetate. Condensation of **8** with pteroic acid analogue **9**[3] via DEPC activation[11] gave **10**, and final alkaline hydrolysis of the methyl and ethyl esters gave the target compound **11**. An initial attempt to prepare **11**, using *tert*-butyl esters as the carboxyl-

protecting group instead of ethyl and methyl esters, failed due to problems with efficient or selective removal of the Z-group. Catalytic hydrogenolysis using 10% palladium on charcoal failed to give any products presumably because of catalyst poisoning by sulphur, and TMSI[12] or EtSH-BF_3 · Et_2O[13] proved non-selective in removing the Z-group.

Scheme 2



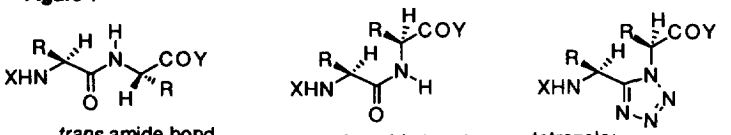
Conditions: (a) i) Et_3N (1.5 eq), $ClCO_2CH_2CH_3$ (1.25 eq), THF, -10°C, 10 min, ii) $NaBH_4$ (3 eq)/MeOH, -10°C to R.T.; (b) Et_3N (1.5 eq), CH_3SO_2Cl (1.2 eq), CH_2Cl_2; (c) Glu(OBu^t)-OBu^t (1eq), 2,6- lutidine, DMF; (d) H_2, 10%Pd/C; (e) DEPC (2.2 eq), Et_3N (2.2 eq), DMF; (f) TFA.

The main literature method for the preparation of methyleneamino isosteric dipeptides involves direct reduction of the amide carbonyl, a reaction which can also lead to considerable reduction of any ester function present.[14] To avoid this problem the methyleneamino dipeptide derivative **18** was prepared through the alternative sequence outlined in Scheme 2.

The mixed anhydride of glutamate **12** was generated *in situ* using ethyl chloroformate/triethylamine, and then reduced with $NaBH_4$/MeOH[15] to the alcohol **13** from which the mesylate **14** was obtained by treatment with triethylamine and mesyl chloride in dichloromethane. Nucleophilic substitution of the primary mesylate by di-*tert*-butyl glutamate in the presence of 2,6-lutidine as a base afforded the CH_2NH isostere **15** in 46% yield. The Z-group was removed by catalytic hydrogenolysis, and the free base **16** was coupled to pteroic acid analogue **9** via DEPC activation. Finally, the *tert*-butyl esters were removed by treatment with TFA to afford **18** as its TFA salt.

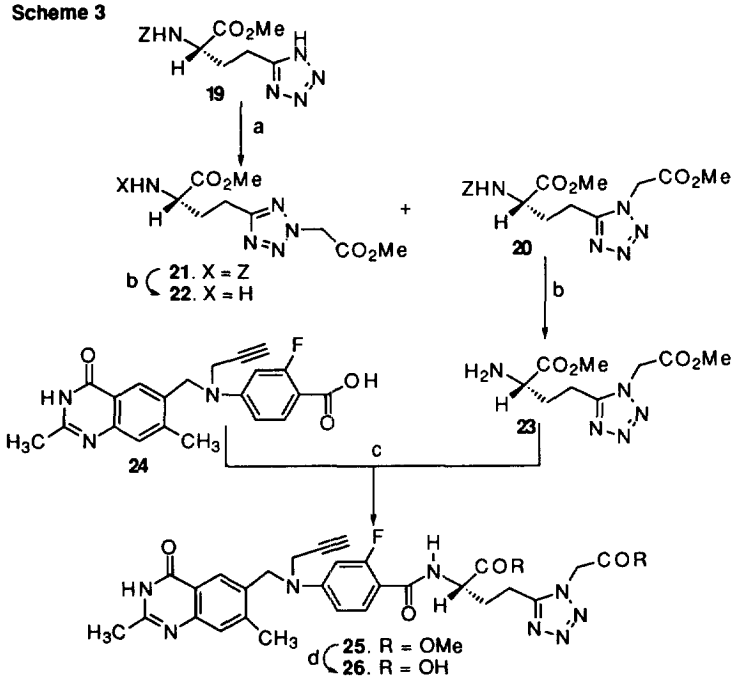
Next the question of replacing the γ-glutamyl amide bond by a *cis* amide bond mimic was addressed. By analogy to the *trans* olefinic group, it was suggested that the *cis* olefinic group might serve as a mimic for the *cis* amide bond.[16]

Figure 1



This approach, however, was thought impossible since it had been found that the *cis* β,γ-unsaturated carbonyl system isomerises to the more stable *trans* α,β-unsaturated system.[16] The tetrazole ring, subsequently proposed by Marshall[17] as an alternative *cis* amide isosteric replacement because it locks a dipeptide into a geometry corresponding to the *cis* conformation (Figure 1), was then investigated.

Scheme 3

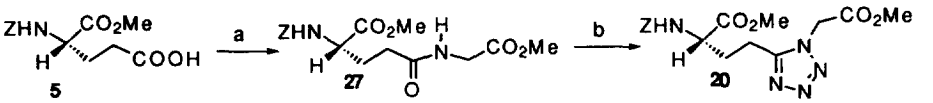


Conditions: (a) Et_3N (1.2 eq), $BrCH_2CO_2Me$ (2 eq), CH_2Cl_2; (b) H_2, 10% Pd/C; (c) DEPC, Et_3N, DMF; (d) 1N aq. NaOH, $MeOH/H_2O$.

Our approach to compound **26** was based on alkylation of the tetrazole ring of methyl (2S) -2-benzyloxycarbonylamino-4-(5-tetrazolyl)butyrate[18] (**19**) with methyl bromoacetate (Scheme 3). This reaction gave a mixture of the two regioisomers (**20** and **21**, 5:3 ratio) which were separable by column chromatography. The less polar 2,5-disubstituted tetrazole derivative **21** was isolated in 30% yield as an oil whilst the more polar 1,5-disubstituted tetrazole derivative **20**, was isolated in 49% yield as a solid. Structure assignment of the two regioisomers was based on their NMR spectra. As expected the *N*-CH_2CO_2Me

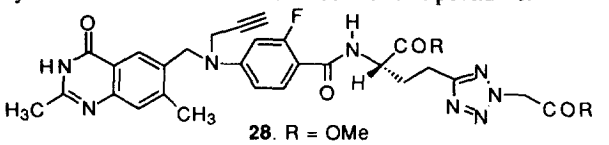
protons of the 2,5-disubstituted analogue **21** were lower field than the corresponding protons of the 1,5-disubstituted analogue **20**. This was unambiguously confirmed by synthesising the 1,5-disubstituted derivative **20** employing Zabrocki's method.[19]

Scheme 4



Conditions: (a) i) NMM (1 eq), $ClCO_2CH_2CH(CH_3)_2$ (1 eq), THF, -20°C, 10 min, ii) HCl Gly-OMe (1eq), NMM (1eq), THF, -20°C to R.T; (b) i) PCl_5, quinoline, $CHCl_3$ii) HN_3.

Thus treatment of Z-L-Glu-OMe-γ-Gly-OMe **27** with quinoline / phosphorus pentachloride and hydrazoic acid (Scheme 4) afforded Z-L-Glu-OMe-γ-ψ[CN_4]-Gly-OMe in 36% yield which had an identical ^1H NMR spectum to that of **20**. Synthesis of the target compound **26** was then accomplished by conversion of **20** to **23** by catalytic hydrogenolysis, condensation of **23** with **24** via DEPC activation to give **25**, and final alkaline hydrolysis of the methyl esters. The 2,5-disubstituted tetrazole derivative **29** was made from regioisomer **21** by a route identical to that described for compound **26**.



28. R = OMe
29. R = OH

The inhibitory activity of the thioamide **11** against TS and L1210 cell growth (Table 1) was similar to that of the parent compound, ICI 198583-γ-Glu (**2**).[3] By contrast, the methyleneamino derivative **17** was ~10-fold and ~100-fold poorer as inhibitor of TS and L1210 cell growth respectively (Table 1). An interesting feature of this compound was the low L1210:1565/L1210 cross-resistance factor, suggesting that **11** does not use the reduced folate carrier (RFC) for cellular uptake. L1210:1565 is a L1210 mutant cell line with impaired reduced folate transport carrier.

Table 1: Inhibitory activities against TS, and L1210, W1L2 cell growth[a]

Compound	TS, IC_{50} (nM)	L1210, IC_{50} (μM)	W1L2, IC_{50} (μM)	L1210:1565/L1210 cross-resistance factor	
2	2.0	0.16±0.069	0.09, 0.074	30	(from ref 3)
11	2.0	0.78, 0.94	0.24	5	
17	26	13, 13	8.8	1	
26	2.4	1.4, 1.2	0.092, 0.096	5	
29	1.2	0.065, 0.042	0.016, 0.019	25	

[a]Methodologies for the inhibition of L1210 TS and L1210, W1L2 cell growth are as described in refs 20-22.

The inhibitory activities of the tetrazole isostere **26** (1,5-disubstituted derivative) were broadly similar to **11**. On the other hand, although the 2,5-disubstituted derivative **29** had a similar TS inhibitory activity to **26**, it

was ~20-fold more potent against L1210 cell growth. This suggest that other factors, such as cellular uptake, may be responsible for this potent activity. The L1210:1565 cell line (impaired RFC) is 25-fold cross-resistant to **29** but only 5-fold cross-resistant to **26**.

In summary, the development of synthetic routes to γ-glutamyl amide bond isosteres, *i.e.*, CSNH, CH_2NH and CN_4, has allowed the synthesis of a series of novel quinazoline antifolates with modified glutamate side chains. These compounds, and in particular, the tetrazole derivatives exhibited potent TS and L1210 cell growth inhibitory activities.

Experimental

Thin layer chromatography (TLC) was performed on precoated sheets of silica $60F_{254}$ (Merck Art 5735). Visualisation 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 (b.p. 60-80°C). Electron impact (EI) and chemical ionisation (CI) mass spectra were determined with a VG 7070H spectrometer and a VG 2235 data system using the direct-insertion method, an ionising voltage of 70eV and a trap current of 100 mA, and an ion-source temperature of 160°C. Fast atom bombardment (FAB) mass spectra were determined with a VG ZAB-SE spectrometer. Electrospray ionisation (ESI) mass spectra were recorded using a TSQ 700 triple quadrupole mass spectrometer (Finnigan MAT) fitted with an electrospray ionisation source (Analytica, USA). 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, USA) at 1 mL/min. Masses were scanned from 200 to 800 amu at a scanning speed of 3 sec/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 Limited, Leicester, UK.

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). 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. 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 and 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 x 100 ml). The combined organic extracts were successively washed with 10% aqueous citric acid (2 x 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.

Diethyl *N*-[α-methyl *N*-(benzyloxycarbonyl)-L-γ-glutamyl]-L-glutamate.

(Z-L-Glu-OMe-γ-L-Glu(OEt)-OEt) (6). The general procedure A was followed using α-methyl *N*-(benzyloxycarbonyl)-L-glutamate (**5**) (2.00 g, 6.77 mmol), dry THF (7 ml), *N*-methylmorpholine (0.684 g, 6.77 mmol), isobutyl chloroformate (0.924 g, 6.77 mmol) and a suspension of diethyl L-glutamate hydrochloride (1.62 g, 6.78 mmol) in dry THF (7 ml) and *N*-methylmorpholine (0.684 g, 6.77 mmol). The crude product was purified by column chromatography using a gradient of ethyl acetate in dichloromethane (40 to 60%) as eluent. Trituration with petrol afforded a white solid which was dried *in vacuo* over P_2O_5 (2.75 g, 84%), m.p. 94-95 °C (Lit[23] 50-52 °C); ^{1}H-NMR (DMSO-d_6) δ 1.16 (t, J = 6.8 Hz, 6H, 2 x $CO_2CH_2CH_3$), 1.80, 1.94 (2 x m, 4H, 2 x β-CH_2), 2.23, 2.36 (2 x t, J = 7.6 Hz, 4H, 2 x γ-CH_2), 3.63 (s, 3H, CO_2CH_3), 4.06 (m, 5H, 2 x $CO_2CH_2CH_3$ and ZHNC*H*), 4.21 (m, 1H, $CH_2CONHCH$), 5.03 (m, 2H, $PhCH_2$), 7.35 (m, 5H, Ar*H*), 7.78 (d, J = 7.8 Hz, 1H, Z*H*NCH), 8.26 (d, J = 7.4 Hz, 1H, $CH_2CONHCH$); MS (FAB, m/z) 481 $(M+H)^+$, 91 $(PhCH_2^+)$; Found C, 57.28; H, 6.58; N, 5.73. $C_{23}H_{32}N_2O_9$ requires C, 57.49; H, 6.71; N, 5.83%.

Ethyl (2S)-2-[(4S)-4-(benzyloxycarbonylamino)-4-(methoxycarbonyl) butanethioamido]-4-(ethoxycarbonyl)butyrate. (Z-L-Glu-OMe-γ-ψ[CSNH]-L-Glu(OEt)-OEt) (7). To a solution of **6** (1.45 g, 3.02 mmol) in dry THF (11 ml) was added Lawesson's reagent (0.60 g, 1.5 mmol) and the flask was fitted with a condenser equipped with a calcium chloride drying tube. The reaction mixture was stirred at room temperature for 2.5 h and then heated at reflux until all the Lawesson's reagent had dissolved. The solution was allowed to cool to room temperature and then silica gel (Merck Art. 7734, 2.5 g) was added. The solvent was removed *in vacuo* and the residue was placed on a silica gel column. Elution with a gradient of ethyl acetate in dichloromethane (15-35%) gave the title compound **7** (1.35 g, 90%) as a colourless viscous oil; ^{1}H-NMR (DMSO-d_6) δ 1.17 (m, 6H, 2 x $CO_2CH_2CH_3$), 1.92-2.20 (m, 4H, 2 x β–CH_2), 2.40 (t, J = 7.4 Hz, 2H, $CH_2CH_2CO_2Et$), 2.66 (m, 2H, CH_2CH_2CSNH), 3.64 (s, 3H, CO_2CH_3), 4.05 (m, 5H, 2 x $CO_2CH_2CH_3$ and ZHNC*H*), 4.83 (m, 1H, CSNHC*H*)), 5.03 (m, 2H, $ArCH_2$), 7.36 (m, 5H, Ar*H*), 7.78 (d, J= 8.0 Hz, 1H, Z*H*NCH, 10.20 (d, J = 6.8 Hz, 1H, CSN*H*CH); MS (FAB, m/z) 497 $(M+H)^+$, 91 $(PhCH_2^+)$; Found C, 55.61; H, 6.50; N, 5.55; S, 6.40. $C_{23}H_{32}N_2O_8S$ requires C, 55.63; H, 6.50; N, 5.64; S, 6.46%.

Ethyl (2S)-2-[(4S)-4-amino-4-(methoxycarbonyl)butanethioamido]-4-(ethoxycarbonyl)

butyrate. (L-Glu-OMe-γ-ψ[CSNH]-L-Glu(OEt)-OEt) (8). A 30% solution of hydrogen bromide in acetic acid (2.0 ml) was added into a flask containing **7** (1.0 g, 2.0 mmol) under an argon atmosphere. The resulting solution was stirred at room temperature for 20 min and then diluted with dry diethyl ether (25 ml). The mixture was then cooled in an ice bath and the supernatant liquid was decanted. This procedure was repeated once more and then the oil was dissolved in saturated aqueous sodium bicarbonate (70 ml). The solution was extracted with ethyl acetate (3 x 50 ml); the ethyl acetate extracts were combined, dried (Na_2SO_4) and the solvent evaporated to give **8** as a pale orange oil (0.443 g, 62%). This was immediately used in the next experiment without further purification.

Ethyl (2S)-2-[(4S)-4-[4-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzamido]-4-(methoxycarbonyl)butanethioamido]-4-(ethoxycarbonyl)butyrate (10). The general procedure C was followed using **8** (0.505 g, 1.40 mmol), 4-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynyl amino]benzoic acid, trifluoroacetate salt (**9**) (0.599 g, 1.30 mmol), DMF (16 ml), diethyl phosphorocyanidate (0.473 g, 2.9 mmol) and triethylamine (0.293 g, 2.9 mmol). The crude product was purified by column chromatography using a gradient of tetrahydrofuran in hexanes (50 to 75%) as eluent. Reprecipitation from dichloromethane / petrol gave the title compound **10** (0.400 g, 44%) as a white solid, m.p. 175-177°C; ^{1}H-NMR (DMSO-d_6) δ 1.16 (m, 6H, 2 x $CO_2CH_2CH_3$), 2.04, 2.38 (2 x m, 6H, 2 x β-CH_2 and $CH_2CH_2CO_2Et$), 2.32 (s, 3H, quinazoline 2-CH_3), 2.67 (t, J = 7.5 Hz, 2H, CH_2CH_2CSNH), 3.23 (s, 1H, C≡C*H*), 3.63 (s, 3H, CO_2CH_3), 4.04 (m, 4H, 2 x $CO_2CH_2CH_3$), 4.34 (s, 2H, $CH_2C{\equiv}CH$), 4.44 (m, 1H, $C_6H_4CONHCH$), 4.78 (m, 3H, quinazoline 6-CH_2 and CSNHC*H*), 6.83 (d, J = 8.8 Hz, 2H, 3′,5′-Ar*H*), 7.54 (d, J = 8.4 Hz, 1H, quinazoline 8-*H*), 7.69 (dd, J = 8.5, 1.5 Hz, 1H, quinazoline 7-*H*), 7.76 (d, J = 8.7 Hz, 2H, 2′,6′-Ar*H*), 7.96 (s, 1H, quinazoline 5-*H*), 8.43 (d, J = 7.6 Hz, 1H, C_6H_4CONH), 10.20 (d, J = 6.9 Hz, 1H, CSN*H*), 12.20 (s, 1H, lactam N*H*); MS (ESI, m/z) 692 $(M+H)^+$, 330 (M-dipeptide)$^+$; Found C, 60.55; H, 5.96; N, 10.22; S, 4.62. $C_{35}H_{41}N_5O_8S$ requires C, 60.77; H, 5.97; N, 10.12; S, 4.63%.

(2S)-2-[(4S)-4-[4-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzamido]-4-(carboxy)butanethioamido]-4-(carboxy)butyric acid (11). To a suspension of **10** (0.100 g, 0.14 mmol) in methanol (3 ml) was added aqueous NaOH (1N, 0.56 ml, 0.56 mmol). The resulting solution was stirred at room temperature for 2 h. More aqueous NaOH (1N, 0.1 ml, 0.1 mmol) was then added followed by an extra portion of NaOH (1N, 0.1 ml, 0.1 mmol) after a total time of 5.5 h. Stirring was continued at room temperature for 1 h and then the solution was diluted with water (20 ml). The organic solvent was removed by evaporation and the alkaline solution was first washed with ethyl acetate (2 x 50 ml) and then acidified to pH 3 with 1N hydrochloric acid. Ethyl acetate (70 ml) was then added; the two layers were separated and the aqueous layer was washed with more ethyl acetate (70 ml). The ethyl acetate extracts were combined, dried (Na_2SO_4) and concentrated *in vacuo*. Reprecipitation from tetrahydrofuran (minimum amount) / petrol gave the title compound **11**, a white solid, which was dried *in vacuo* over P_2O_5 (0.050 g, 56%), m.p. 155°C (dec); ^{1}H-NMR (DMSO-d_6) δ 2.05 (m) and 2.27 (m obscured) (6H, 2 x β-CH_2 and CH_2CH_2COOH), 2.33 (s, 3H, quinazoline 2-CH_3), 2.66 (t, J = 7.2 Hz, 2H, CH_2CH_2CSNH), 3.23 (s, 1H, C≡C*H*), 4.34 (m, 3H, $CH_2C{\equiv}CH$ and $C_6H_4CONHCH$), 4.78 (m, 3H, quinazoline 6-CH_2 and CSNHC*H*), 6.84 (d, J = 8.6 Hz, 2H, 3′,5′-Ar*H*), 7.55 (d, J = 8.3 Hz, 1H, quinazoline 8-*H*), 7.70 (d, J = 8.5 Hz, 1H, quinazoline 7-*H*), 7.77 (d, J = 8.4 Hz, 2H, 2′,6′-Ar*H*), 7.96 (s, 1H, quinazoline 5-*H*), 8.33 (d, J = 7.8 Hz, 1H, C_6H_4CONH), 10.12 (d, J = 7.4 Hz, 1H, CSN*H*); MS (ESI, m/z) 622 $(M+H)^+$, 330 (M-dipeptide)$^+$, 312

$(M+2H)^{2+}$, 174; Found C, 56,52; H, 5.14; N, 10.67; S, 4.84. $C_{30}H_{31}N_5O_8S \cdot H_2O$ requires C, 56.33; H, 5.20; N, 10.95; S, 5.01%.

***tert*-Butyl 5-hydroxy-2-(benzyloxycarbonylamino)pentanoate (13).** To a stirred solution of α-*tert*-butyl *N*-(benzyloxycarbonyl)-L-glutamate (**12**) (6.8 g, 0.020 mol) in dry THF (55 ml) cooled to -10°C was added triethylamine (3.03 g, 0.030 mol) followed by ethyl chloroformate (2.76 g, 0.025 mol). After stirring for 10 min at -10°C the reaction mixture was flushed with argon; then sodium borohydride (2.28 g, 0.06 mol) was added in one portion followed by dropwise addition of methanol (60 ml) over a 15 minute period while the temperature was maintained below 0°C. Stirring was continued at 0°C for 30 min and then the mixture was neutralised with 2N hydrochloric acid. The organic solvents were removed *in vacuo* and the residue extracted with ethyl acetate (2 x 160 ml). The combined ethyl acetate extracts were washed with 5% aqueous sodium bicarbonate and then 10% aqueous citric acid, dried ($MgSO_4$), and concentrated *in vacuo*. Purification by column chromatography, on gradient elution with ethyl acetate in hexanes (40 to 60%), gave the title compound **13** (4.09 g, 63%) as a pale yellow oil; ^{1}H-NMR (DMSO-d_6) δ 1.38-1.79 (m, 13H, C(CH_3)$_3$), 3-CH_2 and 4-CH_2), 3.30 (q, J = 5.7 Hz, 2H, CH_2OH), 3.87 (m, 1H, 2-C*H*), 4.38 (t, J = 5.1 Hz, 1H, CH_2OH, exchangeable with D_2O), 5.03 (m, 2H, ArCH_2), 7.35 (m, 5H, Ar*H*), 7.53 (d, J = 7.5 Hz, 1H, N*H*); MS (CI, m/z) 324 $(M+H)^+$, 268 $(M\text{-}Bu^t)^+$, 224, 91 $(PhCH_2^+)$; Found C, 62.93; H, 7.77; N, 4.23. $C_{17}H_{25}NO_5$ requires C, 63.14; H, 7.79; N, 4.33%. $[\alpha]^{25} = +6.2°$ (c = 1.4, CH_2Cl_2), Lit.[24] $[\alpha]_D = +6.0°$ (c = 1.4, CH_2Cl_2).

***Tert*-butyl 5-methylsulphonyloxy-2-(benzyloxycarbonylamino) pentanoate (14).** To a stirred solution of **13** (3.75 g, 11.61 mmol) in dichloromethane (50 ml) cooled to -10°C was added triethylamine (1.76 g, 17.41 mmol) followed by methanesulphonyl chloride (1.60 g, 14.06 mmol) over a 5 minute period. Stirring was continued for 25 min while the temperature was maintained below 0°C. The reaction mixture was then transferred to a separatory funnel with the aid of more dichloromethane (150 ml) and washed successively with water (150 ml), 10% aqueous citric acid (2 x 150 ml), saturated aqueous sodium bicarbonate (2 x 150 ml) and brine (150 ml), dried (Na_2SO_4), and concentrated *in vacuo*. to leave the title compound **14** (4.50 g, 96%). Part of this sample (1.85 g) was further purified by column chromatography on elution with 1:1 hexane / ethyl acetate to give 1.71 g of the product as a pale yellow oil; ^{1}H-NMR (DMSO-d_6) δ 1.39 (s, 9H, C(CH_3)$_3$), 1.72 (m, 4H, 3-CH_2 and 4-CH_2), 3.14 (s, 3H, OSO_2CH_3), 3.94 (m, 1H, 2-C*H*), 4.19 (t, J = 5.8 Hz, 2H, CH_2OSO_2CH3), 5.04 (s, 2H, ArCH_2), 7.35 (m, 5H, Ar*H*), 7.62 (d, J = 7.4 Hz, 1H, N*H*); MS (CI, m/z) 402 $(M+H)^+$, 346 $(M\text{-}Bu^t)^+$, 306 $(M\text{-}OSO_2CH_3)^+$, 91 $(PhCH_2^+)$; Found C, 53.62; H, 6.79; N, 3.36; S, 7.93. $C_{18}H_{27}NSO_7$ requires C, 53.85; H, 6.78; N, 3.49; S, 7.99%. $[\alpha]^{20} = +8.73°$ (c = 4.9, $CHCl_3$).

***Tert*-butyl (2S)-2-(benzyloxycarbonylamino)-5-[(1S)-1,3-(di-*tert*-butoxycarbonyl) propylamino]pentanoate. (Z-L-Glu-OBu^t-γ-ψ[CH_2NH]-L-Glu(OBu^t)-OBu^t) (15).** To a solution of **14** (0.900 g, 2.24 mmol) in dry DMF (5 ml) was added di-*tert*-butyl L-glutamate (0.870 g, 3.36 mmol) followed by 2,6-lutidine (0.431 g, 4.03 mmol). The resulting solution was stirred at 65°C for 42 h under argon; then cooled to room temperature and diluted with ethyl acetate (120 ml) and water (100 ml). The two layers were separated and the aqueous layer was washed with more ethyl acetate (2 x 80 ml). The combined ethyl acetate extracts were washed with brine (100 ml) and water (100 ml), dried (Na_2SO_4), and concentrated *in vacuo*. Purification by column chromatography, on gradient elution with ethyl acetate in hexanes (30 to 40%), gave the title compound **15** (0.585 g, 46%) as a colourless viscous oil; ^{1}H-NMR (DMSO-d_6), δ, 1.39, 1.40 (2 x s, 27H, 3 x C(CH_3)$_3$), 1.71 (m, 6H, 2 x β-CH_2 and CH_2CH$_2$NH), 2.26, 2.50 (2 x m, 4H, $CH_2CH_2CO_2Bu^t$ and CH_2NH), 2.99 (t, J = 6.8 Hz, 1H, CH_2NHCH), 3.86 (m, 1H, ZHNC*H*), 5.03 (m, 2H, ArCH_2), 7.34 (m, 5H,

Ar*H*), 7.52 (d, J = 7.5 Hz, 1H, CON*H*); MS (CI, m/z) 565 $(M+H)^+$, 463 $(M-CO_2Bu^t)^+$, 91 $(PhCH_2^+)$; Found C, 63.33; H, 8.52; N, 4.90. $C_{30}H_{48}N_2O_8 \cdot 0.25H_2O$ requires C, 63.30; H, 8.59; N, 4.92%.

***Tert*-butyl (2S)-2-amino-5-[(1S)-1,3-(di-*tert*-butoxycarbonyl)propylamino]pentanoate. (L-Glu-OBu^t-γ-ψ[CH_2NH]-L-Glu(OBu^t)-OBu^t) (16).** The general procedure B was followed using **15** (0.640 g, 1.13 mmol), ethyl acetate (100 ml) and 10% Pd/C (0.096 g). The reaction mixture was stirred for 4.5 h under hydrogen atmosphere. Standard work-up afforded the product contaminated with approximately 5% of the starting material. This crude product was used in the next experiment without further purification.

***Tert*-butyl (2S)-2-[4-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzamido]-5-[(1S)-1,3-(di-*tert*-butoxycarbonyl)propylamino]pentanoate (17).** The general procedure C was followed using **16** (0.387 g, 0.9 mmol), 4-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoic acid, trifluoroacetate salt (**9**) (0.461 g, 1.0 mmol), diethyl phosphorocyanidate (0.359 g, 2.2 mmol) and triethylamine (0.222 g, 2.2 mmol). The crude product was purified by column chromatography eluting with 2% methanol in ethyl acetate. Reprecipitation from dichloromethane (minimum amount) / petrol gave the title compound **17** (0.215 g, 31%) as a white solid, m.p. 108-111°C; ^{1}H-NMR (DMSO-d_6) δ 1.37, 1.39 (2 x s, 27H, 3 x C(CH_3)$_3$), 1.73 (m, 6H, 2 x β-CH_2 and CH_2CH_2NH), 2.26, 2.54 (2 x m, 4H, $CH_2CH_2CO_2Bu^t$ and CH_2NH), 2.32 (s, 3H, quinazoline 2-CH_3), 2.99 (t, J = 6.4 Hz, 1H, CH_2NHCH), 3.18 (s, 1H, C≡C*H*), 4.23 (m, 1H, CONHC*H*), 4.32 (s, 2H, $CH_2C{\equiv}C$), 4.77 (s, 2H, quinazoline 6-CH_2), 6.83 (d, J = 8.8 Hz, 2H, 3′,5′-Ar*H*), 7.53 (d, J = 8.4 Hz, 1H, quinazoline 8-*H*), 7.68 (dd, J = 2.0, 9.6 Hz, 1H, quinazoline 7-*H*), 7.73 (d, J = 8.9 Hz, 2H, 2′,6′-Ar*H*), 7.97 (d, J = 1.7 Hz, 1H, quinazoline 5-*H*), 8.13 (d, J= 7.3 Hz, 1H, CON*H*), 12.13 (s, 1H, lactam N*H*); MS (FAB, m/z) 760 $(M+H)^+$, 592 (M-3 x Bu^t)$^+$, 330 (M-dipeptide)$^+$; Found C, 66.13; H, 7.58; N, 9.01. $C_{42}H_{57}N_5O_8$ requires C, 66.38; H, 7.56; N, 9.22%.

(2S)-2-[4-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino] benzamido]-5-[(1S)-1,3-(di-carboxy)propylamino]pentanoic acid (18). The general procedure D was followed using **17** (0.110 g, 0.145 mmol) and trifluoroacetic acid (8.5 ml). The title compound **18** was obtained as a white solid (0.100 g, 83%), m.p. 132°C (dec); ^{1}H-NMR (DMSO-d_6) δ 1.70-2.06 (m, 6H, β-CH_2 and CH_2CH_2NH), 2.35 (s, 3H, quinazoline 2-CH_3), 2.36 (m obscured, 2H, $CH_2CH_2CO_2H$), 2.96 (m, 2H, CH_2NH), 3.19 (s, 1H, C≡C*H*), 3.96 (m, 1H, CH_2NHCH) 4.34 (m, 3H, $CH_2C{\equiv}C$ and CONHC*H*), 4.79 (s, 2H, quinazoline 6-CH_2), 6.85 (d, J= 8.9 Hz, 2H, 3′,5′-Ar*H*), 7.55 (d, J = 8.3 Hz, 1H, quinazoline 8-*H*), 7.74 (m, 3H, 2′,6′-Ar*H* and quinazoline 7-*H*), 7.97 (s, 1H, quinazoline 5-*H*), 8.24 (d, J= 8.2 Hz, 1H, CON*H*); MS (FAB, m/z) 592 $(M+H)^+$, 518, 330 (M-dipeptide)$^+$; Found C, 49.54; H, 4.59; N, 8.19; F, 12.42. $C_{30}H_{33}N_5O_8 \cdot 1.8TFA \cdot H_2O$ requires C, 49.53; H, 4.55; N, 8.59; F, 12.58%.

Dimethyl *N*-[*N*-(benzyloxycarbonyl)-L-γ-glutamyl]-glycinate. (Z-L-Glu-OMe-γ-Gly-OMe) (27). The general procedure A was followed using α-methyl *N*-(benzyloxycarbonyl)-L-glutamate (**5**) (1.0 g, 3.38 mmol), *N*-methylmorpholine (0.341 g, 3.38 mmol), isobutyl chloroformate (0.460 g, 3.38 mmol) and a suspension of methyl glycinate hydrochloride (0.422 g, 3.38 mmol) in dry tetrahydrofuran (5 ml) and *N*-methylmorpholine (0.341 g, 3.38 mmol). Purification of the crude product, on gradient elution with ethyl acetate in dichloromethane (20 to 45%), gave the title compound as a colourless oil (0.853 g, 69%), which solidified on standing at room temperature, m.p. 94-95°C; ^{1}H-NMR (DMSO-d_6), δ, 1.78, 1.94 (2 x m, 2H, β-CH_2), 2.23 (t, J = 7.6 Hz, 2H, γ-CH_2), 3.61, 3.63 (2 x s, 6H, 2 x CO_2CH_3), 3.81 (d, J = 5.8 Hz, 2H, gly α-CH_2), 4.04 (m, 1H, glu α-C*H*), 5.03 (s, 2H, PhCH_2), 7.36 (m, 5H, Ar*H*), 7.78 (d, J = 7.7 Hz, 1H, glu N*H*),

8.31 (t, J = 6.0 Hz, 1H, gly N*H*); MS (CI, m/z), 367 $(M+H)^+$, 307 $(M-CO_2Me)^+$, 259, 91 $(PhCH_2^+)$; Found C, 55.76; H, 6.05; N, 7.64. $C_{17}H_{22}N_2O_7$ requires C, 55.73; H, 6.05; N, 7.65%.

Methyl (2S) -2-(benzyloxycarbonylamino)-4-(2-methoxycarbonylmethyltetrazol-5-yl)butyrate (Z-L-Glu-OMe-γ-ψ[2,5-CN_4]Gly-OMe) (21) and Methyl (2S)-2-(benzyloxycarbonylamino)-4-(1-methoxycarbonylmethyltetrazol-5-yl)butyrate (Z-L-Glu-OMe-γ-ψ[CN_4]Gly-OMe) (20).

Method a.

To a stirred solution of methyl bromoacetate (2.45 g, 16.0 mmol) in anhydrous dichloromethane (35 ml) was added methyl (2S)-2-benzyloxycarbonylamino-4-(tetrazol-5-yl)butyrate (**19**) (2.55 g, 8.0 mmol) and then triethylamine (0.970 g, 9.6 mmol) under argon. The resulting clear solution was stirred at room temperature for 3 h, then diluted with ethyl acetate (200 ml) and washed with saturated aqueous sodium bicarbonate (2 x 150 ml) and water (150 ml), dried (Na_2SO_4) and concentrated *in vacuo*. Purification by column chromatography, on gradient elution with ethyl acetate in dichloromethane (20 to 40%), gave in order of elution:

a. 21 (0.95 g, 30%) as a colourless oil; ^{1}H-NMR (DMSO-d_6) δ 1.98-2.20 (m, 2H, β-CH_2), 2.95 (t, J = 7.0 Hz, 2H, γ-CH_2), 3.64, 3.72 (2 x s, 6H, 2 x CO_2CH_3), 4.15 (m, 1H, glu α-C*H*), 5.05 (m, 2H, PhCH_2), 5.79 (s, 2H, gly α-CH_2), 7.36 (m, 5H, Ar*H*), 7.92 (d, J = 7.8 Hz, 1H, glu N*H*); MS (CI, m/z) 392 $(M+H)^+$, 332 $(M-CO_2Me)^+$, 91 $(PhCH_2^+)$; Found C, 52.02; H, 5.46; N, 17.65. $C_{17}H_{21}N_5O_6$ requires C, 52.17; H, 5.41; N, 17.89%.

b. 20 (1.55 g, 49%) as a white solid, m.p. 91-94°C; ^{1}H-NMR (DMSO-d_6) δ 1.95-2.18 (m, 2H, β-CH_2), 2.92 (m, 2H, γ-CH_2), 3.64, 3.72 (2 x s, 6H, 2 x CO_2CH_3), 4.24 (m, 1H, glu α-C*H*), 5.05 (m, 2H, PhCH_2), 5.51 (s, 2H, gly α-CH_2), 7.36 (m, 5H, Ar*H*), 7.87 (d, J = 7.9 Hz, 1H, glu N*H*); MS (CI, m/z) 392 $(M+H)^+$, 332 $(M-CO_2Me)^+$, 91 $(PhCH_2^+)$; Found C, 52.13; H, 5.43; N, 17.64. $C_{17}H_{21}N_5O_6$ requires C, 52.17; H, 5.41; N, 17.89%.

Method b.

To a stirred solution of phosphorus pentachloride (0.208 g, 1.0 mmol) in chloroform (5 ml) was added quinoline (0.258 g, 2.0 mmol) at room temperature (a white precipitate had formed). The reaction mixture was stirred at room temperature for 20 min and then the dipeptide **27** (0.365 g, 1.0 mmol) was added in portions while the temperature was maintained below 20°C. Stirring was continued at 20°C for 25 min (a clear solution was obtained after a few minutes); then a benzene solution of hydrazoic acid[25] (approx. 2M, 3 ml, *caution* it is poisonous) was added and the orange solution was stirred at room temperature for 1 h and 20 min before being evaporated. The crude residue was partitioned between ethyl acetate (30 ml) and water (30 ml); the two layers were separated and the organic layer was washed with 1N hydrochloric acid (2 x 15 ml), half saturated aqueous sodium bicarbonate (2 x 15 ml), water (25 ml) and brine (25 ml), dried (Na_2SO_4) and concentrated *in vacuo* to leave a pale yellow oil. Purification by column chromatography , on elution with 35% ethyl acetate in dichloromethane, afforded the product as a colourless oil which solidified on standing overnight (0.140 g, 36%), m.p. 93-96°C; ^{1}H-NMR identical with that of **20** prepared using Method a.

Methyl (2S)-2-amino-4-(1-methoxycarbonylmethyltetrazol-5-yl)butyrate.

(L-Glu-OMe-γ-ψ[CN_4]Gly-OMe) (23). The general procedure B was followed using **20** (0.470 g, 1.20 mmol), ethyl acetate (55 ml) and 10% Pd/C (0.120 g). The reaction mixture was stirred at room temperature for 15 h. The product **23** (0.310 g, 98%), a colourless oil, was immediately used in the next experiment without further purification.

Methyl (2S)-2-[4-[*N*-[(3,4-dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop -2-ynylamino]-2-fluorobenzamido]-4-(1-methoxycarbonylmethyltetrazol-5-yl)butyrate (25).

The general procedure C was followed using **23** (0.270 g, 1.05 mmol), 4-[*N*-[(3,4-dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl)-*N*-(prop-2 -ynyl)amino]-2-fluorobenzoic acid, trifluoroacetate salt **24** (0.493 g, 1.0 mmol), diethyl phosphorocyanidate (0.358 g, 2.2 mmol) and triethylamine (0.222 g, 2.2 mmol). The crude product was purified by column chromatography eluting first with 5% methanol in dichloromethane, then with methanol / dichloromethane / chloroform (1:10:9) and finally 5% methanol in chloroform. Reprecipitation from methanol-chloroform (1:5 v / v, minimum amount) / hexanes gave the title compound **25** as a white solid which was dried *in vacuo* over P_2O_5 (0.360 g, 58%), m.p. 249-251°C; ^{1}H-NMR (DMSO-d_6) δ 2.23 (m, 2H, β-CH_2), 2.31, 2.44 (2 x s, 6H, quinazoline 2-CH_3 and quinazoline 7-CH_3), 2.93 (t, J = 8.1 Hz, 2H, γ-CH_2), 3.26 (s, 1H, C≡C*H*), 3.64, 3.69 (2 x s, 6H, 2 x CO_2CH_3), 4.32 (s, 2H, CH_2C≡C), 4.55 (m, 1H, glu α-C*H*), 4.70 (s, 2H, quinazoline 6-CH_2), 5.51 (s, 2H, $CN_4CH_2CO_2$Me), 6.63 (m, 2H, 3',5'-Ar*H*), 7.44 (s, 1H, quinazoline 8-*H*), 7.54 (t, J = 9.0 Hz, 1H, 6'-Ar*H*), 7.68 (s, 1H, quinazoline 5-*H*), 8.30 (dd, J= 7.3, 3.6 Hz, 1H, glu N*H*), 12.10 (s, 1H, lactam); MS (ESI, m/z) 619 $(M+H)^+$, 362 (M-dipeptide)$^+$, 310 $(M+2H)^{2+}$; Found C, 57.27; H, 5.14; N, 17.46; F, 3.12. $C_{30}H_{31}FN_8O_6 \cdot 0.5H_2O$ requires C, 57.41; H, 5.14; N, 17.85; F, 3.03%.

(2S)-2-[4-[*N*-[(3,4-dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzamido]-4-(1-carboxymethyltetrazol-5-yl)butyric acid (26). To a suspension of **25** (0.216 g, 0.35 mmol) in methanol (6 ml) and water (1 ml) was added aqueous NaOH (1N, 1.30 ml, 1.30 mmol). The resulting clear solution was stirred at room temperature for 3 h; then diluted with water (3 ml) and acidified to pH 4 with 1N hydrochloric acid. The precipitate was then collected by filtration, washed with water (20 ml) and dried *in vacuo* over P_2O_5 to a white solid, the product **26** (0.150 g, 73%) m.p. 200 (dec); ^{1}H-NMR (DMSO-d_6) δ 2.21 (m, 2H, β-CH_2), 2.32, 2.44 (2 x s, 6H, quinazoline 2-CH_3 and quinazoline 7-CH_3), 2.90 (m, 2H, γ-CH_2), 3.26 (s, 1H, C≡C*H*), 4.32 (s, 2H, CH_2C≡C), 4.43 (m, 1H, glu α-C*H*), 4.70 (s, 2H, quinazoline 6-CH_2), 5.28 (s, 2H, CN_4CH_2COOH), 6.63 (m, 2H, 3',5'-Ar*H*), 7.44 (s, 1H, quinazoline 8-*H*), 7.55 (t, J = 9.2 Hz, 1H, 6'-Ar*H*), 7.69 (s, 1H, quinazoline 5-*H*), 8.17 (dd, J= 7.0, 4.6 Hz, 1H, glu N*H*); MS (ESI, m/z) 591 $(M+H)^+$, 362 (M-dipeptide)$^+$, 296 $(M+2H)^{2+}$; Found C, 55.38; H, 4.81; N, 18.23; F, 3.15. $C_{28}H_{27}FN_8O_6 \cdot H_2O$ requires C, 55.26; H, 4.80; N, 18.40; F, 3.12%.

Methyl (2S)-2-amino-4-(2-methoxycarbonylmethyltetrazol-5-yl)butyrate.

(L-Glu-OMe-γ-ψ[2,5-CN_4]Gly-OMe) (22). The general procedure B was followed using **21** (0.820 g, 2.10 mmol), ethyl acetate (100 ml) and 10% Pd/C (0.220 g). The reaction mixture was stirred at room temperature for 15 h. The product **22** (0.410 g, 76%), a colourless oil, was immediately used in the next experiment without further purification.

Methyl (2S)-2-[4-[*N*-[(3,4-dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynyl amino]-2-fluorobenzamido]-4-(2-methoxycarbonylmethyltetrazol-5-yl)butyrate (28). The general procedure C was followed using **22** (0.380 g, 1.48 mmol), 4-[*N*-[(3,4-dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoic acid, trifluoroacetate salt **24** (0.690 g, 1.4 mmol), diethyl phosphorocyanidate (0.502 g, 3.08 mmol) and triethylamine (0.312 g, 3.08 mmol). The crude product was purified by column chromatography eluting first with a gradient of methanol in ethyl acetate (1 to 5%) and then methanol/dichloromethane/chloroform (1:10:9). Reprecipitation from methanol-dichloromethane (1:5 v / v, minimum amount) / hexanes gave the title compound **28** as a white solid which was dried *in vacuo*

over P_2O_5 (0.505 g, 58%), m.p. 218°C; ^{1}H-NMR (DMSO-d_6) δ 2.22 (m, 2H, β-CH_2), 2.31, 2.43 (2 x s, 6H, quinazoline 2-CH_3 and quinazoline 7-CH_3), 2.97 (t, J = 7.7 Hz, 2H, γ-CH_2), 3.26 (s, 1H, C≡C*H*), 3.64, 3.72 (2 x s, 6H, CO_2CH_3), 4.31 (s, 2H, CH_2C≡C), 4.48 (m, 1H, glu α-C*H*), 4.70 (s, 2H, quinazoline 6-CH_2), 5.78 (s, 2H, CN4CH_2CO_2Me), 6.63 (m, 2H, 3′,5′-Ar*H*), 7.44 (s, 1H, quinazoline 8-*H*), 7.52 (t, J = 8.9 Hz, 1H, 6′-Ar*H*), 7.69 (s, 1H, quinazoline 5-*H*), 8.33 (dd, J= 7.2, 3.1 Hz, 1H, glu N*H*), 12.11 (s, 1H, lactam NH); MS (ESI, m/z) 619 $(M+H)^+$, 362 (M-dipeptide)$^+$, 310 $(M+2H)^{2+}$, 187; Found C, 56.72; H, 4.98; N, 17.38; F, 3.32. $C_{30}H_{31}FN_8O_6 \cdot H_2O$ requires C, 56.60; H, 5.22; N, 17.60; F, 2.98%.

(2S)-2-[4-[*N*-[(3,4-dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzamido]-4-(2-carboxymethyltetrazol-5-yl)butyric acid (29). To a suspension of **28** (0.215 g, 0.35 mmol) in methanol (6 ml) and water (1 ml) was added aqueous NaOH (1N, 1.30 ml, 1.30 mmol). The resulting clear solution was stirred at room temperature for 3 h; then diluted with water (3 ml) and acidified to pH 3 with 1N hydrochloric acid. The precipitate was then collected by filtration, washed with water (20 ml) and dried *in vacuo* over P_2O_5 to a white solid, the product **29** (0.160 g, 78%) m.p. 225-228°C; ^{1}H-NMR (DMSO-d_6) δ 2.21 (m, 2H, β-CH_2), 2.32, 2.45 (2 x s, 6H, quinazoline 2-CH_3 and quinazoline 7-CH_3), 2.95 (t, J = 7.5 Hz, 2H, γ-CH_2), 3.26 (s, 1H, C≡C*H*), 4.32 (s, 2H, CH_2C≡C), 4.43 (m, 1H, glu α-C*H*), 4.70 (s, 2H, quinazoline 6-CH_2), 5.52 (s, 2H, CN4CH_2COOH), 6.64 (m, 2H, 3′,5′-Ar*H*), 7.45 (s, 1H, quinazoline 8-*H*), 7.55 (t, J = 9.2 Hz, 1H, 6′-Ar*H*), 7.70 (s, 1H, quinazoline 5-*H*), 8.17 (dd, J= 7.0, 4.6 Hz, 1H, glu N*H*); MS (ESI, m/z) 591 $(M+H)^+$, 362 (M-dipeptide)$^+$, 296 $(M+2H)^{2+}$; Found C, 55.14; H, 4.63; N, 18.17; F, 3.15. $C_{28}H_{27}FN_8O_6 \cdot H_2O$ requires C, 55.26; H, 4.80; N, 18.40; F, 3.12%.

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