Chemoenzymatic preparation of the novel antifolate thymidylate synthase inhibitor *N*-(4-{*N*-[(6*S*)-2-methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]-*N*-(prop-2-ynyl)amino}benzoyl)-L-glutamic acid and its glutamyl cleavage product

Jonathan H. Marriott,*[a] Stephen Neidle,[b] Zbigniew Matusiak,[c] Vassilios Bavetsias,[a] Ann L. Jackman,[a] Camille Melin[a] and F. Thomas Boyle[c]

[a] *CRC Centre for Cancer Therapeutics, and* [b] *CRC Biomolecular Structure Unit, The Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey, UK SM2 5NG*
[c] *Zeneca Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire, UK SK10 4TG*

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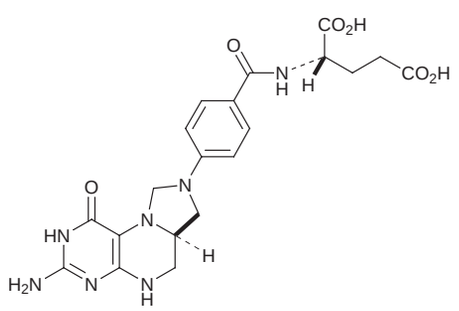
5-Aminoindane was converted in six steps to the cyclopenta[*g*]quinazoline ketone **13**. Condensation of **13** with diethyl 4-aminobenzoyl-L-glutamate, followed by *in situ* reduction, produced the secondary amine **15**. *N*-Propargylation of **15**, followed by deprotection, gave the diacid **17** as a mixture of diastereoisomers. Treatment of **17** with the bacterial enzyme carboxypeptidase G_2 resulted in removal of the L-glutamic acid residue from (6*R*)-**17** to give a chromatographically separable mixture of the monoacid **18** and the antifolate **5** [(6*S*)-**17**], which was assayed as an inhibitor of thymidylate synthase (K_i^{app} = 3 nM). Treatment of isolated diacid **5** with carboxypeptidase G_2 produced the monoacid **19** in *ca*. 98% enantiomeric excess. The (6*S*) stereochemistry of compound **19** has been established by X-ray crystal structure determination of the amide derivative **24**.

Introduction

Thymidylate synthase (TS) catalyses the conversion of 2′-deoxyuridine 5′-monophosphate (dUMP) and the cofactor (6*R*)-N^5,N^{10}-methylene-5,6,7,8-tetrahydrofolate **1** to thymidine



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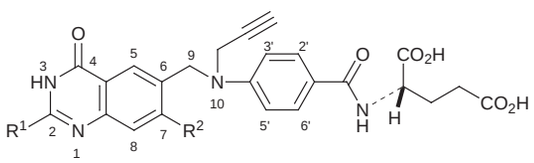
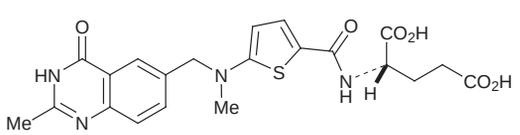


2 $R^1 = NH_2$; R^2 = H
3 a R^1 = Me; R^2 = H
b $R^1 = R^2$ = Me

5′-monophosphate (TMP) and 7,8-dihydrofolate. This is the final step in the *de novo* pathway for synthesis of TMP. TMP is required for DNA synthesis and inhibition of TS has proven to be an effective target for anticancer drug design.[1]

TS inhibition may be effected with quinazoline-based compounds designed to occupy the cofactor site of the enzyme. Efforts to discover a clinical candidate among such compounds led originally to CB3717 **2**;[2] this compound produced antitumour responses in clinical trials but was withdrawn from further development following the observation of nephrotoxicity associated with its low solubility.[3] Structure–activity studies revealed that replacement of the quinazoline 2-amino substituent in CB3717 **2** by a methyl group, as in ICI 198583 **3a**, improved water solubility without excessively reducing TS inhibitory activity.[4] Further structural modifications led to the discovery of raltitrexed (ZD1694, Tomudex) **4**,[1,5,6] which is now in clinical use.



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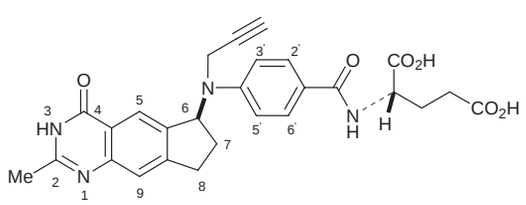

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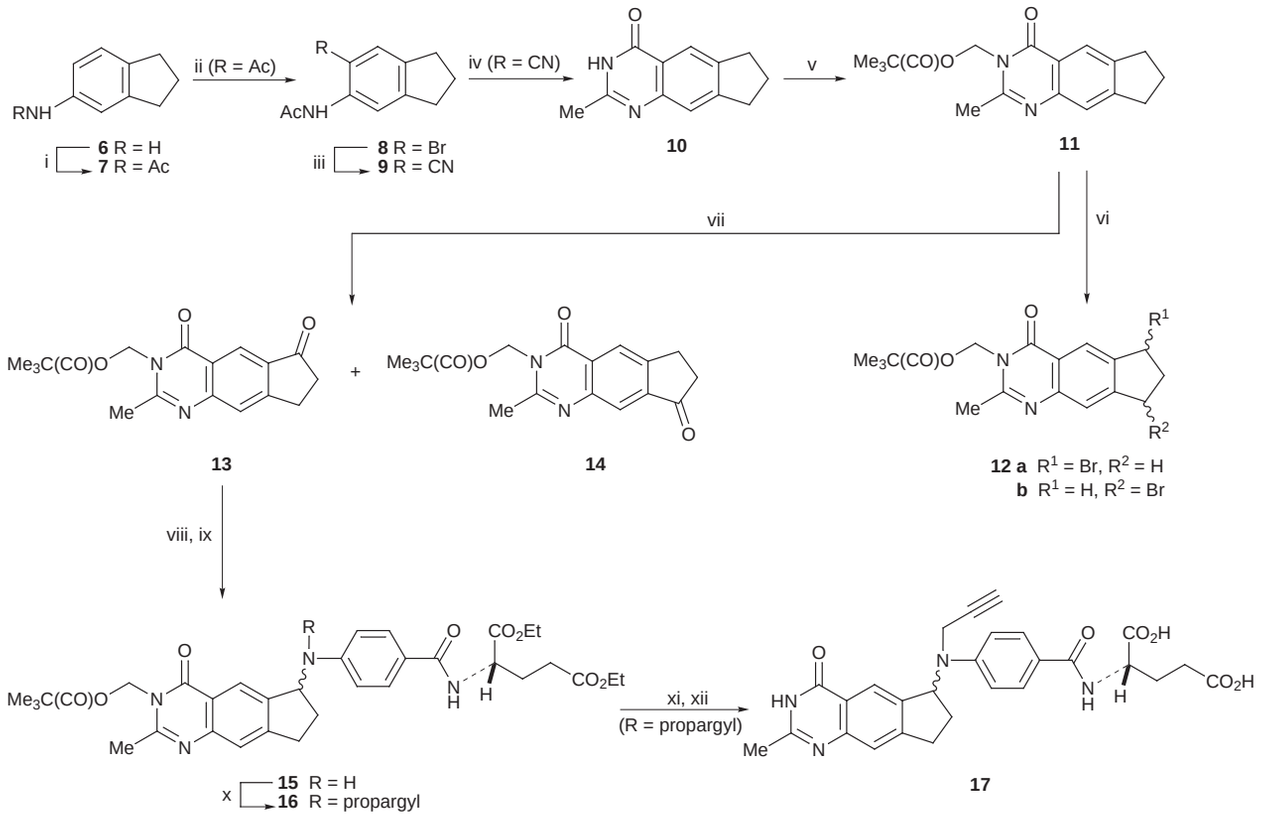
The potent cytotoxicity of raltitrexed **4** is due in part to its active transport into cells by the reduced folate carrier (RFC) and its intracellular metabolism to poly-γ-glutamyl derivatives.[5,7] Our current drug discovery programme is directed towards TS inhibitors whose cytotoxic potency will depend neither on polyglutamation nor on use of the RFC. Optimisation of cytotoxic potency among such compounds depends on optimisation of affinity for TS. Crystallographic studies[8] indicate that in the covalent ternary complex formed from *Escherichia coli* TS, 5-fluoro-2′-deoxyuridine 5′-monophosphate (FdUMP) and the natural cofactor **1**, the bound cofactor has a conformation in which its 4-aminobenzoyl portion extends markedly away from the plane of the tetrahydropteridine ring system. In the crystallised ternary complex of *E. coli* TS with FdUMP and CB3717 **2**, compound **2** is bound in a partially folded conformation with the plane of its 4-amino-



Scheme 1 *Reagents and conditions*: i, Ac_2O, pyridine, EtOAc, room temp. (81%); ii, Br_2, AcOH, room temp. (97%); iii, CuCN, NMP, 125 °C (86%); iv, H_2O_2, NaOH, EtOH–H_2O, 50 °C (83%); v, KOBu^t, chloromethyl pivalate, DMSO, room temp. (51%); vi, NBS, Bz_2O_2, CCl_4, reflux (see text); vii, 70% Bu^{t}OOH, CrO_3, CH_2Cl_2, room temp. (see text); viii, diethyl 4-aminobenzoyl-L-glutamate, TsOH·H_2O, mol. sieves, DME, reflux; ix, $NaBH_3CN$, AcOH, MeOH, room temp. (38% from **13**); x, propargyl bromide, $CaCO_3$, DMA, 100 °C (77%); xi, NaOH, MeOH–H_2O, room temp.; xii, aq. HCl (93% from **16**).

benzoyl residue inclined at 65° to that of the quinazoline ring system.[9] Replacement of the quinazoline 7-H in CB3717 **2** by a methyl group was predicted in modelling studies[10,11] to reinforce the TS-binding conformation, and 7-methyl substitution in compound **3a** and other quinazoline-based antifolates is associated with enhanced TS inhibition.[10–12] In pursuit of further improvements in TS inhibitory potency, we have now prepared compound **5**, in which the quinazoline 7-substituent is cyclised to C-6 in a 5-membered ring, with the 4-aminobenzoyl residue attached to the same side of the plane of the heterocyclic ring system as it lies on in TS-bound **1**[8] and in TS-bound **2**.[9] Compound **5**, and compounds in which the glutamic acid residue of its (6*RS*) modification **17** is replaced by alternative groups, have been found to be potent TS inhibitors.[13,14] In this paper we describe the preparation of compound **5**, and its conversion into its glutamyl cleavage product **19**, by procedures utilising the bacterial enzyme carboxypeptidase G_2 (CPG_2).[15] We also report the results of a TS inhibition assay for compound **5**.

Results and discussion

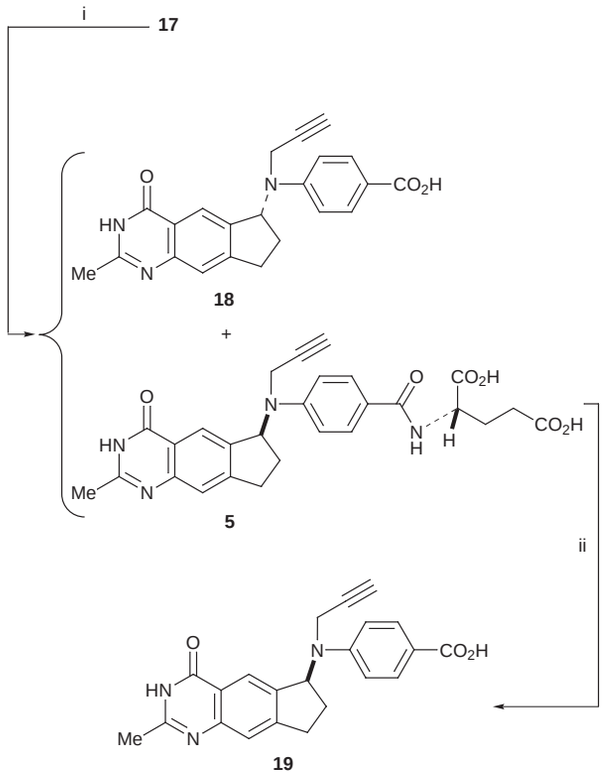
The route to compound **5** is outlined in Schemes 1 and 2. As in an earlier reported synthesis[16] of the cyclopenta[*g*]quinazoline ring system, 5-aminoindane **6** was used as the starting material. Acetylation of the amine **6** produced the amide **7**[17] which was brominated to give compound **8**.[17] Compound **8** reacted smoothly with copper(I) cyanide in 1-methyl-2-pyrrolidone at 125 °C to give the cyano compound **9**. Treatment of **9** with alkaline hydrogen peroxide led, as expected,[18] to cyclisation to give the quinazolin-4-one **10** in good yield.

To improve the solubility of intermediates and to prevent unwanted reactions of the heterocycle, an N^3-pivaloyloxymethyl group was introduced at this point, to give compound **11**. The intermediates **7** to **11** were all isolated in a pure state without use of chromatography.

Two methods of functionalisation of C-6 of the protected quinazolin-4-one **11** were investigated. Treatment of **11** with *N*-bromosuccinimide–dibenzoyl peroxide in refluxing carbon tetrachloride, which had been used[11] for functionalisation of the 6-Me group of 2,6,7-trimethyl-3-pivaloyloxymethylquinazolin-4-one, gave a *ca.* 3:1 mixture of products believed to be the 6-bromo derivative **12a** and its 8-bromo isomer **12b**; the desired isomer **12a** was not completely characterised since it tended to partially decompose during chromatography, and the ketone **13** was found to be a more convenient intermediate. Treatment of compound **11** with 70% aqueous *tert*-butyl hydroperoxide in dichloromethane in the presence of 0.05 equiv. chromium(VI) oxide[19] at ambient temperature produced a crude mixture of 6-oxo and 8-oxo derivatives (**13** and **14** respectively) in an estimated ratio of roughly 3:2. Part of the desired isomer **13** could be isolated from the crude product mixture by direct crystallisation, and further material could be obtained by chromatography (total isolated yield 37%).

Condensation of the ketone **13** with diethyl 4-aminobenzoyl-L-glutamate in the presence of toluene-4-sulfonic acid monohydrate in refluxing 1,2-dimethoxyethane, with removal of water by 3 Å molecular sieve beads, followed directly by treatment of the product mixture with sodium cyanoborohydride, gave compound **15** (38% yield), along with some unchanged **13**. The same product **15** was obtained from the partially purified 6-bromo compound **12a** on heating with diethyl 4-aminobenzoyl-L-glutamate in the presence of calcium carbonate in *N*,*N*-dimethylacetamide.

An N^{10}-propargyl substituent, which generally provides optimum TS binding in quinazoline-based antifolates,[20] was introduced into **15** using propargyl bromide (prop-2-ynyl bromide) and calcium carbonate in DMA at 100 °C, to give com-

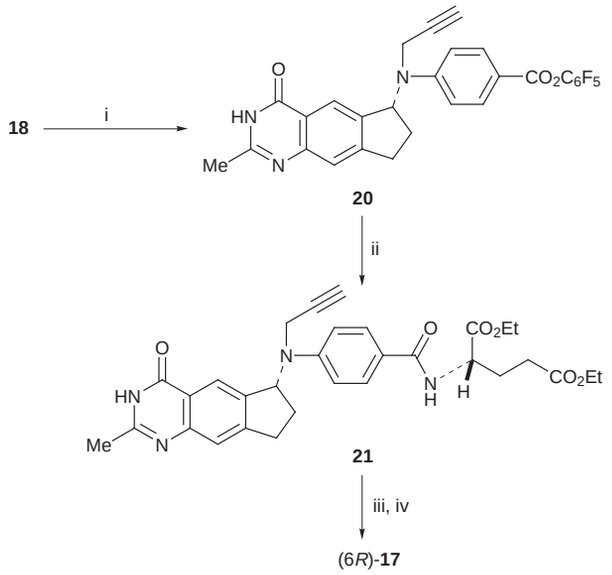


Scheme 2 *Reagents and conditions*: i, carboxypeptidase G_2, Zn^{2+}, aq. trisHCl, pH 7.3, 37 °C, 10 h (see text); ii, carboxypeptidase G_2, Zn^{2+}, aq. trisHCl, pH 7.3, 37 °C, 72 h (80%).

pound **16** in 77% yield. Compounds **15** and **16** were obtained as mixtures of C-6 diastereoisomers; diastereoisomer separation was not accomplished at either stage. Treatment of compound **16** with alkali, followed by acidification, resulted in precipitation of the diacid **17** (Scheme 1), which could be partially resolved into two components by analytical HPLC (see Experimental section).

Computer modelling[10] predicted that the (6*S*)-diastereoisomer of **17** (compound **5**) was preferred. The bacterial enzyme carboxypeptidase G_2 (CPG_2) had been previously shown to cleave the glutamyl residue from both folic acid[15] and various antifolates including raltitrexed **4**.[21] We have now found that treatment of the diastereoisomeric mixture **17** with CPG_2 (Scheme 2, step i) results in a relatively rapid conversion of the (6*R*)-diastereoisomer into the monoacid **18**, with only a small proportion (<10%) of (6*S*)-diastereoisomer cleavage being observed. The reaction was followed by HPLC (see Experimental section), and was stopped by acidification after *ca*. 10 h when (6*R*)-**17** was no longer detectable. The resulting mixture of product **18** and unchanged diacid **5** was then separated by column chromatography. The diacid **5** was thus obtained in 80% yield (based on the quantity present in a 1 : 1 mixture of diastereoisomers **17**), with only *ca*. 1% contamination by unchanged (6*R*)-**17**, and the cleavage product **18** was obtained in near-quantitative yield as a 92 : 8 mixture with its (*S*) enantiomer **19**. A specimen of compound **18** thus prepared was reconverted to its L-glutamic acid conjugate (6*R*)-**17** *via* conversion to the pentafluorophenyl ester **20** using pentafluorophenyl trifluoroacetate,[22] followed by coupling to diethyl L-glutamate (Scheme 3). Deprotection produced compound (6*R*)-**17**, in which HPLC showed, as expected, the presence of an impurity with the same retention time as the (6*S*)-diastereoisomer **5**. The two C-6 diastereoisomers of **17** were not found to be distinguishable through their 250 MHz ^{1}H NMR spectra.

The affinity of antifolate TS inhibitors for TS is enhanced if there is a second α-amino acid residue conjugated to the γ-CO_2H group of the L-glutamic acid residue.[12c,21,23] In order to permit the synthesis of TS inhibitors containing γ-glutamyl



Scheme 3 *Reagents and conditions*: i, pentafluorophenyl trifluoroacetate, pyridine, DMA, room temp. (86%); ii, diethyl L-glutamate hydrochloride, Et_3N, HOBT, DMF, room temp. (86%); iii, NaOH, MeOH–H_2O, room temp.; iv, aq. HCl (82% from **21**).

dipeptide residues and other groups in place of the single L-glutamic acid residue in compound **5**, we required the (*S*)-monoacid **19**. The isolated (6*S*)-glutamic acid conjugate **5** was subjected (Scheme 2, step ii) to prolonged (total 72 h) treatment with CPG_2, and after work-up and column chromatography the desired product **19** was isolated in 80% yield and *ca*. 98% enantiomeric excess (estimated by HPLC). The enantiomeric purity of **19** can be maximised by ensuring (1) that (6*R*)-**17** has been completely consumed before the treatment of (6*RS*)-**17** with CPG_2 is stopped, and (2) that compound **5** is completely freed of compound **18** before the subsequent treatment with CPG_2. In practice, it was found that under the CPG_2 cleavage conditions applied to the diastereoisomeric mixture (6*RS*)-**17**, unwanted cleavage of the (6*S*)-diastereoisomer **5** was so slow that HPLC analysis of the reaction mixture could be repeated conveniently until (6*R*)-**17** was consumed, without serious detriment to the yield of recovered **5**.

To confirm assignment of (6*S*) stereochemistry to compound **19**, an X-ray crystal structure determination was carried out on derivative **24**. The acid **19** was converted (Scheme 4) to its pentafluorophenyl ester **22** which was N^3-methylated before coupling with (*S*)-*sec*-butylamine to give the crystalline amide **24**. The results of the structure determination are shown in Fig. 1.

Diacid **5** [containing *ca*. 1% of diastereoisomer (6*R*)-**17**] has been assayed as an inhibitor of partially purified TS from mouse L1210 cells, and found to have $K_i^{app} = 3$ nM. For comparison, compound **3b**,[10,12a] which differs from compound **5** in having only a 7-methyl group instead of the ring-closing ethano bridge of compound **5**, has TS $K_i^{app} = 17$ nM.

In summary, the novel cyclopenta[g]quinazoline-based antifolate TS inhibitor **5** has been synthesised from 5-aminoindane **6** *via* selective cleavage of the (6*R*)-diastereoisomer in the mixture (6*RS*)-**17** by CPG_2. Treatment of the isolated diacid **5** with CPG_2 provides its glutamyl cleavage product **19** which is a potential precursor for a range of new TS inhibitors.

Experimental

Reactions were monitored by TLC on silica-coated glass plates (Merck no. 5715) visualised under UV light. Column chromatography was carried out on silica gel 60 (Merck nos. 15111, 9385 or 7729). Melting points were determined on a Kofler block and are uncorrected. NMR spectra were recorded on a

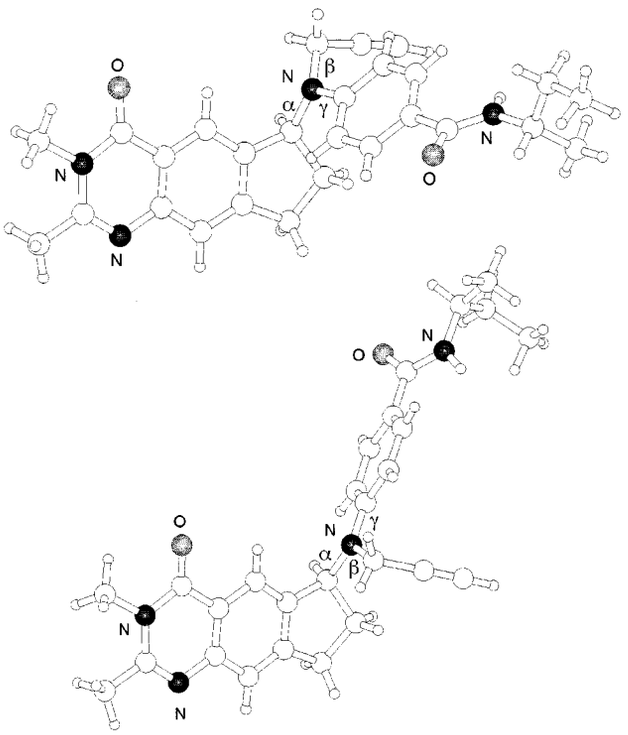
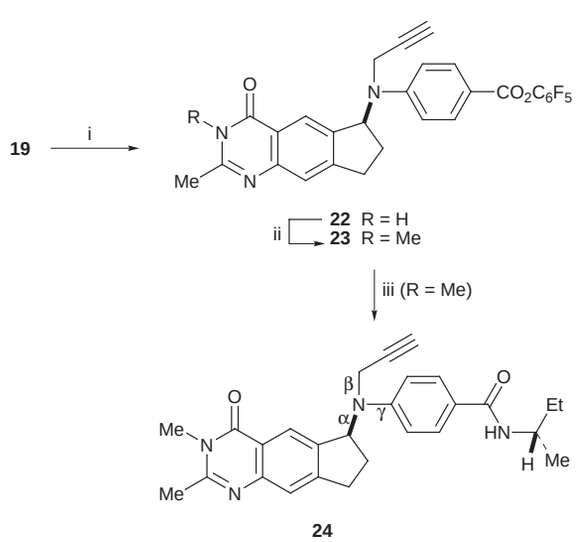


Fig. 1 Computer plots of the two independent molecules in the crystallographic asymmetric unit of compound **24**; labelling of bonds around C(6)–N atom corresponds to that in the structure shown in Scheme 4.



Scheme 4 *Reagents and conditions*: i, pentafluorophenyl trifluoroacetate, pyridine, DMA, room temp. (92%); ii, MeI, Cs_2CO_3, DMF, room temp. (89%); iii, (S)-(+)-sec-butylamine, DMF, room temp. (80%).

Bruker AC250 spectrometer at 250 MHz for ^{1}H spectra and at 235 MHz for ^{19}F spectra. The solvent signal ($\delta_H = 2.5$) was used as internal standard for ^{1}H spectra of samples in $(CD_3)_2SO$, TMS ($\delta_H = 0$) for ^{1}H spectra of samples in $CDCl_3$, and $FCCl_3$ ($\delta_F = 0$) for ^{19}F spectra. IR spectra were recorded on a Perkin-Elmer 1720X spectrometer. Optical rotations were determined on a Perkin-Elmer 141 polarimeter. Elemental analyses were carried out by C.H.N Analysis Ltd., Leicester, UK. Electron impact (EI) mass spectra were obtained using a VG7070H spectrometer. HPLC analyses were performed using a 25 cm × 0.46 cm Astec Cyclobond I (beta) column with 83% 25 mM Na_2HPO_4, 25 mM NaH_2PO_4–17% acetonitrile (isocratic) as eluant at a flow rate of 1 cm^3 min^{-1} and with a Waters 510 solvent delivery system, model 680 automated gradient controller, model U6K injector, and model 490 programmable wavelength detector set to monitor at 230 and 280 nm. Retention times were determined on a Trivector Trilab 3000 multichannel chromatography system. Carboxypeptidase G_2 was supplied by CAMR, Porton Down, Wiltshire, UK, and was handled as a 2 unit mm^{-3} stock solution in 100 mM tris hydrochloride (pH 8), containing 0.26 mM $ZnCl_2$. Compounds **3b** and **5** were tested as inhibitors of partially purified TS isolated from L1210 mouse leukaemia cells that overproduce TS due to amplification of the TS gene. K_i^{app} determinations were performed at an (*RS*)-5,10-methylenetetrahydrofolate concentration of 200 μM. These methods and the derivation of K_i^{app} have previously been published.[24]

5-Acetamidoindane 7

Acetic anhydride (120 cm^3, 1.27 mol) was added during 30 min to a stirred solution of 5-aminoindane (153.9 g, 1.12 mol) in ethyl acetate (770 cm^3) and pyridine (103 cm^3), whilst keeping the reaction mixture below 30 °C (ice–water bath). After 17 h at room temperature the mixture was evaporated to dryness. The residue was triturated with diethyl ether (1230 cm^3). After cooling to 5 °C, the solid was collected, washed successively with cold diethyl ether (770 cm^3) and hexane (770 cm^3) and dried to give 5-acetamidoindane **7** (164.4 g, 81%), mp 107–108 °C (lit.,[17] 108 °C) (Found: C, 75.4; H, 7.5; N, 8.0; $C_{11}H_{13}NO$ requires C, 75.4; H, 7.5; N, 8.0%); $\delta_H[(CD_3)_2SO]$ 2.01 (5 H, m, 2-H, Me), 2.79 (4 H, m, 1-H, 3-H), 7.10 (1 H, d, *J* 8.0, 7-H), 7.25 (1 H, dd, *J* 1.8, 8.0, 6-H), 7.50 (1 H, s, 4-H), 9.80 (1 H, s, N-H); *m*/*z* (FAB) 198 [(M + Na)$^+$, 10%], 176 [(M + H)$^+$, 100], 154 (8), 133 (18).

5-Acetamido-6-bromoindane 8

Bromine (52.5 cm^3, 163 g, 1.02 mol) was added dropwise to a mechanically stirred, cooled (ice–water bath) solution of 5-acetamidoindane **7** (162.2 g, 0.93 mol) in acetic acid (621 cm^3), at such a rate that the reaction mixture remained at 15–20 °C (addition took 80 min). The mixture was stirred for a further 30 min at room temperature, then added to ice–water (4 dm^3). The solid which separated was collected, washed thoroughly with water, and dried to give 5-acetamido-6-bromoindane **8** (227.3 g, 97%) as a white solid which was used without further purification; a recrystallised sample had mp 140–142 °C (from hexane) (lit.,[17] 143 °C) (Found: C, 52.1; H, 4.7; N, 5.5; $C_{11}H_{12}BrNO$ requires C, 52.0; H, 4.8; N, 5.5%); $\delta_H[(CD_3)_2SO]$ 2.04 (5 H, m, 2-H, Me), 2.82 (4 H, m, 1-H, 3-H), 7.37, 7.47 (each 1 H, s, 4-H, 7-H), 9.38 (1 H, s, N-H); *m*/*z* (EI) 255, 253 (M$^+$, each 15%), 213, 211 (41, 43), 174 (100).

5-Acetamido-6-cyanoindane 9

5-Acetamido-6-bromoindane **8** (227 g, 0.89 mol) and copper(I) cyanide (104.1 g, 1.16 mol) were stirred together in 1-methyl-2-pyrrolidone (954 cm^3) at 125 °C under argon for 30 min. The resulting solution was cooled to room temperature and added to a stirred mixture of aqueous NH_3 solution (d 0.88; 1590 cm^3) and ice (4770 cm^3). The resulting mixture was stirred for 15 min. The solid which separated was collected by filtration, washed with water (4770 cm^3), and stirred with dichloromethane (3180 cm^3) for 30 min. The resulting suspension was filtered and the filtrate was washed once with a small volume of water, dried ($MgSO_4$) and evaporated. The residue was triturated with diethyl ether (2384 cm^3) to give 5-acetamido-6-cyanoindane **9** (153.8 g, 86%), mp 174 °C (Found: C, 71.9; H, 6.1; N, 13.9; $C_{12}H_{12}N_2O$ requires C, 72.0; H, 6.0; N, 14.0%); ν_{max}(KBr disc)/cm^{-1} 2240, 1671; $\delta_H[(CD_3)_2SO]$ 2.06 (5 H, m, 2-H, Me), 2.88 (4 H, m, 1-H, 3-H), 7.40, 7.62 (each 1 H, s, 4-H, 7-H), 10.03 (1 H, s, N-H); *m*/*z* (EI) 200 (M$^+$, 11%), 158 (100).

2-Methyl-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4-one 10

Sodium hydroxide pellets (73.6 g, 1.84 mol) were added to a

mechanically stirred, precooled (ice–water bath) mixture of 5-acetamido-6-cyanoindane **9** (218.7 g, 1.09 mol), water (421 cm^3), ethanol (2103 cm^3) and aqueous hydrogen peroxide solution (30%; 841 cm^3) (gas evolution observed). After the initial exotherm (to *ca.* 40 °C) had ceased, the cooling bath was removed and the mixture was slowly warmed to 50–55 °C (hot air gun) and kept at this temperature for 1 h. It was then cooled and evaporated. Water (4.2 dm^3) was added to the residue and the mixture was heated to 50 °C. It was then cooled and acidified to pH 4–5 with 2 M hydrochloric acid whilst being stirred mechanically. The resulting precipitate was collected, washed with water, and dried to give the title compound **10** (181.8 g, 83%), mp 284–286 °C (Found: C, 71.8; H, 6.1; N, 13.9; $C_{12}H_{12}N_2O$ requires C, 72.0; H, 6.0; N, 14.0%); δ_H[$(CD_3)_2SO$] 2.05 (2 H, m, 7-H), 2.31 (3 H, s, 2-Me), 2.95 (4 H, m, 6-H, 8-H), 7.39 (1 H, s, 9-H), 7.87 (1 H, s, 5-H), 12.05 (1 H, s, 3-H); *m/z* (EI) 200 (M^+, 100%), 199 (93), 171 (8), 156 (23).

2-Methyl-3-pivaloyloxymethyl-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4-one 11

Potassium *tert*-butoxide (63.2 g, 0.56 mol) was added in portions during 15 min to a mechanically stirred suspension of **10** (87.9 g, 0.44 mol) in DMSO (691 cm^3) at room temperature (water bath) under argon. After a further 30 min, chloromethyl pivalate (124 cm^3, 0.86 mol) was added dropwise during 30 min whilst keeping the mixture at room temperature. After a further 24 h the mixture was poured into a stirred mixture of ammonium chloride (500 g) and ice–water (3 dm^3). Ethyl acetate (2 dm^3) was added and the mixture was filtered. The ethyl acetate layer was separated and the aqueous layer was extracted with ethyl acetate (400 cm^3). The combined ethyl acetate solution was washed with brine (400 cm^3), dried ($MgSO_4$) and evaporated to *ca.* 1 dm^3. Solid material which had separated was removed by filtration and the filtrate was further evaporated. The residual slurry was triturated with diethyl ether (500 cm^3). After cooling at 0 °C for a few hours, the solid was collected, washed with cold diethyl ether, and dried to give the title compound **11** (69.8 g, 51%), mp 130–132 °C (from hexane) (Found: C, 68.8; H, 7.1; N, 8.9; $C_{18}H_{22}N_2O_3$ requires C, 68.8; H, 7.05; N, 8.9%); ν_{max}(KBr disc)/cm^{-1} 1607, 1683, 1737; δ_H[$(CD_3)_2SO$] 1.13 (9 H, s, CMe_3), 2.06 (2 H, m, 7-H), 2.58 (3 H, s, 2-Me), 2.97 (4 H, m, 6-H, 8-H), 6.05 (2 H, s, 3-CH_2), 7.43 (1 H, s, 9-H), 7.92 (1 H, s, 5-H); *m/z* (EI) 314 (M^+, 21%), 212 (42), 200 (88), 57 (100).

Bromination of compound 11; reaction of major product 12a with diethyl 4-aminobenzoyl-L-glutamate

Compound **11** (5 g, 16 mmol), *N*-bromosuccinimide (3.12 g, 17.5 mmol), and dibenzoyl peroxide (0.01 g) were stirred and heated together in carbon tetrachloride (50 cm^3) under reflux. After 90 min the mixture was cooled to 0 °C and filtered. The filtrate was evaporated and the residue triturated with ether (40 cm^3). After cooling the resulting mixture to 0 °C the precipitate was collected, washed with ether and dried (yield 4.172 g). The NMR spectrum of this material showed two main components in the ratio *ca.* 3:1. The mixture was chromatographed with hexane–ethyl acetate (gradient from 100:0 to 50:50 v/v). The isolated minor (less polar) component was triturated with hexane to give 8-bromo-2-methyl-3-pivaloyloxymethyl-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4-one **12b** (0.751 g, 12%), mp 133–135 °C (Found: C, 55.3; H, 5.4; N, 7.0; $C_{18}H_{21}BrN_2O_3$ requires C, 55.0; H, 5.4; N, 7.1%); δ_H($CDCl_3$) 1.21 (9 H, s, CMe_3), 2.63 (2 H, m, and 3 H, s, superimposed, 7-H, 2-Me), 3.02 (1 H, ddd, *J* 3.2, 7.4, 16.3, 6-H), 3.25 (1 H, m, 6-H), 5.59 (1 H, dd, *J* 2.8, 6.1, 8-H), 6.11 (2 H, AB quartet, J_{AB} 10.5, 3-CH_2), 7.66 (1 H, s, 9-H), 8.15 (1 H, s, 5-H); *m/z* (EI) 392 (M^+, 8%), 313 (66), 199 (100). Fractions containing the major, more polar product were combined and evaporated to leave a residue (1.675 g). Trituration of part of this material (1.223 g) with hexane gave the impure 6-bromo compound **12a** as a solid (1.107 g; purity *ca.* 70% by NMR) containing traces of **12b** and larger amounts of two other impurities [for major component **12a**: δ_H($CDCl_3$) 1.22 (9 H, s, CMe_3), 2.63 (5 H, m, 7-H, 2-Me), 3.03 (1 H, ddd, *J* 3.3, 6.5, 17.2, 8-H), 3.30 (1 H, m, 8-H), 5.64 (1 H, dd, *J* 2.7, 5.5, 6-H), 6.11 (2 H, m, 3-CH_2), 7.49 (1 H, s, 9-H) 8.31 (1 H, s, 5-H)]. This material (1.05 g) was heated with diethyl 4-aminobenzoyl-L-glutamate (2.625 g, 8.1 mmol) in the presence of calcium carbonate (1.375 g, 13.8 mmol) in DMA (10 cm^3) at 110 °C for 30 min. The mixture was cooled and evaporated and the residue partitioned between ethyl acetate (100 cm^3) and water (100 cm^3). The aqueous layer was extracted with ethyl acetate (3 × 25 cm^3) and the combined ethyl acetate solution washed with brine (4 × 25 cm^3), dried ($MgSO_4$) and evaporated. The residue was chromatographed with hexane–ethyl acetate (gradient from 2:1 to 1:3 v/v) and the isolated product material triturated with ether to give compound **15** (0.839 g) as a yellow solid, mp 171 °C (softened from 134 °C), with NMR data as for the material prepared from ketone **13** (see below).

2-Methyl-3-pivaloyloxymethyl-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazoline-4,6-dione 13 and 2-methyl-3-pivaloyloxymethyl-3,4,7,8-tetrahydro-6*H*-cyclopenta[g]quinazolin-4,8-dione 14

tert-Butyl hydroperoxide (70% solution in water; 197.5 cm^3, 1.44 mol) was added during 7 min to a stirred suspension of chromium(VI) oxide (1.05 g, 10.5 mmol) in dichloromethane (413 cm^3) at room temperature (water bath). After complete addition, solid **11** (65.1 g, 0.207 mol) was added in portions during 2 min and the mixture was rapidly stirred at room temperature for 22 h. It was then cooled to 0 °C and 10% aqueous sodium metabisulfite solution (350 cm^3) was added at such a rate that the temperature did not exceed 10 °C. The resulting mixture was stirred for 2 h at room temperature, then partitioned between ethyl acetate (700 cm^3) and brine (500 cm^3). The aqueous layer was extracted with ethyl acetate (3 × 250 cm^3) and the combined organic solution was washed with saturated aqueous sodium hydrogen carbonate (500 cm^3). The sodium hydrogen carbonate solution was back-extracted with ethyl acetate (2 × 50 cm^3) and the organic solutions were combined and further washed successively with saturated brine (2 × 250 cm^3), saturated aqueous sodium hydrogen carbonate (2 × 250 cm^3) and saturated brine (250 cm^3), then dried ($MgSO_4$) and evaporated. The residue was crystallised from ethyl acetate to give a solid (42.68 g) which was found by NMR to be a *ca.* 3:1 mixture of **13** and **14.** Recrystallisation from ethyl acetate afforded pure **13** (17.259 g), mp 203–205 °C (Found C, 65.7; H, 6.2; N, 8.5; $C_{18}H_{20}N_2O_4$ requires C, 65.8; H, 6.1; N, 8.5%); ν_{max}(KBr disc)/cm^{-1} 1612, 1684, 1711, 1729; δ_H[$(CD_3)_2SO$] 1.15 (9 H, s, CMe_3), 2.64 (3 H, s, 3-Me), 2.72 (2 H, m, 7-H), 3.24 (2 H, m, 8-H), 6.06 (2 H, s, 3-CH_2), 7.72 (1 H, s, 9-H), 8.27 (1 H, s, 5-H); *m/z* (EI) 328 (M^+, 25%), 243 (5), 227 (56), 226 (45), 214 (77), 198 (26), 57 (100). Further **13** (7.756 g) was isolated from mother liquors by chromatography with dichloromethane–ethyl acetate (2:1 v/v) as eluant (total yield of **13**, 25.015 g, 37%), which also afforded pure samples of the less polar isomeric ketone **14**, mp 165 °C [from petroleum spirit (bp 60–80 °C)–EtOAc] (Found: C, 65.7; H, 6.1; N, 8.5; $C_{18}H_{20}N_2O_4$ requires C, 65.8; H, 6.1; N, 8.5%); ν_{max}(KBr disc)/cm^{-1} 1610, 1682, 1722, 1738; δ_H[$(CD_3)_2SO$] 1.14 (9 H, s, CMe_3), 2.62 (3 H, s, 2-Me), 2.76 (2 H, m, 7-H), 3.23 (2 H, m, 6-H), 6.07 (2 H, s, 3-CH_2), 7.73 (1 H, s, 9-H), 8.29 (1 H, s, 5-H); *m/z* (EI) 328 (M^+, 32%), 243 (4), 227 (60), 226 (65), 214 (96), 198 (26), 57 (100).

Diethyl *N*-(4-{*N*-[(6*RS*)-2-methyl-4-oxo-3-pivaloyloxymethyl-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]amino}-benzoyl)-L-glutamate 15

A 2-necked flask was charged with the ketone **13** (15.0 g, 45.7

mmol), diethyl 4-aminobenzoyl-L-glutamate (22.1 g, 68.6 mmol), toluene-4-sulfonic acid monohydrate (0.54 g, 2.8 mmol) and 1,2-dimethoxyethane (445 cm^3), and fitted with a pressure compensated dropping funnel containing activated 3 Å molecular sieve beads (4–8 mesh; 203 g). A condenser was fitted to the top of the dropping funnel and the mixture was stirred and heated under argon under reflux so that the condensed solvent passed through the molecular sieves before returning to the reaction flask. After 4 h the mixture was cooled to room temperature and a solution of sodium cyanoborohydride (4.5 g, 72 mmol) in methanol (81 cm^3) was added, followed immediately by acetic acid (4.5 cm^3). After a further 17 h, the solvents were evaporated and the residue was partitioned between ethyl acetate (400 cm^3) and saturated aqueous sodium hydrogen carbonate (400 cm^3). The aqueous layer was extracted with ethyl acetate (3 × 75 cm^3) and the combined organic solution was washed with brine (2 × 75 cm^3), dried ($MgSO_4$) and concentrated. Column chromatography with petroleum spirit (bp 60–80 °C)–ethyl acetate (successively 1:1, 1:2 and 1:3 v/v), followed by crystallisation from ethanol, afforded the title compound **15** (11.12 g, 38%), mp 169–174 °C (Found: C, 64.3; H, 6.65; N, 8.8; $C_{34}H_{42}N_4O_8$ requires C, 64.3; H, 6.7; N, 8.8%); $\delta_H[(CD_3)_2SO]$ 1.12 (9 H, s, CMe_3), 1.18 (6 H, m, 2 × CH_3CH_2), 2.07 (3 H, m, 7-H, glu β-H), 2.43 (2 H, t, *J* 7.4, glu γ-H), 2.60 (3 H, s and 1 H, m, superimposed, 2-Me, 7-H), 3.01 (1 H, m, 8-H), 3.08 (1 H, m, 8-H), 4.03 (4 H, m, 2 × CH_3CH_2), 4.39 (1 H, m, glu α-H), 5.18 (1 H, m, 6-H), 6.04 (2 H, AB quartet, J_{AB} 10.8, 3-CH_2), 6.73 (1 H, d, *J* 8.2, 6-NH), 6.79 (2 H, d, *J* 8.7, 3′-H, 5′-H), 7.50 (1 H, s, 9-H), 7.71 (2 H, d, *J* 8.7, 2′-H, 6′-H), 7.94 (1 H, s, 5-H), 8.31 (1 H, d, *J* 7.4, glu N-H); *m/z* (FAB) 657 [(M + Na)$^+$, 100%], 635 [(M + H)$^+$, 7], 432 (24), 313 (19), 199 (18).

Diethyl *N*-(4-{*N*-[(6*RS*)-2-methyl-4-oxo-3-pivaloyloxymethyl-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]-*N*-(prop-2-ynyl)amino}benzoyl)-L-glutamate 16

Compound **15** (11.57 g, 18.2 mmol), propargyl bromide (80% w/w solution in toluene; 25.7 cm^3, 230 mmol), calcium carbonate (9.2 g, 92 mmol), and *N*,*N*-dimethylacetamide (215 cm^3) were stirred together at 98–105 °C (bath temperature) under argon for 21 h. The mixture was cooled and evaporated and the residue partitioned between ethyl acetate (400 cm^3) and water (400 cm^3). The aqueous layer was extracted with ethyl acetate (4 × 100 cm^3) and the combined ethyl acetate solution washed with brine (4 × 50 cm^3), dried ($MgSO_4$) and evaporated. The residue was chromatographed with hexane–ethyl acetate (gradient from 4:1 to 1:4 v/v) as eluant. Evaporation of appropriate fractions gave the title compound **16** as a glass (9.39 g, 77%) [Found (in material triturated with hexane): C, 65.6; H, 6.6; N, 8.3; $C_{37}H_{44}N_4O_8 \cdot 0.25H_2O$ requires C, 65.6; H, 6.6; N, 8.3%]; $\delta_H[(CD_3)_2SO]$ 1.13 (9 H, s, CMe_3), 1.18 (6 H, m, 2 × CH_3CH_2), 2.05 (2 H, m, glu β-H), 2.22 (1 H, m, 7-H), 2.43 (2 H, t, *J* 7.4, glu γ-H), 2.5 (m, coincides with solvent signal, 7-H), 2.60 (3 H, s, 2-Me), 3.04 (1 H, m, 8-H), 3.15 (2 H, m, 8-H, C≡CH), 3.88 (1 H, m, $CH_2C{\equiv}C$), 4.07 (5 H, m, $CH_2C{\equiv}C$, 2 × CH_3CH_2), 4.40 (1 H, m, glu α-H), 5.78 (1 H, t, *J* 8.0, 6-H), 6.03 (2 H, s, 3-CH_2), 7.01 (2 H, d, *J* 8.7, 3′-H, 5′-H), 7.54 (1 H, s, 9-H), 7.79 (2 H, d, *J* 8.7, 2′-H, 6′-H), 7.84 (1 H, s, 5-H), 8.43 (1 H, d, *J* 7.4, glu N-H); *m/z* (FAB) 695 [(M + Na)$^+$, 23%], 673.3212 [(M + H)$^+$, 33], 470 (48), 313 (100), 199 (78) [calc. for (M + H)$^+$, 673.3237].

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-glutamic acid 17

A stirred solution of compound **16** (17.03 g, 25.3 mmol) in methanol (567 cm^3) was treated with aqueous sodium hydroxide solution (1 M; 114 cm^3) at room temperature under argon. After 24 h the solution was concentrated to *ca.* 80 cm^3, diluted with water (380 cm^3) and acidified to pH 4 with 2 M hydrochloric acid whilst stirring and cooling in ice. The resulting suspension was centrifuged and the precipitate washed several times with water by resuspension and centrifugation, then collected, washed once more, and dried to give the title compound **17** (11.839 g, 93%), mp 173–175 °C (Found: C, 61.6; H, 5.5; N, 10.55; $C_{27}H_{26}N_4O_6 \cdot 1.25H_2O$ requires C, 61.8; H, 5.5; N, 10.7%); $\delta_H[(CD_3)_2SO]$ 2.06 (2 H, m, glu β-H), 2.21 (1 H, m, 7-H), 2.33 (5 H, m, 2-Me, glu γ-H), 2.5 (m, coincides with solvent signal, 7-H), 3.02 (1 H, m, 8-H), 3.14 (2 H, m, 8-H, C≡CH), 3.87 (1 H, m, $CH_2C{\equiv}C$), 4.06 (1 H, m, $CH_2C{\equiv}C$), 4.37 (1 H, m, glu α-H), 5.76 (1 H, t, *J* 8.0, 6-H), 7.02 (2 H, d, *J* 8.3, 3′-H, 5′-H), 7.49 (1 H, s, 9-H), 7.78 (3 H, m, 2′-H, 6′-H, 5-H), 8.32 (1 H, d, *J* 7.7, glu N-H), 12.15 (1 H, s, 3-H), 12.39 (2 H, br s, 2 × CO_2H); *m/z* (FAB) 525 [(M + Na)$^+$, 100%], 503 [(M+H)$^+$, 16], 462 (70), 356 (24); t_R 708, 775 s.

4-{*N*-[(6*R*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]-*N*-(prop-2-ynyl)amino}benzoic acid 18 and *N*-(4-{*N*-[(6*S*)-2-methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[g]quinazolin-6-yl]-*N*-(prop-2-ynyl)amino}benzoyl)-L-glutamic acid 5

Compound **17** (5.614 g, 11.2 mmol) was dissolved in a portion (679 cm^3) of a solution prepared by dissolving tris(hydroxymethyl)aminomethane (tris) (12.11 g, 100 mmol) and anhydrous zinc chloride (0.035 g, 0.26 mmol) in water (950 cm^3). The pH of the resulting solution was adjusted to 7.3 by addition of 2 M hydrochloric acid, and water (15 cm^3) was added to give a total volume *ca.* 715 cm^3 {[tris] = 0.1 M}. The solution was warmed to 37 °C, carboxypeptidase G_2 (2042 units) was added, and the mixture was gently shaken. The reaction was followed by HPLC which indicated progressive disappearance of the diastereoisomer of **17** having the shorter retention time. After shaking for 10 h at 37 °C the mixture was cooled in ice and acidified to pH 4 with glacial acetic acid. The resulting suspension was kept at 5 °C overnight. The precipitate was collected, washed with water, dried, and chromatographed on silica (Merck no. 9385). Elution with dichloromethane–methanol (9:1 followed by 4:1 v/v), evaporation of appropriate fractions, and trituration with diethyl ether, afforded compound **18** [2.142 g; ee 84% (by HPLC)]. To prepare a sample for analysis, compound **18** (0.2 g) was dissolved in a mixture of 0.5 M aqueous sodium hydrogen carbonate (3 cm^3) and 1 M aqueous sodium hydroxide (3 cm^3); the solution was filtered, cooled in ice and acidified with glacial acetic acid and the precipitate was centrifuged, collected by filtration and washed with water to give **18** (0.178 g), mp 255 °C (decomp.) (Found: C, 70.5; H, 5.2; N, 11.1; $C_{22}H_{19}N_3O_3$ requires C, 70.8; H, 5.1; N, 11.25%); $[\alpha]_D^{23}$ +16.5 (*c* 1.0 in 1.0 M aq. NaOH); $\delta_H[(CD_3)_2SO]$ 2.22 (1 H, m, 7-H), 2.34 (3 H, s, 2-Me), 2.5 (m, coincides with solvent signal, 7-H), 3.02 (1 H, m, 8-H), 3.11 (2 H, m, 8-H, C≡CH), 3.87 (1 H, m, $CH_2C{\equiv}C$), 4.03 (1 H, m, $CH_2C{\equiv}C$), 5.74 (1 H, t, *J* 8.0, 6-H), 7.01 (2 H, d, *J* 8.9, 3′-H, 5′-H), 7.48 (1 H, s, 9-H), 7.79 (1 H, s, 5-H), 7.82 (2 H, d, *J* 8.9, 2′-H, 6′-H), 12.09 (2 H, br s, 3-H, CO_2H); *m/z* (FAB) 396 [(M + Na)$^+$, 48%], 374 [(M + H)$^+$, 82], 356 (51), 329 (54), 307 (100); t_R 882 s. Further elution of the column with dichloromethane–methanol–acetic acid (9:1:0.1 followed by 8:2:0.2) gave compound **5** which was re-chromatographed similarly to remove traces of compound **18**. Appropriate fractions were evaporated and the residue was triturated with water. After cooling to 5 °C the solid which had separated was collected, washed with cold water and dried to give **5** as a white solid (2.257 g, 80%), mp 202–205 °C. Material (0.145 g) suitable for analysis was obtained by acidifying a filtered, ice-cooled solution of **5** (0.157 g) in aqueous $NaHCO_3$ solution (0.5 M; 5 cm^3) to pH 4 with 1 M hydrochloric acid, collecting the precipitate and washing with water (Found: C, 62.1; H, 5.4; N, 10.75; $C_{27}H_{26}N_4O_6 \cdot H_2O$ requires C, 62.3; H, 5.4; N, 10.8%); $\delta_H[(CD_3)_2SO]$ 2.01 (2 H, m, glu β-H), 2.24 (1 H, m, 7-H), 2.33

(5 H, m, 2-Me, glu γ-H), 2.5 (m, coincides with solvent signal, 7-H), 3.02 (1 H, m, 8-H), 3.14 (2 H, m, 8-H, C≡CH), 3.87 (1 H, m, $CH_2C{\equiv}C$), 4.05 (1 H, m, $CH_2C{\equiv}C$), 4.36 (1 H, m, glu α-H), 5.76 (1 H, t, *J* 7.9, 6-H), 7.02 (2 H, d, *J* 8.9, 3′-H, 5′-H), 7.49 (1 H, s, 9-H), 7.79 (2 H, d, *J* 8.9, 2′-H, 6′-H), 7.80 (1 H, s, 5-H), 8.24 (1 H, d, *J* 7.5, glu N-H), 12.14 (1 H, s, 3-H), 12.63 (2 H, br s, 2 × CO_2H); *m/z* (FAB) 525 [$(M + Na)^+$, 7%], 503 [$(M + H)^+$, 7], 356 (9), 329 (17), 307 (32), 289 (31), 254 (39), 232 (100); t_R 777 s.

4-{*N*-[(6*S*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]-*N*-(prop-2-ynyl)amino}benzoic acid 19

Compound **5** (2.232 g, 4.4 mmol) was dissolved in an aqueous tris–$ZnCl_2$ solution (prepared as above; 424 cm^3) and the pH was adjusted to 7.3 with 2 M hydrochloric acid. Water (7 cm^3) was added to bring the total volume to *ca*. 446 cm^3 {[tris] = 0.1 M}. Carboxypeptidase G_2 (2233 units) was added and the solution was incubated at 37 °C with gentle shaking. A second portion of carboxypeptidase G_2 (2233 units) was added after 24 h, and a further portion (80 units) after 48 h (total time). After 72 h (total) the mixture was cooled to 0 °C and acidified to pH 4 with glacial acetic acid. The precipitate was isolated by centrifugation and filtration, washed with water, dried and chromatographed. Elution with dichloromethane–methanol (9 : 1 followed by 4 : 1), evaporation of appropriate fractions, and trituration of the residue with diethyl ether, afforded the title compound **19** [1.326 g, 80%; ee *ca*. 98% (by HPLC)]. A specimen (0.145 g) was dissolved in aqueous sodium hydroxide (0.67 M; 15 cm^3), and reprecipitated by acidification with acetic acid after filtering and cooling the solution, to give a sample for analysis (0.137 g) (darkened gradually above 250 °C without distinct mp) (Found: C, 69.6; H, 5.2; N, 11.0; $C_{22}H_{19}N_3O_3\cdot 0.3H_2O$ requires C, 69.75; H, 5.2; N, 11.1%); $[\alpha]_D^{25}$ −22 (*c* 1.0 in 1.0 M aq. NaOH); $\delta_H[(CD_3)_2SO]$ 2.21 (1 H, m, 7-H), 2.33 (3 H, s, 2-Me), 2.5 (m, coincides with solvent signal, 7-H), 3.01 (1 H, m, 8-H), 3.15 (2 H, m, 8-H, C≡CH), 3.87 (1 H, m, $CH_2C{\equiv}C$), 4.04 (1 H, m, $CH_2C{\equiv}C$), 5.76 (1 H, t, *J* 8.1, 6-H), 7.02 (2 H, d, *J* 9.0, 3′-H, 5′-H), 7.49 (1 H, s, 9-H), 7.78 (1 H, s, 5-H), 7.81 (2 H, d, *J* 9.0, 2′-H, 6′-H), 12.13 (1 H, s, 3-H), 12.3 (1 H, br s, CO_2H); *m/z* (FAB) 396 [$(M + Na)^+$, 41%], 374 [$(M + H)^+$, 78], 356 (44), 329 (13), 307 (27); t_R 981 s.

Pentafluorophenyl 4-{*N*-[(6*R*)-2-methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]-*N*-(prop-2-ynyl)amino}benzoate 20

Pyridine (0.9 cm^3, 11 mmol) and pentafluorophenyl trifluoroacetate (1.1 cm^3, 6.4 mmol) were added successively to a stirred solution of compound **18** (ee 84%; 1.705 g, 4.6 mmol) in DMA (170 cm^3) at room temperature. After 3 h further pentafluorophenyl trifluoroacetate (1.1 cm^3) was added. After a further 2 h the solution was evaporated and the residue chromatographed with dichloromethane–ethanol (stepwise gradient from 100 : 0 to 95 : 5) as eluant. The isolated product material was rechromatographed with the same solvents (stepwise gradient to 96 : 4) and finally triturated with ether and dried to give the title compound **20** (2.12 g, 86%), mp 225–227 °C (Found: C, 61.9; H, 3.5; N, 7.65; F, 17.3; $C_{28}H_{18}F_5N_3O_3\cdot 0.25H_2O$ requires C, 61.8; H, 3.4; N, 7.7; F, 17.5%); $\delta_H[(CD_3)_2SO]$ 2.25 (1 H, m, 7-H), 2.33 (3 H, s, 2-Me), 2.54 (1 H, m, 7-H), 3.04 (1 H, m, 8-H), 3.23 (2 H, m, 8-H, C≡CH), 3.97 (1 H, m, $CH_2C{\equiv}C$), 4.13 (1 H, m, $CH_2C{\equiv}C$), 5.87 (1 H, t, *J* 8.0, 6-H), 7.16 (2 H, d, *J* 8.8, 3′-H, 5′-H), 7.50 (1 H, s, 9-H), 7.79 (1 H, s, 5-H), 8.03 (2 H, d, *J* 8.8, 2′-H, 6′-H), 12.16 (1 H, s, 3-H); $\delta_F[(CD_3)_2SO]$ −162.49 (2 F, t, *J* 23, 3-F, 5-F), −158.13 (1 F, t, *J* 23, 4-F), −153.45 (2 F, d, *J* 21, 2-F, 6-F); *m/z* (FAB) 540.1328 [$(M + H)^+$, 21%], 356 (19), 199 (100) [calc. for $(M + H)^+$, 540.1347].

Diethyl *N*-(4-{*N*-[(6*R*)-2-methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]-*N*-(prop-2-ynyl)amino}benzoyl)-L-glutamate 21

A solution of dry triethylamine (0.072 g, 0.71 mmol) in DMF (1.5 cm^3) was added to a stirred mixture of compound **20** (0.093 g, 0.172 mmol), diethyl L-glutamate hydrochloride (0.055 g, 0.229 mmol) and 1-hydroxybenzotriazole (1 mg) at room temperature. After 16 h the mixture was evaporated and the residue dissolved in ethyl acetate (15 cm^3). The solution was washed successively with 10% aqueous citric acid solution (5 cm^3), saturated aqueous sodium hydrogen carbonate (5 cm^3), and brine (5 cm^3), then dried ($MgSO_4$) and evaporated. The residue was chromatographed with dichloromethane–ethanol (stepwise gradient from 100 : 0 to 95 : 5). A colourless glass was obtained which was triturated with hexane and dried to give the title compound **21** (0.083 g, 86%), mp 95 °C (Found: C, 65.9; H, 6.1; N, 9.9; $C_{31}H_{34}N_4O_6\cdot 0.25\ H_2O$ requires C, 66.1; H, 6.2; N, 9.9%); $\delta_H[(CD_3)_2SO]$ 1.18 (6 H, m, 2 × CH_3CH_2), 2.06 (2 H, m, glu β-H), 2.26 (1 H, m, 7-H), 2.33 (3 H, s, 2-Me), 2.42 (2 H, t, *J* 7.4, glu γ-H), 2.5 (m, coincides with solvent signal, 7-H), 3.08 (2 H, m, 8-H, C≡CH), 3.16 (1 H, m, 8-H), 3.88 (1 H, m, $CH_2C{\equiv}C$), 4.06 (5 H, m, $CH_2C{\equiv}C$, 2 × CH_3CH_2), 4.42 (1 H, m, glu α-H), 5.74 (1 H, t, *J* 7.9, 6-H), 7.01 (2 H, d, *J* 8.9, 3′-H, 5′-H), 7.48 (1 H, s, 9-H), 7.80 (3 H, m, 2′-H, 6′-H, 5-H), 8.34 (1 H, d, *J* 7.5, glu N–H), 12.04 (1 H, s, 3-H); *m/z* (FAB) 581 [$(M + Na)^+$, 15%], 559.2541 [$(M + H)^+$, 14], 356 (39), 199 (100) [calc. for $(M + H)^+$, 559.2557].

N-(4-{*N*-[(6*R*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]-*N*-(prop-2-ynyl)amino}benzoyl)-L-glutamic acid (6*R*)-17

Aqueous sodium hydroxide solution (0.4 M; 0.55 cm^3), was added to a stirred suspension of diester **21** (0.068 g, 0.122 mmol) in methanol (2.7 cm^3) at room temperature. After 24 h further aqueous sodium hydroxide (1.0 M; 0.33 cm^3) was added. After a further 2 h the solution was concentrated to low volume, re-diluted with water (5 cm^3), and filtered. It was then acidified to pH 4 with 1 M hydrochloric acid whilst stirring and cooling in ice. The resulting suspension was centrifuged and the precipitate collected, washed with cold water and dried to give the title compound (6*R*)-**17** as a white solid (0.050 g, 82%), mp 171–174 °C, containing *ca*. 10% compound **5** (estimated by HPLC; peaks overlapped) (Found: C, 61.6; H, 5.5; N, 10.7; $C_{27}H_{26}N_4O_6\cdot 1.25H_2O$ requires C, 61.8; H, 5.5; N, 10.7%); $\delta_H[(CD_3)_2SO]$ 2.06 (2 H, m, glu β-H), 2.22 (1 H, m, 7-H), 2.33 (5 H, m, 2-Me, glu γ-H), 2.5 (m, coincides with solvent signal, 7-H), 3.02 (1 H, m, 8-H), 3.09 (2 H, m, 8-H, C≡CH), 3.88 (1 H, m, $CH_2C{\equiv}C$), 4.05 (1 H, m, $CH_2C{\equiv}C$), 4.39 (1 H, m, glu α-H), 5.74 (1 H, t, *J* 7.8, 6-H), 7.00 (2 H, d, *J* 9.0, 3′-H, 5′-H), 7.48 (1 H, s, 9-H), 7.79 (3 H, m, 2′-H, 6′-H, 5-H), 8.24 (1 H, d, *J* 7.7, glu N-H), 12.07 (1 H, s, 3-H), 12.28, (br s, CO_2H); *m/z* (FAB) 525 [$(M + Na)^+$, 81%], 503 [$(M + H)^+$, 69], 356 (100); t_R 711 s (major peak) and 805 s (impurity peak). Addition of this material to (6*RS*)-**17** obtained as above resulted in relative enlargement of the HPLC peak of shorter retention time, and addition of compound **5** to this material resulted in relative enlargement of the impurity peak.

Pentafluorophenyl 4-{*N*-[(6*S*)-2-methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]-*N*-(prop-2-ynyl)amino}-benzoate 22

Pyridine (0.5 cm^3, 6 mmol) and pentafluorophenyl trifluoroacetate (0.7 cm^3, 4 mmol) were added successively to a stirred solution of the acid **19** (1 g, 2.7 mmol) in DMA (110 cm^3) at room temperature. After 3 h further pentafluorophenyl trifluoroacetate (0.6 cm^3, 3.5 mmol) was added. After a further 2 h the products were evaporated and the residue was chromatographed with dichloromethane–ethanol (gradient, 100 : 0 to

90:10) as eluant. The isolated product material was re-chromatographed with dichloromethane–ethanol (gradient, 100:0 to 95:5) as eluant to give the title compound **22** (1.335 g, 92%) as an amorphous solid, mp 223–225 °C [Found (in material triturated with ether): C, 61.7; H, 3.5; N, 7.7; F, 17.5; $C_{28}H_{18}F_5N_3O_3 \cdot 0.25H_2O$ requires C, 61.8; H, 3.4; N, 7.7; F, 17.5%]; δ_H [$(CD_3)_2SO$] 2.26 (1 H, m, 7-H), 2.34 (3 H, s, 2-Me), 2.55 (1 H, m, 7-H), 3.05 (1 H, m, 8-H), 3.19 (2 H, m, 8-H, C≡CH), 3.97 (1 H, m, $CH_2C{\equiv}C$), 4.14 (1 H, m, $CH_2C{\equiv}C$), 5.87 (1 H, t, *J* 7.8, 6-H), 7.16 (2 H, d, *J* 9.1, 3′-H, 5′-H), 7.50 (1 H, s, 9-H), 7.81 (1 H, s, 5-H), 8.03 (2 H, d, *J* 9.1, 2′-H, 6′-H), 12.10 (1 H, s, 3-H); δ_F[$(CD_3)_2SO$] −162.46 (2 F, t, *J* 22, 3-F, 5-F), −158.09 (1 F, t, *J* 23, 4-F), −153.45 (2 F, d, *J* 21, 2-F, 6-F); *m*/*z* (FAB) 539.1290 (M^+, 100%), 356 (94) (calc. for M^+, 539.1268).

Pentafluorophenyl 4-{*N*-[(6*S*)-2,3-dimethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]-*N*-(prop-2-ynyl)amino}benzoate 23

Caesium carbonate (0.201 g, 0.62 mmol) was added to a rapidly stirred mixture of **22** (0.225 g, 0.42 mmol), DMF (1.9 cm^3), and iodomethane (0.283 g, 2 mmol) at room temperature. After 3 h the mixture was evaporated and the residue partitioned between ethyl acetate (50 cm^3) and water (15 cm^3). The aqueous layer was extracted with ethyl acetate (4 × 10 cm^3) and the combined ethyl acetate solution was dried ($MgSO_4$) and evaporated. The residue was chromatographed using dichloromethane–ethanol (100:0, 99:1 and 98:2 in succession) as eluant. Trituration of the isolated product material with hexane gave the title compound **23** (0.206 g, 89%) as a white solid, mp 237–239 °C (Found: C, 61.6; H, 3.7; N, 7.3; $C_{29}H_{20}F_5N_3O_3 \cdot \frac{2}{3}H_2O$ requires C, 61.6; H, 3.8; N, 7.4%); δ_H[$(CD_3)_2SO$] 2.24 (1 H, m, 7-H), 2.57 (1 H, m and 3 H, s, superimposed, 7-H, 2-Me), 3.04 (1 H, m, 8-H), 3.23 (2 H, m, 8-H, C≡CH), 3.51 (3 H, s, 3-Me), 3.97 (1 H, m, $CH_2C{\equiv}C$), 4.13 (1 H, m, $CH_2C{\equiv}C$), 5.89 (1 H, t, *J* 8.0, 6-H), 7.17 (2 H, d, *J* 9.1, 3′-H, 5′-H), 7.51 (1 H, s, 9-H), 7.83 (1 H, s, 5-H), 8.03 (2 H, d, *J* 9.1, 2′-H, 6′-H); δ_F[$(CD_3)_2SO$] −162.44 (2 F, t, *J* 21, 3-F, 5-F), −158.07 (1 F, t, *J* 22, 4-F), −153.44 (2 F, d, *J* 19, 2-F, 6-F); *m*/*z* (FAB) 553.1450 (M^+, 100%), 370 (53) (calc. for M^+, 553.1425).

4-{*N*-[(6*S*)-2,3-Dimethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]-*N*-(prop-2-ynyl)amino}-*N*-[(*S*)-*sec*-butyl]benzamide 24

(*S*)-(+)-*sec*-Butylamine (0.08 g, 1.1 mmol) was added to a stirred solution of **23** (0.104 g, 0.19 mmol) in DMF (1 cm^3) at room temperature. After 3.5 h, the mixture was evaporated and the residue chromatographed using dichloromethane–ethanol (100:0, 98:2, and 97:3 in succession) as eluant. Evaporation of appropriate fractions left the title compound **24** as a glass (0.067 g, 80%). Crystallisation from ethyl acetate gave an analytical specimen, mp 232–234 °C (Found: C, 73.3; H, 6.9; N, 12.7; $C_{27}H_{30}N_4O_2$ requires C, 73.3; H, 6.8; N, 12.7%); δ_H[$(CD_3)_2SO$] 0.86 (3 H, t, *J* 7.4, CH_3CH_2), 1.11 (3 H, d, *J* 6.7, CH_3CH), 1.50 (2 H, m, CH_3CH_2), 2.21 (1 H, m, 7-H), 2.5 (m, coincides with solvent signal, 7-H), 2.56 (3 H, s, 2-Me), 3.03 (1 H, m, 8-H), 3.09 (2 H, m, 8-H, C≡CH), 3.51 (3 H, s, 3-Me), 3.88 (2 H, m, $CH_2C{\equiv}C$, CH_3CH), 4.02 (1 H, m, $CH_2C{\equiv}C$), 5.74 (1 H, t, *J* 8.0, 6-H), 6.98 (2 H, d, *J* 9.0, 3′-H, 5′-H), 7.49 (1 H, s, 9-H), 7.77 (4 H, m, 2′-H, 6′-H, 5-H, N-H); *m*/*z* (FAB) 465 [$(M + Na)^+$, 71%], 443 [$(M + H)^+$, 47], 370 (100).

Crystallographic studies

Compound **24** was recrystallised from ethanol as small elongated plates. Persistent attempts at data collection with laboratory X-ray sources did not produce data sets with >20% reflections being observed. A satisfactory data set was finally obtained at the Daresbury Synchrotron Facility using a Bruker SMART CCD area detector system on station 9.8, with φ and ω scans and a crystal of maximum dimensions 0.1 mm cooled to −123 °C. The X-ray wavelength was 0.68750 Å, and data was collected to θ max of 27.4°. The space group was uniquely assigned as $P2_12_12_1$, with cell dimensions $a = 8.9541(1)$, $b = 14.9705(2)$, $c = 35.6640(3)$ Å; the cell volume of 4780.66 $Å^3$ suggested that the asymmetric unit contains two independent molecules, which was confirmed by the subsequent analysis. A total of 17904 reflections were measured, which were merged to 9834 unique reflections; R_{merge} was 0.045. 7663 reflections had $I > 2\sigma(I)$. The structure was solved by non-routine application of direct methods, and refined by full-matrix least-squares methods. The positions of all hydrogen atoms were revealed in subsequent ΔF maps, and their positional and isotropic thermal parameters were included in the final rounds of refinement. At convergence, the final *R* factor for the 7663 significant data with $F > 4\sigma(F)$ was 0.0543. Atomic coordinates, temperature factors and derived geometric parameters are available from the Cambridge Crystallographic Data Centre.†

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† CCDC reference number 207/324. See http://www.rsc.org/suppdata/p1/1999/1495 for crystallographic files in .cif format.

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