Design and Synthesis of Cyclopenta[*g*]quinazoline-Based Antifolates as Inhibitors of Thymidylate Synthase and Potential Antitumor Agents[†,‡]

Vassilios Bavetsias,*[,¶] Jonathan H. Marriott,[¶] Camille Melin,[¶] Rosemary Kimbell,[¶] Zbigniew S. Matusiak,[§] F. Thomas Boyle,[§] and Ann L. Jackman[¶]

CRC Centre for Cancer Therapeutics at The Institute of Cancer Research, Cancer Research Campaign Laboratories, 15 Cotswold Road, Sutton, Surrey SM2 5NG, England, and Zeneca Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, England

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Following the development of raltitrexed, the synthesis of nonpolyglutamatable inhibitors of TS that do not use the reduced folate carrier (RFC) for cellular entry should provide compounds which overcome mechanisms of resistance to folate-based inhibitors of TS that are associated with decreased/altered folylpolyglutamate synthetase (FPGS) expression and/or an impaired RFC. Examination of a computer graphics model of the humanized *Escherichia coli* TS enzyme with quinazoline inhibitors of TS, such as **1** bound in the active site of the enzyme, suggested that conformational restriction introduced by bridging the C9 with C7 to form a pentacycle may be beneficial for binding to TS. That led to the synthesis of a series of potent cyclopenta[*g*]quinazoline-based inhibitors of the enzyme in which the glutamyl residue associated with classical antifolates was replaced with a variety of glutamate-derived ligands; the most potent inhibitor being the L-Glu-γ-D-GluT^{α} derivative **7j**. In the mouse L1210:1565 cell line (mutant RFC), the majority of these compounds had activity equal or only slightly greater compared with the parental L1210 cell line, indicating a reduced dependence on the RFC for cellular uptake in the L1210 cell line.

Introduction

Over the last two decades there has been extensive interest in the thymidylate synthase (TS) enzyme as a target in cancer chemotherapy in particular since the discovery of CB 3717 (Chart 1), a folate-based inhibitor of TS that reached Phase I clinical trials in early 1980s.[1−3] Although this compound was withdrawn from the clinic due to undesirable nephrotoxicity, its antitumor activity prompted many research groups to intensify their search for a clinically suitable alternative inhibitor of the enzyme. As a result, raltitrexed, a polyglutamatable inhibitor of TS developed jointly by the Institute of Cancer Reseach and Zeneca Pharmaceuticals, is now widely registered for the treatment of advanced colorectal cancer.[4−7] ZD9331, a nonpolyglutamatable inhibitor of TS which like raltitrexed utilizes the reduced folate carrier (RFC) for cellular entry,[8] is currently under clinical evaluation. In addition, other folate-based inhibitors of TS[9−13] (i.e., LY231514, GW1843, and the lipophilic inhibitor Thymitaq which utilizes neither the RFC nor FPGS) have reached the stage of clinical investigation.[14−18] Our present research program is focused mainly on the synthesis of nonpolyglutamatable inhibitors of the enzyme that do not rely on the reduced folate carrier for cellular entry. Such compounds should circumvent mechanisms of resistance to folate-based inhibitors of TS associated with a decreased/altered folylpolyglutamate synthetase (FPGS) expression and/or a decreased reduced-folate carrier expression. With this aim, we identified a novel class of cyclopenta[*g*]quinazoline-based inhibitors of the enzyme that displayed a low dependency on the RFC for cellular uptake in the L1210 cells. We now report here the synthesis of 13 cyclopenta[*g*]quinazoline-based inhibitors of the TS enzyme.

Design and Synthesis

In the design of this series, the cyclopenta[*g*]quinazoline moiety was chosen because the conformational restriction introduced by the presence of the pentacycle is believed to be favorable for binding to TS.[19] The crystal structure of *Escherichia coli* TS ternary complex with FdUMP and CB3717 indicates that the folate analogue binds in a partially folded conformation with the *p*-aminobenzoate (PABA) moiety inclined at 65° to the quinazolin-4-one ring.[20,21] Furthermore, molecular mechanics analysis of CB3717 using SCANOPT and semiempirical quantum mechanical energy calculations (AMPAC) indicated that a C7-methyl substitution reinforces the binding conformation.[22] Indeed, the 7-Me derivative of ICI 198583 was a more potent inhibitor of TS by 2-fold compared with ICI 198583.[22] This improvement was also seen with compound **1** (Figure 1) which was also a 2-fold better inhibitor of TS compared with

[†] Part of this work has been presented in preliminary form, see: Bavetsias, V.; Marriott, J. H.; Melin, C.; Kimbell, R.; Boyle, F. T.; Jackman, A. L. Synthesis and Antitumor Activity of Cyclopenta[*g*]-quinazoline-Based Antifolates, a Novel Class of Thymidylate Synthase (TS) Inhibitors. In *Chemistry and Biology of Pteridines and Folates*; Pfleiderer, W., Rokos, H., Eds.; Blackwell Science: Berlin, 1997; pp 205−208.

[‡] Abbreviations: TS, thymidylate synthase; FPGS, folylpolyglutamyl synthetase; RFC, reduced folate carrier; DEPC, diethyl phosphorocyanidate; Z, benzyloxycarbonyl; Glu, glutamic acid, Ala, alanine; TFA, trifluoroacetic acid; EDCI 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide; DMAP, 4-(dimethylamino)pyridine; PyBOP, 1*H*-1,2,3-benzotriazol-1-yloxy-tris[pyrrolidino]-phosphonium hexafluorophosphate; CPG_2, carboxypeptidase G_2; MCPBA, *m*-chloroperbenzoic acid; AlaT, 1-(5-tetrazolyl)ethylamine; Meglu, *N*-methylglutamic acid; DIEA, diisopropylethylamine.

[¶] CRC Centre for Cancer Therapeutics.

[§] Zeneca Pharmaceuticals.

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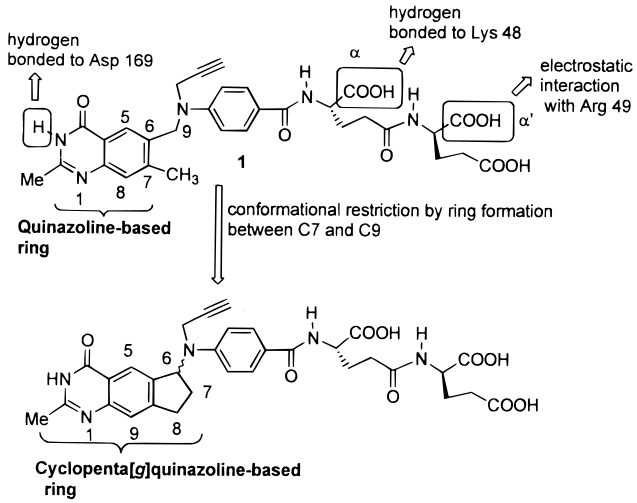
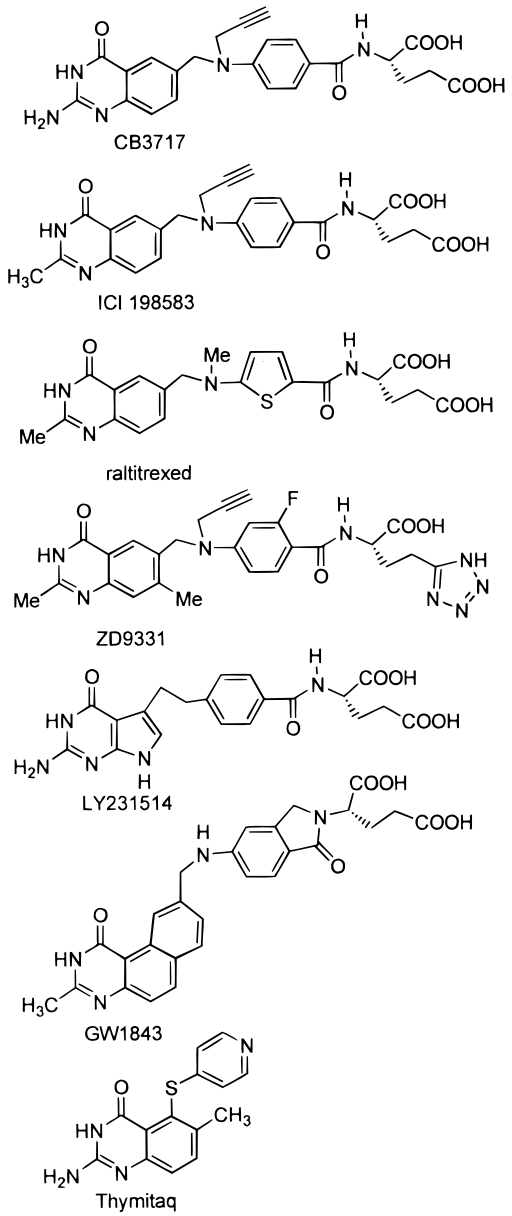


Figure 1. From quinazoline to cyclopenta[*g*]quinazoline-based inhibitors of TS.

Chart 1



its C7-unsubstituted counterpart,[23] leading us to speculate that further conformational restriction in compounds of this type by ring formation between C7 and C9 (Figure 1) would have a beneficial effect on binding to TS. On the basis of our previous experience with the quinazoline-based inhibitors of TS and in particular γ-linked dipeptide derivatives of ICI 198583, two factors governed the choice of the glutamate-derived ligand.[23−25] First, the α-carboxyl of the first Glu residue plays a crucial role for binding to TS since it is hydrogen bonded to Lys48 through a molecule of water while the α-carboxyl of the second (distal) residue in a dipeptide derivative such as **1** interacts electrostatically with Arg49 (Figure 1).[23] Second, some quinazoline-based antifolates bearing ligands such as **5e** and **5k** (Scheme 1) showed low dependency on RFC for cellular uptake.[24,25]

Our approach to the synthesis of this class of compounds is outlined in Scheme 1. In this convergent route, antifolates **7a**−**m** were synthesized by coupling of the acid **3** to the appropriate glutamate-derived ligand **5** via DEPC, pentafluorophenyl ester, or PyBOP activation, followed by the removal of the protecting groups (Scheme 1).

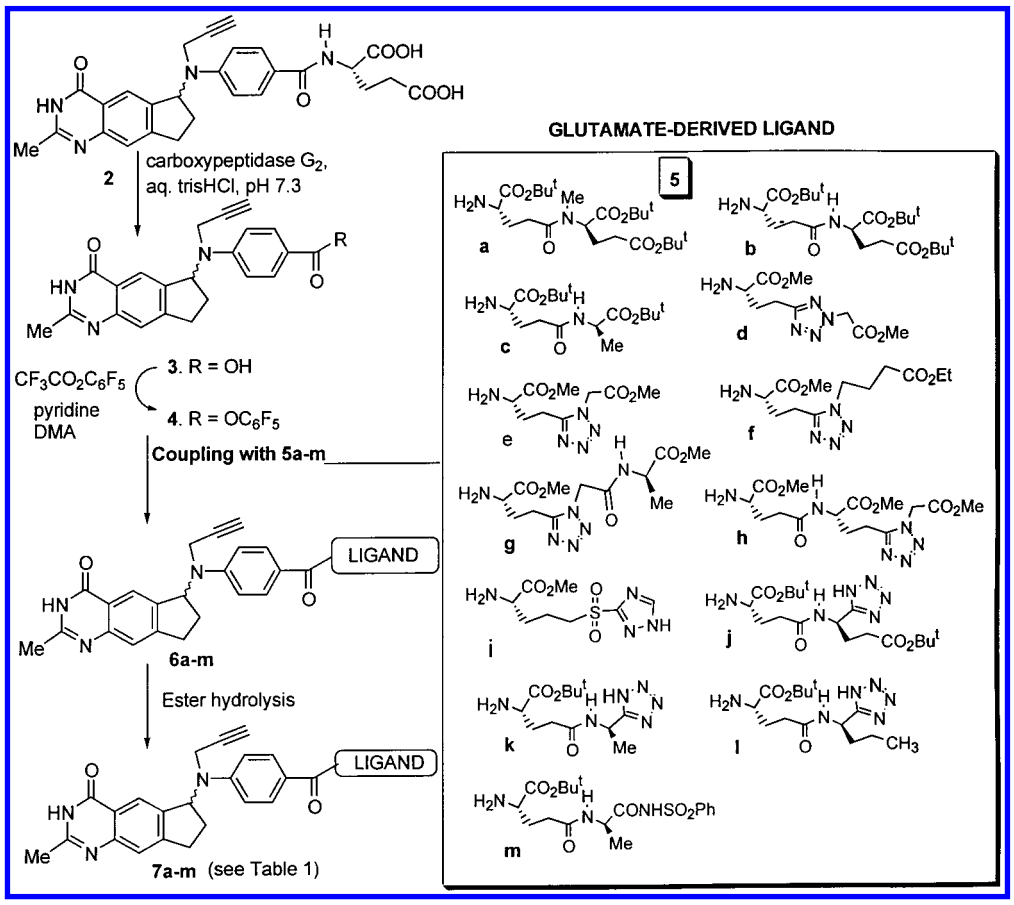
4-[*N*-((6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoic acid (**3**) was obtained from **2** by the enzymatic removal of the glutamyl residue with carboxypeptidase G_2.[26] It should be noted that **3** was a racemate, and hence each final antifolate in this study was obtained as a mixture of two diastereoisomers, in a ratio of approximately 1:1. Chiral HPLC (ASTEC CYCLOBOND I, BETA column) indicated two peaks for compound **3** in a ratio of approximately 1:1. In addition, a number of final compounds were analyzed by chiral HPLC and also gave two peaks in an approximate ratio of 1:1.

The synthetic strategy to antifolates **7a**−**m** required the development of synthetic routes to each individual glutamate-derived ligand.

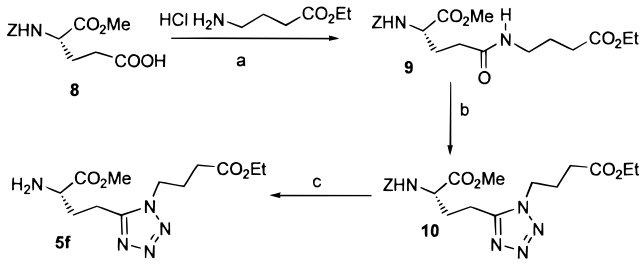
Syntheses of **5a**−**e**, which were required for the preparation of **7a**−**e**, respectively, were as previously described.[23,25,27]

The synthesis of the 1,5-disubstituted tetrazolyl derivative **5f** is shown in Scheme 2. First the γ-glutamyl amide derivative **9** was prepared from α-methyl *N*-(benzyloxycarbonyl)glutamate (**8**) and ethyl γ-aminobutyrate hydrochloride salt via isobutyl mixed anhydride

Scheme 1



Scheme 2[a]



[a] Conditions: (a) $ClCO_2CH_2CH(CH_3)_2$, NMM, THF; (b) PCl_5, quinoline, $CHCl_3$, HN_3 in benzene; (c) H_2, 10% Pd/C.

coupling. The γ-glutamyl amide bond of **9** was converted into the tetrazole ring by treatment with PCl_5, quinoline, and hydrazoic acid as the source of the azide anion.[28] At the final step the Z-group was removed by catalytic hydrogenolysis to give the desired amine **5f**.

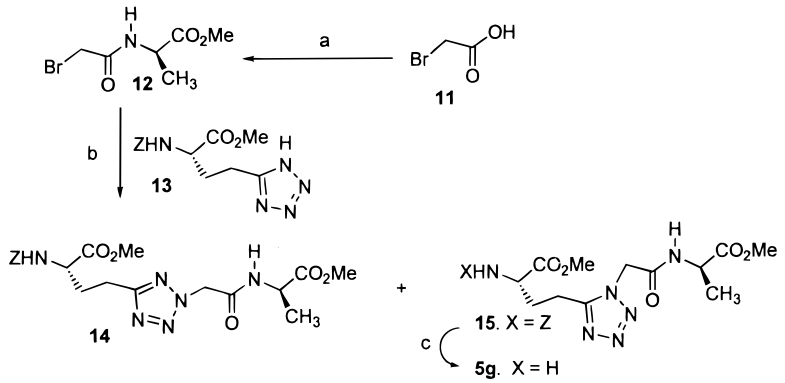
A different approach was employed to prepare the 1,5-disubstituted tetrazolyl derivative **5g** (Scheme 3). The synthesis started with bromoacetic acid (**11**) which was condensed with D-alanine α-methyl ester via isobutyl mixed anhydride activation to give **12**. Subsequent alkylation of the tetrazole ring of **13**[29] with the bromoacetyl derivative **12** resulted in a mixture of two regioisomers **14** and **15**, separable by column chromatography. At the final step, removal of the Z-group from **15** by catalytic hydrogenolysis afforded the desired amine **5g**.

The glutamate-derived ligand **5h** was prepared in two steps from **5e** (Scheme 4). First **5e** was condensed with **8** via isobutyl anhydride coupling to give **16**. Subsequent removal of the Z-group by catalytic hydrogenolysis afforded **5h**.

The route to methyl (2*S*)-2-amino-5-(1*H*-1,2,4-triazol-3-ylsulfonyl)pentanoate (**5i**) is shown in Scheme 5. The primary alcohol **17** was obtained from α-methyl *N*-(benzyloxycarbonyl)glutamate in 52% yield by reducing the ethyl mixed anhydride of **8**, generated in situ, with $NaBH_4$/MeOH.[30] Substitution of the mesylate by 1*H*-1,2,4-triazole-3-thiol in the presence of Et_3N in DMF afforded the sulfide **19** which was oxidized to the sulfone **20** with 2 equiv of MCPBA in $CHCl_3$. Complete removal of the Z-group did not occur during catalytic hydrogenolysis but was achieved satisfactorily by treatment of **20** with 30% HBr in AcOH.

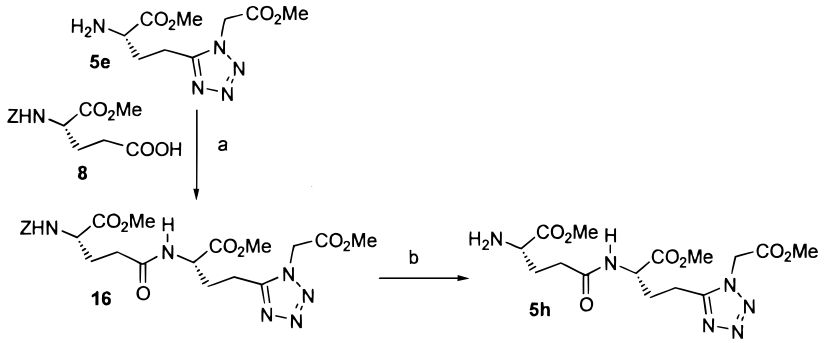
The synthesis of the tetrazolyl acid mimics **5j**−**l** is shown in Scheme 6. The key step to these compounds was the construction of the tetrazole ring which was effected by treatment of the appropriate nitrile with NaN_3/NH_4Cl in DMF.[31] For the synthesis of L-Glu-OBu^t-γ-D-AlaT (**5k**),[32] the starting material was Z-D-Ala (**21k**) which was first converted to the amide **22k** by treatment of the isobutyl mixed anhydride of **21k**, generated in situ, with gaseous ammonia. Subsequent dehydration with *p*-toluenesulfonyl chloride and pyridine in CH_2Cl_2 to the nitrile **23k** followed by its conversion to Z-D-AlaT (**24k**) by treatment with NaN_3 and NH_4Cl in DMF. Z-D-AlaT had an optical rotation of +38 (c = 1, MeOH), virtually identical to that reported by Grzonka and Liberek (+34.5, c = 1, MeOH) who obtained optically pure Z-D-AlaT by resolving Z-DL-AlaT using L-tyrosine hydrazide as the resolving agent.[33] Removal of the Z-group was achieved by catalytic hydrogenolysis, and

Scheme 3[a]



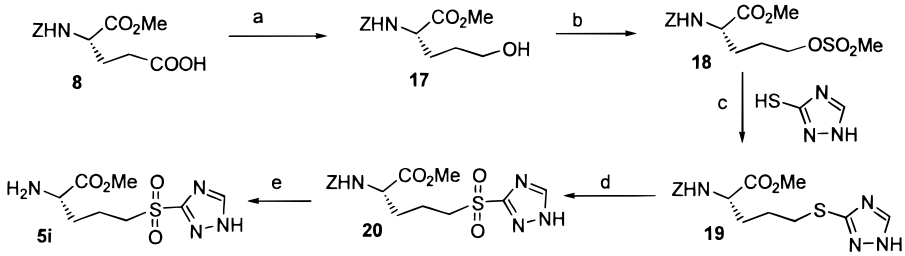
[a] Conditions: (a) (i) $ClCO_2CH_2CH(CH_3)_2$, NMM, THF, (ii) HCl·Ala-OMe, Et_3N, (b) Et_3N, CH_2Cl_2; (c) H_2, 10% Pd/C, AcOEt/EtOH.

Scheme 4[a]



[a] Conditions: (a) $ClCO_2CH_2CH(CH_3)_2$, NMM, THF; (b) H_2, 10% Pd/C, AcOEt.

Scheme 5[a]



[a] Conditions: (a) (i) $ClCO_2Et$, Et_3N, THF, (ii) $NaBH_4$, MeOH; (b) CH_3SO_2Cl, Et_3N, CH_2Cl_2; (c) Et_3N, DMF; (d) MCPBA (2 equiv), $CHCl_3$; (e) H_2, 10% Pd/C, EtOH or 30% HBr in AcOH.

the resultant amine **25k** was coupled to Z-Glu-OBu^t via isobutyl mixed anhydride activation to give the dipeptide analogue **26k**. The target compound **5k** was finally obtained from **26k** by removing the Z-group by catalytic hydrogenolysis (10% palladium on charcoal) in EtOH. Tetrazolyl derivatives **5j,l** were prepared in a similar manner from Z-D-Glu(OBu^t)-OH (**21j**) and norvaline (**21l**), respectively, though the amide **22j** was dehydrated to the nitrile **23j** by using $POCl_3$/pyridine in CH_2Cl_2.

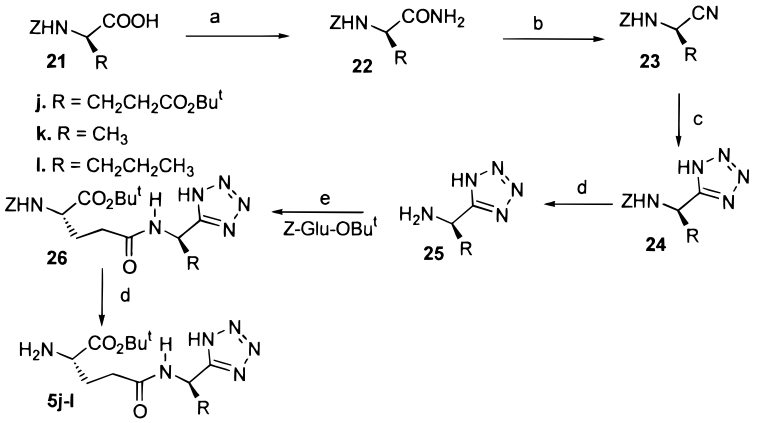
The synthesis of the acyl sulfonamide derivative **5m** is shown in Scheme 7. A variety of coupling reagents failed to yield sulfonamide **27** from Z-D-Ala and benzenesulfonamide. This condensation was finally effected by using EDCI as coupling reagent with catalytic amounts of DMAP in CH_2Cl_2. Catalytic hydrogenolysis of **27** afforded the D-alanine derivative **28** which was coupled to Z-Glu-OBu^t via isobutyl mixed anhydride activation.

Biological Evaluation

The antifolates listed in Table 1 were tested as inhibitors of partially purified TS from L1210 mouse leukemia cells that overproduce TS due to amplification of the TS gene. The partial purification and assay method used in this study was as previously described.[34] Kiapps were performed with CB3717 as a control (mean Kiapp over several experiments is 20 nM). First an inverse relative potency is obtained (Kiapp of test compound/Kiapp of CB3717). This is then multiplied by 20 (the mean Kiapp of CB3717), allowing comparisons of Kiapps to be made between experiments.

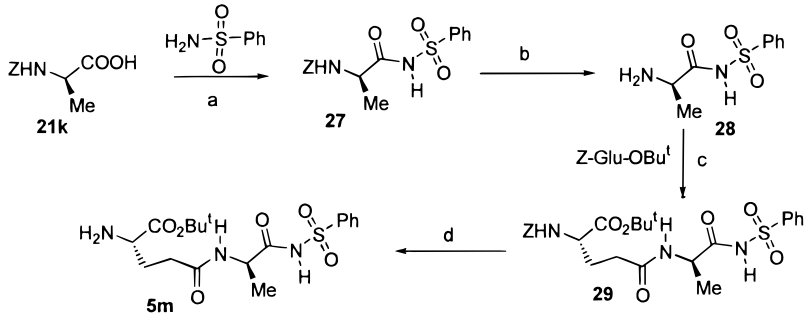
Inhibition of L1210 and L1210:1565 cell growth was also determined as previously described.[35] L1210:1565 is a variant L1210 cell line with a deficient folate/MTX transport via the RFC.[35] This cell line was made resistant to CI-920, a compound that uses the RFC transport system.[36] The L1210:1565 cell line harbors a single mutation in the open reading frame of RFC1 which results in premature stop at amino acid 26. [37]

Scheme 6[a]



[a] Conditions: (a) (i) $ClCO_2CH_2CH(CH_3)_2$, NMM, THF, (ii) NH_3; (b) pyridine, *p*-toluenesulfonyl chloride, CH_2Cl_2 (for the preparation of **23j**, $POCl_3$/pyridine in CH_2Cl_2 was employed); (c) NaN_3, NH_4Cl, DMF; (d) H_2, 10% Pd/C, EtOH; (e) $ClCO_2CH_2CH(CH_3)_2$, NMM, THF.

Scheme 7[a]

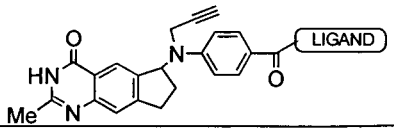


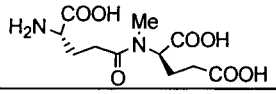
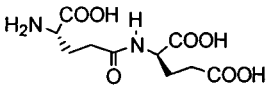
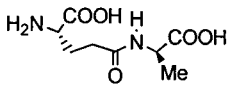
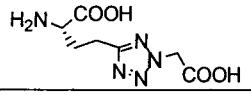
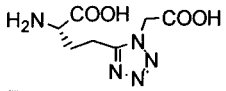
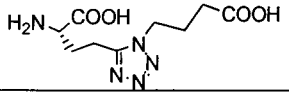
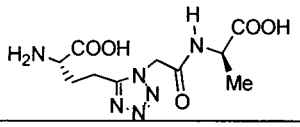
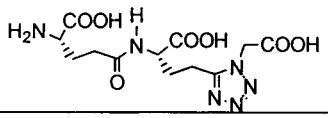
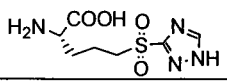
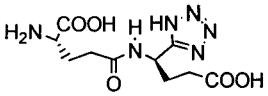
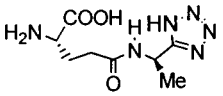
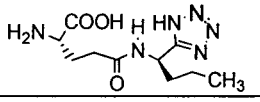
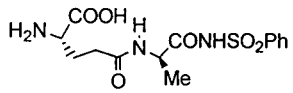
[a] Conditions: (a) DMAP, EDCl, CH_2Cl_2; (b) H_2, Pd/C, MeOH; (c) $ClCO_2CH_2CH(CH_3)_2$, NMM, THF; (d) H_2, Pd/C, EtOH.

Results and Discussion

The cyclopenta[*g*]quinazoline-based L-Glu-γ-D-Glu dipeptide derivative **7b** was a very potent inhibitor of TS (Kiapp = 0.42 nM, Table 1), 5-fold more potent than its quinazoline-based counterpart **1** (TS Kiapp = 2.0 nM),[23] suggesting that the conformational restriction introduced by the presence of the pentacycle was beneficial on binding to TS. Despite this increased activity, **7b** showed a similar inhibitory activity against L1210 cell growth compared with **1** but was ~5-fold more potent than **1** against the L1210:1565 cell line (resistance factor of 7), suggesting that **7b** may use the RFC less efficiently than **1**. However, both these compounds have low affinities for the RFC as measured by the inhibition of [^{3}H]MTX uptake (for **1** K_i = ~200 μM, for **7b** K_i = ~150 μM, for MTX K_i = 3.5 μM). Indeed, it is a feature of this class of dipeptide analogues that the affinity for the RFC does not always correlate with the activity in the L1210:1565 cell line. This suggests that there may be a poor relationship between affinity for the RFC and rate of internalization via the RFC. Subsequently, compound **7b** served as the main structural template for the exploration of the SAR, in particular with regard to overcoming resistance in the L1210:1565 cell line. Replacement of the D-Glu of **7b** with D-Ala gave **7c** that displayed similar TS and L1210 cell growth inhibitory activities to **7b** and a low L1210:1565/L1210 resistance factor (3). Similarly, replacement of the γ-amidic hydrogen of **7b** with a methyl group (to give compound **7a**) resulted in a ~7-fold increase in the L1210 IC_{50} and a low resistance factor (2), most probably due to a low rate of uptake via the RFC. One of the most interesting results was obtained when the α-carboxyl of the distal glutamyl residue in **7b** was replaced with a tetrazolyl ring to give compound **7j**, the most potent inhibitor of TS in this series. This compound displayed equal potency in both cell lines, clearly overcoming resistance in the L1210 cells (K_i for the inhibition of [^{3}H]-MTX = 106 μM). However, no advantage in terms of TS inhibition was observed when the same modification (tetrazolyl ring) was introduced in the L-Glu-γ-D-Ala dipeptide derivative **7c** to give compound **7k** (Table 1). A different acid mimic, the acylsulfonamide derivative **7m** was also synthesized, and although it was as potent against TS as **7k**, it had reduced activity against the L1210:1565 cell line giving a significant level of cross-resistance (ratio of 5). However, the K_i values for the inhibition of [^{3}H]MTX uptake were higher for **7m** (K_i = 40 μM) than for **7k** (K_i = 14 μM). The SAR on the tetrazolyl acid mimics series was further explored by replacing the propanoic chain of **7j** with a propyl group to give **7l**. This compound was a less potent inhibitor of TS (~10-fold) but more potent against L1210 cells. The Ll210:1565/L1210 resistance factor of 7 and the relatively low K_i for the inhibition of [^{3}H]MTX uptake (10 μM) suggest that this may be because of increased cellular uptake via the RFC. The 1,2,4-triazole derivative **7i** was interesting in that it was both potent against L1210 cells (IC_{50} = 0.49 μM) and

Table 1. Cyclopenta[*g*]quinazoline-based Inhibitors of TS



Compound	Ligand	L1210TS Kiapp, (nM)	L1210 IC_{50} (μM)	L1210:1565 IC_{50}, (μM) resistance factors in parentheses
1	for structure see figure 1	2.0	0.22±0.22	14,8 (50)
7a		0.78	2.3, 1.8	4.4, 3.0 (2)
7b		0.42	0.3 ± 0.07	2.2 ± 0.28 (7)
7c		1.1	0.42 ± 0.09	1.4 ± 0.39 (3)
7d		1.7	0.18+0.026	1.3, 0.84 (8)
7e		2.4, 1.8	1.7, 2.0	3.5, 4.1 (2)
7f		0.9, 2.0	2.7 ± 0. 33	2.3 + 0.12 (1)
7g		2.2	19 ± 6.1	13 ± 3.0 (0.7)
7h		0.26	7.2, 7.2	3.9, 3.0 (0.5)
7i		0.78	0.49 ± 0.18	0.68 ± 0.24 (1)
7j		0.2	1.5 ±0.54	1.3 ± 0.45 (1)
7k		2.2	0.64±0.11	1.2, 1.6 (2)
7l		2.6	0.44±0.067	3.6, 2.7 (7)
7m		2.4	1.9±0.26	8.2, 7.8 (5)

completely overcame resistance in the L1210:1565 cells (resistance factor of 1). Nevertheless, the K_i for inhibition of [^{3}H]MTX uptake was only 6 μM, which again illustrates lack of correlation between these two measurements.

In the quinazoline-based series of inhibitors of TS it was observed that compounds bearing **5d**, and in particular **5e**, as a glutamate-derived ligand displayed rather low L1210:1565/L1210 resistance factors.[25] This prompted the synthesis of 2,5- and 1,5-disubstituted

tetrazolyl derivatives **7d** and **7e**, respectively. Although **7d** and **7e** displayed similar TS inhibitory activities it appeared that they differ in their transport properties since **7e** was ~10-fold less potent against L1210 cells than **7d**. The L1210:1565/L1210 resistance factor of 2 suggests that this lower potency in L1210 cells may be due to a low rate of uptake via RFC. Subsequently, the SAR was further explored by synthesising three more 1,5-disubstituted tetrazolyl derivatives, **7f**−**h**. All three compounds, and in particular **7h**, were potent inhibitors of the TS enzyme and had L1210:1565/L1210 resistance factors close to 1 which indicates low (if any) reliance on RFC for cellular uptake (**7h** K_i for inhibition of [^{3}H]-MTX uptake = 69 μM). However, this apparent loss of interaction with RFC is at the expense of low cytotoxic potency; compounds **7e** and **7f** were the most potent in this series.

Regarding FPGS activity, we believe that for the majority of these antifolates polyglutamation is not occurring since the glutamate moiety associated with classical antifolates is now structurally modified. Indeed, previous studies from our laboratories with quinazoline-based antifolates bearing glutamate-derived ligands (e.g., L-Glu-γ-D-Glu) indicated no cross-resistance in the L1210:R^{D1694} cell line. In this cell line the predominant mechanism of resistance is a decreased ability to polyglutamate synthetic antifolates.[38]

In conclusion, by utilizing our understanding of how quinazoline-based inhibitors of TS bind to the active site of the humanized *E. coli* thymidylate synthase, we rationally designed and synthesized a series of cyclopenta[*g*]quinazoline-based inhibitors. We used the L1210 and L1210:1565 (inoperative RFC) cell lines to evaluate both cytotoxic potency and whether the new analogues overcame resistance. It was found that some of the compounds presented in this study do not apparently use the RFC as a transport mechanism in mouse L1210 cells. The mechanism by which these compounds enter cells it is still not clear but is being investigated. These compounds are also being evaluated in human cell lines which display different levels of RFC and other folate transporters.

Experimental Section

Thin-layer chromatography (TLC) was performed on precoated sheets of silica $60F_{254}$ (Merck Art 5735). Visualization was achieved by UV or Arnold's base (4,4′-methylenebis-*N*,*N*-dimethylaniline) reagent which was prepared and used as follows: Arnold's base (0.19 g) was dissolved in glacial acetic acid (30 mL) and the solution was diluted with water (500 mL). To this solution was added potassium iodide (1 g). First the TLC plate was placed into a chlorine atmosphere for 3−5 min. The chlorine atmosphere was generated in a desiccator by the addition of a few drops of concentrated HCl to $KMnO_4$ contained in a 25 mL beaker. After the excess chlorine had been removed by drying with a hair drier, the TLC plate was sprayed with Arnold's base solution, left for a few seconds, and finally dried well using a hair drier. Primary or secondary amines usually show up as blue spots. Merck silica 60 (Art 15111) was used in low-pressure column chromatography. Petrol refers to light petroleum (bp 60−80 °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). 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.

4-[*N*-((6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoic Acid (3). Compound **2**[26] (1.21 g, 2.4 mmol) was dissolved in a solution of tris (1.83 g, 15.1 mmol) in H_2O (137 mL). $ZnCl_2$ (6.4 mg) was added and the pH adjusted to 7.3 with 2 M HCl (~4 mL required). The resulting homogeneous solution was made up to 151 mL with H_2O. A solution of CPG_2 (404 units) in 0.9% aqueous NaCl (1.1 mL) was then added, and the resulting solution was incubated with shaking at 37 °C (in a 250 mL flask). After 31 h a further portion of CPG_2 (424 units) in 0.9% aqueous NaCl (1.9 mL) was added, and incubation with shaking at 37 °C was continued for a further 38 h. TLC of supernatant (Bu^nOH−AcOH−H_2O, v/v/v 5:2:3) indicated the presence of the product and no starting material. The mixture was cooled in an ice bath and acidified to pH 4 with glacial AcOH, and the resulting suspension was centrifuged. The precipitate was washed three times by resuspension in H_2O, centrifugation, and removal of the supernatant. The final precipitate was frozen in an ice−salt bath, thawed, centrifuged, and dried after pipetting off further H_2O which separated (0.913 g): mp >325 °C; ^{1}H NMR (DMSO-d_6) 2.21 (m, 1H, 7-H), 2.33 (s, 3H, 2-CH_3), 2.50 (m, 1H, 7-H-obscured by solvent signal), 3.01 (m, 1H, 8-H), 3.16 (m, 2H, 8-H and C≡CH), 3.87 (m, 1H, CH_2C≡C), 4.05 (m, 1H, CH_2C≡C), 5.77 (t, J = 8.0 Hz, 1H, 6-H), 7.03 (d, J = 9.1 Hz, 2H, 3′,5′-ArH), 7.49 (s, 1H, 9-H), 7.78 (s, 1H, 5-H), 7.81 (d, J = 8.9 Hz, 2H, 2′,6′-ArH), 12.14 (br s, 1H, NH), 12.30 (br s, 1H, COOH); MS (FAB, *m*/*z*) 374 (M + H)$^+$. Anal. ($C_{22}H_{19}N_3O_3$·0.5H_2O) C, H, N.

Pentafluorophenyl 4-[*N*-((6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoate (4). To a stirred solution of **3** (0.169 g, 0.45 mmol) in dry DMA (17 mL) under argon was added dry pyridine (0.08 mL, 1.0 mmol) followed by pentafluorophenyl trifluoroacetate (0.11 mL, 0.6 mmol). Stirring was continued at room temperature for 100 min, then a fresh portion of pentafluorophenyl trifluoroacetate (0.11 mL, 0.6 mmol) was added. After 4 h total reaction time the reaction mixture was concentrated in vacuo to an oily residue. Purification by column chromatography, on elution with a gradient of EtOH in CH_2Cl_2 (0 to 4%), afforded a white solid. This was triturated with hexanes, collected by filtration, and dried in vacuo over P_2O_5 to afford the title compound **4** as a white solid (0.193 g, 79%): mp 250−252 °C; ^{1}H NMR (DMSO-d_6) 2.29 (m, 1H, 7-H), 2.34 (s, 3H, 2-CH_3), 2.50 (m partly obscured, 1H, 7-H), 3.04 (m, 1H, 8-H), 3.15 (m, 1H, 8-H), 3.39 (m, 1H, C≡CH), 4.05 (AB system, 2H, J = 20.4 Hz, CH_2C≡C), 5.88 (t, J = 8.0 Hz, 1H, 6-H), 7.17 (d, J = 9.1 Hz, 2H, 2′,6′-ArH), 8.03 (d, J = 9.0 Hz, 2H, 3′,5′-ArH), 7.51 (s, 1H, 9-H), 7.80 (s, 1H, 5-H), 12.19 (br s, 1H, N^3-H); MS (FAB, *m*/*z*) 540 (M + H)$^+$. Anal. ($C_{28}H_{18}F_5N_3O_3$·0.3H_2O) C, H, N, F.

Preparation of Glutamate-Derived Ligands 5. Ethyl 4-[α-Methyl *N*-(Benzyloxycarbonyl)-L-γ-glutamyl]aminobutyrate (9). To a stirred under argon solution of α-methyl *N*-benzyloxycarbonyl-L-glutamate (2.66 g, 9.0 mmol) in anhydrous THF (18 mL) cooled to −20 °C was added NMM (0.909 g, 9.0 mmol) followed by i-BuOCOCl (1.23 g, 9.0 mmol). The resulting white suspension was stirred at −20 °C for 10 min, and then a slurry of ethyl 4-aminobutyrate hydrochloride (1.51 g, 9.0 mmol) in NMM (0.909 g, 9.0 mmol) and THF (15 mL) was added into the reaction mixture. Stirring was continued at −20 °C for 10 min, then the dry ice−acetone bath was removed, and the reaction mixture was allowed to stir for a further 2 h. The *N*-methylmorpholine hydrochloride was removed by filtration and the filtrate was concentrated in vacuo to give an orange oily residue. Purification by column

chromatography, on elution first with 30% AcOEt in CH_2Cl_2 and then 50% AcOEt in CH_2Cl_2, gave the title compound **9** as a white solid (2.65 g, 72%), mp 80−81 °C: ^{1}H NMR (DMSO-d_6) 1.18 (t, J = 7.03 Hz, 3H, OCH_2CH_3), 1.56−2.05 (m, 4H, $CHCH_2CH_2CONH$ and $CONHCH_2CH_2CH_2CO_2Et$), 2.15 (t, J = 7.23 Hz, $CHCH_2CONH$), 2.28 (t, J = 7.45 Hz, $CONHCH_2CH_2CH_2CH_2CO_2Et$), 3.04 (q, J = 6.60 Hz, 2H, $CONHCH_2CH_2CH_2CO_2Et$), 3.63 (s, 3H, CO_2Me), 4.03 (m, 3H, $CO_2CH_2CH_3$, α-CH), 5.04 (s, 2H, $PhCH_2$), 7.36 (m, 5H, Ph), 7.78 (d, J = 7.7 Hz, 1H, OCONH), 7.86 (t, J = 5.06 Hz, 1H, $CH_2CONHCH_2$); MS (FAB, m/z) 431 (M + Na)$^+$. Anal. ($C_{20}H_{28}N_2O_7$) C, H, N.

Methyl 2-[*N*-(Benzyloxycarbonyl)amino]-4-(1-ethoxycarbonylpropyltetrazol-5-yl)butyrate (10). To a stirred under argon mixture of PCl_5 (1.13 g, 5.2 mmol) in $CHCl_3$ (12 mL) was added quinoline (1.33 g, 10.3 mmol); a pale yellow precipitate had formed. Stirring was continued at room temperature for 20 min under argon, and then a solution of **9** (2.1 g, 5.2 mmol) in $CHCl_3$ (10 mL) was slowly added into the reaction mixture while the temperature was maintained below 20 °C. Stirring was continued for 25 min at a temperature below 20 °C, then a freshly prepared solution of HN_3 in benzene[39] (15 mL, *caution*: it is poisonous) was added, and the yellow solution was stirred at room temperature for 2 h. More HN_3 in benzene (6 mL) was added, and the reaction mixture was stirred for a further 1.5 h before being concentrated in vacuo. The oily residue was partitioned between AcOEt (150 mL) and H_2O (150 mL). The two layers were separated, and the organic layer was washed with 1 N HCl (150 mL), half-saturated $NaHCO_3$ solution (150 mL), and H_2O (150 mL), dried (Na_2SO_4), and concentrated in vacuo to an orange oily residue. Purification by column chromatography, on elution with a gradient of AcOEt in hexanes (50 to 60%), afforded the title compound **10** as a colorless gum (0.63 g, 28%): ^{1}H NMR (DMSO-d_6) 1.15 (t, J = 7.12 Hz, 3H, OCH_2CH_3), 1.95−2.20 (m, 4H, $CHCH_2CH_2$ and $CN_4CH_2CH_2CH_2CO_2Et$), 2.36 (t, J = 7.28 Hz, 2H, $CN_4CH_2CH_2CH_2CO_2Et$) 2.94 (t, J = 7.92 Hz, 2H, $CHCH_2CN_4$), 3.64 (s, 3H, CO_2Me), 4.00 (q, J = 7.07 Hz, 2H, $CO_2CH_2CH_3$), 4.21 (m, 1H, CONHC*H*), 4.33 (t, J = 7.05 Hz, 2H, $CN_4CH_2CH_2CH_2CO_2Et$), 5.04 (s, 2H, $PhCH_2$), 7.36 (m, 5H, Ph), 7.89 (d, J = 7.9 Hz, 1H, OCONH); MS (FAB, m/z) 456 (M + Na)$^+$. Anal. ($C_{20}H_{27}N_5O_6$) C, H, N.

Methyl 2-Amino-4-(1-ethoxycarbonylpropyltetrazol-5-yl)butyrate (5f). To a solution of **10** (0.240 g, 0.55 mmol) in AcOEt (30 mL) was added 10%Pd/C (60 mg). The resulting mixture was stirred for 4 h under H_2 (balloon). The catalyst was removed by filtration, and the filtrate was concentrated in vacuo to give the title compound **5f** as a colorless oil (0.164 g, 100%): ^{1}H NMR (DMSO-d_6) 1.17 (t, J = 6.93 Hz, 3H, OCH_2CH_3), 1.80−2.15 (m, 4H, $CHCH_2CH_2$ and $CN_4CH_2CH_2CH_2CH_2CO_2Et$), 2.39 (t, J = 7.2 Hz, 2H, $CHCH_2CN_4$), 2.96 (t, J = 7.5 Hz, 2H, $CN_4CH_2CH_2CH_2CO_2Et$), 3.39 (dd, J = 4.9, 8.4 Hz, 1H, α-C*H*), 3.62 (s, 3H, CO_2Me), 4.05 (q, J = 7.10 Hz, 2H, $CO_2CH_2CH_3$), 4.37 (t, J = 6.9 Hz, 2H, $CN_4CH_2CH_2CH_2CO_2Et$); MS (FAB, m/z) 322 (M + Na)$^+$.

Methyl (2*R*)-2-[*N*-(Bromoacetyl)amino]propanoate (12). To a stirred solution of bromoacetic acid (1.00 g, 7.2 mmol) in anhydrous THF (10 mL) cooled to −10 °C and under argon was added NMM (0.72 g, 7.2 mmol) followed by i-BuOCOCl (0.98 g, 7.2 mmol) (a white precipitate had formed). Stirring was continued at −10 °C for 7 min, and then a slurry of D-alanine hydrochloride methyl ester (1.00 g, 7.2 mmol) in anhydrous THF (12 mL) and NMM (0.720 g, 7.2 mmol) was added into the reaction mixture. Stirring was continued at −10 °C for 10 min, then the dry ice/acetone bath was removed, and the reaction mixture was allowed to stir for a further 15 min. The *N*-methylmorpholine hydrochloride was removed by filtration, and the filtrate was concentrated in vacuo to give a pale yellow oil. Purification by column chromatography, on elution with 40% AcOEt in hexanes, afforded a colorless oil which solidified on standing at room temperature to afford the title compound **12** as a white solid (1.0 g, 63%), mp 51−52 °C: ^{1}H NMR (DMSO-d_6) 1.28 (d, J = 7.3 Hz, 3H, $CHCH_3$), 3.63 (s, 3H, OCH_3), 3.88 (s, 2H, $BrCH_2$), 4.27 (m, 1H, C*H*CH$_3$), 8.76 (d, J = 6.9 Hz, 1H, CONH); MS (FAB, m/z) 224, 226 [(M + H)$^+$, bromine isotopic pattern]. Anal. ($C_6H_{10}BrNO_3$) C, H, N; Br: calcd 35.66; found 34.96.

Methyl (2*S*)-2-[*N*-(Benzyloxycarbonyl)amino]-4-{1-[((1*R*)-1-(methoxycarbonyl)ethyl)carbamoylmethyl]-tetrazol-5-yl}butyrate (15). To a stirred solution of **12** (0.270 g, 1.2 mmol) in anhydrous CH_2Cl_2 (2 mL) was added methyl (2*S*)-2-(benzyloxycarbonylamino)-4-(tetrazol-5-yl)butyrate[29] (**13**) (0.319 g, 1.0 mmol) followed by Et_3N (0.121 g, 1.2 mmol). Stirring was continued at room temperature for 24 h under argon (a white precipitate was obtained). The reaction mixture was then diluted with AcOEt (10 mL), and the white precipitate was filtered off and washed with more AcOEt (∼15 mL). The filtrate was concentrated in vacuo to an oily residue which was purified by column chromatography using a gradient of AcOEt in hexanes (60 to 80%) as eluant. There was thus obtained in order of elution:

(1) Methyl (2*S*)-2-[*N*-(benzyloxycarbonyl)amino]-4-{2-[((1R)-1-(methoxycarbonyl)ethyl)carbamoylmethyl]tetrazol-5-yl}-butyrate (**14**) as a gum which solidified on standing at room temperature to a white solid (0.105 g, 23%): mp 106−107 °C; ^{1}H NMR (DMSO-d_6) 1.32 (d, J = 7.3 Hz, 3H, $CHCH_3$), 1.90−2.20 (m, 2H, $CHCH_2CH_2$), 2.91 (t, J = 6.7 Hz, 2H, $CHCH_2CH_2$), 3.63 (s, 6H, 2 × CO_2CH_3), 4.16 (m, 1H, ZHNC*H*), 4.31 (m, 1H, C*H*CH$_3$), 5.04 (s, 2H, $PhCH_2$), 5.44 (s, 2H, NCH_2CONH), 7.36 (m, 5H, Ph), 7.93 (d, J = 7.8 Hz, 1H, Z*H*NCH), 8.97 (d, J = 7.0 Hz, 1H, N*H*CHCH$_3$); MS (FAB, m/z) 463 (M + H)$^+$.

(2) The desired product methyl (2*S*)-2-[*N*-(benzyloxycarbonyl)amino]-4-{1-[((1*R*)-1-(methoxycarbonyl)ethyl)carbamoylmethyl]-tetrazol-5-yl}butyrate (**15**) as a gum which was solidified on standing at room temperature. This was triturated with CH_2Cl_2/hexanes to give a white solid which collected by filtration (0.242 g, 52%), mp 153−154 °C: ^{1}H NMR (DMSO-d_6) 1.31 (d, J = 7.3 Hz, 3H, $CHCH_3$), 1.95−2.20 (m, 2H, $CHCH_2CH_2$), 2.88 (t, J = 6.6 Hz, 2H, $CHCH_2CH_2$), 3.62, 3.64 (2 × s, 6H, 2 × CO_2CH_3), 4.15−4.40 (m, 2H, ZHNC*H* and C*H*CH$_3$), 5.04 (s, 2H, $PhCH_2$), 5.21 (s, 2H, NCH_2CONH), 7.36 (m, 5H, Ph), 7.90 (d, J = 7.9 Hz, 1H, Z*H*NCH), 8.99 (d, J = 7.0 Hz, 1H, N*H*CHCH$_3$); MS (FAB, m/z) 463 (M + H)$^+$.

Anal. ($C_{20}H_{26}N_6O_7$) C, H, N.

Methyl (2*S*)-2-Amino-4-{1-[((1*R*)-1-(methoxycarbonyl)-ethyl)carbamoylmethyl]tetrazol-5-yl}butyrate (5g). To a stirred solution of **15** (0.266 g, 0.58 mmol) in AcOEt (25 mL) and EtOH (10 mL) was added 10% Pd/C (0.050 g). The mixture was stirred at 24 °C for 4 h under H_2. The palladium catalyst was removed by filtration, the filtrate was concentrated in vacuo, and the resulting residue was dried in vacuo over P_2O_5 to afford the title compound **5g** as a white solid (0.169 g, 90%), mp 87−89 °C: ^{1}H NMR (DMSO-d_6) 1.32 (d, J = 7.3 Hz, 3H, $CHCH_3$), 1.90, 2.02 (2 × m, 2H, $CHCH_2CH_2$), 2.88 (t, J = 7.3 Hz, 2H, $CHCH_2CH_2$), 3.38 (dd(obscured), J = 4.8, 8.7 Hz, 1H, H_2NCH), 3.61, 3.63 (2 × s, 6H, 2 × CO_2CH_3), 4.30 (m, 1H, C*H*CH$_3$), 5.21 (ABq, J = 16.7 Hz, 2H, NCH_2CONH), 8.99 (d, J = 7.0 Hz, 1H, N*H*CHCH$_3$); MS (FAB, m/z) 329 (M + H)$^+$.

Methyl (2*S*)-2-{*N*-[α-Methyl-*N*-(benzyloxycarbonyl)-L-γ-glutamyl]amino}-4-(1-methoxycarbonylmethyltetrazol-5-yl)butyrate (16). To a stirred solution of α-methyl *N*-(benzyloxycarbonyl)-L-glutamate (0.295 g, 1.0 mmol) in dry THF (5 mL) and NMM (0.100 g, 1.0 mmol) cooled to −20 °C was added i-BuOCOCl (0.137 g, 1.0 mmol) (a white precipitate had formed). Stirring was continued at −20 °C for 10 min, and then a solution of methyl (2*S*)-2-amino-4-(1-methoxycarbonylmethyltetrazol-5-yl)butyrate[25] (**5e**) (0.260 g, 1.0 mmol) in dry THF (4 mL) was added into the reaction mixture which was stirred at −20 °C for 10 min and then at room temperature for 1.5 h. The *N*-methylmorpholine hydrochloride was removed by filtration, and the filtrate was concentrated in vacuo to a colorless viscous oil. This was twice chromatographed, first on elution with 1% MeOH in AcOEt and then on elution with 30% CH_2Cl_2 in AcOEt. The title compound **16** was obtained as a viscous oil (0.467 g, 87%) which solidified on standing at −20 °C for a few weeks: mp 64−65 °C; ^{1}H NMR (DMSO-d_6) 1.80, 1.90−2.20 (2 × m, 4H, 2 × $CHCH_2CH_2$), 2.24 (t, J = 7.5 Hz, 2H, $CHCH_2CH_2CONH$), 2.87 (t, J = 8.0 Hz, 2H, $CHCH_2CH_2$), 3.61, 3.62, 3.72 (3 × s, 9H, 3 × CO_2Me), 4.04 (m, 1H, ZHNC*H*CO_2-

Me), 4.40 (m, 1H, $CH_2CONHCHCO_2Me$), 5.01 (s, 2H, $PhCH_2$), 5.50 (s, 2H, NCH_2CO_2Me), 7.36 (m, 5H, Ph), 7.78 (d, J = 7. 9 Hz) and 8.36 (d, J = 7.6 Hz), 2H, 2 × CONH); MS (CI, m/z) 535 $(M + H)^+$. Anal. ($C_{23}H_{30}N_6O_9$) C, H, N.

Methyl (2*S*)-2-[*N*-(α-Methyl-L-γ-glutamyl)amino]-4-(1-methoxycarbonylmethyltetrazol-5-yl)butyrate (5h). To a solution of **16** (0.309 g, 0.58 mmol) in AcOEt (25 mL) was added 10% Pd/C (0.046 g). The mixture was stirred at room temperature (11 °C) for 7 h under H_2. TLC (20% CH_2Cl_2 in AcOEt) indicated incomplete reaction. More catalyst (0.045 g) was added, and stirring was continued at 22 °C for 16 h under H_2. The catalyst was then removed by filtration, and the filtrate was concentrated in vacuo to give the title compound **5h** (0.220 g, 96%) as a viscous oil: ^{1}H NMR (DMSO-d_6) 1.60, 1.80, 2.10 (3 × m, 4H, 2 × $CHCH_2CH_2$), 2.23 (t, J = 8.0 Hz, 2H, $CHCH_2CH_2CO$), 2.88 (t, J = 8.0 Hz, 2H, $CHCH_2CH_2$), 3.29 (dd, J = 5.2, 8.1 Hz, 1H, H_2NCHCO_2Me), 3.62, 3.73 (2 × s, 9H, 3 × CO_2Me), 4.38 (m, 1H, $CH_2CONHCHCO_2Me$), 5.52 (s, 2H, NCH_2CO_2Me), 8.37 (d, J = 7.6 Hz), 1H, CONH); MS (ESI, m/z) 401 $(M + H)^+$.

Methyl (2*S*)-2-[*N*-(Benzyloxycarbonyl)amino]-5-(hydroxy)pentanoate (17). To a stirred solution of α-methyl *N*-(benzyloxycarbonyl)-L-glutamate (4.01 g, 13.6 mmol) in dry THF (33 mL) cooled to −10 °C and under argon was added Et_3N (2.05 g, 20.3 mmol) followed by EtOCOCl (1.83 g, 17.0 mmol). After stirring at −10 °C for 10 min, $NaBH_4$ (1.54 g, 40.7 mmol) was added in one portion followed by dropwise addition of MeOH (40 mL) over a 15 min period while the temperature was maintained below 0 °C. Stirring was continued at 0 °C for 40 min, and then the reaction mixture was neutralized with 1 N aqueous NaOH. The organic solvents were then removed in vacuo, and the residue was extracted with AcOEt (2 × 180 mL). The combined AcOEt extracts washed with saturated aqueous $NaHCO_3$ (2 × 100 mL) and H_2O (100 mL), dried (Na_2SO_4), and concentrated in vacuo to an oily residue. This was purified by column chromatography using a gradient of AcOEt in hexanes (50 to 90%) as eluant to afford the title compound **17** (1.98, 52%) as a colorless oil: ^{1}H NMR (DMSO-d_6) 1.40−1.80 (m, 4H, 3-CH_2 and 4-CH_2), 3.37 (q (obscured), 2H, J = 5.9 Hz, CH_2OH), 3.62 (s, 3H, CO_2Me), 4.02 (m, 1H, 2-CH), 4.47 (t, J = 5.2 Hz, CH_2OH, exchangeable with D_2O), 5.03 (s, 2H, $PhCH_2$), 7.35 (m, 5H, Ph), 7.77 (d, J = 7.7 Hz, 1H, CONH); MS (FAB, m/z) 304 $(M + Na)^+$, 282 $(M + H)^+$. Anal. ($C_{14}H_{19}NO_5$) C, H, N.

Methyl (2*S*)-2-[*N*-(Benzyloxycarbonyl)amino]-5-(methylsulfonyloxy)pentanoate (18). To a solution of **17** (1.84 g, 7.0 mmol) in CH_2Cl_2 (27 mL) cooled to −10 °C were added Et_3N (1.057 g, 10.47 mmol) and then MsCl (0.99 g, 8.7 mmol) over a 2 min period. Stirring was continued for 35 min while the temperature was maintained below 0 °C. The reaction mixture was then diluted with CH_2Cl_2 (200 mL) and washed with H_2O (100 mL), 10% aqueous citric acid (2 × 100 mL), saturated aqueous $NaHCO_3$ (100 mL), and dilute brine (100 mL), dried (Na_2SO_4), and concentrated in vacuo to a yellow oily residue. Purification by column chromatography, on elution with 1:1 v/v AcOEt/hexanes, afforded the title compound **18** as a colorless viscous oil (2.40 g, 96%): ^{1}H NMR (DMSO-d_6) 1.72 (m, 4H, 3-CH_2 and 4-CH_2), 3.15 (s, 3H, OSO_2Me), 3.64 (s, 3H, CO_2Me), 4.08 (m, 1H, 2-CH), 4.19 (t, J = 5.3 Hz, CH_2OSO_2Me), 5.05 (s, 2H, $PhCH_2$), 7.35 (m, 5H, Ph), 7.78 (d, J = 7.8 Hz, 1H, CONH); MS (FAB, m/z) 360 $(M + H)^+$. Anal. ($C_{15}H_{21}NO_7S$) C, H, N, S.

Methyl (2*S*)-2-[*N*-(Benzyloxycarbonyl)amino]-5-(1*H*-1,2,4-triazol-3-ylthio)pentanoate (19). To a stirred solution of **18** (2.35 g, 6.5 mmol) in anhydrous DMF (6.5 mL) under argon was added 1*H*-1,2,4-triazole-3-thiol (0.86 g, 8.5 mmol) followed by Et_3N (0.86 g, 8.5 mmol). The reaction mixture was stirred at room temperature for 90 h, then it was diluted with AcOEt (200 mL), and the resulting solution was washed with 10% aqueous citric acid (100 mL), brine (100 mL), and H_2O (100 mL), dried (Na_2SO_4), and concentrated in vacuo to a yellow oily residue. Purification by column chromatography, on gradient elution with AcOEt in hexanes (40 to 80%), afforded a gum (1.84 g, 77%) which solidified on standing at room temperature to afford the title compound **19** as a white solid: mp 99−100 °C; ^{1}H NMR (DMSO-d_6) 1.63−1.90 (m, 4H, 3-CH_2 and 4-CH_2), 3.06 (t, J = 6.3 Hz, 2H, CH_2S-), 3.61 (s, 3H, CO_2Me), 4.05 (m, 1H, 2-CH), 5.03 (s, 2H, $PhCH_2$), 7.35 (m, 5H, Ph), 7.80 (d, J = 7.8 Hz, 1H, CONH), 8.4 (br s, N=CH); MS (FAB, m/z) 365 $(M + H)^+$. Anal. ($C_{16}H_{20}N_4O_4S$) C, H, N, S.

Methyl (2*S*)-2-[*N*-(Benzyloxycarbonyl)amino]-5-(1*H*-1,2,4-triazol-3-ylsulfonyl)pentanoate (20). To a stirred solution of **19** (0.660 g, 1.8 mmol) in $CHCl_3$ (8 mL) cooled to −10 °C under argon was added a suspension of MCPBA (technical 80−90%, 0.775 g, ∼3.6 mmol) in $CHCl_3$ (8 mL) (precooled to −10 °C) with the aid of $CHCl_3$ (4 mL). Stirring was continued at −10 °C for 5 min, and then the reaction mixture was allowed to stand at −20 °C for 23 h. The white solid was filtered off, and the filtrate was concentrated in vacuo to a semisolid residue which was purified by column chromatography using a gradient of AcOEt in hexanes (50 to 100%) as eluant. The title compound **20** was obtained as a gummy solid (0.410 g, 58%): ^{1}H NMR (DMSO-d_6) 1.63−1.90 (m, 4H, 3-CH_2 and 4-CH_2), 3.42 (m, 2H, CH_2SO_2-), 3.61 (s, 3H, CO_2-Me), 4.06 (m, 1H, 2-CH), 5.03 (s, 2H, $PhCH_2$), 7.36 (m, 5H, Ph), 7.75 (d, J = 7.8 Hz, 1H, CONH), 8.9 (s, 1H, N=CH); MS (FAB, m/z) 397 $(M + H)^+$. Anal. ($C_{16}H_{20}N_4O_6S$) C, H, N, S.

Methyl (2*S*)-2-Amino-5-(1*H*-1,2,4-triazol-3-ylsulfonyl)-pentanoate (5i). To a solution of **20** (0.330 g, 0.83 mmol) in EtOH (24 mL) was added 10% Pd/C (0.350 g). The mixture was stirred at 26 °C for 4 h under H_2. More catalyst (0.050 g) was then added, and stirring was continued at 26 °C for a further 2 h. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo to a gummy residue which dried in vacuo over P_2O_5 to give a white solid (0.182 g), a mixture of the starting material and the desired poduct (ratio 0.6:1, as judged by ^{1}H NMR). This was used in the next experiment without any further purification.

***tert*-Butyl (4*R*)-4-(Benzyloxycarbonylamino)-4-carbamoylbutyrate (22j).** A solution of i-BuOCOCl (1.92 g, 0.014 mol) in dry THF (11 mL) was added during 5 min to a stirred, precooled (to −15 °C) solution of *N*-(benzyloxycarbonyl)-D-glutamic acid γ-*tert*-butyl ester (4.75 g, 0.014 mol) and dry Et_3N (1.97 mL, 0.014 mol) in THF (28 mL) under argon. After a further 10 min at −15 °C, NH_3 was bubbled through the solution for 30 min while maintaining the temperature between −5 and +5 °C. The mixture was then allowed to warm to room temperature. The precipitate was removed by filtration and washed with THF. The combined filtrate and washings were evaporated, and the residue was dissolved in AcOEt (150 mL). The solution was washed successively with saturated aqueous $NaHCO_3$ (2 × 35 mL), H_2O (35 mL), 10% citric acid (35 mL), and H_2O (2 × 35 mL), then dried (Na_2SO_4), and evaporated. The white solid residue was redissolved in the minimum volume of CH_2Cl_2 and the solution added dropwise to stirred hexane (300 mL). The resulting precipitate was collected, washed with hexane, and dried to give the title compound **22j** as a white powder (3.638 g, 77%): mp 138−140 °C; ^{1}H NMR (DMSO-d_6 + D_2O) δ 1.36 (s, 9H, Bu^t), 1.69, 1.84 (2 × m, each 1H, 3-CH_2), 2.21 (t, J = 7.7 Hz, 2H, 2-CH_2), 3.90 (m, 1H, 4-CH), 5.01 (AB quartet, J = 12.7 Hz, 2H, $PhCH_2$), 7.34 (m, 5H, Ph); MS (FAB, m/z) 359 [$(M + Na)^+$], 337 [$(M + H)^+$]. Anal. ($C_{17}H_{24}N_2O_5$) C, H, N.

***tert*-Butyl (4*R*)-4-(Benzyloxycarbonylamino)-4-cyanobutyrate (23j).** A solution of $POCl_3$ (1.25 mL, 13.4 mmol) in CH_2Cl_2 (5.5 mL) was added during 20 min to a stirred solution of **22j** (3.0 g, 8.9 mmol) in dry pyridine (16 mL) at −5 °C under argon. The reactants were then allowed to warm to room temperature. After a further 15 h the mixture was partitioned between cold H_2O (110 mL) and AcOEt (40 mL). The aqueous layer was extracted with further AcOEt (3 × 40 mL), and the combined AcOEt solution was washed successively with 10% citric acid solution (4 × 15 mL) and H_2O (25 mL), then dried (Na_2SO_4), and evaporated. PhMe (4 × 25 mL) was added and evaporated, and the residue was chromatographed using a gradient of AcOEt in hexane (0 to 33%) as eluant to give the title compound **23j** as a pale yellow oil (2.433 g, 86%): ^{1}H NMR

(DMSO-d_6) δ 1.39 (s, 9H, Bu^t), 1.97 (m, 2H, 3-CH_2), 2.34 (t, J = 7.4 Hz, 2H, 2-CH_2), 4.61 (m, 1H, 4-CH), 5.08 (s, 2H, PhCH_2), 7.37 (m, 5H, Ph), 8.21 (d, J = 8.0 Hz, 1H, NH); MS (FAB, m/z) 341 [(M + Na)$^+$], 319 [(M + H)$^+$]. Anal.($C_{17}H_{22}N_2O_4$) C, H, N.

***tert*-Butyl (4*R*)-4-(Benzyloxycarbonylamino)-4-(5-tetrazolyl)butyrate (24j).** Compound **23j** (1.53 g, 4.8 mmol), NH_4Cl (0.28 g, 5.2 mmol), NaN_3 (0.345 g, 5.3 mmol), and DMF (6 mL) were stirred together at 90−95 °C under argon for 20 h. The mixture was cooled and concentrated, and the residue was taken up in H_2O (40 mL) and AcOEt (30 mL). The resulting two-phase mixture was cooled in ice, and sufficient 10% citric acid solution was added to acidify the aqueous phase to pH 3. The AcOEt phase was separated, and the aqueous phase was extracted with further AcOEt (3 × 30 mL). The combined AcOEt solution was washed with H_2O (4 × 25 mL), dried ($MgSO_4$), and evaporated. A solution of the residue in CH_2Cl_2 was filtered and evaporated. The residual solid was redissolved in CH_2Cl_2 (3 mL) and the solution added dropwise to stirred hexane (20 mL). After cooling at 5 °C overnight, the precipitate was collected, washed with hexane, and dried to give the title compound **24j** (1.462 g, 84%): mp 99−101 °C; $[\alpha]_D^{18}$ +34.0 (c = 1, $CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 1.39 (s, 9H, Bu^t), 2.15 (m, 2H, 3-CH_2), 2.31 (m, 2H, 2-CH_2), 5.04 (m, 3H, 4-CH, PhCH_2), 7.36 (m, 5H, Ph), 7.97 (d, J = 8.0 Hz, 1H, CONH); MS (FAB) m/z 384 [(M + Na)$^+$], 362 [(M + H)$^+$]. Anal. ($C_{17}H_{23}N_5O_4$) C, H, N.

***tert*-Butyl (4*R*)-4-Amino-4-(5-tetrazolyl)butyrate (25j).** A solution of **24j** (1.2 g, 3.3 mmol) in EtOH (77 mL) was stirred with 10% Pd−C (0.166 g) under H_2 (balloon) at room temperature for 16 h. The catalyst was removed by filtration, and the solution was evaporated. CH_2Cl_2 was added to the residue and evaporated, and the white solid obtained was triturated with hexane and dried to give the title compound **25j** (0.687 g, 91%): mp 175 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ 1.38 (s, 9H, Bu^t), 2.07 (m, 2H, 3-CH_2), 2.26 (m, 2H, 2-CH_2), 4.44 (dd, J = 6.2, 7.7 Hz, 1H, 4-CH), 8.28 (br. s, 3H, NH_3^+); MS (FAB, m/z) 250 [(M + Na)$^+$], 228 [(M + H)$^+$].

***tert*-Butyl (4*R*)-4-{*N*-[*N*-(Benzyloxycarbonyl)-α-*tert*-butyl-L-γ-glutamyl]amino}-4-(5-tetrazolyl)butyrate (26j).** A stirred solution of *N*-(benzyloxycarbonyl)-L-glutamic acid α-*tert*-butyl ester (0.891 g, 2.6 mmol) in dry THF (10 mL) was cooled to −20 °C under argon, and dry NMM (0.29 mL, 2.6 mmol) and i-BuOCOCl (0.34 mL, 2.6 mmol) were added successively. After 10 min, **25j** (0.60 g, 2.6 mmol) and further THF (5 mL) were added. After a further 15 min at −20 °C, the mixture was allowed to come to room temperature. After a further 4.5 h, the mixture was filtered and the filtrate concentrated. The residue was dissolved in AcOEt (100 mL) and the solution washed successively with 10% citric acid solution (50 mL) and brine (3 × 20 mL), dried ($MgSO_4$), and evaporated. The residue was chromatographed using 0 to 10% EtOH in CH_2Cl_2 (stepwise gradient) as eluant. The isolated product material was triturated with hexane and dried to give the title compound **26j** (0.948 g, 66%): mp 143−145 °C; ^{1}H NMR (DMSO-d_6) δ 1.38, 1.39 (2 × s, total 18H, Bu^t), 1.7−2.2 (m, 4H, butyryl 3-CH_2, glu β-CH_2), 2.29 (m, 4H, butyryl 2-CH_2, glu γ-CH_2), 3.90 (m, 1H, glu α-CH), 5.03 (AB quartet, J = 12.5 Hz, 2H, PhCH_2), 5.17 (m, 1H, butyryl 4-CH), 7.35 (m, 5H, Ph), 7.58 (d, J = 7.7 Hz, 1H, glu α-NH), 8.43 (d, J = 7.9 Hz, 1H, butyryl 4-NH); MS (FAB, m/z) 569 [(M + Na)$^+$], 547 [(M + H)$^+$]. Anal. ($C_{26}H_{38}N_6O_7$) C, H, N.

***tert*-Butyl (4*R*)-4-[*N*-(α-*tert*-Butyl-L-γ-glutamyl)amino]-4-(5-tetrazolyl)butyrate (5j).** A solution of **26j** (0.320 g, 0.59 mmol) in EtOH (35 mL) was stirred with 10% Pd−C (0.12 g) under H_2 (balloon) at room temperature. After 16 h, further 10% Pd−C (0.09 g) was added, and after a further 6 h, the catalyst was removed by filtration and the solution evaporated. Several portions of dry CH_2Cl_2 were added and evaporated, and the final residue was dried in vacuo over P_2O_5 to give the slightly impure title compound **5j** as a crisp glass (0.24 g) which was used without further purification: ^{1}H NMR (DMSO-d_6) δ 1.38 (s, 9H, Bu^t), 1.45 (s, 9H, Bu^t), 1.91 (m, 4H, butyryl 3-CH_2, glu γ-CH_2), 2.19 (m, 4H, butyryl 2-CH_2, glu γ-CH_2), 3.76 (t, J = 6.4 Hz, 1H, glu α-CH), 5.08 (m, 1H, butyryl 4-CH), 6.1 (v. br, 3H, NH_3^+), 8.29 (d, J = 8.4, 1H, CONH); MS (FAB, m/z) 413 [(M + H)$^+$].

(2*R*)-2-(Benzyloxycarbonylamino)propionamide (22k). Et_3N (9.3 mL, 67 mmol) and i-BuOCOCl (8.7 mL, 67 mmol) were added successively to a stirred, cooled (−15 °C) solution of *N*-(benzyloxycarbonyl)-D-alanine (15.0 g, 67 mmol) in THF (135 mL). After 10 min, NH_3 was bubbled through the mixture (slowly at first) for 30 min, with continued cooling. The mixture was then allowed to come to room temperature. After 1 h (from the end of the period of ammonia treatment) the mixture was filtered and the precipitate washed with a little THF. The combined filtrate and washings were evaporated, and the white solid residue was crystallized from AcOEt (60 mL) to give the title compound **22k** (9.949 g, 67%): mp 132−134 °C; ^{1}H NMR (DMSO-d_6 + D_2O) δ 1.19 (d, J = 7.2 Hz, 3H, CH_3), 3.94 (m, 1H, 2-CH), 5.00 (s, 2H, PhCH_2), 7.34 (m, 5H, Ph); MS (CI, m/z) 223 (M + H)$^+$. Anal. ($C_{11}H_{14}N_2O_3$) C, H, N.

(2*R*)-2-(Benzyloxycarbonylamino)propionitrile (23k). *p*-Toluenesulfonyl chloride (10.68 g, 56 mmol) was added to a stirred mixture of **22k** (9.6 g, 43 mmol), dry pyridine (32 mL), and dry CH_2Cl_2 (20 mL) at 0 °C under argon. The mixture was stirred at 0−5 °C for 0.5 h and at ambient temperature for 3 h. It was then re-cooled (ice−salt bath), and H_2O (2.5 mL) was added. The products were partitioned between AcOEt (200 mL) and water (200 mL). The aqueous layer was extracted with AcOEt (100 mL + 3 × 50 mL), and the combined AcOEt solution was washed successively with HCl (0.5 M; 3 × 200 mL), saturated aqueous $NaHCO_3$ (100 mL), and H_2O (100 mL), then dried ($MgSO_4$), and evaporated. PhMe was added and evaporated, and the residue was chromatographed using hexane−AcOEt (100:0, 80:20, and 75:25 in succession) as eluant. The solid thus isolated was triturated with hexane and dried to give the title compound **23k** (7.574 g, 86%): mp 82−83 °C; ^{1}H NMR (DMSO-d_6) δ 1.41 (d, J = 7.2 Hz, 3H, CH_3), 4.60 (m, 1H, 2-CH), 5.08 (s, 2H, PhCH_2), 7.37 (m, 5H, Ph), 8.19 (d, J = 7.4 Hz, 1H, NH); MS (CI, m/z) 205 (M + H)$^+$. Anal. ($C_{11}H_{12}N_2O_2$) C, H, N.

(1*R*)-*N*-(Benzyloxycarbonyl)-1-(5-tetrazolyl)ethylamine (24k). Compound **23k** (7.0 g, 34 mmol), NH_4Cl (2.01 g, 37 mmol), NaN_3 (2.38 g, 37 mmol), and dry DMF (44 mL) were stirred together under argon at 90 °C (bath temperature) for 19 h. The mixture was cooled and filtered, the solids were washed with DMF, and the combined filtrate and washings were evaporated. A rapidly stirred mixture of the residue with H_2O (325 mL) was acidified to pH 3 with 1 M HCl, and the resulting mixture was extracted with AcOEt (3 × 300 mL). The combined AcOEt solution was washed with H_2O (2 × 220 mL), dried ($MgSO_4$), and evaporated. The white solid residue was crystallized from AcOEt (44 mL) to give the title compound **24k** (5.139 g, 61%): mp 141−142 °C; $[\alpha]_D^{21}$ +38 (c = 1, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.50 (d, J = 7.1 Hz, 3H, CH_3), 5.04 (m, 3H, PhCH_2 and 1-CH), 7.37 (m, 5H, Ph), 8.07 (d, J = 7.4 Hz, 1H, 1-NH), 13.46 (br. s, 1H, tetrazole NH); MS (ESI, m/z) 248 (M + H)$^+$. Anal. ($C_{11}H_{13}N_5O_2$) C, H, N.

(1*R*)-1-(5-Tetrazolyl)ethylamine (25k). A solution of **24k** (3.06 g, 12.4 mmol) in EtOH (270 mL) was stirred with 10% Pd−C (0.45 g) under H_2 (balloon) at room temperature for 19 h. The catalyst was removed by filtration through Celite, and the filtrate was evaporated. The residue was triturated with ether and dried to give the title compound **25k** (1.371 g, 98%): mp 268−270 °C (decomp.); ^{1}H NMR (DMSO-d_6) δ 1.51 (d, J = 6.8 Hz, 3H, CH_3), 4.51 (q, J = 6.8 Hz, 1H, 1-CH), 8.27 (br. s, 3H, NH_3^+).

(1*R*)-*N*-[*N*-(Benzyloxycarbonyl)-α-*tert*-butyl-L-γ-glutamyl]-1-(5-tetrazolyl)ethylamine (26k). Dry NMM (1.16 mL, 10.6 mmol) and i-BuOCOCl (1.37 mL, 10.6 mmol) were added successively to a stirred, cooled (−20 °C) solution of *N*-(benzyloxycarbonyl)-L-glutamic acid α-*tert*-butyl ester (3.57 g, 10.6 mmol) in dry THF (90 mL) under argon. After 10 min, a suspension of **25k** (1.26 g, 11.1 mmol) in THF (165 mL) was added while keeping the mixture at −20 °C. After a further 10 min, the mixture was brought to room temperature, stirred for a further 4 h, then filtered. The filtrate was evaporated, and a solution of the residue in AcOEt (400 mL)

was washed successively with 10% citric acid (2 × 450 mL) and brine (500 mL), then dried (Na_2SO_4), and evaporated. The residue was chromatographed using $CHCl_3$−MeOH (gradient, 100:0 to 75:25) as eluant. The isolated product material was dissolved in a small volume of CH_2Cl_2 and the solution was added dropwise to cooled (ice−salt bath), stirred hexane (300 mL). The resulting precipitate was rechromatographed with CH_2Cl_2−EtOH (100:0 to 75:25) and precipitated similarly at −20 °C to give the title compound **26k** (2.656 g, 58%): mp 78−83 °C; ^{1}H NMR (DMSO-d_6) δ 1.39 (s, 9H, Bu^t), 1.42 (d, J = 7.0 Hz, 3H, CH_3), 1.84 (m, 2H, glu β-CH_2), 2.20 (t, J = 7.5 Hz, 2H, glu γ-CH_2), 3.88 (m, 1H, glu α-CH), 5.02 (m, 2H, PhCH_2), 5.16 (m, 1H, CH_3CH), 7.35 (m, 5H, Ph), 7.66 (d, J = 7.6 Hz, 1H), 8.40 (d, J = 7.6 Hz, 1H) (2 × CONH); MS (ESI, m/z) 455 (M + Na)$^+$, 433 (M + H)$^+$. Anal. ($C_{20}H_{28}N_6O_5$) C, H, N.

(1*R*)-*N*-(α-*tert*-Butyl-L-γ-glutamyl)-1-(5-tetrazolyl)ethylamine (5k). A solution of **26k** (0.360 g, 0.83 mmol) in EtOH (50 mL) was stirred with 10% Pd−C (0.093 g) under H_2 (balloon) at ambient temperature for 18 h. The catalyst was removed by filtration through Celite, and the filtrate was evaporated. CH_2Cl_2 was added to the residue and evaporated, and the resulting white solid was triturated with hexane and dried to give the title compound **5k** (0.169 g, 68%): mp 113−115 °C, which was used without further purification; ^{1}H NMR (DMSO-d_6) δ 1.40 (d, J = 6.9 Hz, 3H, CH_3), 1.45 (s, 9H, Bu^t), 1.91 (m, 2H, glu β-CH_2), 2.26 (m, 2H, glu γ-CH_2), 3.64 (t, J = 6.4 Hz, 1H, glu α-CH), 5.13 (m, 1H, CH_3CH), 5.99 (br. s, 3H, NH_3^+), 8.27 (d, J = 7.9 Hz, 1H, CONH); MS (ESI, m/z) 299 (M + H)$^+$.

(2*R*)-2-(Benzyloxycarbonylamino)pentanamide (22l). To a stirred solution of *N*-(benzyloxycarbonyl)-D-norvaline (**21l**) (4.0 g, 16 mmol) in anhydrous THF (35 mL) under argon cooled to −15 °C was added Et_3N (1.61 g, 15.9 mmol) followed by i-BuOCOCl (2.17 g, 15.9 mmol). Stirring was continued at −15 °C for 10 min, and then anhydrous gaseous NH_3 was passed through the suspension over a 30 min period while the temperature was maintained at −15 °C. The reaction mixture was then stirred for 1.25 h while it was allowed to warm to room temperature. The white precipitate was filtered off, the filtrate was concentrated in vacuo to give a white solid which was dissolved in CH_2Cl_2/MeOH, and to this solution silica gel (Art Merck 7734, 8 g) was added. The solvent was removed in vacuo to give a white powder which was placed on a silica gel column made up in 10% CH_2Cl_2 in AcOEt. The column was eluted with 10% CH_2Cl_2 in AcOEt to afford the title compound **22l** as a white solid which was dried in vacuo over P_2O_5 (2.77 g, 70%): mp 138−143 °C; ^{1}H NMR (DMSO-d_6) 0.85 (t, J = 7.27 Hz, 3H, CH_3), 1.30 (m, 2H, 4-CH_2), 1.52 (m, 2H, 3-CH_2), 3.92 (m, 1H, 2-CH), 5.02 (s, 2H, PhCH_2), 6.96 (s, 2H, CONH_2, exchangeable with D_2O), 7.24−7.37 (m, 6H, Ph and CONH); MS (FAB, m/z) 251 (M + H)$^+$. Anal. ($C_{13}H_{18}N_2O_3$) C, H, N.

(2*R*)-2-(Benzyloxycarbonylamino)pentanonitrile (23l). To a stirred solution of **22l** (2.5 g, 10 mmol) in anhydrous pyridine (7.7 mL, 95.0 mmol) and anhydrous CH_2Cl_2 (5 mL) cooled in an ice bath under argon was added *p*-toluenesulfonyl chloride (2.48 g, 13.0 mmol). The resulting yellow solution was stirred at 0 °C for 30 min; the ice bath was then removed, and stirring was continued for 4 h. The reaction mixture was partitioned between AcOEt (150 mL) and H_2O (150 mL). The two layers were separated, and the aqueous layer was extracted with AcOEt (2 × 150 mL). The combined AcOEt extracts were washed with 0.5 N HCl (3 × 80 mL) and H_2O (200 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was dissolved in CH_2Cl_2, and to this solution silica gel (Art Merck 7734, 7 g) was added. The solvent was concentrated in vacuo, and the white free running powder was placed on a silica gel column made up in 10% AcOEt in hexanes. Elution of the column with a gradient of AcOEt in hexanes (10 to 30%) afforded the title compound **23l** as a white solid which was dried in vacuo over P_2O_5 (1.95 g, 84%): mp 90 °C; ^{1}H NMR (DMSO-d_6) 0.89 (t, J = 7.31 Hz, 3H, CH_3), 1.37 (m, 2H, 4-CH_2), 1.70 (m, 2H, 3-CH_2), 4.51 (q, J = 7.54 Hz, 1H, 2-CH), 5.08 (s, 2H, PhCH_2), 7.36 (m, 5H, Ph), 8.17 (d, J = 7.59 Hz, CONH); MS (FAB, m/z) 233 (M + H)$^+$. Anal. ($C_{13}H_{16}N_2O_2$) C, H, N.

(1*R*)-*N*-(Benzyloxycarbonyl)-1-(5-tetrazolyl)butylamine (24l). To a stirred solution of **23l** (1.65 g, 7.1 mmol) in anhydrous DMF (10 mL) under nitrogen was added NH_4Cl (0.418 g, 7.82 mmol) followed by NaN_3 (0.508 g, 7.82 mmol). The reaction mixture was then heated at 90 °C for 20 h under nitrogen; then it was allowed to cool to room temperature. The precipitate was filtered off, washed with DMF (8 mL), and concentrated in vacuo. The residue was treated with water (80 mL) and acidified to pH ∼4 with 1 N HCl, and the mixture was extracted with AcOEt (3 × 90 mL). The combined AcOEt extracts were washed with H_2O (2 × 80 mL), dried (Na_2SO_4), and concentrated in vacuo to a white solid which was recrystallized from AcOEt−hexanes (v/v, 1:1). The white solid was collected by filtration and dried in vacuo over P_2O_5 (1.25 g, 64%): mp 128 °C; ^{1}H NMR (DMSO-d_6) 0.87 (t, J = 7.39 Hz, 3H, CH_3), 1.30 (m, 2H, 3-CH_2), 1.83 (m, 2H, 2-CH_2), 4.89 (q, J = 7.74 Hz, 1H, 1-CH), 5.03 (ABq, J = 12.44 Hz, 2H, PhCH_2), 7.36 (m, 5H, Ph), 8.02 (d, J = 7.79 Hz, CONH); MS (FAB, m/z) 276 (M + H)$^+$. Anal. ($C_{13}H_{17}N_5O_2$) C, H, N.

(1*R*)-1-(5-Tetrazolyl)butylamine (25l). To a solution of **24l** (1.01 g, 3.6 mmol) in EtOH (80 mL) was added 10% Pd/C (0.150 g). The mixture was stirred for 22 h under H_2 (balloon). The catalyst was then removed by filtration, and the filtrate was concentrated in vacuo to give a white solid which was triturated with AcOEt, collected by filtration, and dried in vacuo over P_2O_5 (0.475 g, 93%): mp 255 °C (dec); ^{1}H NMR (DMSO-d_6) 0.85 (t, J = 7.39 Hz, 3H, CH_3), 1.24 (m, 2H, 3-CH_2), 1.82 (m, 2H, 2-CH_2), 4.36 (dd, J = 6.10, 7.94 Hz, 1H, 1-CH), 8.18 (br s, 2H, NH_2, exchangeable with D_2O); MS (FAB, m/z) 142 (M + H)$^+$.

(1*R*)-*N*-[α-*tert*-Butyl *N*-(Benzyloxycarbonyl)amino-L-γ-glutamyl]-1-(5-tetrazolyl)butylamine (26l). To a solution of Z-L-Glu-OBu^t (0.910 g, 2.7 mmol) in anhydrous THF (20 mL) cooled to −20 °C (under argon) was added NMM (0.272 g, 2.7 mmol) followed by i-BuOCOCl (0.369 g, 2.7 mmol). The reaction mixture was stirred for 10 min at −20 °C, and then a suspension of **25l** (0.388 g, 2.75 mmol) in anhydrous THF (35 mL) was added. Stirring was continued at −20 °C for 10 min, then the dry ice/acetone bath was removed, and the reaction mixture was stirred for a further 3.5 h. The white precipitate was removed by filtration, the filtrate was concentrated in vacuo, and the oily residue was partitioned between AcOEt (250 mL) and 10% aqueous citric acid (100 mL). The two layers were separated, and the organic layer was washed with 10% citric acid (100 mL) and dilute brine (100 mL), dried (Na_2SO_4), and concentrated in vacuo to an oily residue. Purification by column chromatography, on elution with 5% MeOH in CH_2Cl_2, afforded the title compound **26l** which was reprecipitated from CH_2Cl_2/hexanes. The white solid was collected by filtration, washed with hexanes, and dried in vacuo over P_2O_5 (0.95 g, 77%): mp 140 °C; ^{1}H NMR (DMSO-d_6) 0.87 (t, J = 7.15 Hz, 3H, CH_3), 1.31 (m, 2H, 3-CH_2), 1.39 (s, 9H, Bu^t), 1.72−1.95 (m, 4H, 2-CH_2 and glu β-CH_2), 2.23 (m, 2H, glu γ-CH_2), 3.87 (m, 1H, glu α-CH), 5.03 (s, 2H, PhCH_2), 5.11 (q obscured, J = 6.46 Hz, 1H, 1-CH), 7.35 (m, 5H, Ph), 7.63 (d, J = 7.70 Hz, 1H, glu NH), 8.48 (d, J = 7.74 Hz, 1H, CH_2-CH_2CONH), 13.48 (br s, 1H, tetrazolyl NH); MS (FAB, m/z) 483 (M + Na)$^+$. Anal. ($C_{22}H_{33}N_6O_5$) C, H, N.

(1*R*)-*N*-[α-*tert*-Butyl L-γ-glutamyl]-1-(5-tetrazolyl)butylamine (5l). To a solution of **26l** (0.170 g, 0.37 mmol) in EtOH (11 mL) was added 10% Pd/C (20 mg). The mixture was stirred for 15 h under H_2. The catalyst was then removed by filtration, and the filtrate was concentrated in vacuo over P_2O_5 to afford the title compound **5l** as a white solid: (0.120 g, 100%), mp 108−111 °C; ^{1}H NMR (DMSO-d_6) 0.84 (t, J = 7.40 Hz, 3H, CH_3), 1.39 (s, 9H, Bu^t), 1.27 (m), 1.60−2.00 (m) (6H, CH_2CH_2-CH_3 and glu β-CH_2), 2.27 (t, J = 7.41 Hz, 2H, glu γ-CH_2), 3.69 (t, J = 6.26 Hz, 1H, glu α-CH), 5.08 (q, J = 6.57 Hz, 1H, 1-CH), 8.28 (d, J = 8.43 Hz, 1H, CH_2CH_2CONH); MS (FAB, m/z) 349 (M + Na)$^+$.

(1*R*)-*N*-(Benzyloxycarbonyl)-1-(phenylsulfonylcarbamoyl)ethylamine (27). To a stirred solution of Z-D-Ala (0.640

g, 2.87 mmol) in dry CH_2Cl_2 (20 mL) under argon was added benzenesulfonamide (2.25 g, 14.3 mmol), 4-DMAP (90 mg), and finally EDCI (dried in vacuo over P_2O_5 at 60 °C prior to use, 0.549 g, 2.87 mmol). The resulting mixture was stirred at room temperature for 24 h under argon before being partitioned between AcOEt (120 mL) and 1 N aqueous HCl (100 mL). The organic layer was washed with half-saturated brine (2 × 100 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was treated with CH_2Cl_2 (∼30 mL), and the insoluble white solid was filtered off. The filtrate was concentrated in vacuo, and the residue was dissolved in CH_2Cl_2/MeOH. To this solution silica gel (Merck Art 7734, 3 g) was added, the solvents were removed in vacuo, and the free running powder was placed on a silica gel column made up in 30% EtOAc in hexanes. The column was eluted with a gradient of AcOEt in hexanes (30 to 100%) to give the product contaminated with benzenesulfonamide. This impure product was treated with CH_2Cl_2 (20 mL); the insoluble solid was filtered off, and the filtrate was concentrated in vacuo. The residue was rechromatographed on elution with a gradient of AcOEt in CH_2Cl_2 (10 to 30%) to give the title compound **27** as a white solid (0.246 g, 24%): mp 136−139 °C; ^{1}H NMR (DMSO-d_6) 1.13 (d, J = 7.18 Hz, 3H, CH_3), 4.03 (m, 1H, *CH*CH_3), 4.97 (s, 2H, $PhCH_2$), 7.32, 7.65, 7.90 (3 × m, aromatics, CONH), 12.24 (brs, 1H, CON*H*SO_2Ph); MS (ESI, *m*/*z*) 385 $(M + Na)^+$. Anal. ($C_{17}H_{18}N_2O_5S$) C, H, N, S.

(1*R*)-1-(Phenylsulfonylcarbamoyl)ethylamine (28). To a solution of **27** (0.500 g, 1.38 mmol) in MeOH (50 mL) was added 10% Pd/C (0.105 g). The mixture was stirred at room temperature for 3.5 h under H_2 (balloon). The catalyst was removed by filtration, and the filtrate was concentrated in vacuo to give the title compound **28** as a gray solid (0.32 g, 100%) that was used in the next experiment without any further purification. ^{1}H NMR (DMSO-d_6) 1.24 (d, J = 7.04 Hz, 3H, CH_3), 3.39 (q, J = 7.08 Hz, 1H, *CH*CH_3), 7.40 (m), 7.60 (br s, exchangeable with D_2O), 7.78 (m) (7H, NH_2 and aromatics); MS (ESI, *m*/*z*) 251 $(M + Na)^+$.

(1*R*)-*N*-[α-*tert*-Butyl *N*-(Benzyloxycarbonyl)amino-L-γ-glutamyl]-1-(phenylsulfonylcarbamoyl)ethylamine (29). To a solution of Z-Glu-OBu^t (0.428 g, 1.27 mmol) in anhydrous THF (3.5 mL) cooled to −20 °C was added NMM (0.128 g, 1.27 mmol) followed by i-BuOCOCl (0.174 g, 1.27 mmol). The reaction mixture was stirred at −20 °C for 10 min under argon, and then a suspension of **28** (0.289 g, 1.27 mmol) in anhydrous THF (22 mL) was added. The reaction mixture was stirred at −20 °C for 5 min, then the acetone/dry ice bath was removed, and stirring was continued for a further 4 h. The white precipitate was removed by filtration and washed with THF, and the filtrate was concentrated in vacuo. The residue was dissolved in CH_2Cl_2/MeOH, and to this solution silica gel (Art Merck 7734, 3.0 g) was added. The solvents were removed in vacuo, and the free running powder was placed on a silica gel column made up in 2% MeOH in CH_2Cl_2. Elution with a gradient of MeOH in CH_2Cl_2 (2 to 6%) afforded the title compound **29** as a white solid (0.252 g, 84%): mp >70 °C (softens); ^{1}H NMR (DMSO-d_6) 1.11 (d, J = 7.12 Hz, 3H, C*H*$_3$), 1.38 (s, 9H, CO_2Bu^t), 1.60−1.90 (m, 2H, $CHCH_2CH_2$), 2.14 (t, J = 7.83 Hz, 2H, $CHCH_2CH_2CONH$), 3.84 (m, 1H, C*H*CH_2-CH_2), 4.16 (quintet, J = 6.97 Hz, 1H, *CH*CH_3), 5.02 (m, 2H, $PhCH_2$), 7.35 (m, 5H, $PhCH_2$), 7.60 (m) and 7.88 (d, J = 8.07 Hz) (6H, SO_2Ph, OCONH), 8.06 (d, J = 6.19 Hz, 1H, CONH); MS (ESI, *m*/*z*) 570 $(M + Na)^+$; measured 570.1876, calculated for $C_{26}H_{33}N_3O_8SNa$ $(M + Na)^+$ 570.1886. Anal. ($C_{26}H_{33}N_3O_8S$) C, H, N, S.

(1*R*)-*N*-[α-*tert*-Butyl L-γ-Glutamyl]-1-(phenylsulfonylcarbamoyl)ethylamine (5m). To a solution of **29** (0.200 g, 0.36 mmol) in EtOH (11 mL) was added 10% Pd/C (26 mg). The mixture was stirred at room temperature for 3 h under H_2. The catalyst was then removed by filtration, and the filtrate was concentrated in vacuo to give the title compound **5m** as a white solid (0.135 g, 91%): mp 115−117 °C; ^{1}H NMR (DMSO-d_6) 1.11 (d, J = 7.08 Hz, 3H, C*H*$_3$), 1.44 (s, 9H, CO_2-Bu^t), 1.80−2.00 (m, 2H, $CHCH_2CH_2$), 2.23 (m, 2H, $CHCH_2CH_2$-CONH), 3.84 (t, J = 5.93 Hz, 1H, C*H*CH_2CH_2), 3.94 (quintet, J = 7.35 Hz, 1H, *CH*CH_3), 7.36 (m), 7.72 (m exchangeable with D_2O) (6H, SO_2Ph, CH_2CON*H*); MS (ESI, *m*/*z*) 436 $(M + Na)^+$.

Preparation of Antifolate Esters. Tri-*tert*-butyl *N*-{*N*-{4-[*N*-((6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-L-γ-glutamyl}-*N*-methyl-L-glutamate (6a). DEPC (0.16 g, 1.0 mmol) and Et_3N (0.10 g, 1.0 mmol) were added successively to a stirred mixture of **3** (0.171 g, 0.45 mmol), tri-*tert*-butyl L-γ-glutamyl *N*-methyl-L-glutamate (**5a**) (0.267, 0.58 mmol), and DMF (2.4 mL) at 0 °C. After 5 min the mixture was allowed to warm to room temperature and stirred in the dark for 5 h. It was then partitioned between AcOEt (30 mL) and H_2O (30 mL). The aqueous layer was extracted with AcOEt (4 × 15 mL), and the combined AcOEt solution was washed successively with 10% citric acid solution (2 × 15 mL), saturated aqueous $NaHCO_3$, and half-saturated brine (4 × 30 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by column chromatography on gradient elution with EtOH in CH_2Cl_2 (0 to 5%). The obtained glass was triturated with hexanes to give the title compound **6a** as a solid (0.238 g, 65%): mp 108−110 °C; ^{1}H NMR (DMSO-d_6) 1.37, 1.38, 1.41 (3 × s, 27H, $C(CH_3)_3$), 1.87, 2.00, 2.17 (3 × m, 7H, glu β-CH_2, Meglu β-CH_2, Meglu γ-CH_2, 7-H), 2.33 (s, 3H, 2-CH_3), 2.5 (m (obscured by the DMSO peak), 3H, glu γ-CH_2, 7-H), 2.63, 2.82 (2 × s, 3H, N-CH_3), 3.02−3.13 (m, 3H, C≡CH, 8-CH_2), 3.83 (m, 1H, $CH_2C{\equiv}C$), 4.09 (m, 1H, $CH_2C{\equiv}C$), 4.30 (m, 1H, glu α-CH), 4.51, 4.82 (2 × m, 1H, Meglu α-CH), 5.76 (t, J = 8.0 Hz, 1H, 6-H), 7.01 (d, J = 8.8 Hz, 2H, 3′,5′-ArH), 7.49 (s, 1H, 9-H), 7.80 (m, 3H, 2′,6′-ArH, 5-H), 8.33 (m, 1H, glu NH), 12.14 (s, 1H, N^3-H); MS (FAB, *m*/*z*) 836 $(M + Na)^+$, 814 $(M + H)^+$. Anal. ($C_{45}H_{59}N_5O_9$) C, H, N.

Tri-*tert*-butyl *N*-{*N*-{4-[*N*-((6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-L-γ-glutamyl}-D-glutamate (6b). A mixture of **4** (0.1 g, 0.2 mmol), tri-*tert*-butyl L-γ-glutamyl-D-glutamate[23] (**5b**) (0.165 g, 0.37 mmol), *N*-hydroxybenzotriazole (0.01 g), Et_3N (0.27 mL, 1.9 mmol), and DMA (10 mL) was stirred at room temperature for 18 h. The mixture was evaporated, and the residue was partitioned between AcOEt (100 mL) and water (100 mL). The solvent was then removed in vacuo, and the residue was purified by column chromatography on elution with a gradient of MeOH in AcOEt (0 to 10%) to afford the title compound **6b** as a gum which was used without further purification: ^{1}H NMR ($CDCl_3$) 1.45 (s, 27H, $C(CH_3)_3$), 1.7 (m, 4H, glu β-CH_2), 2.2 (t, 1H, C≡CH), 2.3 (m, 4H, glu γ-CH_2), 2.52 (s, 3H, 2-CH_3), 2.55 (m, 1H, 7-H), 3.0 (m,-1H, 7-H), 3.25 (m, 1H, 8-H), 3.36 (2 d's,1H, 8-H), 3.82 (2 d's, 1H, $CH_2C{\equiv}C$), 4.02 (2 d's, 1H, $CH_2C{\equiv}C$), 4.49 (m, 1H, glu α-CH), 4.75 (m, 1H, glu α-CH), 5.65 (t, 1H, 6-H), 6.65 (d, 1H, CONH), 7.0 (d, 2H, 3′,5′-ArH), 7.06 (d, 1H, CONH), 7.58 (s, 1H, cyclopenta[*g*]quinazoline 9-H), 7.81 (d, 2H, 2′,6′-ArH), 8.1 (s, 1H, 5-H).

Di-*tert*-butyl *N*-{*N*-{4-[*N*-((6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-L-γ-glutamyl}-D-alaninate (6c). The method followed that used to prepare **6a** but using di-*tert*-butyl L-γ-glutamyl-D-alaninate[23] (0.171 g, 0.52 mmol) in anhydrous DMF (3.5 mL), **3** (0.150 g, 0.40 mmol), DEPC (0.143 g, 0.88 mmol), and Et_3N (0.089 g, 0.88 mmol). Purification by column chromatography, on elution with AcOEt (∼200 mL) and then 2% MeOH in $CHCl_3$ afforded a pale yellow solid. This was rechromatographed using a gradient of MeOH in AcOEt (0 to 4%) as eluant. The product, a white solid, was reprecipitated from CH_2Cl_2 (∼5 mL)/hexanes to afford the title compound **6c** as a white solid (0.195 g, 71%): mp 145−148 °C (softens); ^{1}H NMR (DMSO-d_6) 1.20 (d, J = 7.3 Hz, 3H, ala-CH_3), 1.38, 1.41 (2 × s, 18H, 2 × $C(CH_3)_3$), 1.80−2.28 (m, 6H, glu β-CH_2, glu γ-CH_2 and 7-CH_2), 2.33 (s, 3H, 2-CH_3), 2.97, 3.13 (2 × m, 3H, 8-CH_2 and C≡CH), 3.95 (ABq, J = 19.3 Hz, 2H, $CH_2C{\equiv}C$), 4.09 (m(obscured), 1H, ala α-CH), 4.26 (m, 1H, glu α-CH), 5.75 (t, J = 8.1 Hz, 1H, 6-CH), 7.01 (d, J = 8.9 Hz, 2H, 3′,5′-ArH), 7.48 (s, 1H, 9-H), 7.79 (d, J = 8.4 Hz, 3H, 2′, 6′-ArH and 5-H), 8.21 (d, J = 7.0 Hz, 1H, ala NH), 8.35 (d, J = 7.3 Hz, 1H, glu

NH), 12.10 (s, 1H, N^3-H); MS (FAB, *m*/*z*) 708 $(M + Na)^+$. Anal. ($C_{38}H_{47}N_5O_7$ $0.5H_2O$) C, H, N.

Methyl (2*S*)-2-[4-[*N*-((6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzamido]-4-(2-methoxycarbonylmethyltetrazol-5-yl)butyrate (6d). The method followed that used to prepare **6b** but using **5d**[25] (0.074 g, 0.29 mmol) in DMF (2 mL), **4** (0.114 g, 0.21 mmol), and 1-hydroxybenzotriazole (3.2 mg). The reaction mixture was stirred at room temperature for 3 days under argon, and then it was concentrated in vacuo to a white solid. This was dissolved in CH_2Cl_2/MeOH, and to the resulting solution was added silica gel (Art Merck 7734, 1.5 g). The solvents were removed in vacuo, and the white free running powder was placed on a silica gel column made up in 1% MeOH in CH_2Cl_2. The column was eluted with a gradient of MeOH in CH_2Cl_2 (1 to 4%). The product was reprecipitated from CH_2Cl_2−MeOH/hexanes to give a white solid (0.098 g, 76%): mp 206−207 °C; ^{1}H NMR (DMSO-d_6) 2.10−2.27 (m) and 2.50 (m obscured) (4H, CHCH_2-CH_2 and 7-CH_2), 2.33 (s, 3H, 2-CH_3), 2.95−3.20 (m, 5H, $CHCH_2CH_2$, 8-CH_2 and C≡CH), 3.64, 3.72 (2 × s, 6H, 2 × CO_2-Me), 3.97 (ABq, *J* = 18.99 Hz, 2H, $CH_2C{\equiv}C$), 4.48 (m, 1H, C*H*CH_2CH_2), 5.79 (m, 3H, N-CH_2CO_2Me and 6-CH), 7.03 (d, *J* = 7.62 Hz, 2H, 3′,5′-ArH), 7.49 (s, 1H, 9-H), 7.82 (d, *J* = 9.14 Hz, 3H, 5-H and 2′,6′-ArH), 8.57 (d, *J* = 7.31 Hz, 1H, CONH), 12.12 (s, 1H, N^3-H); MS (FAB, *m*/*z*) 635 $(M + Na)^+$. Anal. ($C_{31}H_{32}N_8O_6$ H_2O) C, H, N.

Methyl (2*S*)-2-[4-[*N*-((6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzamido]-4-(1-methoxycarbonylmethyltetrazol-5-yl)butyrate (6e). The method followed that used to prepare **6a** but using **5e**[25] (0.142 g, 0.55 mmol) in anhydrous DMF (5 mL), **3** (0.149 g, 0.40 mmol), DEPC (0.143 g, 0.88 mmol), and Et_3N (0.089 g, 0.88 mmol). The crude product was dissolved in CH_2Cl_2/MeOH, and to the resulting solution was added silica gel (Art Merck 7734, 1.5 g). The solvents were removed in vacuo, and the yellow free running powder was placed on a silica gel column made up in 2% MeOH in AcOEt. The column was eluted with 2% MeOH in AcOEt (∼300 mL) and then 2% MeOH in $CHCl_3$. The product was reprecipitated from CH_2Cl_2 (8 mL)−MeOH (1.5 mL)/hexanes to give a white solid (0.096 g, 39%): mp 219−221 °C; ^{1}H NMR (DMSO-d_6) 2.23 (m) and 2.50 (m obscured) (4H, CHC$H_2$$CH_2$ and 7-CH_2), 2.33 (s, 3H, 2-CH_3), 2.93−3.13 (m, 5H, $CHCH_2CH_2$, 8-CH_2 and C≡CH), 3.65, 3.70 (2 × s, 6H, 2 × CO_2Me), 3.97 (ABq, *J* = 19.04 Hz, 2H, $CH_2C{\equiv}C$), 4.55 (m, 1H, C*H*CH_2CH_2), 5.52 (s, 2H, N-CH_2CO_2Me), 5.76 (t, *J* = 7.9 Hz, 1H, 6-CH), 7.02 (d, *J* = 8.90 Hz, 2H, 3′,5′-ArH), 7.49 (s, 1H, 9-H), 7.81 (d, *J* = 8.90 Hz, 3H, 5-H, 2′,6′-ArH), 8.55 (d, *J* = 7.55 Hz, 1H, CONH), 12.12 (s, 1H, N^3-H); MS (FAB, *m*/*z*) 635 $(M + Na)^+$. Anal. ($C_{31}H_{32}N_8O_6$ $0.5H_2O$) C, H, N.

Methyl (2*S*)-2-[4-[*N*-((6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzamido]-4-(1-ethoxycarbonylpropyltetrazol-5-yl)butyrate (6f). The method followed that used to prepare **6a** but using **5f** (0.158 g, 0.53 mmol) in anhydrous DMF (5 mL), **3** (0.149 g, 0.40 mmol), DEPC (0.143 g, 0.88 mmol), and then Et_3N (0.089 g, 0.88 mmol). Purification by column chromatography, on elution with 2% MeOH in AcOEt and then 2% MeOH in $CHCl_3$, gave a white gel which was reprecipitated from CH_2Cl_2/hexanes. The precipitate was collected by filtration, washed with hexanes, and dried in vacuo over P_2O_5 to give the title compound **6f** as a white solid (0.160 g, 61%): mp 176−180 °C; ^{1}H NMR (DMSO-d_6) 1.14 (t, *J* = 7.05 Hz, 3H, $CO_2CH_2CH_3$), 2.00−2.40 (m), 2.50 (m obscured) (8H, $CN_4CH_2CH_2CH_2$, $CH_2CH_2CN_4$, and 7-CH_2), 2.33 (s, 3H, 2-CH_3), 2.90−3.25 (m, 5H, $CHCH_2CH_2$, 8-CH_2 and C≡CH), 3.65 (s, 3H, CO_2Me), 3.80−4.13 (m, 4H, $CH_2C{\equiv}C$ and CO_2CH_2-CH_3), 4.34 (t, *J* = 7.11 Hz, 2H, $CN_4CH_2CH_2$), 4.55 (m, 1H, C*H*CH_2CH_2), 5.76 (t, *J* = 7.9 Hz, 1H, 6-CH), 7.02 (d, *J* = 8.70 Hz, 2H, 3′,5′-ArH), 7.49 (s, 1H, 9-H), 7.79 (s, 1H, 5-H), 7.81 (d, *J* = 8.62 Hz, 2H, 2′,6′-ArH), 8.56 (d, *J* = 7.60 Hz, 1H, CONH), 12.12 (s, 1H, N^3-H); MS (FAB, *m*/*z*) 677 $(M + Na)^+$; FAB−HRMS: measured 655.2952, calculated for $C_{34}H_{39}N_8O_6$ $(M + H)^+$ 655.2993.

Methyl (2*S*)-2-{4-[*N*-(6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzamido}-4-{1-[(1*R*)-1-(methoxycarbonyl)ethyl)carbamoylmethyl]tetrazol-5-yl}butyrate (6g). The method followed that used to prepare **6a** but using **5g** (0.165 g, 0.50 mmol) in anhydrous DMF (3.5 mL), **3** (0.171 g, 0.46 mmol), DEPC (0.164 g, 1.01 mmol), and then Et_3N (0.102 g, 1.01 mmol). The crude product was dissolved in CH_2-Cl_2/MeOH, and to the resulting solution was added silica gel (Art Merck 7734, 1.5 g). The solvents were removed in vacuo, and the yellow free running powder was placed on a silica gel column made up in AcOEt. The column was eluted with 2% MeOH in AcOEt (∼300 mL) and then a gradient of MeOH in $CHCl_3$ (1 to 3%). Reprecipitation from MeOH (2 mL)−CH_2Cl_2 (7 mL)/hexanes afforded the title compound **6g** as a white solid (0.130 g, 42%): mp 228−230 °C; ^{1}H NMR (DMSO-d_6) 1.27 (d, *J* = 7.3 Hz, 3H, $CHCH_3$), 2.24 (m), 2.50 (m obscured) (4H, CHCH_2CH_2), and 7-CH_2), 2.32 (s, 3H, 2-CH_3), 2.91 (t, *J* = 7.9 Hz, 2H, $CHCH_2CH_2$), 2.97−3.20 (m, 3H, 8-CH_2 and C≡CH), 3.60, 3.64 (2 × s, 6H, 2 × CO_2Me), 3.96 (ABq, *J* = 18.8 Hz, 2H, $CH_2C{\equiv}C$), 4.27 (m, 1H, C*H*CH_3), 4.54 (m, 1H, -C_6H_4-CONHC*H*), 5.21 (s, 2H, NCH_2CONH), 5.75 (t, *J* = 7.9 Hz, 6-CH), 7.00 (d, *J* = 8.7 Hz, 2H, 3′,5′-ArH), 7.48 (s, 1H, 9-H), 7.77 (s, 1H, 5-H), 7.79 (d, *J* = 8.6 Hz, 2H, 2′,6′-ArH), 8.55 (d, *J* = 7.5 Hz, 1H, -C_6H_4-CONH), 8.97 (d, *J* = 6.9 Hz, 1H, *N*-CH_2-CON*H*), 12.13 (s, 1H, N^3-H); MS (FAB, *m*/*z*) 684 $(M + H)^+$. Anal. ($C_{34}H_{37}N_9O_7$·$0.5H_2O$) C, H, N.

Methyl (2*S*)-2-{*N*-{*N*-{4-[*N*-((6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-α-methyl-L-γ-glutamyl}amino}-4-(1-methoxycarbonylmethyltetrazol-5-yl)butyrate (6h). The method followed that used to prepare **6a** but using **5h** (0.220 g, 0.58 mmol) in anhydrous DMF (3.5 mL), **3** (0.171 g, 0.46 mmol), DEPC (0.164 g, 1.01 mmol), and Et_3N (0.102 g, 1.01 mmol). Purification by column chromatography, on elution with AcOEt (∼100 mL) and then with a gradient of MeOH in $CHCl_3$ (2 to 3%), afforded a pale yellow solid which reprecipitated from CH_2Cl_2 (10 mL)−MeOH (2 mL)/hexanes to give a white solid (0.143 g). Because of the low yield, the initial aqueous washing and the citric acid washings, obtained during the workup, were combined and then extracted with AcOEt (2 × 150 mL), dried (Na_2SO_4), and concentrated in vacuo to a white solid. Purification as described above afforded an additional 0.060 g of the product: mp 197−200 °C; ^{1}H NMR (DMSO-d_6) 1.83−2.30 (m), 2.50 (m obscured) (8H, 2 × CHCH_2-CH_2, $CHCH_2CH_2CONH$ and 7-CH_2), 2.33 (s, 3H, 2-CH_3), 2.88 (t, *J* = 7.9 Hz, 2H, $CHCH_2CH_2$), 2.94−3.24 (m, 3H, 8-CH_2 and C≡CH), 3.61, 3.64, 3.72 (3 × s, 9H, 3 × CO_2Me), 3.96 (ABq, *J* = 19.8 Hz, 2H, $CH_2C{\equiv}C$), 4.40 (m, 2H, 2 × C*H*CH_2CH_2), 5.52 (s, 2H, N-CH_2CO_2Me), 5.76 (t, *J* = 7.9 Hz, 1H, 6-H), 7.00 (d, *J* = 9.0 Hz, 2H, 3′,5′-ArH), 7.49 (s, 1H, 9-H), 7.77 (s, 1H, 5-H), 7.79 (d, *J* = 8.9 Hz, 1H, 2′,6′-ArH), 8.40 (d, *J* = 7.6 Hz) and 8.49 (d, *J* = 7.5 Hz) (2H, 2 × CONH), 12.13 (s, 1H, N^3-H). MS (FAB, *m*/*z*) 756 $(M + H)^+$. Anal. ($C_{37}H_{41}N_9O_9$·$0.8H_2O$) C, H, N.

Methyl (2*S*)-2-{4-[*N*-(6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopena[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzamido}-5-(1*H*-1,2,4-triazol-3-ylsulfonyl)-pentanoate (6i). To a stirred solution of methyl (2*S*)-2-amino-5-(1*H*-1,2,4-triazol-3-ylsulfonyl)pentanoate (**5i**) (0.182 g, supposedly 0.36 mmol of free amine) in anhydrous DMF (2.5 mL) cooled to 0 °C under argon was added **3** (0.171 g, 0.46 mmol) followed by PyBOP, (0.163 g, 0.32 mmol) and then DIEA (0.116 g, 0.9 mmol). A clear solution was obtained after ∼1 min. This was stirred at 0 °C for 5 min, the ice bath was then removed, and stirring was continued for a further 3 h before the reaction mixture being concentrated in vacuo to a gummy residue. This was triturated with CH_2Cl_2 (5 mL), the precipitated brown solid was filtered off, and the filtrate was concentrated in vacuo to a brownish oily residue which was purified by column chromatography using a gradient of MeOH in $CHCl_3$ (2 to 7%) as eluant. The product, still impure, was rechromatographed using 10% MeOH in CH_2Cl_2 as eluant to give a white solid

which was triturated with hexanes, collected by filtration, and washed with hexanes to give the title compound **6i** as a white solid (0.050 g, 27%): mp 174−178 °C (softens); ^{1}H NMR (DMSO-d_6) 1.60−2.00, 2.21 (2 × m, 6H, 3-CH_2 and 4-CH_2 and 7-CH_2), 2.33 (s, 3H, 2-CH_3), 2.90−3.20 (m, 3H, 7-CH_2 and C≡CH), 3.44 (m, 2H, CH_2SO_2), 3.96 (ABq, J = 18.94 Hz, 2H, CH_2C≡C), 3.61 (s, 3H, CO_2Me), 4.39 (m, 1H, 2-CH), 5.76 (t, J = 7.5 Hz, 6-CH), 7.02 (d, J = 8.0 Hz, 2H, 3′,5′-ArH), 7.49 (s, 1H, 9-H), 7.75 (d, J = 8.9 Hz, 2H, 2′,6′-ArH), 7.79 (s, 2H, 5-H), 8.45 (d, J = 7.4 Hz, 1H, CONH), 8.85 (s, 1H, N=CH); MS (FAB, m/z) 618 (M + H)$^+$; FAB−HRMS measured 640.1965, calculated for $C_{30}H_{31}N_7O_6SNa$ (M + Na)$^+$ 640.1962.

***tert*-Butyl (4*R*)-4-{*N*-{*N*-{4-[*N*-((6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-α-*tert*-butyl-L-γ-glutamyl}-amino}-4-(5-tetrazolyl)butyrate (6j).** The method followed that used to prepare **6i** but using PyBOP (0.30 g, 0.6 mmol), DIEA (0.30 mL, 1.7 mmol), **3** (0.190 g, 0.50 mmol), **5j** (0.23 g, 0.6 mmol), and dry DMF (3 mL). After 5 min the mixture was allowed to come to room temperature, and after a further 2.5 h it was partitioned between AcOEt (75 mL) and 10% aqueous citric acid solution (75 mL). The aqueous layer was extracted with AcOEt (2 × 50 mL), and the combined AcOEt solution was washed successively with 10% citric acid (75 mL) and brine (4 × 25 mL), then dried (Na_2SO_4), and evaporated. The residue was chromatographed with CH_2Cl_2−EtOH (gradient, up to 100% EtOH) and rechromatographed with the same system (gradient, up to 75% EtOH). The more polar product was isolated as a glass which was triturated with hexane, then dissolved in CH_2Cl_2 (5 mL). The solution was added to stirred hexane (30 mL), and the resulting precipitate was collected, washed with hexane, and dried to give the title compound **6j** (0.213 g, 55%): mp 154−156 °C; ^{1}H NMR (DMSO-d_6) δ 1.37, 1.38, 1.41 (3 × s, total 18H, 2 × Bu^t), 1.94, 2.24 (2 × m, total 9H, glu β-CH_2, glu γ-CH_2, butyryl 2,3-CH_2, 7-H), 2.33 (s, 3H, 2-CH_3), 2.5 (m, presumed 1H, coincides with solvent signal, 7-H), 3.02 (m, 1H, 8-H), 3.13 (m, 2H, 8-H, C≡CH), 3.88 (m, 1H, CH_2C≡CH), 4.06 (m, 1H, CH_2C≡CH), 4.29 (m, 1H, glu α-CH), 5.14 (m, 1H, butyryl 4-CH), 5.76 (t, J = 8.0 Hz, 1H, 6-H), 7.02 (d, J = 9.0 Hz, 2H, 3′,5′-H), 7.49 (s, 1H, 9-H), 7.79 (m, 3H, 2′,6′-H, 5-H), 8.40 (m, 2H, glu NH, butyryl 4-NH), 12.14 (s, 1H, N3-H); MS (FAB, m/z) 790.3640, (M + Na)$^+$ requires 790.3653. Anal. ($C_{40}H_{49}N_9O_7$·1.8H_2O) C, H; N; calcd, 15.75; found, 16.36.

(1*R*)-*N*-{α-*tert*-Butyl-*N*-{4-[*N*-((6*RS*)-2-methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-L-γ-glutamyl}-1-(5-tetrazolyl)ethylamine (6k). The method followed that used to prepare **6b** but using **4** (0.165 g, 0.31 mmol), **5k** (0.114 g, 0.38 mmol), 1-hydroxybenzotriazole (0.002 g, 0.02 mmol), and dry DMF (1.5 mL). The crude product was purified by column chromatography using CH_2Cl_2−EtOH (stepwise gradient from 100:0 to 0:100) as eluant. The isolated product material was triturated with hexane and dried to give the title compound **6k** (0.188 g): mp 180 °C; ^{1}H NMR (DMSO-d_6) δ 1.41 (m, 12H, CH_3CH, Bu^t), 1.99 (m, 2H, glu β-CH_2), 2.24 (m, 3H, glu γ-CH_2, 7-H), 2.34 (s, 3H, CH_3), 2.5 (m, presumed 1H, coincides with solvent signal, 7-H), 3.02 (m, 1H, 8-H), 3.10 (m, 2H, 8-H, C≡CH), 3.88 (m, 1H, CH_2C≡C), 4.05 (m, 1H, CH_2C≡C), 4.25 (m, 1H, glu α-CH), 5.14 (m, 1H, CH_3CH), 5.75 (t, J = 8.0 Hz, 1H, 6-H), 7.01 (d, J = 8.9 Hz, 2H, 3′,5′-H), 7.48 (s, 1H, 9-H), 7.79 (m, 3H, 2′,6′-H, 5-H), 8.16 (d, J = 7.8 Hz, 1H), 8.40 (d, J = 7.2 Hz, 1H) (dipeptide CONH × 2), 12.10 (br. s, 1H, N^3-H); MS (FAB, m/z) 654.3160, $C_{34}H_{40}N_9O_5$ [(M + H)$^+$] requires 654.3152.

(1*R*)-*N*-{α-*tert*-Butyl-*N*-{4-[*N*-((6*RS*)-2-methyl-4-oxo-3,4,7,8-tetrahydro-6H-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-L-γ-glutamyl}-1-(5-tetrazolyl)butylamine (6l). The method followed that used to prepare **6b** but using **5l** (0.106 g, 0.33 mmol), **4** (0.145 g, 0.27 mmol), anhydrous DMF (2.5 mL), and 1-hydroxybenzotriazole (3.4 mg). Purification of the crude product by column chromatography, on elution first with a gradient of MeOH in CH_2Cl_2 (2 to 10%) and then with a gradient of MeOH in $CHCl_3$ (10 to 15%) afforded a white solid, still impure by TLC (15% MeOH in $CHCl_3$). This was dissolved in MeOH/CH_2Cl_2, and to this solution was added silica gel (Merck Art 7734, 1.5 g). The solvent was removed in vacuo, and the white free-running powder was placed on a silica gel column made up in 5% MeOH in $CHCl_3$. The column was eluted with a gradient of MeOH in $CHCl_3$ (5 to 15%) to afford the title compound **6l** as a white solid that dried in vacuo over P_2O_5 (0.120 g, 66%): mp 200 °C (dec); ^{1}H NMR (DMSO-d_6) 0.82 (t, J = 7.4 Hz, 3H, CH_2-CH_2CH_3), 1.20 (m), 1.70 (m) 2.00 (m), 2.22 (m), and 2.50 (m obscured by DMSO peak) (10H, CH_2CH_2CONH, CH_2CH_2CH$_3$, and 7-CH_2), 1.40 (s, 9H, CO_2Bu^t), 2.33 (s, 3H, 2-CH_3), 2.94−3.21 (m, 3H, 8-CH_2 and C≡CH), 3.96 (ABq, J = 19.11 Hz, 2H, CH_2C≡C), 4.20 (m, 1H, CHCH$_2$CH$_2$CONH), 5.07 (q, J = 7.09 Hz, 1H, CH$_2$CH$_2$CONHCH), 5.75 (t, J = 8.06 Hz, 1H, 6-H), 7.02 (d, J = 8.89 Hz, 2H, 3′,5′-ArH), 7.48 (s, 1H, 9-H), 7.78 (d, J = 8.83 Hz, 3H, 2′,6′-ArH and 5-H), 8.17 (d, J = 8.40 Hz) 8.43 (d, J = 7.23 Hz), (2H, 2 × CONH), 12.14 (s, 1H, N^3-H); MS (ESI, m/z) 704 (M + Na)$^+$, 682 [(M + H)$^+$; FAB−HRMS found 682.3455, calculated for $C_{36}H_{44}N_9O_5$ (M + H)$^+$ 682.3465.

(1*R*)-*N*-{α-*tert*-Butyl-*N*-{4-[*N*-((6*RS*)-2-methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-L-γ-glutamyl}-1-(phenylsulfonylcarbamoyl)ethylamine (6m). The method followed that used to prepare **6b** but using **5m** (0.102 g, 0.25 mmol) in anhydrous DMF (2 mL), **4** (0.127 g, 0.24 mmol), and 1-hydroxybenzotriazole (4.5 mg). Purification of the crude product by column chromatography, on elution with a gradient of MeOH in $CHCl_3$ (2 to 7%) afforded a white solid which was reprecipitated from MeOH (few drops)−CH_2Cl_2/hexanes. The precipitate was collected by filtration, washed with hexanes, and dried in vacuo over P_2O_5 (0.104 g, 57%): mp >205 °C (dec); ^{1}H NMR (DMSO-d_6) 1.10 (d, J = 6.9 Hz, 3H, CONHCHCH_3), 1.40 (s, 9H, CO_2Bu^t), 1.94 (m), 2.17 (m) 2.50 (m obscured by DMSO peak) (6H, CHCH_2CH_2, and 7-CH_2), 2.34 (s, 3H, 2-CH_3), 2.90−3.20 (m, 3H, 8-CH_2 and C≡CH), 3.96 (Abq obscured, J = 18.40 Hz, 2H, CH_2C≡C), 3.80 (m obscured, 1H, CONH-CHCH$_3$), 4.17 (m, 1H, CONHCHCH$_2$CH$_2$), 5.75 (t, J = 7.93 Hz, 1H, 6-H), 7.02 (d, J = 8.94 Hz, 2H, 3′,5′-ArH), 7.35 (m), 7.49 (m obscured) and 7.72 (m obscured) (5H, SO_2Ph), 7.48 (s, 1H, 9-H), 7.78 (d, J = 9.16 Hz, 3H, 2′,6′-ArH and 5-H), 8.35 (d, J = 7.02 Hz, 1H, CONHCHCH$_2$CH$_2$), 12.10 (s, 1H, N^3-H); MS (ESI, m/z) 807 (M + K)$^+$, 791 (M + Na)$^+$. Anal. ($C_{40}H_{44}N_6O_8S$·0.5H_2O) C, H, N.

Preparation of Antifolate Acids. *N*-{*N*-{4-[*N*-((6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-L-γ-glutamyl}-*N*-methyl-L-glutamic Acid (7a). A solution of **6a** (0.159 g, 0.20 mmol) in TFA (8.4 mL) was stirred at ambient temperature in the dark for 75 min, then concentrated in vacuo. The residual glass was triturated with Et_2O, dried, and dissolved in 0.5 M aqueous $NaHCO_3$ (3 mL). The solution was filtered and acidified to pH 4 with 1 M HCl while cooling in ice. The resulting suspension was centrifuged, and the precipitate was washed four times by resuspension in water, centrifugation, and removal of the supernatant, then dried to a white solid (0.096 g, 73%): mp 168 °C; ^{1}H NMR (DMSO-d_6) 1.75−2.3 (m, 7H, glu β-CH_2, Meglu β-CH_2, Meglu γ-CH_2, 7-H), 2.33 (s, 3H, 2-CH_3), 2.5 (m (obscured by the DMSO peak), 3H, glu γ-CH_2, 7-H), 2.65, 2.82 (2 × s, 3H, N-CH_3), 3.02−3.14 (m, 3H, C≡CH, 8-CH_2), 3.83 (m, 1H, CH_2C≡C), 4.09 (m, 2H, CH_2C≡C), 4.35 (m, 1H, glu α-CH), 4.57, 4.92 (2 × m, 1H, Meglu α-CH), 5.76 (t, J = 8.0 Hz, 1H, 6-H), 7.01 (d, J = 7.8 Hz, 2H, 3′,5′-ArH), 7.49 (s, 1H, 9-H), 7.80 (m, 3H, 2′,6′-ArH, 5-H), 8.35 (m, 1H, glu NH), 12.15 (s, 1H, N^3-H); MS (FAB, m/z) 646 (M + H)$^+$. Anal. ($C_{33}H_{35}N_5O_9$·1.5H_2O) C, H, N.

***N*-{*N*-{4-[*N*-((6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]-benzoyl}-L-γ-glutamyl}-D-glutamic Acid (7b).** A mixture of **6b** (0.220 g, 0.28 mmol), TFA (2 mL), and CH_2Cl_2 (20 mL) was stirred at room temperature for 16 h. The mixture was evaporated, and the residue was dissolved in a saturated aqueous $NaHCO_3$ (20 mL). The solution was acidified to pH 4 by the addition of 2 N aqueous HCl. The precipitate was

isolated by filtration, washed with H_2O, and dried in vacuo to give the title compound **7b** (0.066 g) as a solid: mp 184 °C; ^{1}H NMR (DMSO-d_6) 2.0 (m, 4H, glu β-CH_2), 2.3 (m, 5H, glu γ-CH_2, 7-H), 2.35 (s, 3H, 2-CH_3), 2.55 (m, 1H, 7-H), 3.0 (s, 1H, C≡CH), 3.05 (m, 1H, 8-H), 3.2 (m, 1H, 8-H), 3.85 (d, 1H, CH_2C≡C), 4.1 (d, 1H, CH_2C≡C), 4.25 (2 d's, 1H, glu α-CH), 4.4 (2 d's, 1H, glu α-CH), 5.75 (t, 1H, 6-H), 7.05 (d, 2H, 3′,5′-ArH), 7.5 (s, 1H, 9-H), 7.82 (d, 2H, 2′,6′-ArH), 7.85 (s, 1H, 5-H), 8.12 (d, 1H, CONH), 8.32 (d, 1H, CONH), 12.05 (s, 1H, N^3-H); MS (FAB, *m*/*z*) 654 (M + Na)$^+$. Anal. ($C_{32}H_{33}N_5O_9$ 3H_2O) C, H, N.

***N*-{*N*-{4-[*N*-((6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-L-γ-glutamyl}-D-alanine (7c).** The method followed that used to prepare **7a** but using **6c** (0.138 g, 0.2 mmol) and TFA (7 mL). After acidification the precipitated white solid was collected by filtration, washed with H_2O (∼5 mL), and dried in vacuo over P_2O_5 to afford the title compound **7c** as a white solid: mp 185 °C (dec); ^{1}H NMR (DMSO-d_6) 1.23 (d, J = 7.3 Hz, 3H, ala-CH_3), 1.83−2.28 (m, 6H, glu β-CH_2, glu γ-CH_2 and 7-CH_2), 2.33 (s, 3H, 2-CH_3), 2.97, 3.15 (2 × m, 3H, 8-CH_2 and C≡CH), 3.96 (ABq, J = 19.0 Hz, 2H, CH_2C≡C), 4.18 (m (obscured), 1H, ala α-CH), 4.35 (m, 1H, glu α-CH), 5.76 (t, J = 7.9 Hz, 1H, 6-CH), 7.02 (d, J = 8.9 Hz, 2H, 3′,5′-ArH), 7.49 (s, 1H, 9-H), 7.81 (d, J = 8.5 Hz, 3H, 2′,6′-ArH and 5-H), 8.17 (d, J = 7.0 Hz, 1H, ala NH), 8.33 (d, J = 7.4 Hz, 1H, glu NH), 12.10 (s, 1H, N^3-H); MS (FAB, *m*/*z*) 574 (M + H)$^+$. Anal. ($C_{30}H_{31}N_5O_7$·1.5H_2O) C, H, N.

(2*S*)-2-[4-[*N*-((6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzamido]-4-(2-carboxymethyltetrazol-5-yl)butyric Acid (7d). To a suspension of **6d** (0.079 g, 0.13 mmol) in MeOH (2 mL) was slowly added 1 N aqueous NaOH (0.52 mL, 0.5 mmol) followed by H_2O (2 mL). The resulting clear solution was stirred at room temperature for 2.5 h, and then more 1 N NaOH (0.2 mL) was added. Stirring was continued at room temperature for 1 h, then the reaction mixture was diluted with H_2O (5 mL), and the solution was acidified to pH ∼4 with 1 N HCl. The white precipitate was collected by filtration, washed with water, and dried in vacuo over P_2O_5 to afford the title compound **7d** as a white solid (0.063 g, 84%): mp 173 °C (dec); ^{1}H NMR (DMSO-d_6) 2.10−2.30 (m) and 2.50 (m obscured) (4H, CHCH_2CH$_2$ and 7-CH_2), 2.33 (s, 3H, 2-CH_3), 2.90−3.20 (m, 5H, CHCH$_2$CH_2, 8-CH_2 and C≡CH), 3.97 (ABq, J = 18.8 Hz, 2H, CH_2C≡C), 4.43 (m, 1H, CHCH$_2$CH$_2$), 5.62 (s, 2H, N-CH_2CO_2H), 5.76 (t, J = 7.9 Hz, 1H, 6-CH), 7.03 (d, J = 8.22 Hz, 2H, 3′,5′-ArH), 7.49 (s, 1H, 9-H), 7.82 (d, J = 8.2 Hz, 3H, 5-H and 2′,6′-ArH), 8.44 (d, J = 9.0 Hz, 1H, CONH), 12.14 (s, 1H, N^3-H); MS (FAB, *m*/*z*) 607 (M + Na)$^+$. Anal. ($C_{29}H_{28}N_8O_6$·1.8H_2O) C, H, N.

(2S)-2-[4-[*N*-((6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzamido]-4-(1-carboxymethyltetrazol-5-yl)butyric Acid (7e). The method followed that used to prepare **7d** but using **6e** (0.080 g, 0.13 mmol) in MeOH (2 mL), 1 N aqueous NaOH (0.52 mL, 0.52 mmol), and H_2O (2 mL). The title compound **7e** was obtained as a white solid (0.060 g, 80%): mp 179 °C (dec); ^{1}H NMR (DMSO-d_6) 2.10−2.30 (m), 2.50 (m obscured) (4H, CHCH_2CH$_2$ and 7-CH_2), 2.33 (s, 3H, 2-CH_3), 2.90−3.22 (m, 5H, CHCH$_2$CH_2, 8-CH_2 and C≡CH), 3.97 (ABq, J = 19.8 Hz, 2H, CH_2C≡C), 4.51 (m, 1H, CHCH$_2$CH$_2$), 5.38 (s, 2H, N-CH_2CO_2H), 5.76 (t, J = 7.7 Hz, 1H, 6-CH), 7.02 (d, J = 8.8 Hz, 2H, 3′,5′-ArH), 7.49 (s, 1H, 9-H), 7.79 (s, 1H, 5-H), 7.81 (d, J = 8.89 Hz, 2H, 2′,6′-ArH), 8.46 (d, J = 7.86 Hz, 1H, CONH), 12.14 (s, 1H, N^3-H); MS (FAB, *m*/*z*) 607 (M + Na)$^+$. Anal. ($C_{29}H_{28}N_8O_6$·1.5H_2O) C, H, N.

(2*S*)-2-{4-[*N*-(6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzamido}-4-{1-[((1*R*)-1-(carboxy)ethyl)carbamoylmethyl]tetrazol-5-yl}butyric Acid (7g). The method followed that used to prepare **7d** but using **6g** (0.085 g, 0.12 mmol) in MeOH (2.0 mL), 1 N aqueous NaOH (0.5 mL, 0.5 mmol), and H_2O (1 mL). The title compound **7g** was obtained as a white solid (0.062 g, 77%): mp 182−189 °C; ^{1}H NMR (DMSO-d_6) 1.27 (d, J = 7.3 Hz, 3H, CHCH_3), 2.24 (m), 2.50 (m obscured) (4H, CHCH_2CH$_2$ and 7-CH_2), 2.33 (s, 3H, 2-CH_3), 2.89−3.25 (m, 5H, CHCH$_2$CH_2, 8-CH_2 and C≡CH), 3.96 (ABq, J = 19.0 Hz, 2H, CH_2C≡C), 4.18 (m, 1H, CHCH$_3$), 4.46 (m, 1H, -C_6H_4-CONHCH), 5.21 (s, 2H, NCH_2CONH), 5.76 (t, J = 8.4 Hz, 6-CH), 7.01 (d, J = 8.0 Hz, 2H, 3′,5′-ArH), 7.49 (s, 1H, 9-H), 7.78 (s, 1H, 5-H), 7.80 (d, J = 8.6 Hz, 2H, 2′,6′-ArH), 8.44 (d, J = 7.8 Hz, 1H, -C_6H_4-CONH), 8.86 (d, J = 7.2 Hz, 1H, N-CH_2CONH), 12.14 (s, 1H, N^3-H); MS (FAB, *m*/*z*) 656 (M + H)$^+$. Anal. ($C_{32}H_{33}N_9O_7$·1.5H_2O) C, H, N.

(2*S*)-2-{*N*-{*N*-{4-[*N*-((6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-L-γ-glutamyl}amino}-4-(1-carboxypropyltetrazol-5-yl)butyric Acid (7f). The method followed that used to prepare **7d** but using **6f** (0.094 g, 0.14 mmol) in MeOH (2 mL), 1 N aqueous NaOH (0.56 mL, 0.56 mmol), and H_2O (2 mL). The title compound (**7f**) was obtained as a white solid (0.070 g, 85%): mp 160 °C (softens); ^{1}H NMR (DMSO-d_6) 1.95−2.31 (m), 2.50 (m obscured) (8H, CHCH_2CH$_2$, $CN_4CH_2CH_2CH_2$ and 7-CH_2), 2.33 (s, 3H, 2-CH_3), 2.95−3.21 (m, 5H, CHCH$_2$CH_2, 8-CH_2 and C≡CH), 3.96 (ABq, J = 19.44 Hz, 2H, CH_2C≡C), 4.34 (t, J = 7.10 Hz, 2H, CN_4CH_2CH$_2$), 4.47 (m, 1H, CHCH$_2$CH$_2$), 5.76 (t, J = 7.9 Hz, 1H, 6-CH), 7.02 (d, J = 8.64 Hz, 2H, 3′,5′-ArH), 7.49 (s, 1H, 9-H), 7.79 (s, 1H, 5-H), 7.81 (d, J = 8.87 Hz, 2H, 2′,6′-ArH), 8.43 (d, J = 7.79 Hz, 1H, CONH), 12.12 (s, 1H, N^3-H); MS (FAB, *m*/*z*) 635 (M + Na)$^+$. Anal. ($C_{31}H_{32}N_8O_6$·1.2H_2O) C, H, N.

(2*S*)-2-{*N*-{*N*-{4-[*N*-((6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-L-γ-glutamyl}amino}-4-(1-carboxymethyltetrazol-5-yl)butyric Acid (7h). The method followed that used to prepare **7d** but using **6h** (0.120 g, 0.16 mmol) in MeOH (3.2 mL) and 1 N aqueous NaOH (0.96 mL, 0.96 mmol). The title compound **7h** was obtained as a white solid (0.090 g, 79%): mp 176 °C (dec); ^{1}H NMR (DMSO-d_6) 1.80−2.27 (m), 2.50 (m obscured) (8H, 2 × CHCH_2CH$_2$, CHCH$_2$CH_2CONH and 7-CH_2), 2.34 (s, 3H, 2-CH_3), 2.87 (t, J = 7.8 Hz, 2H, CHCH$_2$CH_2), 2.94−3.20 (m, 3H, 8-CH_2 and C≡CH), 3.96 (ABq, J = 18.3 Hz, 2H, CH_2C≡C), 4.35 (m, 2H, 2 × CHCH$_2$CH$_2$), 5.35 (s, 2H, N-CH_2CO_2Me), 5.75 (t, J = 7.6 Hz, 1H, 6-H), 7.01 (d, J = 8.8 Hz, 2H, 3′,5′-ArH), 7.49 (s, 1H, 9-H), 7.80 (s, 1H, 5-H), 7.82 (d, J = 6.8 Hz, 1H, 2′,6′-ArH), 8.24 (d, J = 7.8 Hz) and 8.34 (d, J = 7.7 Hz) (2H, 2 × CONH), 12.09 (s, 1H, N^3-H); MS (FAB, *m*/*z*) 714 (M + H)$^+$. Anal. ($C_{34}H_{35}N_9O_9$·1.5H_2O) C, H, N.

(2*S*)-2-{4-[*N*-(6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopena[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzamido}-5-(1*H*-1,2,4-triazol-3-ylsulfonyl)pentanoic Acid (7i). The method followed that used to prepare **7d** but using **6i** (0.038 g, 0.06 mmol) in MeOH (1 mL), 1 N aqueous NaOH (0.15 mL, 0.15 mmol), and H_2O (1 mL). The title compound **7i** was obtained as a white solid (0.021 g, 57%): mp 180 °C (dec); ^{1}H NMR (DMSO-d_6) 1.60−2.00, 2.20 (2 × m), 2.50 (m obscured) (6H, 3-CH_2 and 4-CH_2 and 7-CH_2), 2.34 (s, 3H, 2-CH_3), 2.90−3.23 (m, 3H, 7-CH_2 and C≡CH) 3.40 (m, 2H, CH_2SO_2-), 3.96 (ABq, J = 18.53 Hz, 2H, CH_2C≡C), 4.32 (m, 1H, 2-CH), 5.76 (t, J = 7.2 Hz, 6-CH), 7.02 (d, J = 8.3 Hz, 2H, 3′,5′-ArH), 7.49 (s, 1H, 9-H), 7.77 (d, J = 8.9 Hz, 2H, 2′, 6′-ArH), 7.80 (s, 2H, 5-H), 8.32 (d, J = 7.7 Hz, 1H, CONH), 8.87 (s, 1H, N=CH); MS (FAB, *m*/*z*) 604 (M + H)$^+$. Anal. ($C_{29}H_{29}N_7O_6S$·1.5H_2O) C, H, N.

(4*R*)-4-{*N*-{*N*-{4-[*N*-((6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-L-γ-glutamyl}amino}-4-(5-tetrazolyl)butyric Acid (7j). TFA (9 mL) was added dropwise during 8 min to a stirred, cooled (ice−water bath) suspension of **6j** (0.080 g, 0.10 mmol) in H_2O (3.75 mL). The resulting solution was protected from light and allowed to come to room temperature. After 3.5 h, further TFA (9 mL) was added, and after a further 1 h the solution was evaporated. TFA (3 × 5 mL) was added to the residue and evaporated. A solution of the final residue in aqueous NaOH solution (0.5 M; 5 mL) was filtered and acidified to pH 4 with 1 M HCl while stirring and cooling in ice. The resulting precipitate was isolated by

centrifugation and filtration, washed with H_2O, and dried to give the title compound **7j** (0.040 g, 58%): mp 178−180 °C; ^{1}H NMR (DMSO-d_6) δ 1.95, 2.20 (2 × m) and 2.33 (s) (overlapping, total 12H, glu β-CH_2, glu γ-CH_2, butyryl 2,3-CH_2, 2-Me, 7-H), 2.5 (m, presumed 1H, coincides with solvent signal, 7-H), 3.02 (m, 1H, 8-H), 3.14 (m, 2H, 8-H, C≡CH), 3.88 (m, 1H, CH_2C≡CH), 4.06 (m, 1H, CH_2C≡CH), 4.41 (m, 1H, glu α-CH), 5.18 (m, 1H, butyryl 4-CH), 5.77 (t, J = 8.0 Hz, 1H, 6-H), 7.03 (d, J = 9.0 Hz, 2H, 3′,5′-H), 7.49 (s, 1H, 9-H), 7.81 (m, 3H, 2′,6′-H, 5-H), 8.44 (d, J = 7.8 Hz, 1H), 8.55 (d, J = 7.7 Hz, 1H) (glu α-CH, butyryl 4-CH), 12.15 (s, 1H, N^3-H); MS (FAB, m/z) 678 [(M + Na)$^+$]. Anal. ($C_{32}H_{33}N_9O_7$·$2H_2O$) C, H, N.

(1*R*)-*N*-{*N*-{4-[*N*-((6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-L-γ-glutamyl}-1-(5-tetrazolyl)ethylamine (7k). TFA (7.5 mL) was added to a stirred suspension of **6k** (0.170 g, 0.26 mmol) in H_2O (3.1 mL) at room temperature. The resulting solution was stirred in the dark, and after 3.25 h, further TFA (7.5 mL) was added. Workup as described for **7j** afforded the title compound **7k** (0.063 g, 39%): mp 157−160 °C; ^{1}H NMR (DMSO-d_6) δ 1.46 (d, J = 7.1 Hz, 3H, CH_3-CH), 1.93 (m, 1H, glu β-H), 2.21, 2.28 (2 × m, overlapping, total 4H, glu β-H, glu γ-CH_2, 7-H), 2.34 (s, 3H, 2-CH_3), 2.5 (m, presumed 1H, coincides with solvent signal, 7-H), 3.02 (m, 1H, 8-H), 3.14 (m, 2H, C≡CH, 8-H), 3.88 (m, 1H, CH_2C≡C), 4.06 (m, 1H, CH_2C≡C), 4.41 (m, 1H, glu α-CH), 5.22 (m, 1H, CH_3C*H*), 5.77 (t, J = 7.9 Hz, 1H, 6-H), 7.02 (d, J = 8.9 Hz, 2H, 3′,5′-H), 7.49 (s, 1H, 9-H), 7.81 (m, 3H, 2′,6′-H, 5-H), 8.43 (d, J = 7.7 Hz, 1H), 8.59 (d, J = 7.4 Hz, 1H) (dipeptide CONH × 2), 12.17 (br. s, 1H), 12.6 (br s, 1H) (N^3-H, CO_2H or tetrazole NH); MS (FAB) m/z 620 [(M + Na)$^+$], 598 [(M + H)$^+$]. Anal. ($C_{30}H_{31}N_9O_5$·$1.5H_2O$) C, H, N.

(1*R*)-*N*-{*N*-{4-[*N*-((6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-L-γ-glutamyl}-1-(5-tetrazolyl)butylamine (7l). To a mixture of **6l** (0.102 g, 0.15 mmol) and H_2O (3.1 mL) was added TFA (7.5 mL), and the solution was stirred at room temperature for 2^3/$_4$ h with protection from the light. More TFA (7.5 mL) was then added, and stirring was continued at this temperature for a further 1.5 h. Workup as described for **7j** afforded the title compound **7l** as a white solid (0.056 g, 60%): mp 176−180 °C (dec); ^{1}H NMR (DMSO-d_6) 0.86 (t, J = 7.35 Hz, 3H, $CH_2CH_2CH_3$), 1.30 (m), 1.63−2.30 (m), 2.50 (m obscured by DMSO peak) (10H, CH_2CH_2CONH, $CH_2CH_2CH_3$, and 7-CH_2), 2.34 (s, 3H, 2-CH_3), 2.95−3.21 (m, 3H, 8-CH_2 and C≡CH), 3.96 (ABq, J = 20.23 Hz, 2H, CH_2C≡C), 4.42 (m, 1H, CONHC*H*CH_2CH_2), 5.13 (q, J = 5.87 Hz, 1H, CONHC*H*), 5.76 (t, J = 7.92 Hz, 1H, 6-H), 7.02 (d, J = 8.95 Hz, 2H, 3′,5′-ArH), 7.49 (s, 1H, 9-H), 7.82 (d, J = 8.98 Hz, 3H, 5-H and 2′,6′-ArH), 8.35 (d, J = 7.86 Hz), 8.47 (d, J = 7.56 Hz) (2H, 2 × CONH), 12.08 (s, 1H, N^3-H); MS (FAB, m/z) 648 (M + Na)$^+$, 626 (M + H)$^+$; FAB−HRMS found 626.2830; calculated for $C_{32}H_{36}N_9O_5$ (M + H)$^+$ 626.2839.

(1*R*)-*N*-{*N*-{4-[*N*-((6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoyl}-L-γ-glutamyl}-1-(phenylsulfonylcarbamoyl)ethylamine (7m). A solution of **6m** (0.080 g, 0.10 mmol) in TFA (8 mL) and H_2O (0.8 mL) was stirred at room temperature for 2 h with protection from the light. Workup as described for **7j** afforded the title compound **7m** as a white solid (0.064 g, 86%): mp 175−176 °C (dec); ^{1}H NMR (DMSO-d_6) 1.10 (d, J = 6.80 Hz, 3H, CONHCHCH_3), 1.80−2.25 (m), and 2.50 (m obscured by DMSO peak) (6H, CHCH_2CH_2, and 7-CH_2), 2.33 (s, 3H, 2-CH_3), 2.90−3.21 (m, 3H, 8-CH_2 and C≡C*H*), 3.96 (ABq, J = 18.69 Hz, 2H, CH_2C≡C), 4.18 (m obscured, 1H, CONHC*H*CH_3), 4.31 (m, 1H, CONHC*H*CH_2-CH_2), 5.75 (t, J = 7.85 Hz, 1H, 6-H), 7.01 (d, J = 8.51 Hz, 2H, 3′,5′-ArH), 7.48 (s, 1H, 9-H), 7.57−7.72 (m) and 7.89 (d) (5H, SO_2Ph), 7.78 (s, 1H, 5-H), 7.79 (d, J = 7.81 Hz, 2H, 2′,6′-ArH), 8.13 (d, J = 6.56 Hz, CONH), 8.31 (d, J = 7.51 Hz, 1H, CON*H*CHCOOH), 12.13 (s, 1H, N^3-H); MS (FAB, m/z) 735 (M + Na)$^+$. Anal. ($C_{36}H_{36}N_6O_8S$·$1.8H_2O$) C, H, N.

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