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Targeting the α-folate receptor with cyclopenta[*g*]quinazoline-based inhibitors of thymidylate synthase

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Abstract—The α-FR has been reported to be overexpressed in many carcinomas, in particular those of the ovary and uterus. The high expression of α-FR in some tumours compared with normal tissues has been exploited over the last decade for folate-mediated targeting of macromolecules, anticancer drugs, imaging agents and nucleic acids to cancer cells. CB300638, a cyclopenta[*g*]quinazoline-based inhibitor of thymidylate synthase (TS), has been reported to have high affinity for the receptor and selectivity for α-FR overexpressing tumour cell lines. In this study, the structural features of the molecule, in particular modifications at the 2-position, have been investigated with respect to TS inhibition, affinity for the α-FR and reduced folate carrier (RFC) and activity in A431-FBP cells (transfected with human α-FR) compared with neo-transfected A431 cells. Compounds **1a**,**b**, **2a**,**b** and **3a**,**b** were synthesised utilising multistep sequences. It was found that the 2-substituent does not affect the affinity for the α-FR; however, it greatly affects selectivity for A431-FBP cells, and suggests that there are factors other than TS inhibition and α-FR affinity that are important for the activity of these compounds. Compound **2b** (2-CH_2OH derivative) displayed the highest selectivity for the A431-FBP cells compared with A431 cells.

1. Introduction

The majority of folates are transported into cells by the ubiquitously expressed reduced folate carrier (RFC). Likewise, most classical antifolates utilise the RFC to enter cells.[1] In addition, the α-folate receptor (α-FR) can function as a high affinity, low capacity, folate transporter via a receptor-mediated endocytosis process.[2,3] This may play a role in the activity of some antifolate drugs in tumours that overexpress the receptor.[4]

The FR (sometimes referred to as the membrane folate binding protein (FBP)) is a glycosyl-phosphatidylinositol (GPI)-anchored cell surface glycoprotein that exists in three isoforms, α, β and γ.[5] Whilst displaying restricted expression on the apical membrane surface of some normal tissues (predominantly kidney and choroid plexus), the α-FR has been reported to be overexpressed in many carcinomas.[5–9] In ovarian cancer, the α-FR is expressed to a high level in ~90% of cases.[6,8] The β-isoform is very limited in its expression and is generally non-functional for folate binding, although it is reported to be overexpressed in some pathogenic cells including synovial macrophages and myeloid leukaemic cells.[8] The γ-isoform is a restricted lineage marker on myeloid stem cells and is secreted.[10] The high expression of α-FR in some tumours compared with normal tissues has been exploited over the last decade for folate-mediated targeting of macromolecules, anticancer drugs, imaging agents and nucleic acids to cancer cells.[11–16] By using this approach, the folate receptor has been utilised as a vehicle for the transfer of these agents (conjugated in large to folic acid) selectively into cancer cells.[11–17]

Our approach has been to discover folate-based thymidylate synthase (TS) inhibitors that are selectively transported via the α-FR and not the RFC. It is predicted

Abbreviations: TS, thymidylate synthase; α-FR, α-folate receptor; FBP, folate binding protein; RFC, reduced folate carrier; DEPC, diethyl phosphorocyanidate; DIEA, diisopropylethylamine; TFA, trifluoroacetic acid; Glu, glutamic acid; Pg, propargyl.

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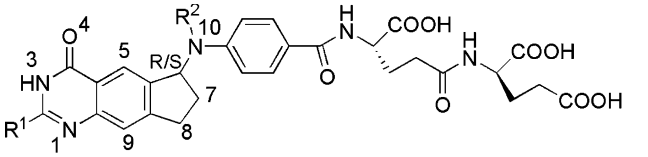
that such compounds would be less toxic than classical antifolate drugs, that is, should accumulate to low levels in normal proliferating tissues compared with α-FR overexpressing tumours.

Thymidylate synthase is an essential enzyme that catalyses the conversion of 2′-deoxyuridine 5′-monophosphate (dUMP) to thymidine 5′-monophosphate (dTMP), and is an established target in cancer chemotherapy.[18] A number of inhibitors of TS, synthesised in our laboratory, had high affinity for α-folate receptor including a class of cyclopenta[*g*]quinazoline-based antifolates.[19–21] These were very potent inhibitors of the TS enzyme and some were poor substrates for the RFC. In particular, CB300638 (Fig. 1) displayed high affinity for the α-FR and low affinity for the RFC.[21] In addition, CB300638 was ~300-fold more potent at inhibiting in vitro cell growth of the A431-FBP cell line (transfected with α-FR) compared with the A431 cell line (neo-transfected).[21] Indeed, this activity was reduced when 1 μM folic acid was added to the medium, consistent with CB300638 being an α-FR-mediated TS inhibitor. These findings prompted us to investigate the structural features of the molecule that influence affinity for the α-folate receptor and the cellular uptake via folate receptor-mediated endocytosis. In particular, we were interested in studying the effect of changing the substituent at the 2-position of CB300638 (e.g., change to NH_2 and CH_2OH) on affinity for the α-FR. This stems from the observation in quinazoline-based antifolates that substituents at the 2-position appeared to influence affinity for both α-FR and RFC.[22]

By undertaking this study, it was hoped to identify compounds that specifically utilise the folate receptor for cellular entry. It was expected that molecules displaying this property could be delivered with a degree of selectivity to those tumours overexpressing the receptor compared with the normal tissues. It was therefore envisaged that cyclopenta[*g*]quinazoline-based inhibitors of TS that utilise the α-folate receptor to enter cells could be selectively delivered to folate receptor overexpressing cells and then exert their anticancer activity by inhibiting TS.

2. Chemistry

The target molecules for this study are shown in Figure 1. For their synthesis, a linear synthetic approach was followed by first synthesising the cyclopenta[*g*]quinazoline ring. C^6–N^{10} bond formation then allowed the introduction of the *p*-aminobenzoate moiety followed by the N^{10}-substituent. Finally, attachment of the dipeptide unit and removal of the protecting groups afforded the desired products as a mixture of diastereoisomers since the chiral centre at 6-position is racemic. Although the synthesis and activity of **1b** (CB300638) was earlier reported by us,[19] this compound was re-synthesised by the new route utilised to prepare the 2-NH_2 and 2-CH_2OH counterparts of **1b**, and it is presented in this study.



1a. R^1 = Me, R^2 = Me; **1b (CB300638).** R^1 = Me, R^2 = Pg

2a. R^1 = CH_2OH, R^2 = Me; **2b.** R^1 = CH_2OH, R^2 = Pg

3a. R^1 = NH_2, R^2 = Me; **3b.** R^1 = NH_2, R^2 = Pg

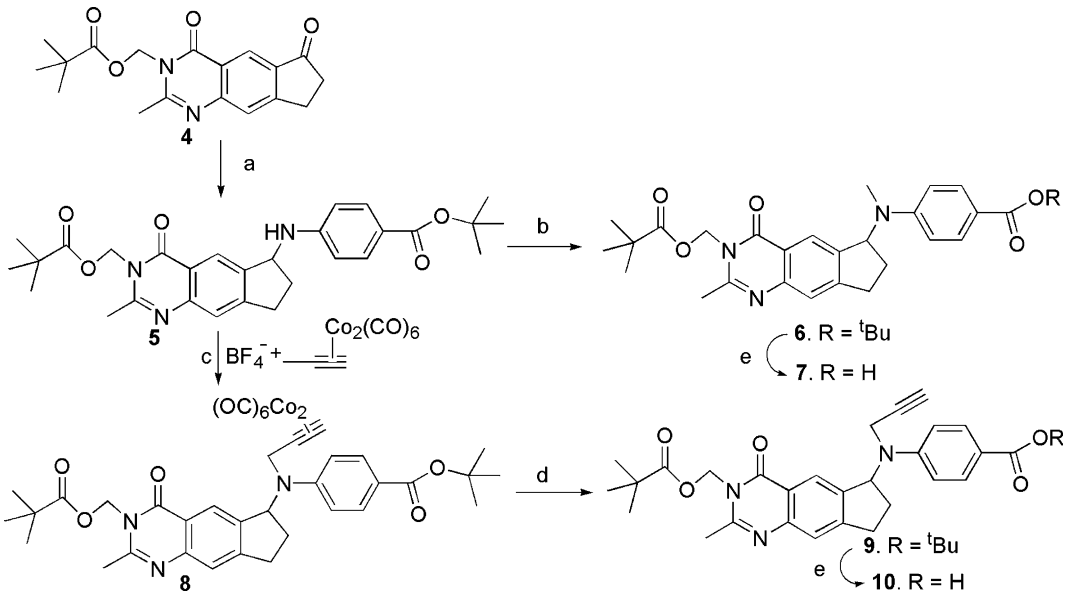
Figure 1. Cyclopenta[*g*]quinazoline-based inhibitors of TS.

The syntheses of **1a**,**b** are shown in Schemes 1 and 2. The synthetic sequence starts with the 6-oxo-cyclopenta[*g*]quinazolin-4-one derivative **4**.[23] Reductive amination of the ketone utilising *tert*-butyl 4-aminobenzoate as the amine component afforded **5** in 29% yield. Methylation of the N^{10}–H was achieved upon treatment with 37% aq HCHO/AcOH/$NaBH_3CN$ (Scheme 1). N^{10}-Propargylation was carried out under mild conditions (CH_2Cl_2, DIEA and rt) by reacting **5** with the (propargyl)$Co_2(CO)_6^+BF_4^-$ salt followed by decomplexation with $Fe(NO_3)_3$ in EtOH, an elegant modification of the Nicholas reaction.[24,25]

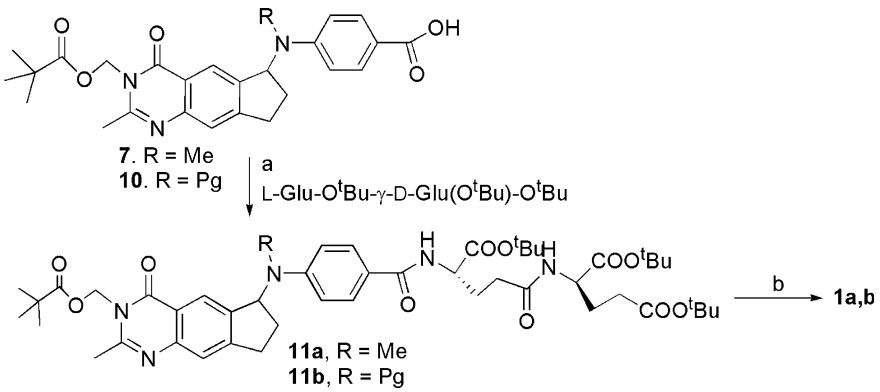
The acids **7** and **10**, obtained from **6** and **9**, respectively, by TFA hydrolysis, were coupled with L-Glu-*O*^{t}Bu-γ-D-Glu(*O*^{t}Bu)-*O*^{t}Bu[26] via carboxyl activation using DEPC (Schemes 1, and 2). Finally, removal of the protecting groups by treatment with TFA and then alkaline hydrolysis afforded the desired products **1a**,**b** (Scheme 2).

Our initial efforts to synthesise the 2-hydroxymethylcyclopenta[*g*]quinazoline-based derivatives **2a**,**b** were based on the linear synthetic sequence utilised for the synthesis of their 2-methyl counterparts **1a**,**b**, and in this route the 6-oxo-cyclopenta[*g*]quinazolin-4-ones **24a**,**b** were key intermediates (Scheme 3). The synthesis starts with 5-amino-6-carboxyindane[27] which was cyclised to **13** upon treatment with chloroacetonitrile and MeOH/Na. Displacement of the chloride was accomplished by treating **13** with caesium acetate in DMF; and the resulting acetate ester was hydrolysed to the 2-hydroxymethylcyclopenta[*g*]quinazolin-4-one **14**. The hydroxy functionality was then protected as either an acetate or trimethylacetate ester, and it was found that the use of the trimethylacetate (pivaloyl) ester as a protecting group leads to an improvement in solubility making these compounds easier to handle in subsequent steps. Oxidation of **15a** with CrO_3/*tert*-butyl hydroperoxide was problematic on this occasion. However, by replacing CrO_3 with $(Ph_3SiO)_2CrO_2$,[28] the 6-oxo-cyclopenta[*g*]quinazolin-4-one **24a** was obtained in 20% yield, and **24b** in 45% yield (Scheme 3).

Initial attempts to construct the C^6–N^{10} bond by conventional reductive amination conditions (i.e., 4-TsOH, DME, then $NaBH_3CN$, MeOH and AcOH) failed, prompting us to investigate alternative reductive amination conditions and also to attempt constructing the C^6–N^{10} bond by a displacement reaction via the bromocyclopenta[*g*]quinazolin-4-one derivative **18**. The displacement of the bromide in **18** with *tert*-butyl



Scheme 1. Reagents and conditions: (a) i—4-toluenesulfonic acid monohydrate, DME, *tert*-butyl 4-aminobenzoate, ii—$NaBH_3CN$, AcOH, MeOH; (b) aq HCHO, AcOH, $NaBH_3CN$; (c) CH_2Cl_2, rt; (d) $Fe(NO_3)_3$, EtOH, rt; (e) TFA, rt.



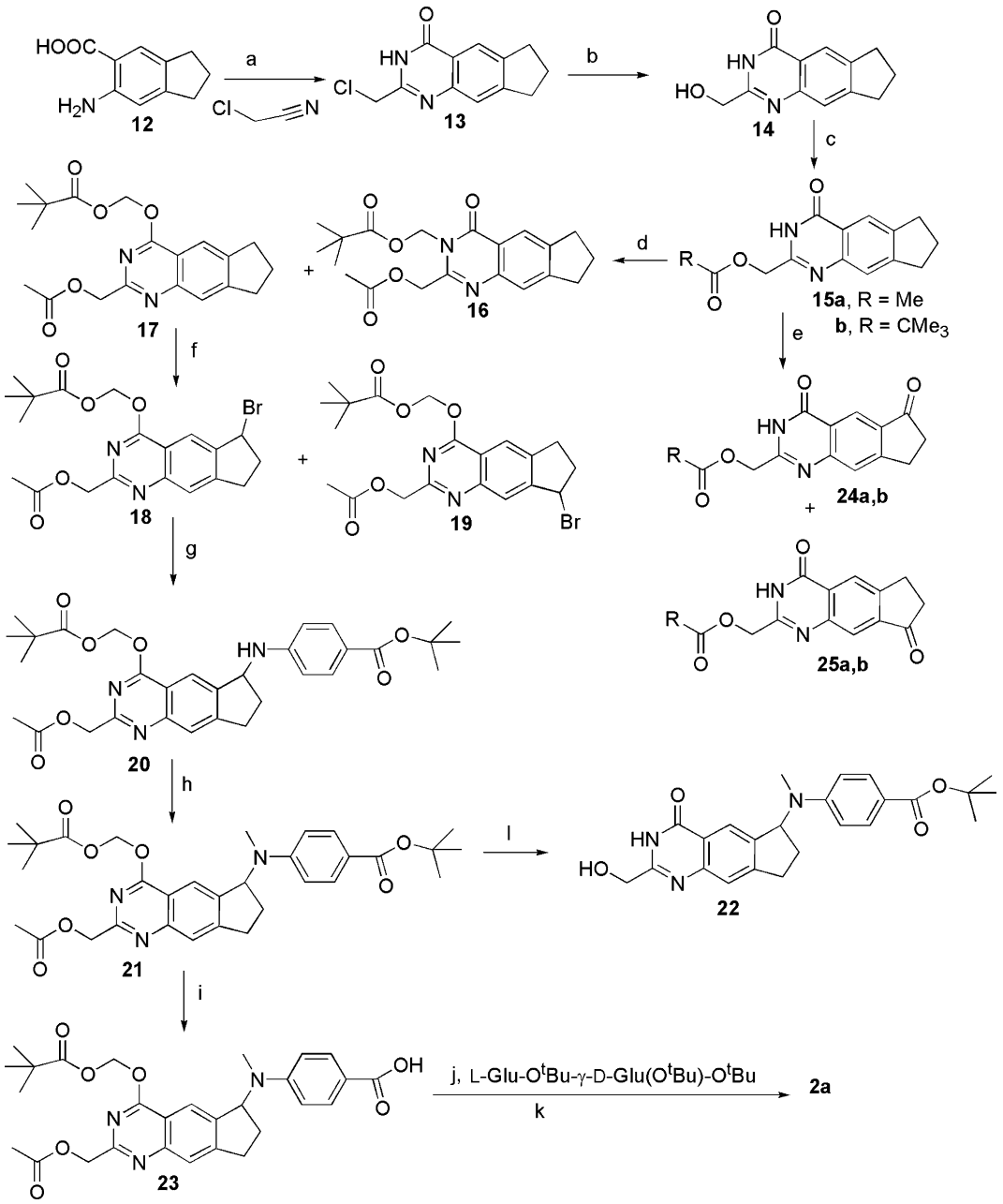
Scheme 2. Reagents and conditions: (a) DEPC, DMF, rt; (b) i—TFA, ii—$NaOH/H_2O$.

4-aminobenzoate was accomplished using excess DIEA in DMF to give **20** in low yield (19%). N^{10}-Methylation, TFA hydrolysis of the *tert*-butyl ester, coupling to L-Glu-γ-D-Glu(*O*^{t}Bu)-*O*^{t}Bu, and removal of the protecting groups afforded the desired target compound **2a** (Scheme 3).

Our persistence in seeking a higher yielding reaction for the formation of the C^6–N^{10} bond via a reductive amination procedure was rewarded, since the ketones **24a**,**b** were finally aminated with *tert*-butyl 4-aminobenzoate in the presence of decaborane[29] (Scheme 4). Subsequent N^{10}-propargylation, TFA hydrolysis of the *tert*-butyl ester, coupling to the dipeptide L-Glu-γ-D-Glu(*O*^{t}Bu)-*O*^{t}Bu,[26] and removal of the protecting groups gave the desired target compound **2b** (Scheme 4). Compound **2b** was also prepared by coupling the 2-CH_2OH unprotected form of **30b** (readily obtained from **29b** by TFA and alkaline hydrolysis of the *tert*-butyl and trimethylacetate esters, respectively) to the dipeptide L-Glu-γ-D-Glu(*O*^{t}Bu)-*O*^{t}Bu followed by TFA *tert*-butyl ester hydrolysis. It should be noted that N^{10}-methylation of **26b** followed by alkaline hydrolysis of the trimethylacetate ester in **27** afforded the 2-hydroxymethyl derivative **22** (Scheme 4) that had an ^{1}H NMR identical with that of a sample produced as described in Scheme 3.

For the synthesis of the 2-aminocyclopenta[*g*]quinazolin-4-one derivatives **3a**,**b** a similar linear approach was followed (Scheme 5). The synthesis started with 5-amino-6-carboxyindane, which was converted into isatoic anhydride **32** upon treatment with triphosgene in THF. Ring-opening of the anhydride in refluxing MeOH gave 5-amino-6-methoxycarbonylindane (**33**). It was found that the 2-aminocyclopenta[*g*]quinazolin-4-one **34** was most efficiently formed when **33** was treated with chloroformamidine hydrochloride in dimethylsulfone at 150 °C (Scheme 5).[30] Chloroformamidine hydrochloride was prepared by passing HCl gas through a solution of NH_2CN in Et_2O.[31]

Next, the amino functionality was protected as a pivalamide by reacting with trimethylacetic anhydride in DMF at 100 °C. The conversion of **35** to the 8-oxo and 6-oxo derivatives **36** and **37**, respectively, was achieved by utilising *tert*-butyl hydroperoxide/CrO_3 as

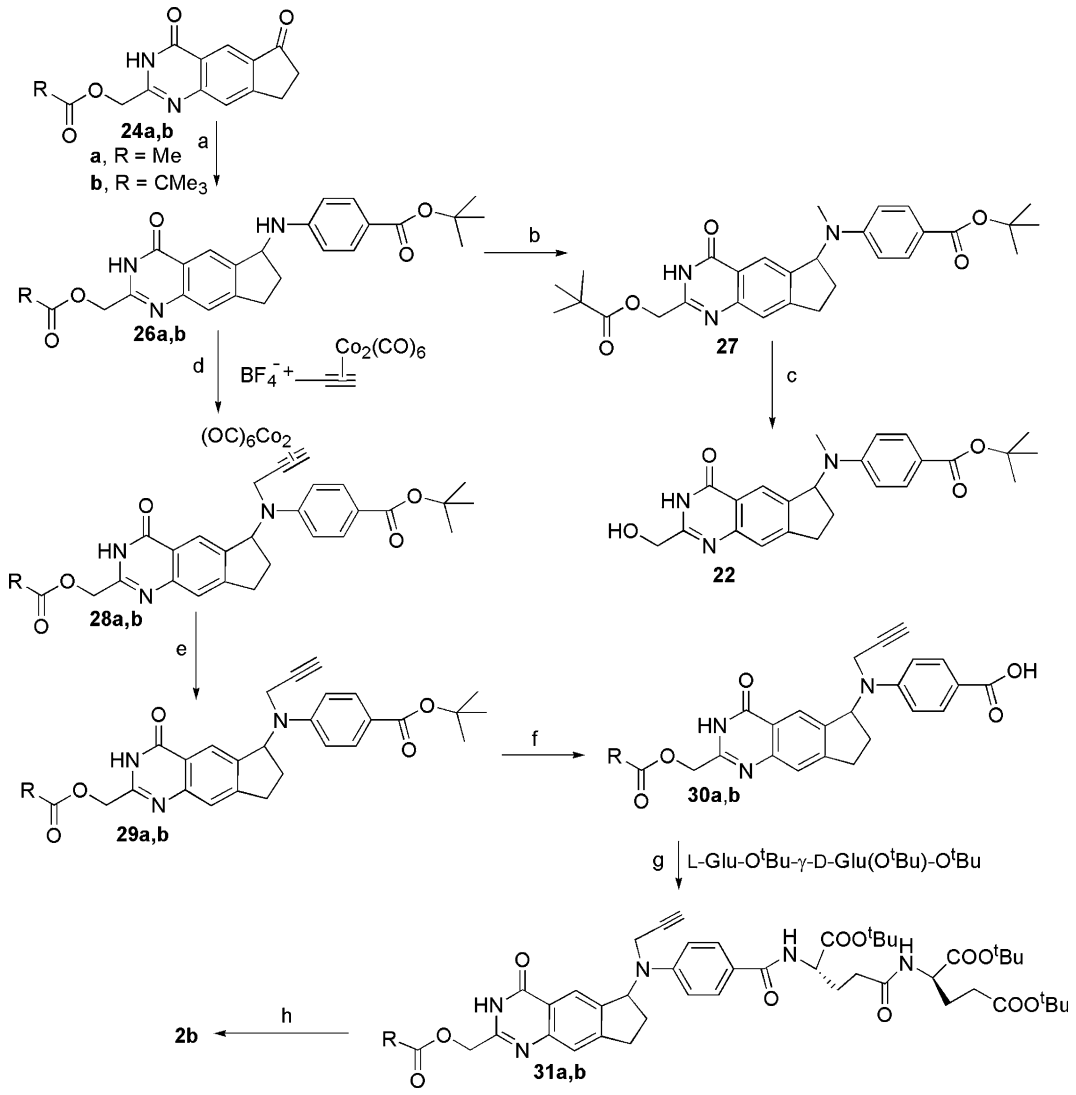


Scheme 3. Reagents and conditions: (a) Na/MeOH, rt, then 80 °C; (b) i—CH_3COOCs, DMF, 60–80 °C, ii—NaOH, $MeOH/H_2O$; (c) acetic anhydride or trimethylacetic anhydride, Et_3N, DMAP, CH_2Cl_2, rt; (d) chloromethyl pivalate, K_2CO_3, DMF, rt; (e) $(Ph_3SiO)_2CrO_2$, *tert*-butyl hydroperoxide; (f) NBS, CCl_4, $(PhCO)_2O_2$, heat; (g) *tert*-butyl 4-aminobenzoate, DIEA, DMF, 80 °C (h) i—37% aq HCHO, AcOH, rt, ii—$NaBH_3CN$, rt; (i) TFA, rt; (j) DEPC, DMF, rt; (k) i—TFA, ii—$NaOH/H_2O$; (l) NaOH, $MeOH/H_2O$.

the oxidation reagent. The C^6–N^{10} bond was formed via a reductive amination reaction under conditions similar with those utilised for the preparation of the 2-methyl counterpart **5** (Scheme 1). N^{10}-Propargylation and N^{10}-methylation were accomplished by a variation of the methodologies utilised for the synthesis of the 2-methylcyclopenta[*g*]quinazolin-4-one counterparts. Subsequent TFA hydrolysis of the *tert*-butyl ester, coupling to the dipeptide L-Glu-γ-D-Glu(*O*^{t}Bu)-*O*^{t}Bu, and removal of the protecting groups gave the desired products **3a**,**b**.

One of the questions to be answered in the synthetic sequences shown in Schemes 3–5 was related to the assignment of the regiochemistry during the oxidation or bromination of the cyclopenta[*g*]quinazolin-4-one analogues to form the 6-oxo- and 8-oxo-cyclopenta[*g*]quinazolin-4-one or 6-bromo- and 8-bromocyclopenta[*g*]quinazolin-4-one derivatives, respectively. The initial assignment for these regioisomers was based on comparing the ^{1}H NMRs of the oxidation products with the ^{1}H NMRs of their 2-methyl counterparts,[23] and in particular looking at the chemical shift pattern for the 5-H and 9-H protons.

Additional evidence was provided by NOESY ^{1}H NMR experiments. First, the 6-oxo-derivatives **24a**,**b** and **37** were derivatised through the 4-position by reacting with



Scheme 4. Reagents and conditions: (a) *tert*-butyl 4-aminobenzoate, decaborane, MeOH, CH_2Cl_2 rt, (b) i—37% aq HCHO, AcOH, THF, rt, ii—$NaBH_3CN$, rt; (c) NaOH, MeOH/H_2O; (d) CH_2Cl_2, DIEA, rt; (e) $Fe(NO_3)_3$, EtOH, rt; (f) TFA, rt; (g) DEPC, DMF, rt, (h) i—TFA; ii—NaOH/H_2O.

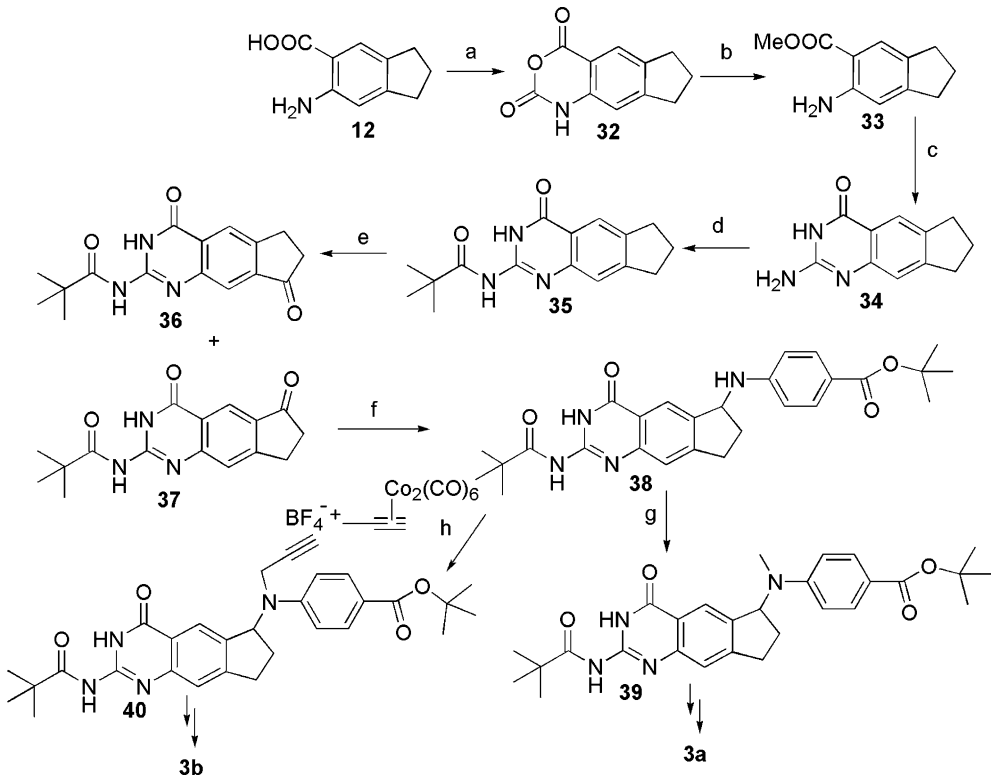
chloromethyl pivalate (Scheme 6). For compound **42**, the NOESY ^{1}H NMR showed strong interaction of H-5 with 7-CH_2 and the 4-OCH_2, and also a weak interaction with 8-CH_2, whereas H-9 showed strong interaction with 8-CH_2. The NOESY ^{1}H NMR for 2-acetoxymethyl, 6-oxo derivative **41** showed a similar pattern. Consistent with the assigned structure the NOESY ^{1}H NMR for the 6-bromo derivative **18** (Scheme 3) showed an interaction of H-5 with 4-OCH_2 and H-6, and also a strong interaction of H-9 with 8-CH_2. The NOESY ^{1}H NMR for the 8-bromo derivative **19** (Scheme 3) showed an interaction of H-5 with 4-OCH_2 and 6-CH_2, and also a strong interaction between H-9 and H-8. It should also be noted that the ^{1}H NMR of compound **22** produced via the bromide intermediate **18** (Scheme 3) was identical to a sample produced via the ketone **24b** (Scheme 4). Likewise, in the case of the 2-amino derivative **43**, the NOESY ^{1}H NMR showed strong interaction of H-5 with 7-CH_2 and the 4-OCH_2, and also a weak interaction with 8-CH_2. For the H-9 strong interaction with 8-CH_2 has been observed. In addition, expansion of the ^{1}H NMR revealed a splitting for the H-9, whereas the H-5 appeared as a singlet.

3. Biological evaluation

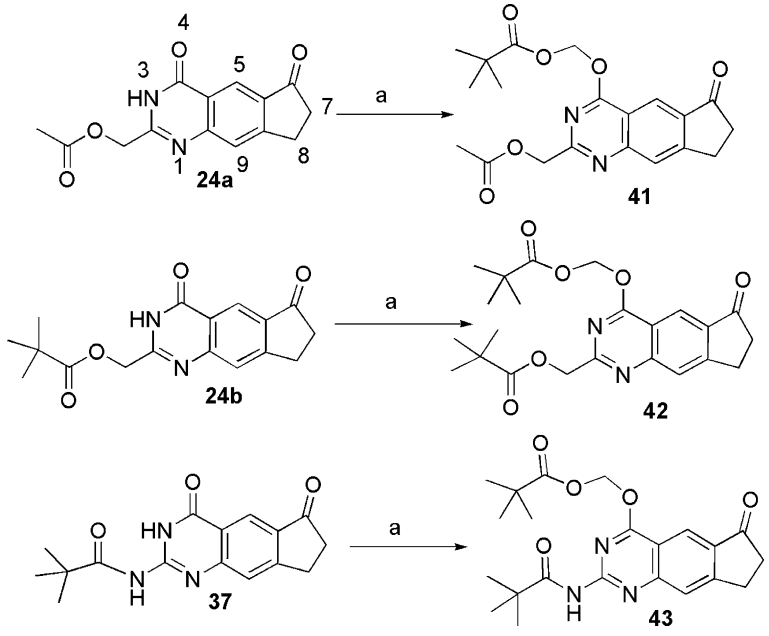
All compounds (i.e., **1a**,**b**, **2a**,**b**, and **3a**,**b**) were tested to determine TS inhibitory activity, affinities for both the α-FR and RFC and the inhibition of cell growth in A431 and A431-FBP cell lines. These studies were performed as previously described.[21]

Regarding inhibition of TS, in the N^{10}-methyl series, compound **1a** with a 2-methyl substituent was a potent inhibitor of the enzyme ($K_i$app = 14 nM). The 2-amino derivative **3a** showed similar potency against TS as **1a**, whereas the 2-hydroxymethyl derivative **2a** was the least potent of the series ($K_i$app = 43 nM; Table 1).

Likewise, in the N^{10}-propargyl series, the 2-methyl and 2-amino derivatives (compounds **1b** and **3b**) displayed



Scheme 5. Reagents and conditions: (a) triphosgene, THF, rt; (b) MeOH, DMAP, reflux; (c) $ClC(NH)NH_2 \cdot HCl$, dimethylsulfone, 150 °C; (d) trimethylacetic anhydride, DMF, 100 °C; (e) *tert*-butyl hydroperoxide, CrO_3, CH_2Cl_2, rt; (f) i—*tert*-butyl 4-aminobenzoate, 4-TsOH, DME, 120 °C, ii—$NaBH_3CN$, MeOH, AcOH, rt; (g) i—37 °C aq HCHO, AcOH, THF, rt; ii—$NaBH_3CN$, rt; (h) i—CH_2Cl_2, DIEA, rt, ii—$Fe(NO_3)_3$, EtOH, rt.

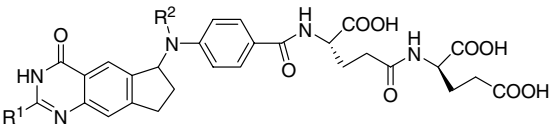


Scheme 6. Reagents and condition: (a) chloromethyl pivalate, K_2CO_3, DMF, rt.

similar TS inhibitory activities, with the 2-hydroxymethyl derivative **2b** being the least potent (Table 1).

The N^{10}-propargyl cyclopenta[*g*]quinazoline derivatives **1b**, **2b** and **3b** (Table 1) were approximately 10- to 20-fold more potent inhibitors of TS than the analogous N^{10}-methyl compounds (compounds **1a**, **2a** and **3a**; Table 1). This is in agreement with what has been observed with the quinazoline-based inhibitors of TS, where it was found that N^{10}-propargyl derivatives were approximately 10-fold more active than the N^{10}-methyl analogues.[26]

Table 1. Cyclopenta[*g*]quinazoline-based antifolates: inhibition of TS



Compound	R^1 (2-substituent)	R^2 (N^{10}-substituent)	Inhibition of L1210 TS, $K_i$app (nM)
1a	Me	Me	14
1b	Me	Pg	0.42[a]
2a	CH_2OH	Me	43
2b	CH_2OH	Pg	2.6, 3.3
3a	NH_2	Me	20
3b	NH_2	Pg	0.32

Results represent individual experiments.
[a] Ref. 19.

With regard to affinity for the RFC, all analogues showed very low affinity (often greater than 250 μM, Table 2). The affinity of compounds for the RFC was determined using a competition assay with tritiated methotrexate. Antifolates utilising the RFC to enter cells usually display an affinity of ~1–5 μM (K_i for inhibition of methotrexate transport).[21]

The affinity of compounds for the α-FR was determined using a competition assay with tritiated folic acid.[21] The nature of the substituent at the 2-position in both the N^{10}-methyl and N^{10}-propargyl series appears to have little effect on the affinity for the α-FR; all compounds displayed similar affinity to folic acid (taken to be 1).

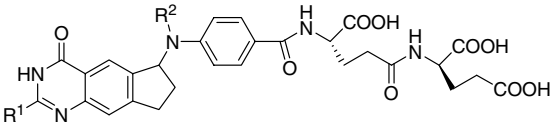
To identify inhibitors of TS that utilise the α-FR for cellular entry, all compounds were tested for their ability to inhibit A431 and A431-FBP tumour cell growth in vitro. The A431 cell line are neo-transfected human vulval carcinoma cells with a functional RFC, but no detectable α-FR expression and the A431-FBP cell line is transfected with human α-FR which it expresses highly.[3,32] The selectivity of compounds for A431-FBP cells was determined by two methods. The first method compares the growth inhibitory activity of the compounds in the two cell lines and the second method compares activity in A431-FBP cells in the absence and presence of an excess of 1 μM folic acid which competitively inhibits binding to the α-FR. Folic acid does not affect binding to the RFC because of its low affinity for the transporter. All of the A431 cell-based assays were performed in folic acid-free culture medium containing physiological levels of reduced folate (20 nM leucovorin).

In the N^{10}-methyl series, compound **1a** was the most potent inhibitor of A431 cell growth (IC_{50} = ~4 μM) with compounds **2a** and **3a** being the least potent (IC_{50} >50 μM; Table 3). Compound **3a** exhibited cell growth inhibition of greater than 50 μM in both the A431 and A431-FBP, that is, no selectivity was observed. Compound **1a** was ~2-fold more potent in inhibiting the A431-FBP cell growth than the A431, and compound **2a** was at least 2-fold more active in A431-FBP than in A431 (Table 3).

The N^{10}-propargyl derivatives **1b**, **2b** and **3b** were more potent inhibitors of A431 cell growth than their N^{10}-methyl counterparts (Table 3), consistent with their increased inhibition of TS. The 2-methyl analogue **1b** displayed a similar potency in A431-FBP as the 2-hydroxymethyl analogue **2b**, but the 2-amino derivative **3b** was approximately 1000-fold less potent in A431-FBP than compounds **1b** and **2b** (Table 3).

Regarding the inhibition of the A431 cell growth, the 2-hydroxymethyl derivative **2b** was approximately 10-fold less potent than **1b** (IC_{50} = 9.8 and 1.4 μM, respectively). This translated into compound **2b** having a greater selectivity (~4900-fold) for cells that express the α-FR (A431-FBP) than those without the α-FR (A431) compared with **1b** (selectivity of 215-fold). The activity of compound **2b** in the A431-FBP cell line was reversed by the addition of folic acid, confirming that the α-FR is an important mechanism of transport. The IC_{50} of compound **2b** (6.4 μM) in the A431-FBP cell line in the presence of 1 μM folic acid was similar to the IC_{50} in the A431 cell line (IC_{50} = 9.8 μM). Likewise, com-

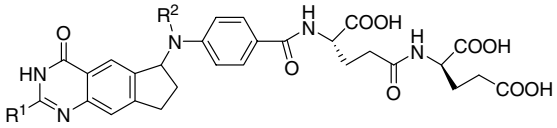
Table 2. Cyclopenta[*g*]quinazoline-based compounds: affinity for α-FR and RFC



Compound	R^1 (2-substituent)	R^2 (N^{10}-substituent)	RFC affinity K_i (μM) L1210 cells	α-FR affinity L1210-FBP cells
1a	Me	Me	>250	0.89, 1. 0
1b	Me	Pg	166 ± 34	0.60 ± 0.06
2a	CH_2OH	Me	>250	1.16 ± 0.16
2b	CH_2OH	Pg	>250	0.73 ± 0.06
3a	NH_2	Me	>250	0.97, 1. 0
3b	NH_2	Pg	>250	0.73 ± 0.05

Results are means of at least three experiments (± SD) or individual values.
RFC affinity was determined by measuring the K_i for inhibition of tritiated methotrexate transport.[21]
Affinities are expressed relative to that of folic acid, relative affinity = 1. Compounds with values greater and less than 1 bind with higher and lower affinities than folic acid, respectively.

Table 3. Inhibition of cell growth in human A431 and A431-FBP cells



Compound	R^1	R^2	Cell growth inhibition, IC_{50} (μM)			
			A431	A431 +1 μM FA	A431-FBP	A431-FBP +1 μM FA
1a	Me	Me	6, 3	5.4, 3.3	4.4, 1.0	6.0, 3.1
1b	Me	Pg	1.4 ± 0.23	1.4 ± 0.25	0.0065 ± 0.00012	0.87 ± 0.29
2a	CH_2OH	Me	>50	>50	21	25
2b	CH_2OH	Pg	9.8 ± 3.4	9.3 ± 3.5	0.002 ± 0.001	6.4 ± 0.87
3a	NH_2	Me	>50	>50	>50	>50
3b	NH_2	Pg	27, 23	22, 25	8.3 ± 0.64	7.1 ± 1.0

Results are means of at least three experiments (±SD) or individual values.

pound **1b** displayed the same trend when an excess of folic acid was present in A431-FBP cells (Table 3).

In summary, the N^{10}-methyl series of compounds (Table 1) were approximately 10-fold less potent inhibitors of TS than the corresponding N^{10}-propargyl derivatives. This trend was also observed in the cell growth inhibitory properties in which the N^{10}-propargyl compounds showed greater potency. With regard to receptor affinities, all of the compounds synthesised were shown to have low affinity for the RFC (no less than 166 μM) and high affinity for the α-FR. This study indicated that in the cyclopenta[*g*]quinazoline-based antifolates the 2-substituent does not affect the affinity for the α-FR, in contrast with compounds in the quinazoline series.[22] However, the substituent at the 2-position greatly affects both the activity and selectivity of this type of compound for α-FR-mediated uptake, and suggests that there are other factors (such as the rate of compound trafficking through the endosome and endosomal unloading), in addition to the affinity for the α-FR, that are important in the activity of these compounds.[33] The possible mechanisms by which folate molecules are captured and transferred to intracellular compartments by endocytosis, and potential barriers to efficient delivery are reviewed by Sabharanjak and Mayor.[34] The 2-hydroxymethyl derivative **2b** displayed the greatest selectivity for A431-FBP cells. Compound **2b** (designated BGC 945) is currently being studied in in vivo models, and further studies are to be undertaken in an attempt to determine other characteristics of this series of compounds that affect internalisation.

4. Experimental

Anhydrous solvents were obtained from Aldrich and used without further purification unless otherwise stated. All other reagents were obtained from commercial suppliers, and used without further purification.

Thin layer chromatography (TLC) was performed on pre-coated aluminium sheets of silica (60 F_{254}, Merck) and visualised with short-wave UV analysis. Merck silica 60 (Art 15111) was used in low-pressure column chromatography unless stated otherwise. High-performance liquid chromatography (HPLC) analyses were performed using a Waters system. The system used a model 510 solvent delivery system, model 680 automated gradient controller, model U6K injector and model M-490 programmable wavelength detector set to monitor at 230 and 280 nm. Retention times were determined on a Trivector Trilab 3000 multichannel chromatography data system. Chiral separations were performed on either a 25 × 0.46 cm Astec cyclobond I (cyclodextrin β) column or Astec cyclobond II (cyclodextrin γ) column (Astec, Advanced Separation Technologies Ltd., UK) and eluted isocratically with different ratios of (25 mM Na_2HPO_4/25 mM NaH_2PO_4 1:1 v/v)/CH_3CN and a flow rate of 1 ml/min.

LC–MS analysis was conducted using gradient elution (MeOH/0.1% HCO_2H in H_2O) and a Supelco Discovery C18 HPLC column (5 × 0.46 cm, 5 μm). Samples were injected using a Gilson 215 liquid handler. The HPLC system employed a Thermoseparations P4000 quaternary pump and UV 2000 detector operating at 254 nm. HPLC eluent passed directly into an LCQ ion trap mass spectrometer (Finnigan LCQ) operating in electrospray ionisation mode. Fast atom bombardment (FAB) mass spectra were determined with VG ZAB-SE spectrometer. ^{1}H NMR spectra were recorded at 250 MHz using a Bruker AC250 spectrometer with the residual solvent (in DMSO-d_6) or tetramethylsilane (in $CDCl_3$) as standard. 2D ^{1}H NMR spectra were recorded at 400 MHz using a Bruker spectrometer (at Kings College, University of London). Field strengths are expressed in units of δ (parts per million) and peak multiplicities are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; br s, broad singlet; m, multiplet. Infrared spectra were obtained using a Perkin-Elmer 1720X FT-IR. Melting points were determined on a Kofler block and are uncorrected. Optical rotations were obtained using a Perkin-Elmer Model 141 Polarimeter with a sodium lamp as radiation source. Elemental analyses were determined by C.H.N. Analysis Limited, Leicester and Warwick Analytical Service, Warwick.

4.1. *tert*-Butyl 4-[*N*-((6*RS*)-2-methyl-3-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)amino]benzoate (5)

4.1.1. Method 1. 2-Methyl-3-(2,2-dimethylpropionyloxymethyl)-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4,6-dione[23] (0.60 g, 1.81 mmol), *tert*-butyl *p*-aminobenzoate (0.54 g, 2.80 mmol), *p*-toluenesulfonic acid monohydrate (0.0070 g, 0.040 mmol) and anhydrous 1,2-dimethoxyethane (20 ml) were heated, in a round-bottomed flask fitted with a pressure-equalising dropping funnel, containing activated molecular sieves, and a condenser, to 120 °C under argon for 5 h. The suspension was cooled to room temperature, sodium cyanoborohydride (0.095 g, 0.95 mmol) in MeOH (2 ml) was added dropwise followed immediately by AcOH (0.10 ml). The resulting suspension was stirred at room temperature under argon for 17 h. The solvents were removed in vacuo and the residue partitioned between EtOAc (40 ml) and saturated aqueous $NaHCO_3$ (40 ml). The aqueous layer was extracted with EtOAc (3× 15 ml). The combined organic extracts were washed with brine (40 ml), dried (Na_2SO_4) and the solvent removed in vacuo. The residue was purified by column chromatography eluting with a gradient of 10–30% EtOAc in CH_2Cl_2 to yield the desired product as a clear oil (0.090 g, 29%).

4.1.2. Method 2. A suspension of 2-methyl-3-(2,2-dimethylpropionyloxymethyl)-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4,6-dione (0.20 g, 0.60 mmol) in anhydrous MeOH (15 ml) and anhydrous CH_3CN (5 ml) was treated with *tert*-butyl *p*-aminobenzoate (0.14 g, 0.73 mmol) followed by decaborane (0.029 g, 0.24 mmol) and the mixture stirred at room temperature under argon for 18 h. The solvent was removed in vacuo and the residue purified by column chromatography (50 g of silica gel) eluting with 10% EtOAc in CH_2Cl_2 to yield the desired product as a white solid (0.143 g, 46%); mp 128–130 °C; ^{1}H NMR ($CDCl_3$) δ 1.21 (s, 9H, Me_3C), 1.58 (s, 9H, CO_2CMe_3), 1.89–2.12 (m, 1H, 7-H), 2.65 (s, 3H, 2-CH_3), 2.71–2.81 (m, 1H, 7-H), 2.90–3.27 (m, 2H, 8-H), 5.14 (t, J = 7.01 Hz, 1H, 6-H), 6.11 (q, J = 4.62 Hz, 2H, 3-CH_2), 6.67 (d, J = 8.87 Hz, 2H, 3′-H, 5′-H), 7.52 (s, 1H, 9-H), 7.86 (d, J = 6.94 Hz, 2H, 2′-H, 6′-H), 8.22 (s, 1H, 5-H); MS (ESI, *m*/*z*): 506 [(M+H)$^+$, 100%], 528 [(M+Na)$^+$, 100%]. Found: C, 68.81; H, 7.07; N, 8.24. $C_{29}H_{35}N_3O_5$ requires: C, 68.89; H, 6.98; N, 8.31.

4.2. *tert*-Butyl 4-[*N*-((6*RS*)-2-methyl-3-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]benzoate (6)

tert-Butyl 4-[*N*-((6*RS*)-2-methyl-3-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)amino]benzoate (0.20 g, 0.39 mmol) was dissolved in THF (17 ml). AcOH (9 ml) was added followed by 37% aqueous formaldehyde (0.35 ml, excess) and the resulting solution was stirred at room temperature for 6 h. After this time, sodium cyanoborohydride (0.057 g, 0.92 mmol) was slowly added and the solution stirred at room temperature for 20 h. The solvent was removed in vacuo and the residue partitioned between EtOAc (20 ml) and H_2O (20 ml). The aqueous layer was extracted with EtOAc (15 ml). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (20 ml), H_2O (20 ml), dried (Na_2SO_4) and the solvent removed in vacuo. The residue was purified by column chromatography (15 g of silica gel) eluting with 30% EtOAc in CH_2Cl_2 to yield the product as a yellow oil (0.20 g, 97%); mp 158–160 °C; ^{1}H NMR (DMSO-d_6) δ 1.14 (s, 9H, Me_3C), 1.53 (s, 9H, CO_2CMe_3), 2.00–2.21 (m, 1H, 7-H), 2.60 (s, 3H, 2-CH_3), 2.68 (s, 3H, N–CH_3), 2.71–3.25 (m, 3H, 7-H, 8-H), 5.80 (t, 1H, 6-H), 6.04 (s, 2H, 3-CH_2), 6.99 (d, J = 7.87 Hz, 2H, 3′-H, 5′-H), 7.53 (s, 1H, 9-H), 7.73 (d, J = 7.71 Hz, 2H, 2′-H, 6′-H), 7.77 (s, 1H, 5-H); MS (FAB, *m*/*z*): 520 [(M+H)$^+$, 12%], 313 [(M−$NMeC_6H_4COO^tBu$), 95%]; HRMS: measured 520.2792; calculated for $C_{30}H_{38}N_3O_5$ (M+H)$^+$: 520.2811. Found: C, 67.76; H, 7.34; N, 7.63. $C_{30}H_{37}N_3O_5 \cdot 3/4H_2O$ requires: C, 67.59; H, 7.28; N, 7.88.

4.3. 4-[*N*-((6*RS*)-2-Methyl-3-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]benzoic acid (7)

A solution of *tert*-butyl 4-[*N*-((6*RS*)-2-methyl-3-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]benzoate (0.20 g, 0.39 mmol) in TFA (11 ml) was stirred at room temperature with protection from the light for 2 h. The solvent was removed in vacuo and the residue was triturated with Et_2O to yield the product as the TFA salt, a pale brown solid (0.16 g, TFA salt); mp 250–253 °C; ^{1}H NMR (DMSO-d_6) δ 1.13 (s, 9H, Me_3C), 1.97–2.20 (m, 1H, 7-H), 2.60 (s, 3H, 2-CH_3), 2.68 (s, 3H, N–CH_3), 2.77–3.32 (m, 3H, 7-H, 8-H), 5.81 (t, J = 9.01 Hz, 1H, 6-H), 6.04 (s, 2H, 3-CH_2), 7.00 (d, J = 8.99 Hz, 2H, 3′-H, 5′-H), 7.53 (s, 1H, 9-H), 7.73 (s, 1H, 5-H), 7.80 (d, J = 8.91 Hz, 2H, 2′-H, 6′-H); MS (FAB, *m*/*z*): 486 [(M+Na)$^+$, 25%]; HRMS: measured 486.2012; calculated for $C_{26}H_{29}N_3O_5Na$ (M+Na)$^+$: 486.2005. Found: C, 62.71; H, 5.74; N, 8.19. $C_{26}H_{29}N_3O_5 \cdot 1/2$ TFA requires: C, 62.30; H, 5.67; N, 8.08.

4.4. Tri-*tert*-butyl *N*-{4-[*N*-((6*RS*)-2-methyl-3-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]benzoyl}-L-γ-glutamyl-D-glutamate (11a)

4-[*N*-((6*RS*)-2-Methyl-3-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]benzoic acid (0.14 g, 0.30 mmol) and tri-*tert*-butyl L-γ-glutamyl-D-glutamate[26] (0.21 g, 0.47 mmol) were dissolved in anhydrous DMF (8 ml) under argon with protection from the light. DEPC (0.090 ml, 0.56 mmol) was added followed by triethylamine (0.080 ml, 0.55 mmol) and the solution stirred at room temperature under argon with protection from the light for 2.5 h then partitioned between EtOAc (50 ml) and H_2O (50 ml). The aqueous layer was extracted with EtOAc (2× 40 ml). The combined organic extracts were washed with 10% aqueous citric acid

(2× 25 ml), saturated aqueous $NaHCO_3$ (50 ml) dilute brine (50 ml), dried (Na_2SO_4) and the solvent removed in vacuo. The residue was purified by column chromatography (15 g of silica gel) eluting with a gradient of 10–50% EtOAc in CH_2Cl_2 to yield the product as a clear oil (0.21 g, 79%). A portion of the product was triturated with hexanes to yield a white solid for analysis: mp 102–104 °C; ^{1}H NMR (DMSO-d_6) δ 1.13 (s, 9H, Me_3C), 1.39, 1.42 (2× s, 27H, 3× CO_2CMe_3), 1.50–2.14 (m, 5H, 2× Glu β-CH_2, 7-CH), 2.17–2.42 (m, 5H, 2× Glu γ-CH_2, 7-CH), 2.60 (s, 3H, 2-CH_3), 2.68 (s, 3H, N^{10}–CH_3), 2.90–3.22 (m, 2H, 8-CH_2), 4.13, 4.29 (2× m, 2H, 2× Glu α-CH), 5.81 (t, J = 8.21 Hz, 1H, 6-H), 6.04 (s, 2H, 3-CH_2), 7.00 (d, J = 8.92 Hz, 2H, 3′-H, 5′-H), 7.52 (s, 1H, 9-H), 7.73 (s, 1H, 5-H), 7.81 (d, J = 8.79 Hz, 2H, 2′-H, 6′-H), 8.12 (d, J = 7.61 Hz, 1H), 8.28 (d, 1H), (2× CONH); MS (FAB, *m/z*): 890 [(M+H)$^+$, 65%], 912 [(M+Na)$^+$, 100%]; HRMS: measured 890.4946; calculated for $C_{48}H_{68}N_5O_{11}$ (M+H)$^+$: 890.4915. Found: C, 64.72; H, 7.69; N, 7.52. $C_{48}H_{67}N_5O_{11}$ requires: C, 64.77; H, 7.59; N, 7.87.

4.5. *N*-{4-[*N*-((6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]benzoyl}-L-γ- glutamyl-D-glutamic acid (1a)

A solution of tri-*tert*-butyl *N*-{4-[*N*-((6*RS*)-2-methyl-3-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]-benzoyl}-L-γ-glutamyl-D-glutamate (0.10 g, 0.11 mmol) in TFA (5 ml) was stirred at room temperature with protection from the light for 2 h. The solvent was removed in vacuo and the residue was dissolved in MeOH (3 ml). The pH of the solution was adjusted to 10.5 with 1 M NaOH then solid NaOH (0.0090 g, 0.22 mmol) in H_2O (3 ml) was added and the solution stirred at room temperature for 3 h. The pH of the solution was adjusted to 3.5 with 0.5 M HCl, cooled to 0 °C and the product collected by filtration as an off-white solid (0.039 g, 57%); mp 184–187 °C; ^{1}H NMR (DMSO-d_6) δ 1.72–2.17 (m, 5H, 2× Glu β-CH_2, 7-CH), 2.20–2.50 (m, 5H, 2× Glu γ-CH_2, 7-CH), 2.33 (s, 3H, 2-CH_3), 2.67 (s, 3H, N^{10}–CH_3), 2.79–3.22 (m, 2H, 8-CH_2), 4.20, 4.34 (2× m, 2H, 2× Glu α-CH), 5.79 (t, 1H, 6-H), 7.00 (d, J = 9.01 Hz, 2H, 3′-H, 5′-H), 7.47 (s, 1H, 9-H), 7.70 (s, 1H, 5-H), 7.81 (d, J = 8.93 Hz, 2H, 2′-H, 6′-H), 8.08 (d, 1H), 8.26 (d, 1H), (2× CONH), 12.09 (br s, 1H, N^3–H); MS (FAB, *m/z*): 630 [(M+Na)$^+$, 40%]; HRMS: measured 630.2161; calculated for $C_{30}H_{33}N_5O_9Na$ (M+Na)$^+$: 630.2176. HPLC: Cyclobond I column, eluting with 88% phosphate buffer/12% CH_3CN. Retention time: 739 s, purity = 98.6.

4.6. *tert*-Butyl 4-[*N*-((6*RS*)-2-methyl-3-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoate (9)

A suspension of (propargyl)$Co_2(CO)_6^+BF_4^-$[24] (0.16 g, 0.39 mmol) in anhydrous CH_2Cl_2 (14 ml, distilled over CaH_2) was treated with *tert*-butyl 4-[*N*-((6*RS*)-2-methyl-3-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)amino]benzoate (0.18 g, 0.36 mmol) and the red solution stirred at room temperature under argon for 10 min. Diisopropylethylamine (0.13 ml, 0.75 mmol) was added and the mixture was stirred at room temperature under argon for 1 h then partitioned between EtOAc (50 ml) and brine (30 ml). The organic extract was dried (Na_2SO_4) and the solvent removed in vacuo. The residue was purified by column chromatography (20 g of silica gel) eluting with a gradient of 0–20% EtOAc in CH_2Cl_2 to yield the complex **8** as a red oil (0.18 g, 62%); ^{1}H NMR ($CDCl_3$) δ 1.21 (s, 9H, Me_3C), 1.67 (s, 9H, CO_2CMe_3), 2.30 (m, 1H, 7-H), 2.60 (m, 1H, 7-H), 2.63 (s, 3H, 2-CH_3), 3.05, 3.20 (2× m, 2H, 8-H), 4.54 (AB system, J = 16.92 Hz, 2H, propargyl CH_2), 5.63 (t, J = 8.10 Hz, 1H, 6-H), 5.97 (s, 1H, propargyl CH), 6.10 (q, J = 8.78 Hz, 2H, POM-CH_2), 6.93 (d, J = 9.01 Hz, 2H, 3′-H, 5′-H), 7.53 (s, 1H, 9-H), 7.90 (d, J = 8.99 Hz, 2H, 2′-H, 6′-H), 8.02 (s, 1H, 5-H).

A solution of this complex **8** (0.17 g, 0.21 mmol) in EtOH (7 ml) was treated with $Fe(NO_3)_3{\cdot}9H_2O$ (1.60 g, excess) and the solution stirred at room temperature for 1 h. The solution was partitioned between EtOAc (25 ml) and H_2O (25 ml). The organic extract was washed with brine (25 ml), dried (Na_2SO_4) and the solvent removed in vacuo. The residue was purified by column chromatography (20 g of silica gel) eluting with 20% EtOAc in CH_2Cl_2 to yield the desired product as a white solid (0.11 g, 94%); ^{1}H NMR ($CDCl_3$) δ 1.21 (s, 9H, Me_3C), 1.58 (s, 9H, CO_2CMe_3), 2.19 (s, 1H, propargyl CH), 2.36 (m, 1H, 7-H), 2.58 (m, 1H, 7-H), 2.63 (s, 3H, 2-CH_3), 3.06, 3.20 (2× m, 2H, 8-H), 3.91 (AB system, J = 18.53 Hz, 2H, propargyl CH_2), 5.63 (t, J = 8.11 Hz, 1H, 6-H), 6.09 (s, 2H, POM-CH_2), 6.96 (d, J = 9.08 Hz, 2H, 3′-H, 5′-H), 7.52 (s, 1H, 9-H), 7.92 (d, J = 9.11 Hz, 2H, 2′-H, 6′-H), 8.11 (s, 1H, 5-H); MS (ESI, *m/z*): 543 [(M)$^+$, 45%], 565 [(M+Na)$^+$, 100%].

4.7. 4-[*N*-((6*RS*)-2-Methyl-3-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoic acid (10)

A solution of *tert*-butyl 4-[*N*-((6*RS*)-2-methyl-3-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoate (0.095 g, 0.17 mmol) in TFA (4 ml) was stirred at room temperature with protection from the light for 2 h. The solvent was removed in vacuo and the residue was triturated with Et_2O to yield the product as the TFA salt, a white solid (0.12 g, TFA salt); mp 100–103 °C; ^{1}H NMR ($CDCl_3$) δ 1.22 (s, 9H, Me_3C), 1.90–2.15 (m, 1H, 7-H), 2.62–2.80 (m, 1H, 7-H), 2.80 (s, 3H, 2-CH_3), 3.11 (s, 1H, propargyl CH), 3.00–3.32 (m, 2H, 8-H), 3.92 (AB system, J = 20.00 Hz, 2H, propargyl CH_2), 5.68 (t, 1H, 6-H), 6.11 (s, 2H, 3-CH_2), 6.99 (d, J = 9.09 Hz, 2H, 3′-H, 5′-H), 7.71 (s, 1H, 9-H), 8.02 (d, J = 9.02 Hz, 2H, 2′-H, 6′-H), 8.14 (s, 1H, 5-H); MS (ESI, *m/z*): 488 [(M+H)$^+$, 90%], 510 [(M+Na)$^+$, 40%].

4.8. Tri-*tert*-butyl *N*-{4-[*N*-((6*RS*)-2-methyl-3-(2,2-dimethylpropionyloxymethyl)-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 (11b)

4-[*N*-((6*RS*)-2-Methyl-3-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoic acid (0.085 g, 0.15 mmol) and tri-*tert*-butyl L-γ-glutamyl-D-glutamate (0.13 g, 0.30 mmol) were dissolved in anhydrous DMF (5 ml) under argon and with protection from the light. DEPC (0.050 ml, 0.31 mmol) was added followed by triethylamine (0.050 ml, 0.33 mmol) and the solution was stirred at room temperature under argon with protection from the light for 3 h then partitioned between EtOAc (30 ml) and H_2O (30 ml). The aqueous layer was extracted with EtOAc (2× 20 ml). The combined organic extracts were washed with 10% aqueous citric acid (2× 40 ml), saturated aqueous $NaHCO_3$ (40 ml) and dilute brine (40 ml), dried (Na_2SO_4) and the solvent removed in vacuo. The residue was purified by column chromatography (25 g of silica gel) eluting with a gradient of 30–60% EtOAc in CH_2Cl_2 to yield the product as a white solid (0.12 g, 85%); mp 94–96 °C; ^{1}H NMR ($CDCl_3$) δ 1.21 (s, 9H, Me_3C), 1.43, 1.47, 1.48 (3s, 27H, 3× CO_2CMe_3), 1.91–2.19 (m, 5H, 2× Glu β-CH_2, 7-CH), 2.20 (s, 1H, propargyl CH), 2.22–2.60 (m, 5H, 3× Glu γ-CH_2, 7-CH), 2.63 (s, 3H, 2-CH_3), 3.01–3.27 (m, 2H, 8-CH_2), 3.92 (AB system, J = 20.32 Hz, 2H, propargyl CH_2), 4.50, 4.77 (2× m, 2H, 2× Glu α-CH), 5.63 (t, J = 8.61 Hz, 1H, 6-H), 6.10 (s, 2H, 3-CH_2), 6.99 (d, J = 8.92 Hz, 2H, 3′-H, 5′-H), 7.03 (d, J = 9.24 Hz, 1H), 7.11 (d, J = 5.58 Hz, 1H), (2× CONH), 7.53 (s, 1H, 9-H), 7.80 (d, J = 8.90 Hz, 2H, 2′-H, 6′-H), 8.12 (s, 1H, 5-H); MS (ESI, *m*/*z*): 914 [(M+H)$^+$, 100%]. Found: C, 64.65; H, 7.52; N, 7.44. $C_{50}H_{67}N_5O_{11}\cdot 1H_2O$ requires: C, 64.43; H, 7.46; N, 7.51.

4.9. *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 (1b)

A solution of tri-*tert*-butyl *N*-{4-[*N*-((6*RS*)-2-methyl-3-(2,2-dimethylpropionyloxymethyl)-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 (0.10 g, 0.11 mmol) in TFA (4 ml) was stirred at room temperature with protection from the light for 2 h then the solvent was removed in vacuo. The residue was dissolved in MeOH (2 ml) and H_2O (2 ml). The pH of the solution was adjusted to pH 10 with 1 M NaOH and stirred at room temperature for 3 h then the solution was acidified to pH 4 with 1 M HCl and cooled to 0 °C. The product was collected by filtration and dried under vacuum over P_2O_5 to yield an off-white solid (0.043 g, 62%); mp 184–186 °C; ^{1}H NMR (DMSO-d_6) δ 1.74–2.00 (m, 5H, 2× Glu β-CH_2, 7-CH), 2.02–2.43 (m, 5H, 3× Glu γ-CH_2, 7-CH), 2.25 (s, 3H, 2-CH_3), 3.10 (s, 1H, propargyl CH), 2.90–3.15 (m, 2H, 8-CH_2), 3.95 (AB system, J = 19.30 Hz, 2H, propargyl CH_2), 4.18, 4.34 (2× m, 2H, 2× Glu α-CH), 5.75 (t, J = 7.63 Hz, 1H, 6-H), 7.01 (d, J = 8.97 Hz, 2H, 3′-H, 5′-H), 7.48 (s, 1H, 9-H), 7.78 (s, 1H, 5-H), 7.80 (d, J = 8.63 Hz, 2H, 2′-H, 6′-H), 8.12 (d, J = 7.82 Hz, 1H), 8.32 (d, J = 7.74 Hz, 1H), (2× CONH); MS (ESI, *m*/*z*): 632 [(M+H)$^+$, 100%]. Found: C, 57.17; H, 5.28; N, 10.36. $C_{32}H_{33}N_5O_9\cdot 2H_2O$ requires: C, 57.57; H, 5.59; N, 10.49.

4.10. 2-Chloromethyl-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4-one (13)

Sodium (0.39 g, 17.10 mmol) was added to anhydrous MeOH (215 ml) under argon at room temperature and stirred for 30 min. To this solution was added chloroacetonitrile (7.50 ml, 141.23 mmol) dropwise and the suspension was stirred for 30 min. 5-Amino-6-carboxyindane (15.00 g, 84.72 mmol) in anhydrous MeOH (340 ml) was then added via a cannula and the mixture stirred at room temperature under argon for 1 h, then refluxed for 3 h. The solvent was removed in vacuo and the residue triturated with cold EtOAc (3× 200 ml) and dried in vacuo over P_2O_5 to yield the product as a grey solid (19.10 g, 96%); ^{1}H NMR (DMSO-d_6) δ 2.08 (quin, J = 7.47 Hz, 2H, 7-H), 2.99 (q, J = 5.48 Hz, 4H, 6-H, 8-H), 4.52 (s, 2H, 2-CH_2), 7.49 (s, 1H, 9-H), 7.93 (s, 1H, 5-H); MS (ESI, *m*/*z*): 235 [(M+H)$^+$(^{35}Cl), 100%], 237 [(M+H)$^+$(^{37}Cl), 30%].

4.11. 2-Hydroxymethyl-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4-one (14)

A solution of caesium acetate (14.40 g, 75.18 mmol) in anhydrous DMF (40 ml) was heated to 60 °C under argon for 30 min. The mixture was cooled to 40 °C and a suspension of 2-chloromethyl-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4-one (2.20 g, 9.42 mmol) in anhydrous DMF (60 ml) was added via a cannula. The mixture was heated to 80 °C under argon for 16 h, cooled to room temperature and the solvent was removed in vacuo. The residue was suspended in H_2O (50 ml) and MeOH (20 ml) and the pH was adjusted to 12.5 with 1 M NaOH solution. The brown suspension was stirred for 2 h at room temperature, filtered to remove the insoluble brown solid and the resulting solution was acidified to pH 5 with 1 M HCl. The precipitate was collected by filtration, washed with acidified H_2O and dried in vacuo over P_2O_5 to yield the product as a pale yellow solid (1.17 g, 58%); mp 205–210 °C; ^{1}H NMR (DMSO-d_6) δ 2.07 (quin, J = 7.43 Hz, 2H, 7-H), 2.98 (q, J = 6.95 Hz, 4H, 6-H and 8-H), 4.38 (s, 2H, 2-CH_2), 7.46 (s, 1H, 9-H), 7.92 (s, 1H, 5-H); MS (FAB, *m*/*z*): 217 [(M+H)$^+$, 100%]; HRMS: measured 217.0977; calculated for $C_{12}H_{13}N_2O_2$ (M+H)$^+$: 217.0977.

4.12. 2-Acetoxymethyl-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4-one (15a)

2-Hydroxymethyl-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]-quinazolin-4-one (1.60 g, 7.41 mmol), triethylamine (1.28 ml, 9.20 mmol), DMAP (0.080 g, 0.65 mmol) and anhydrous CH_2Cl_2 (80 ml) were mixed in a flask under argon. Acetic anhydride (0.96 ml, 10.22 mmol) was added dropwise and the suspension stirred at room temperature under argon for 5 h. The solvent was removed in vacuo and the residue partitioned between EtOAc

(100 ml) and saturated aqueous $NaHCO_3$ (100 ml). The organic extract was washed with saturated aqueous $NaHCO_3$ (75 ml), H_2O (75 ml) and brine (75 ml), dried (Na_2SO_4) and the solvent removed in vacuo. The residue was triturated with hexanes (60 ml) and the product collected by filtration as an off-white solid (1.72 g, 90%); mp 222–224 °C; ^1H NMR (DMSO-d_6) δ 1.84 (quin, $J = 7.32$ Hz, 2H, 7-H), 1.90 (s, 3H, $COCH_3$), 2.75 (q, $J = 5.04$ Hz, 4H, 6-H and 8-H), 4.69 (s, 2H, 2-CH_2), 7.22 (s, 1H, 9-H), 7.68 (s, 1H, 5-H); MS (ESI, *m*/*z*): 259 [$(M+H)^+$, 100%]. Found: C, 64.60; H, 5.42; N, 10.91. $C_{14}H_{14}N_2O_3 \cdot 1/10H_2O$ requires: C, 64.67; H, 5.47; N, 10.78.

4.13. 2-Acetoxymethyl-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4,6-dione (24a) and 2-acetoxymethyl-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4,8-dione (25a)

To a stirred solution of $(Ph_3SiO)_2CrO_2$[28] (0.32 g, 0.50 mmol) in CH_2Cl_2 (170 ml) were added sequentially aqueous 70% *tert*-butyl hydroperoxide (8.50 ml, 61.50 mmol) and 2-acetoxymethyl-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4-one (2.50 g, 9.67 mmol). The mixture was stirred at room temperature with protection from the light for 48 h. The solvents were removed in vacuo and the residue was purified by column chromatography (50 g of silica gel) eluting with a gradient of 20–80% EtOAc in CH_2Cl_2 to yield in order of elution:

(a) 2-Acetoxymethyl-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4,8-dione (**25a**), a white solid (0.34 g, 13%): ^1H NMR (DMSO-d_6) δ 2.14 (s, 3H, $COCH_3$), 2.75 (m, 2H, 6-H), 2.96 (m, 1H, 7-H), 3.25 (m, 1H, 7-H), 4.97 (s, 2H, 2-CH_2), 7.77 (s, 1H, 9-H), 8.29 (s, 1H, 5-H).

(b) All fractions containing 2-acetoxymethyl-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4,6-dione (**24a**) were combined and recrystallised from EtOAc/MeOH to yield a pale yellow solid (0.52 g, 20%): mp 287–289 °C; ^1H NMR (DMSO-d_6) δ 2.14 (s, 3H, $COCH_3$), 2.72 (m, 2H, 8-H), 3.24 (m, 2H, 7-H), 4.98 (s, 2H, 2-CH_2), 7.75 (s, 1H, 9-H), 8.28 (s, 1H, 5-H); MS (ESI, *m*/*z*): 273 [$(M+H)^+$, 100%]. Found: C, 61.81; H, 4.50; N, 10.26. $C_{14}H_{12}N_2O_4$ requires: C, 61.76; H, 4.44; N, 10.29.

4.14. 2-(2,2-Dimethylpropionyloxymethyl)-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4-one (15b)

2-Hydroxymethyl-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4-one (1.00 g, 4.62 mmol), triethylamine (0.77 ml, 5.61 mmol), DMAP (0.050 g, 0.44 mmol) and anhydrous CH_2Cl_2 (50 ml) were mixed in a flask under argon. Trimethylacetic anhydride (1.20 ml, 6.00 mmol) was added dropwise and the suspension stirred at room temperature under argon for 5 h. The solvent was removed in vacuo and the residue partitioned between EtOAc (100 ml) and saturated aqueous $NaHCO_3$ (100 ml). The organic extract was washed with saturated aqueous $NaHCO_3$ (70 ml), H_2O (70 ml) and brine (70 ml), dried (Na_2SO_4) and the solvent was removed in vacuo. The residue was triturated with hexanes (60 ml) and the product collected by filtration as a yellow solid (1.21 g, 87%); mp 185–190 °C; ^1H NMR (DMSO-d_6) δ 1.22 (s, 9H, CMe_3), 2.07 (quin, $J = 7.37$ Hz, 2H, 7-H), 2.98 (q, $J = 5.72$ Hz, 4H, 6-H and 8-H), 4.94 (s, 2H, 2-CH_2), 7.42 (s, 1H, 9-H), 7.92 (s, 1H, 5-H), 12.20 (br, 1H, N^3–H); MS (FAB, *m*/*z*): 301 [$(M+H)^+$, 100%]; HRMS: measured 301.1539; calculated for $C_{17}H_{21}N_2O_3$ $(M+H)^+$: 301.1552. Found: C, 67.65; H, 6.54; N, 9.54. $C_{17}H_{20}N_2O_3$ requires: C, 67.98; H, 6.71; N, 9.33.

4.15. 2-(2,2-Dimethylpropionyloxymethyl)-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4,6-dione (24b) and 2-(2,2-dimethylpropionyloxymethyl)-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4,8-dione (25b)

To a stirred solution of $(Ph_3SiO)_2CrO_2$[28] (0.011 g, 0.017 mmol) in CH_2Cl_2 (5 ml) were added sequentially aqueous 70% *tert*-butyl hydroperoxide (0.18 ml, 1.32 mmol) and 2-(2,2-dimethylpropionyloxymethyl)-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4-one (0.10 g, 0.33 mmol). The mixture was stirred at room temperature with protection from the light for 24 h then the solvents were removed in vacuo and the residue purified by column chromatography (20 g of silica gel) eluting with a gradient of 10–30% EtOAc in $CHCl_3$ to yield, in order of elution:

(a) 2-(2,2-Dimethylpropionyloxymethyl)-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4,8-dione (**25b**) a white solid (0.032 g, 31%); mp 254 °C; ^1H NMR (DMSO-d_6) δ 1.23 (s, 9H, CMe_3), 2.76 (m, 2H, 7-H), 3.26 (m, 2H, 8-H), 4.98 (s, 2H, 2-CH_2), 7.72 (s, 1H, 9-H), 8.29 (s, 1H, 5-H), 12.30 (br, 1H, N^3–H); MS (ESI, *m*/*z*): 315 [$(M+H)^+$, 85%]. Found: C, 64.39; H, 5.77; N, 8.72. $C_{17}H_{18}N_2O_4 \cdot 2/10H_2O$ requires: C, 64.23; H, 5.79; N, 8.82.

(b) 2-(2,2-Dimethylpropionyloxymethyl)-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4,6-dione (**24b**) as a white solid (0.047 g, 45%); mp 185–190 °C; ^1H NMR (DMSO-d_6) δ 1.23 (s, 9H, CMe_3), 2.72 (m, 2H, 7-H), 3.25 (m, 2H, 8-H), 5.00 (s, 2H, 2-CH_2), 7.70 (s, 1H, 9-H), 8.29 (s, 1H, 5-H), 12.20 (br, 1H, N^3–H); MS (FAB, *m*/*z*): 315 [$(M+H)^+$, 100%], 337 [$(M+Na)^+$, 75%]; HRMS: measured 315.1360; calculated for $C_{17}H_{19}N_2O_4$ $(M+H)^+$: 315.1345. Found: C, 64.18; H, 5.72; N, 8.81. $C_{17}H_{18}N_2O_4 \cdot 2/10H_2O$ requires: C, 64.23; H, 5.79; N, 8.82.

4.16. 2-Acetoxymethyl-4-(2,2-dimethylpropionyloxymethyloxy)-7,8-dihydro-6*H*-cyclopenta[*g*]quinazoline (17) and 2-acetoxymethyl-3-(2,2-dimethylpropionyloxymethyl)-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4-one (16)

To a stirred solution of 2-acetoxymethyl-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4-one (2.60 g, 10.08 mmol) in anhydrous DMF (130 ml) under argon was added K_2CO_3 (3.48 g, 24.40 mmol) and the suspension stirred at room temperature for 30 min. Chloromethyl

pivalate (2.24 ml, 15.72 mmol) was added and the suspension stirred at room temperature under argon for 16 h then the solvent was removed in vacuo. The residue was suspended in CH_2Cl_2 (100 ml), filtered to remove insoluble inorganics and then purified by column chromatography (35 g silica gel) eluting with 40% Et_2O in hexanes to yield, in order of elution:

(a) 2-Acetoxymethyl-4-(2,2-dimethylpropionyloxymethyloxy)-7,8-dihydro-6*H*-cyclopenta[*g*]quinazoline (**17**), a clear oil (1.20 g, 32%): ^{1}H NMR (DMSO-d_6) δ 1.14 (s, 9H, POM-CMe_3), 2.10 (m, 2H, 7-H), 2.18 (s, 3H, $COCH_3$), 3.06 (m, 4H, 6-H and 8-H), 5.21 (s, 2H, 2-CH_2), 6.24 (s, 2H, POM-CH_2), 7.73 (s, 1H, 9-H), 7.89 (s, 1H, 5-H); MS (ESI, *m*/*z*): 373 [$(M+H)^+$, 15%], 395 [$(M+Na)^+$, 100%]. Found: C, 64.16; H, 6.48; N, 7.49. $C_{20}H_{24}N_2O_5$ requires: C, 64.50; H, 6.50; N, 7.52.

(b) 2-Acetoxymethyl-3-(2,2-dimethylpropionyloxymethyl)-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4-one (**16**), a white solid (0.90 g, 24%): mp 145 °C; ^{1}H NMR ($CDCl_3$) δ 1.20 (s, 9H, POM-CMe_3), 2.16 (m, 2H, 7-H), 2.20 (s, 3H, $COCH_3$), 3.03 (m, 4H, 6-H and 8-H), 5.19 (s, 2H, 2-CH_2), 6.13 (s, 2H, POM-CH_2), 7.52 (s, 1H, 9-H), 8.10 (s, 1H, 5-H); MS (ESI, *m*/*z*): 373 [$(M+H)^+$, 10%], 395 [$(M+Na)^+$, 75%]. Found: C, 64.47; H, 6.54; N, 7.48. $C_{20}H_{24}N_2O_5$ requires:C, 64.50; H, 6.50; N, 7.52.

4.17. 2-Acetoxymethyl-6-bromo-4-(2,2-dimethylpropionyloxymethyloxy)-7,8-dihydro-6*H*-cyclopenta[*g*]quinazoline (18) and 2-acetoxymethyl-8-bromo-4-(2,2-dimethylpropionyloxymethyloxy)-6,7-dihydro-8*H*-cyclopenta[*g*]quinazoline (19)

To a stirred suspension of 2-acetoxymethyl-4-(2,2-dimethylpropionyloxymethyloxy)-7,8-dihydro-6*H*-cyclopenta[*g*]quinazoline (1.28 g, 3.42 mmol) in anhydrous CCl_4 (25 ml) under argon was added *N*-bromosuccinimide (0.64 g, 3.64 mmol) followed by dibenzoyl peroxide (0.002 g). The suspension was heated to 95 °C under argon for 3 h while illuminating with a 40 W bulb. The solvent was removed in vacuo and the residue purified by column chromatography (60 g of silica gel) eluting with a gradient of 10–30% EtOAc and in hexanes to yield in order of elution:

(a) The 8-bromo derivative **19** as a colourless oil (0.29 g, 19%); ^{1}H NMR (DMSO-d_6) δ 1.14 (s, 9H, Me_3C), 2.18 (s, 3H, $COCH_3$), 2.45–2.80 (m, 2H, 7-H), 3.10–3.38 (m, 2H, 6-H), 5.22 (s, 2H, 2-CH_2), 5.96 (m, 1H, 8-H), 6.25 (m, 2H, 4-OCH_2), 7.92 (s, 1H, 9-H), 8.01 (s, 1H, 5-H).

(b) The desired product, 6-bromo derivative **18**, as a yellow oil (0.79 g, 51%); ^{1}H NMR (DMSO-d_6) δ 1.15 (s, 9H, Me_3C), 2.18 (s, 3H, $COCH_3$), 2.45–2.80 (m, 2H, 7-H), 3.10–3.38 (m, 2H, 8-H), 5.25 (s, 2H, 2-CH_2), 6.02 (m, 1H, 6-H), 6.27 (m, 2H, 4-OCH_2), 7.84 (s, 1H, 9-H), 8.13 (s, 1H, 5-H); MS (ESI, *m*/*z*): 451, 453 [$(M+H)^+$, 10%], 473, 475 [$(M+Na)^+$, 100%].

4.18. *tert*-Butyl 4-[*N*-((6*RS*)-2-acetoxymethyl-4-(2,2-dimethylpropionyloxymethyloxy)-7,8-dihydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)amino]benzoate (20)

A solution of 2-acetoxymethyl-6-bromo-4-(2,2-dimethylpropionyloxymethyloxy)-7,8-dihydro-6*H*-cyclopenta[*g*]quinazoline (0.60 g, 1.30 mmol), *tert*-butyl *p*-aminobenzoate (0.54 g, 2.01 mmol) and diisopropylethylamine (11.70 ml, 67.00 mmol) in DMF (30 ml) was placed in a pre-heated oil-bath at 60 °C and heated at this temperature under argon for 18 h. The solvent was removed in vacuo and the residue was purified by column chromatography (40 g of silica gel) eluting with 25% EtOAc in hexanes to yield the desired product as a yellow solid (0.14 g, 19%); mp 62 °C; ^{1}H NMR ($CDCl_3$) δ 1.01 (s, 9H, Me_3C), 1.41 (s, 9H, $COOCMe_3$), 1.75–1.90 (m, 1H, 7-H), 2.08 (s, 3H, N–CH_3), 2.50–2.63 (m, 1H, 7-H), 2.88–3.12 (m, 2H, 8-H), 4.24 (br s, 1H, N^{10}–H), 5.01 (m, 1H, 6-H), 5.13 (s, 2H, 2-CH_2), 6.07 (m, 2H, 4-OCH_2), 6.53 (d, J = 8.92 Hz, 2H, 3′-H, 5′-H), 7.62 (s, 1H, 9-H), 7.70 (d, J = 8.89 Hz, 2H, 2′-H, 6′-H), 7.90 (s, 1H, 5-H); MS (FAB, *m*/*z*): 564 [$(M+H)^+$, 100%], 586 [$(M+Na)^+$, 75%]. Found: C, 64.94; H, 6.69; N, 7.12. $C_{31}H_{37}N_3O_7.1/2H_2O$ requires C, 65.02; H, 6.69; N, 7.34.

4.19. *tert*-Butyl 4-[*N*-((6*RS*)-2-acetoxymethyl-4-(2,2-dimethylpropionyloxymethyloxy)-7,8-dihydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]benzoate (21)

tert-Butyl 4-[*N*-((6*RS*)-2-acetoxymethyl-4-(2,2-dimethylpropionyloxymethyloxy)-7,8-dihydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)amino]benzoate (0.13 g, 0.23 mmol) was dissolved in THF (10 ml). AcOH (5.20 ml) was added followed by 37% aqueous formaldehyde (0.20 ml, excess) and the solution was stirred at room temperature for 3 h. After this time, sodium cyanoborohydride (0.033 g, 0.52 mmol) was slowly added and the solution stirred at room temperature for 18 h. The solvent was removed in vacuo and the residue partitioned between EtOAc (40 ml) and H_2O (30 ml). The aqueous layer was extracted with EtOAc (20 ml). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (30 ml), H_2O (30 ml), dried (Na_2SO_4) and the solvent removed in vacuo. The residue was purified by column chromatography (25 g of silica gel) eluting with $CHCl_3$ to yield the product as a clear oil (0.11 g, 83%); ^{1}H NMR ($CDCl_3$) δ 1.11 (s, 9H, CMe_3), 1.52 (s, 9H, CO_2CMe_3), 2.10 (m, 1H, 7-H), 2.18 (s, 3H, $COCH_3$), 2.51 (m, 1H, 7-H), 2.69 (s, 3H, N^{10}–CH_3), 2.90–3.28 (m, 2H, 8-H), 5.23 (s, 2H, 2-CH_2), 5.63 (t, J = 8.19 Hz, 1H, 6-H), 6.21 (m, 2H, POM-CH_2), 6.81 (d, J = 9.01 Hz, 2H, 3′-H, 5′-H), 7.73 (s, 1H, 9-H), 7.82 (d, J = 8.99 Hz, 2H, 2′-H, 6′-H), 7.86 (s, 1H, 5-H); MS (ESI, *m*/*z*): 578 [$(M+H)^+$, 80%], 600 [$(M+Na)^+$, 100%].

4.20. 4-[*N*-((6*RS*)-2-Acetoxymethyl-4-(2,2-dimethylpropionyloxymethyloxy)-7,8-dihydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]benzoic acid (23)

A solution of *tert*-butyl 4-[*N*-((6*RS*)-2-acetoxymethyl-4-(2,2-dimethylpropionyloxymethyloxy)-7,8-dihydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]benzoate

(0.10 g, 0.17 mmol) in TFA (7 ml) was stirred at room temperature with protection from the light for 2.5 h. The solvent was removed in vacuo to yield the product as the TFA salt, yellow oil (0.10 g, TFA salt); ^{1}H NMR ($CDCl_3$) δ 1.13 (s, 9H, Me_3C), 2.05–2.20 (m, 1H, 7-H), 2.18 (s, 3H, $COCH_3$), 2.48–2.67 (m, 1H, 7-H), 2.73 (s, 3H, N–CH_3), 3.03–3.37 (m, 2H, 8-H), 5.39 (s, 2H, 2-CH_2), 5.70 (t, J = 8.51 Hz, 1H, 6-H), 6.18–6.37 (m, 2H, 4-OCH_2), 6.86 (d, J = 8.90 Hz, 2H, 3′-H, 5′-H), 7.90 (s, 1H, 9-H), 7.96 (d, J = 8.93 Hz, 2H, 2′-H, 6′-H), 8.05 (s, 1H, 5-H); MS (ESI, *m*/*z*): 522 [$(M+H)^+$, 40%], 544 [$(M+Na)^+$, 100%]. Found: C, 53.69; H, 4.69; N, 6.42. $C_{28}H_{31}N_3O_7\cdot 11/2$ TFA requires: C, 53.76; H, 4.73; N, 6.06.

4.21. Tri-*tert*-butyl *N*-{4-[*N*-((6*RS*)-2-acetoxymethyl-4-(2,2-dimethylpropionyloxymethyloxy)-7,8-dihydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]benzoyl}-L-γ-glutamyl-D-glutamate

4-[*N*-((6*RS*)-2-Acetoxymethyl-4-(2,2-dimethylpropionyloxymethyloxy)-7,8-dihydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]benzoic acid (0.090 g, 0.17 mmol) and tri-*tert*-butyl L-γ-glutamyl-D-glutamate (0.12 g, 0.27 mmol) were dissolved in anhydrous DMF (5 ml) under argon and with protection from the light. DEPC (0.060 ml, 0.37 mmol) was added followed by triethylamine (0.060 ml, 0.41 mmol) and the solution was stirred at room temperature under argon with protection from the light for 3 h then partitioned between EtOAc (30 ml) and H_2O (30 ml). The aqueous layer was extracted with EtOAc (2× 20 ml). The combined organic extracts were washed with 10% aqueous citric acid (2× 25 ml), saturated aqueous $NaHCO_3$ (30 ml) and dilute brine (50 ml), dried (Na_2SO_4) and the solvent removed in vacuo. The residue was purified by column chromatography eluting with a gradient of 0–40% EtOAc in CH_2Cl_2 to yield the product as a yellow oil (0.089 g, 55%). A portion of the product was triturated with hexanes to yield a pale yellow solid for analysis: mp 119 °C; ^{1}H NMR ($CDCl_3$) δ 1.01 (s, 9H, Me_3C), 1.26, 1.30, 1.32 (3× s, 27H, 3× CO_2CMe_3), 1.68–2.00 (m, 5H, 2× Glu β-CH_2, 7-CH), 2.08 (s, 3H, $COCH_3$), 2.10–2.45 (m, 5H, 2× Glu γ-CH_2, 7-CH), 2.59 (s, 3H, N^{10}–CH_3), 2.82–3.18 (m, 2H, 8-CH_2), 4.31, 4.60 (2× m, 2H, 2× Glu α-CH), 5.13 (s, 2H, 2-CH_2), 5.53 (t, J = 8.24 Hz, 1H, 6-H), 6.08 (q, J = 7.90 Hz, 2H, 4-OCH_2) 6.75 (d, J = 8.91 Hz, 2H, 3′-H, 5′-H), 6.90 (d, J = 7.48 Hz, 1H), 6.97 (d, J = 7.93 Hz, 1H), (2× CONH), 7.63 (d, J = 8.58 Hz, 2H, 2′-H, 6′-H), 7.64 (s, 1H, 9-H), 7.72 (s, 1H, 5-H); MS (ESI, *m*/*z*): 948 [$(M+H)^+$, 100%], 970 [$(M+Na)^+$, 60%]. Found: C, 62.95; H, 7.32; N, 7.30. $C_{50}H_{69}N_5O_{13}$ requires: C, 63.34; H, 7.33; N, 7.39.

4.22. *N*-{4-[*N*-((6*RS*)-2-Hydroxymethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]benzoyl}-L-γ-glutamyl-D-glutamic acid (2a)

A solution of tri-*tert*-butyl *N*-{4-[*N*-((6*RS*)-2-acetoxymethyl-4-(2,2-dimethylpropionyloxymethyloxy)-7,8-dihydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]benzoyl}-L-γ-glutamyl-D-glutamate (0.080 g, 0.084 mmol) in TFA (5 ml) was stirred at room temperature with protection from the light for 2.5 h. The solvent was removed in vacuo then the residue was dissolved in MeOH (2.3 ml) and H_2O (2 ml). The pH of the solution was adjusted to pH 10 with 1 M NaOH and the solution was stirred at room temperature for 3 h then acidified to pH 3.5 with 0.5 M HCl and cooled to 0 °C. The product was collected by filtration, washed with H_2O and dried under vacuum over P_2O_5 to yield a pale yellow solid (0.030 g, 57%); mp 179–181 °C; ^{1}H NMR (DMSO-d_6) δ 1.71–2.34 (m, 8H, 2× Glu β-CH_2, 2× Glu γ-CH_2), 2.65 (s, 3H, N^{10}–CH_3), 2.90–3.28 (m, 4H, 7-CH_2, 8-CH_2), 4.14 (s, 2H, 2-CH_2OH), 4.20, 4.32 (2× m, 2H, 2× Glu α-CH), 5.60 (br s, 1H, NH), 5.81 (t, J = 7.83 Hz, 1H, 6-H), 7.00 (d, J = 8.94 Hz, 2H, 3′-H, 5′-H), 7.53 (s, 1H, 9-H), 7.71 (s, 1H, 5-H), 7.80 (d, J = 8.77 Hz, 2H, 2′-H, 6′-H), 8.13 (d, J = 7.72 Hz, 1H), 8.32 (d, J = 7.30 Hz, 1H), (2× CONH); MS (ESI, *m*/*z*): 624 [$(M+H)^+$, 100%], 646 [$(M+Na)^+$, 40%]. Found: C, 53.12; H, 5.44; N, 10.18. $C_{30}H_{33}N_5O_{10}\cdot 3H_2O$ requires: C, 53.18; H, 5.80; N, 10.34.

4.23. *tert*-Butyl 4-[*N*-((6*RS*)-2-acetoxymethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)amino]benzoate (26a)

A suspension of 2-acetoxymethyl-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4,6-dione (0.24 g, 0.88 mmol) in anhydrous MeOH (20 ml) and anhydrous CH_2Cl_2 (10 ml) was treated with *tert*-butyl *p*-aminobenzoate (0.20 g, 1.05 mmol) followed by decaborane (0.042 g, 1.05 mmol) and the mixture stirred at room temperature under argon for 17 h. The solvent was removed in vacuo and the residue purified by column chromatography (50 g of silica gel) eluting with $CHCl_3$ to yield the desired product as a white solid (0.23 g, 58%); mp 222 °C; ^{1}H NMR ($CDCl_3$) δ 1.58 (s, 9H, CMe_3), 2.00 (m, 1H, 7-H), 2.23 (s, 3H, $COCH_3$), 2.72 (m, 1H, 7-H), 3.08 (m, 2H, 8-H), 5.13 (s, 2H, 2-CH_2), 5.15 (m, 1H, 6-H), 6.68 (d, J = 8.82 Hz, 2H, 3′-H, 5′-H), 7.58 (s, 1H, 9-H), 7.87 (d, J = 8.81 Hz, 2H, 2′-H, 6′-H), 8.23 (s, 1H, 5-H); MS (ESI, *m*/*z*): 450 [$(M+H)^+$, 40%], 472 [$(M+Na)^+$, 100%]. Found: C, 64.99; H, 5.91; N, 9.06. $C_{25}H_{27}N_3O_5\cdot 1/4H_2O$ requires: C, 64.86; H, 6.16; N, 9.08.

4.24. *tert*-Butyl 4-[*N*-((6*RS*)-2-acetoxymethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoate (29a)

A suspension of (propargyl)$Co_2(CO)_6^+BF_4^-$ (0.21 g, 0.50 mmol) in anhydrous CH_2Cl_2 (18 ml, distilled over CaH_2) was treated with *tert*-butyl 4-[*N*-((6*RS*)-2-acetoxymethyl-4-oxo-3, 4,7,8-tetrahydro-6*H*-cyclopenta[*g*]-quinazolin-6-yl)amino]benzoate (0.18 g, 0.39 mmol) and the red solution stirred at room temperature under argon for 30 min. Diisopropylethylamine (0.14 ml, 0.80 mmol) was added and the mixture stirred at room temperature under argon for 2 h. The mixture was partitioned between EtOAc (70 ml) and brine (70 ml). The organic extract was dried (Na_2SO_4) and the solvent removed in vacuo. The residue was purified by column chromatography (50 g of silica gel) eluting with a gradient of 0–20% EtOAc in CH_2Cl_2 to yield the complex as a red oil (0.18 g, 59%); ^{1}H NMR ($CDCl_3$) δ 1.59 (s, 9H,

CMe_3), 2.14 (s, 3H, $COCH_3$), 2.35 (m, 1H, 7-H), 2.63 (m, 1H, 7-H), 3.15 (m, 2H, 8-H), 4.57 (AB system, J = 15.92 Hz, 2H, propargyl CH_2), 5.12 (s, 2H, 2-CH_2), 5.65 (t, J = 8.29 Hz, 1H, 6-H), 5.99 (s, 1H, propargyl CH), 6.93 (d, J = 9.01 Hz, 2H, 3′-H, 5′-H), 7.62 (s, 1H, 9-H), 7.90 (d, J = 8.90 Hz, 2H, 2′-H, 6′-H), 7.98 (s, 1H, 5-H), 11.04 (br s, 1H, N^3–H).

A solution of this complex (0.18 g, 0.23 mmol) in ethanol (25 ml) was treated with $Fe(NO_3)_3.9H_2O$ (1.00 g, excess) and the solution stirred at room temperature for 2 h. The solution was partitioned between EtOAc (60 ml) and H_2O (60 ml). The organic extract was washed with brine (60 ml), dried (Na_2SO_4) and the solvent removed in vacuo. The residue was purified by column chromatography (40 g of silica gel) eluting with $CHCl_3$ to yield the desired product as a white solid (0.082 g, 74%); mp 112–114 °C; ^{1}H NMR ($CDCl_3$) δ 1.58 (s, 9H, CMe_3), 2.23 (s, 3H, $COCH_3$), 2.24 (s, 1H, propargyl CH), 2.37 (m, 1H, 7-H), 2.61 (m, 1H, 7-H), 3.19 (m, 2H, 8-H), 3.93 (AB system, J = 18.43 Hz, 2H, propargyl CH_2), 5.12 (s, 2H, 2-CH_2), 5.65 (t, J = 8.20 Hz, 1H, 6-H), 6.96 (d, J = 9.13 Hz, 2H, 3′-H, 5′-H), 7.61 (s, 1H, 9-H), 7.93 (d, J = 9.02 Hz, 2H, 2′-H, 6′-H), 8.09 (s, 1H, 5-H); MS (ESI, m/z): 487 $[(M+H)^+$, 25%], 510 $[(M+Na)^+$, 100%]. Found: C, 68.24; H, 5.98; N, 8.43. $C_{28}H_{29}N_3O_5{\cdot}3/10H_2O$ requires C, 68.24; H, 6.01; N, 8.53.

4.25. 4-[*N*-((6*RS*)-2-Acetoxymethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)-amino]benzoic acid (30a)

A solution of *tert*-butyl 4-[*N*-((6*RS*)-2-acetoxymethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoate (0.067 g, 0.14 mmol) in TFA (5 ml) was stirred at room temperature with protection from the light for 2 h. The solvent was removed in vacuo and the residue triturated with 1:1 Et_2O and hexanes to yield the desired product as a white solid (0.053 g, 89%); ^{1}H NMR (DMSO-d_6) δ 2.12 (s, 3H, $COCH_3$), 2.21 (m, 1H, 7-H), 2.99 (m, 1H, 7-H), 3.14 (s, 1H, propargyl CH), 3.36 (m, 2H, 8-H), 3.96 (AB system, J = 18.94 Hz, 2H, propargyl CH_2), 4.94 (s, 2H, 2-CH_2), 5.78 (t, J = 8.32 Hz, 1H, 6-H), 7.02 (d, J = 9.11 Hz, 2H, 3′-H, 5′-H), 7.55 (s, 1H, 9-H), 7.80 (d, J = 7.32 Hz, 2H, 2′-H, 6′-H), 7.81 (s, 1H, 5-H); MS (ESI, m/z): 432 $[(M+H)^+$, 100%], 454 $[(M+Na)^+$, 10%].

4.26. Tri-*tert*-butyl *N*-{4-[*N*-((6*RS*)-2-acetoxymethyl-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 (31a)

A solution of 4-[*N*-((6*RS*)-2-acetoxymethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoic acid (0.047 g, 0.11 mmol) in anhydrous DMF (5 ml) was treated with tri-*tert*-butyl L-γ-glutamyl-D-glutamate (0.074 g, 0.16 mmol), DEPC (0.040 ml, 0.26 mmol) and triethylamine (0.040 ml, 0.25 mmol). The solution was stirred at room temperature under argon with protection from the light for 3.5 h then partitioned between EtOAc (25 ml) and H_2O (25 ml). The aqueous layer was extracted with EtOAc (2× 15 ml). The combined organic extracts were washed with 10% aqueous citric acid (2× 20 ml), saturated aqueous $NaHCO_3$ (20 ml) and dilute brine (30 ml), dried (Na_2SO_4) and the solvent removed in vacuo. The residue was purified by column chromatography (20 g of silica gel) eluting with a gradient of 30–100% EtOAc in CH_2Cl_2 to yield the desired product as a white solid (0.078 g, 83%); mp 104 °C; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H, $COOCMe_3$), 1.47 (s, 9H, $COOCMe_3$), 1.48 (s, 9H, $COOCMe_3$), 1.80–2.15 (m, 5H, 2× Glu β-CH_2, 7-CH), 2.20 (s, 3H, $COCH_3$), 2.20–2.50 (m, 5H, 2× Glu γ-CH_2, propargyl CH), 2.59 (m, 1H, 7-H), 3.08 (m, 1H, 8-H), 3.20 (m, 1H, 8-H), 3.92 (AB system, J = 19.92 Hz, 2H, propargyl CH_2), 4.45, 4.75 (2× m, 2H, 2× Glu α-CH), 5.10 (s, 2H, 2-CH_2), 5.64 (t, J = 8.30 Hz, 1H, 6-H), 6.99 (d, J = 8.86 Hz, 2H, 3′-H, 5′-H), 7.16 (m, 2H, 2× CONH), 7.61 (s, 1H, 9-H), 7.81 (d, J = 8.84 Hz, 2H, 2′-H, 6′-H), 8.10 (s, 1H, 5-H); MS (ESI, m/z): 858 $[(M+H)^+$, 100%], 880 $[(M+Na)^+$, 50%]. Found: C, 63.16; H, 6.90; N, 7.95. $C_{46}H_{59}N_5O_{11}{\cdot}1H_2O$ requires: C, 63.07; H, 7.02; N, 7.99.

4.27. *N*-{4-[*N*-((6*RS*)-2-Hydroxymethyl-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 (2b)

Tri-*tert*-butyl *N*-{4-[*N*-((6*RS*)-2-acetoxymethyl-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 (0.065 g, 0.08 mmol) was dissolved in TFA (5ml) and stirred at room temperature with protection from the light for 2 h. The solvent was removed in vacuo and the residue was triturated with diethyl ether. The product was collected by filtration, and dissolved in MeOH (3 ml) and H_2O (2.5 ml). The pH of the solution was adjusted to pH ~10 with 1 M NaOH solution and stirred at room temperature for 2 h then acidified to pH 4 with 1 M HCl and cooled to 0 °C. The precipitate was collected by filtration and dried in vacuo over P_2O_5 to yield the desired product as a brown solid (0.016 g, 32%); mp 174 °C; ^{1}H NMR (DMSO-d_6) δ 1.60–2.10 (m, 5H, 2× Glu β-CH_2, 7-CH), 2.15–2.40 (m, 5H, 2× Glu γ-CH_2, 7-H), 2.99 (m, 1H, 8-H), 3.12 (s, 1H, propargyl CH), 3.16 (m, 1H, 8-H), 3.96 (AB system, J = 18.84 Hz, 2H, propargyl CH_2), 4.18, 4.30 (2× m, 2H, 2× Glu α-CH), 4.36 (s, 2H, 2-CH_2), 5.58 (br s, 1H, –OH), 5.77 (t, J = 7.89 Hz, 1H, 6-H), 7.01 (d, J = 8.93 Hz, 2H, 3′-H, 5′-H), 7.54 (s, 1H, 9-H), 7.80 (d, J = 8.51 Hz, 2H, 2′-H, 6′-H), 7.82 (s, 1H, 5-H), 8.15 (d, J = 7.50 Hz, 1H), 8.35 (d, J = 7.18 Hz, 1H) (2× CONH); MS (ESI, m/z): 648 $[(M+H)^+$, 100%], 434 [(M−cyclopenta[*g*]quinazoline ring)$^+$, 25%], HRMS: measured 648.2313; calculated for $C_{32}H_{35}N_5O_{10}$ $(M+H)^+$: 648.2306.

4.28. *tert*-Butyl 4-[*N*-((6*RS*)-2-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)amino]benzoate (26b)

A suspension of 2-(2,2-dimethylpropionyloxymethyl)-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4,6-dione (0.47 g, 1.50 mmol) in anhydrous MeOH (33 ml) and anhydrous CH_2Cl_2 (5 ml) was treated with *tert*-butyl *p*-aminobenzoate (0.34 g, 1.78 mmol) followed by decabo-

rane (0.070 g, 0.58 mmol) and the mixture stirred at room temperature under argon for 18 h. The solvent was removed in vacuo and the residue purified by column chromatography (50 g of silica gel) eluting with 30% EtOAc in CH_2Cl_2 to yield the desired product as a white solid (0.43 g, 58%); mp 231 °C; ^{1}H NMR ($CDCl_3$) δ 1.26 (s, 9H, CMe_3), 1.58 (s, 9H, CO_2CMe_3), 2.00 (m, 1H, 7-H), 2.72 (m, 1H, 7-H), 3.08 (m, 2H, 8-H), 5.10 (s, 2H, 2-CH_2), 5.15 (m, 1H, 6-H), 6.67 (d, J = 8.82 Hz, 2H, 3′-H, 5′-H), 7.58 (s, 1H, 9-H), 7.87 (d, J = 8.84 Hz, 2H, 2′-H, 6′-H), 8.24 (s, 1H, 5-H); MS (ESI, m/z): 492 [$(M+H)^+$, 25%], 514 [$(M+Na)^+$, 100%]. Found: C, 68.37; H, 6.86; N, 8.35. $C_{28}H_{33}N_3O_5$ requires: C, 68.41; H, 6.77; N, 8.55.

4.29. *tert*-Butyl 4-[*N*-((6*RS*)-2-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]benzoate (27)

tert-Butyl 4-[*N*-((6*RS*)-2-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)amino]benzoate (0.050 g, 0.10 mmol) was dissolved in THF (5 ml). AcOH (2 ml) was added followed by 37% aqueous formaldehyde (0.08 ml, excess). The resulting solution was stirred at room temperature for 8 h. After this time, sodium cyanoborohydride (0.014 g, 0.22 mmol) was slowly added and the solution was stirred at room temperature for 17 h. The solvent was removed in vacuo and the residue partitioned between EtOAc (20 ml) and H_2O (20 ml). The aqueous layer was extracted with EtOAc (20 ml). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (20 ml), H_2O (20 ml), dried (Na_2SO_4) and the solvent removed in vacuo to yield the product as a white solid (0.050 g, 97%); mp 231 °C; ^{1}H NMR ($CDCl_3$) δ 1.27 (s, 9H, CMe_3), 1.59 (s, 9H, CO_2CMe_3), 2.11, 2.53 (2× m, 2H, 7-H), 2.75 (s, 3H, N^{10}–CH_3), 3.00–3.25 (m, 2H, 8-H), 5.10 (s, 2H, 2-CH_2) 5.67 (t, J = 8.42 Hz, 1H, 6-H), 6.86 (d, J = 9.08 Hz, 2H, 3′-H, 5′-H), 7.68 (s, 1H, 9-H), 7.90 (d, J = 9.01 Hz, 2H, 2′-H, 6′-H), 8.05 (s, 1H, 5-H), 10.20 (br s, 1H, N^3–H); MS (ESI, m/z): 506 [$(M+H)^+$, 10%], 528 [$(M+Na)^+$, 100%]. Found: C, 67.75; H, 6.82; N, 7.90. $C_{29}H_{35}N_3O_5{\cdot}1/2H_2O$ requires: C, 67.69; H, 7.05; N, 8.17.

4.30. *tert*-Butyl 4-[*N*-((6*RS*)-2-hydroxymethyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]benzoate (22)

4.30.1. Method A. A solution of *tert*-butyl 4-[*N*-((6*RS*)-2-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]benzoate (**27**) (0.050 g, 0.011 mmol) in H_2O (1.5 ml) and MeOH (2.5 ml) was basified to pH 10 with 1 M NaOH solution and stirred at room temperature for 2.5 h. The solution was acidified to pH 4 with 1 M HCl, cooled to 0 °C and the desired product collected by filtration as a pale brown solid (0.030 g, 72%); mp 186 °C; ^{1}H NMR (DMSO-d_6) δ 1.51 (s, 9H, CO_2CMe_3), 2.07 (m, 1H, 7-H), 2.44 (m, 1H, 7-H), 2.67 (s, 3H, N^{10}–H), 3.06 (m, 2H, 8-H), 4.38 (s, 2H, 2-CH_2), 5.80 (t, J = 7.63 Hz, 1H, 6-H), 6.99 (d, J = 9.14 Hz, 2H, 3′-H, 5′-H), 7.54 (s, 1H, 9-H), 7.71 (d, J = 9.67 Hz, 2H, 2′-H, 6′-H), 7.76 (s, 1H, 5-H); MS (ESI, m/z): 422 [$(M+H)^+$, 35%], 444 [$(M+Na)^+$, 85%]. Found: C, 64.40; H, 6.43; N, 9.34. $C_{24}H_{27}N_3O_4{\cdot}11/2H_2O$ requires C, 64.27; H, 6.74; N, 9.37.

4.30.2. Method B. A solution of *tert*-butyl 4-[*N*-((6*RS*)-2-acetoxymethyl-4-(2,2-dimethylpropionyloxymethyloxy)-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]benzoate (**21**) (0.10 g, 0.17 mmol) in H_2O (2.5 ml) and MeOH (4 ml) was basified to pH 10 with 1 N NaOH solution. The solution was stirred at room temperature for 2.5 h then acidified to pH 4 with 1 N HCl and the precipitate was collected by centrifugation and dried in vacuo to yield the desired product as a pale brown solid (0.046 g, 63%): ^{1}H NMR was identical with that of a sample produced from compound **27** as described above.

4.31. *tert*-Butyl 4-[*N*-((6*RS*)-2-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoate (29b)

A suspension of (propargyl)$Co_2(CO)_6^+BF_4^-$ (0.21 g, 0.52 mmol) in anhydrous CH_2Cl_2 (25 ml, distilled over CaH_2) was treated with *tert*-butyl 4-[*N*-((6*RS*)-2-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)amino]benzoate (0.20 g, 0.41 mmol) and the red solution stirred at room temperature under argon for 15 min. Diisopropylethylamine (0.15 ml, 0.86 mmol) was added and stirring was continued at room temperature under argon for 1 h. The mixture was partitioned between EtOAc (30 ml) and brine (30 ml). The organic extract was dried (Na_2SO_4) and the solvent removed in vacuo. The residue was purified by column chromatography (20 g of silica gel) eluting with a gradient of 0–10% EtOAc in CH_2Cl_2 to yield the complex **28b** as a red oil (0.19 g, 58%); ^{1}H NMR ($CDCl_3$) δ 1.26 (s, 9H, CMe_3), 1.59 (s, 9H, CO_2CMe_3), 2.31 (m, 1H, 7-H), 2.62 (m, 1H, 7-H), 3.13 (m, 2H, 8-H), 4.57 (AB system, J = 16.94 Hz, 2H, propargyl CH_2), 5.09 (s, 2H, 2-CH_2), 5.63 (t, J = 8.30 Hz, 1H, 6-H), 5.98 (s, 1H, propargyl CH), 6.91 (d, J = 8.88 Hz, 2H, 3′-H, 5′-H), 7.61 (s, 1H, 9-H), 7.90 (d, J = 8.91 Hz, 2H, 2′-H, 6′-H), 8.14 (s, 1H, 5-H), 10.25 (br s, 1H, N^3–H).

A solution of this complex **28b** (0.19 g, 0.23 mmol) in EtOH (30 ml) was treated with $Fe(NO_3)_3.9H_2O$ (1.10 g, excess) and the solution was stirred at room temperature for 2 h then partitioned between EtOAc (30 ml) and H_2O (30 ml). The organic extract was washed with brine (30 ml), dried (Na_2SO_4) and the solvent removed in vacuo. The residue was purified by column chromatography (20 g of silica gel) eluting with 10% EtOAc in CH_2Cl_2 to yield the desired product as a white solid (0.094 g, 78%); mp 134 °C; ^{1}H NMR ($CDCl_3$) δ 1.32 (s, 9H, CMe_3), 1.61 (s, 9H, CO_2CMe_3), 2.23 (s, 1H, propargyl CH), 2.38 (m, 1H, 7-H), 2.62 (m, 1H, 7-H), 3.07 (m, 1H, 8-H), 3.25 (m, 1H, 8-H), 3.94 (AB system, J = 18.60 Hz, 2H, propargyl CH_2), 5.12 (s, 2H, 2-CH_2), 5.68 (t, J = 8.19 Hz, 1H, 6-H), 6.99 (d, J = 9.12 Hz, 2H, 3′-H, 5′-H), 7.63 (s, 1H, 9-H), 7.95 (d, J = 9.04 Hz, 2H, 2′-H, 6′-H), 8.16 (s, 1H,

5-H), 9.55 (br s, 1H, N^3–H); MS (ESI, *m*/*z*): 530 [(M+H)$^+$, 15%], 552 [(M+Na)$^+$, 100%]. Found: C, 70.14; H, 6.80; N, 7.73. $C_{31}H_{35}N_3O_5$ requires: C, 70.30; H, 6.66; N, 7.93.

4.32. 4-[*N*-((6*RS*)-2-(2,2-Dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoic acid (30b)

A solution of *tert*-butyl 4-[*N*-((6*RS*)-2-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoate (0.080 g, 0.15 mmol) in TFA (5 ml) was stirred at room temperature with protection from the light for 1.5 h. The solvent was removed in vacuo and the residue triturated with 1:1 Et_2O and hexanes to yield the desired product as a white solid (0.081 g, TFA salt); mp 133 °C; ^{1}H NMR (DMSO-d_6) δ 1.23 (s, 9H, CO_2CMe_3), 2.22 (m, 1H, 7-H), 2.50 (m, 1H, 7-H), 3.03 (m, 2H, 8-H), 3.14 (s, 1H, propargyl CH), 3.97 (AB system, J = 18.82 Hz, 2H, propargyl CH_2), 4.95 (s, 2H, 2-CH_2), 5.79 (t, J = 8.61 Hz, 1H, 6-H), 7.03 (d, J = 8.98 Hz, 2H, 3′-H, 5′-H), 7.51 (s, 1H, 9-H), 7.81 (d, J = 6.60 Hz, 2H, 2′-H, 6′-H), 7.83 (s, 1H, 5-H); MS (ESI, *m*/*z*): 474 [(M+H)$^+$, 100%], 496 [(M+Na)$^+$, 55%].

4.33. Tri-*tert*-butyl *N*-{4-[*N*-((6*RS*)-2-(2,2-dimethylpropionyloxymethyl)-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 (31b)

A solution of 4-[*N*-((6*RS*)-2-(2,2-dimethylpropionyloxymethyl)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoic acid (0.080 g, 0.15 mmol) in anhydrous DMF (7 ml) was treated with tri-*tert*-butyl L-γ-glutamyl-D-glutamate (0.15 g, 0.33 mmol), DEPC (0.060 ml, 0.40 mmol) and triethylamine (0.058 ml, 0.40 mmol). The solution was stirred at room temperature under argon with protection from the light for 2.5 h then partitioned between EtOAc (25 ml) and H_2O (25 ml). The aqueous layer was extracted with EtOAc (2× 20 ml). The combined organic extracts were washed with 10% aqueous citric acid (2× 30 ml), saturated aqueous $NaHCO_3$ (30 ml) and dilute brine (30 ml), dried (Na_2SO_4) and the solvent removed in vacuo. The residue was purified by column chromatography (30 g of silica gel) eluting with 40% EtOAc in CH_2Cl_2 to yield the desired product as a white solid (0.094 g, 62%); mp 109 °C; ^{1}H NMR ($CDCl_3$) δ 1.29 (s, 9H, $COCMe_3$), 1.43 (s, 9H, $COOCMe_3$), 1.47 (s, 9H, $COOCMe_3$), 1.48 (s, 9H, $COOCMe_3$), 1.60–2.10 (m, 5H, 2× Glu β-CH_2, 7-CH), 2.21 (s, 1H, propargyl CH), 2.22–2.50 (m, 4H, 2× Glu γ-CH_2), 2.59 (m, 1H, 7-H), 3.08 (m, 1H, 8-H), 3.20 (m, 1H, 8-H), 3.92 (AB system, J = 19.02 Hz, 2H, propargyl CH_2), 4.48, 4.76 (2× m, 2H, 2× Glu α-CH), 5.12 (s, 2H, 2-CH_2), 5.64 (t, J = 8.13 Hz, 1H, 6-H), 6.99 (d, J = 8.76 Hz, 2H, 3′-H, 5′-H), 7.07 (m, 2H, 2× CONH), 7.64 (s, 1H, 9-H), 7.80 (d, J = 8.81 Hz, 2H, 2′-H, 6′-H), 8.13 (s, 1H, 5-H); MS (ESI, *m*/*z*): 900 [(M+H)$^+$, 50%], 922 [(M+Na)$^+$, 100%]. Found: C, 64.85; H, 7.23; N, 7.33. $C_{49}H_{65}N_5O_{11}\cdot 1/2H_2O$ requires C, 64.76; H, 7.27; N, 7.71.

4.34. *N*-{4-[*N*-((6*RS*)-2-Hydroxymethyl-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 (2b)

Tri-*tert*-butyl *N*-{4-[*N*-((6*RS*)-2-(2,2-dimethylpropionyloxymethyl)-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 (0.080 g, 0.093 mmol) was dissolved in TFA (5 ml) and stirred at room temperature with protection from the light for 1 h. The solvent was removed in vacuo and the residue dissolved in MeOH (3 ml) and H_2O (3 ml). The pH of the solution was adjusted to pH 12 with 1M NaOH solution and stirred at room temperature for 6 h. The solution was acidified to pH 4 with 1 M HCl and cooled to 0 °C. The precipitate was collected by filtration and dried in vacuo over P_2O_5 to yield the desired product as a pale brown solid (0.027 g, 47%); mp 172 °C; ^{1}H NMR (DMSO-d_6) δ 1.60–2.10 (m, 5H, 2× Glu β-CH_2, 7-CH), 2.15–2.40 (m, 5H, 2× Glu γ-CH_2, 7-H), 2.99 (m, 1H, 8-H), 3.12 (s, 1H, propargyl CH), 3.16 (m, 1H, 8-H), 3.98 (AB system, J = 19.89 Hz, 2H, propargyl CH_2), 4.18, 4.30 (2× m, 2H, 2× Glu α-CH), 4.36 (s, 2H, 2-CH_2), 5.58 (br s, 1H, OH), 5.77 (t, J = 7.90 Hz, 1H, 6-H), 7.01 (d, J = 8.86 Hz, 2H, 3′-H, 5′-H), 7.54 (s, 1H, 9-H), 7.80 (d, J = 8.54 Hz, 2H, 2′-H, 6′-H), 7.82 (s, 1H, 5-H), 8.15 (d, J = 7.51 Hz, 1H), 8.35 (d, J = 7.22 Hz, 1H) (2× CONH); MS (FAB, *m*/*z*): 670 [(M+Na)$^+$, 10%]; HRMS: measured 670.2135; calculated for $C_{32}H_{33}N_5O_{10}Na$ (M+Na)$^+$: 670.2125. HPLC: Cyclobond I column, eluting with 88% phosphate buffer/12% CH_3CN. Retention times: 630 and 707 s, ratio ~1:1, purity (6*R* + 6*S*) = 96.4.

In a modification of the above methodology, compound **2b** was also prepared as follows: Both protecting groups in compound **29b** were removed, the trimethylacetyl group by alkaline hydrolysis, and the *tert*-butyl ester by TFA hydrolysis. Coupling of the resulting compound with tri-*tert*-butyl L-γ-glutamyl-D-glutamate via DEPC activation, followed by purification, and then TFA hydrolysis of the *tert*-butyl esters afforded compound **2b**: ^{1}H NMR (DMSO-d_6): as reported as above; MS (ESI, *m*/*z*): 648 [(M+H)$^+$, 100%], 670 [(M+Na)$^+$, 80%], 434 [(M−cyclopenta[*g*]quinazoline ring)$^+$, 70%]; HPLC-Cyclobond I chiral column: purity (6*R*+6*S*) = 95.

4.35. 1,2,3,5-Tetrahydro-7-oxa-5-aza-cyclopenta[*b*]naphthalene-6,8-dione (32)

A solution of 5-amino-6-carboxyindane[27] (3.00 g, 16.91 mmol) in anhydrous THF (70 ml) under argon was treated with triphosgene (1.68 g, 5.65 mmol) and stirred at room temperature for 7 h. The suspension was cooled to 0 °C and filtered to yield the product as a white solid (2.43 g, 70%); mp 265 °C (decomposed); ^{1}H NMR (DMSO-d_6) δ 2.03 (quin, J = 7.42 Hz, 2H, 2-H), 2.90 (m, 4H, 1-H and 3-H), 7.00 (s, 1H, 9-H), 7.72 (s, 1H, 5-H), 11.59 (br s, 1H, NH); MS (FAB, *m*/*z*): 204 [(M+H)$^+$, 100%]; HRMS; measured 204.0650; calculated for $C_{11}H_{10}NO_3$ (M+H)$^+$: 204.0661. Found: C, 64.99; H, 4.49; N, 6.90. $C_{11}H_9NO_3$ requires: C, 65.02; H, 4.46; N, 6.89.

4.36. 5-Amino-6-methoxycarbonylindane (33)

A suspension of 1,2,3,5-tetrahydro-7-oxa-5-aza-cyclopenta[*b*]naphthalene-6,8-dione (2.80 g, 13.79 mmol) and DMAP (0.17 g, 1.38 mmol) in anhydrous MeOH (100 ml) was heated to 80 °C under argon for 3.5 h. The solution was cooled to room temperature and the solvent removed in vacuo. The residue was dissolved in EtOAc (100 ml) and washed with 0.1M HCl (2× 50 ml). The organic extract was dried (Na_2SO_4) and the solvent removed in vacuo to yield the product as a yellow solid (2.60 g, 99%); mp 84 °C; ^{1}H NMR (DMSO-d_6) δ 1.94 (m, 2H, 2-H), 2.70 (t, J = 7.32 Hz, 2H), 2.75 (t, J = 7.41 Hz, 2H) (1-CH_2, 3-CH_2), 3.75 (s, 3H, O–CH_3), 6.45 (br s, 2H, 2-NH_2), 6.63 (s, 1H, 9-H), 7.53 (s, 1H, 5-H); MS (FAB, *m*/*z*): 191 [$(M)^+$, 100%]: HRMS; measured 191.0960; calculated for $C_{11}H_{13}NO_2$ $(M)^+$: 191.0946. Found: C, 68.75; H, 6.83; N, 7.28. $C_{11}H_{13}NO_2$ requires: C, 69.09; H, 6.85; N, 7.32.

4.37. Chloroformamidine hydrochloride (prepared according to literature procedure[31])

Cyanamide (2.00 g, 47.61 mmol) was dissolved in Et_2O (150 ml) and anhydrous hydrogen chloride gas was passed through the solution, via a wide glass tube, for 2.5 h, a white precipitate formed. The product was collected by filtration, washed well with Et_2O and dried in vacuo to yield a white solid (5.31 g, 97%); mp 130 °C (decomposed), then melted at 175 °C; ^{1}H NMR (DMSO-d_6) δ 6.52 (br s, 2H, NH_2), 11.00 (br s, 1H, NH); MS (FAB, *m*/*z*): 77 [$(M-HCl)^+$, 100%]. Found: C, 10.70; H, 3.48; N, 24.24; Cl, 61.87. $CH_3N_2Cl{\cdot}HCl$ requires C, 10.45; H, 3.51; N, 24.37; Cl, 61.68.

4.38. 2-Amino-3,4,7,8-tetrahydro-cyclopenta[*g*]quinazolin-4-one (34)

5-Amino-6-methoxycarbonylindane (7.50 g, 39.00 mmol), chloroformamidine hydrochloride (6.75 g, 58.97 mol) and dimethylsulfone (22.50 g, excess) were heated to 150 °C under argon for 1 h. The solid was cooled to room temperature, diluted with H_2O (100 ml) and basified with liquid ammonia (d = 0.88, 75 ml). The resulting suspension was stirred vigorously for 2 h. The product was collected by filtration, washed well with H_2O and dried in vacuo to yield a pale yellow solid (7.71 g, 98%); mp >350 °C (decomposed); ^{1}H NMR (DMSO-d_6) δ 2.04 (m, 2H, 7-H), 2.87 (q, J = 7.62 Hz, 4H, 6-H and 8-H), 6.16 (br s, 2H, 2-NH_2), 7.04 (s, 1H, 9-H), 7.69 (s, 1H, 5-H), 10.74 (br s, 1H, N^3–H); MS (FAB, *m*/*z*); 202 [$(M+H)^+$, 100%]; HRMS: measured 202.0986; calculated for $C_{11}H_{12}N_3O$ $(M+H)^+$: 202.0980. Found: C, 64.00; H, 5.46; N, 20.42. $C_{11}H_{11}N_3O{\cdot}1/2H_2O$ requires C, 64.22; H, 5.63; N, 20.42.

4.39. 2-(2,2-Dimethylpropionylamino)-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4-one (35)

To a suspension of 2-amino-3,4,7,8-tetrahydro-cyclopenta[*g*]quinazolin-4-one (0.50 g, 2.51 mmol) in anhydrous DMF (5 ml) under argon was added trimethylacetic anhydride (0.55 ml, 3.01 mmol) and the suspension heated to 100 °C for 1.5 h. The solution was cooled to room temperature and the solvent removed in vacuo. The resulting solid was partitioned between EtOAc (30 ml) and saturated aqueous $NaHCO_3$ (30 ml) and the aqueous layer was extracted with EtOAc (30 ml). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (25 ml), H_2O (25 ml), dried (Na_2SO_4) and the solvent removed in vacuo to yield the product as a pale yellow solid (0.66 g, 94%); mp 246 °C; ^{1}H NMR (DMSO-d_6) δ 1.25 (s, 9H, CMe_3), 2.06 (quin, J = 7.42 Hz, 2H, 7-H), 2.96 (q, J = 7.69 Hz, 4H, 6-H and 8-H), 7.35 (s, 1H, 9-H), 7.87 (s, 1H, 5-H), 10.93 (br s, 1H, CONH), 12.04 (br s, 1H, CONH); MS (FAB, *m*/*z*); 286 [$(M+H)^+$, 100%], 202 [$(M\text{-}COC(CH_3)_3)^+$, 30%]; HRMS: measured 286.1561; calculated for $C_{16}H_{20}N_3O_2$ $(M+H)^+$: 286.1556. Found: C, 67.03; H, 6.58; N, 14.60. $C_{16}H_{19}N_3O_2$ requires: C, 67.35; H, 6.71; N, 14.73.

4.40. 2-(2,2-Dimethylpropionylamino)-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4,6-dione (37) and 2-(2,2-dimethylpropionylamino)-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4,8-dione (36)

To a vigorously stirred suspension of chromium (VI) oxide (0.14 g, 1.36 mmol) in CH_2Cl_2 (200 ml), cooled to 0 °C, was added dropwise *tert*-butyl hydroperoxide (25.60 ml, 196.00 mmol). The red mixture was allowed to warm to room temperature and stirred for 30 min. 2-(2,2-Dimethylpropionylamino)-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4-one (4.00 g, 13.38 mmol) was slowly added and the mixture stirred at room temperature for 72 h. The suspension was cooled to 0 °C and 10% aqueous sodium metabisulfite solution (44 ml) was added slowly then allowed to warm to room temperature and stirred for 2 h then partitioned between EtOAc (400 ml) and half-saturated brine (250 ml). The aqueous layer was extracted with EtOAc (100 ml). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (250 ml), brine (250 ml) and saturated aqueous $NaHCO_3$ (250 ml), dried (Na_2SO_4) and the solvent removed in vacuo. The residue was purified by column chromatography eluting with a gradient of 0–50% EtOAc in CH_2Cl_2 to yield, in order of elution:

(a) 2-(2,2-Dimethylpropionylamino)-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4,8-dione, a white solid (0.27 g, 7%); mp 280 °C; ^{1}H NMR (DMSO-d_6) δ 1.26 (s, 9H, CMe_3), 2.75 (t, J = 5.93 Hz, 2H, 7-H), 3.22 (t, J = 3.10 Hz, 2H, 8-H), 7.66 (s, 1H, 9-H), 8.24 (s, 1H, 5-H), 11.20 (br s, 1H, CONH), 12.22 (br s, 1H, CONH); MS (FAB, *m*/*z*): 300 [$(M+H)^+$, 100%], HRMS: measured 300.1363; calculated for $C_{16}H_{18}N_3O_3$ $(M+H)^+$: 300.1348. Found: C, 63.90; H, 5.73; N, 13.96. $C_{16}H_{17}N_3O_3$ requires: C, 64.20; H, 5.72; N, 14.04.

(b) 2-(2,2-Dimethylpropionylamino)-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4,6-dione, a white solid; mp 306 °C; ^{1}H NMR (DMSO-d_6) δ 1.27 (s, 9H, CMe_3), 2.70 (t, J = 6.14 Hz, 2H, 7-H), 3.20 (t, J = 4.78 Hz, 2H, 8-H), 7.59 (s, 1H, 9-H), 8.24 (s, 1H, 5-H), 11.32 (br s, 1H, CONH), 12.10 (br s, 1H, CONH); MS (FAB, *m*/*z*): 300 [$(M+H)^+$, 100%]; 216

[M−COC(CH_3), 45%]; HRMS: measured 300.1337; calculated for $C_{16}H_{18}N_3O_3$ $(M+H)^+$: 300.1348. Found: C, 64.05; H, 5.68; N, 14.03. $C_{16}H_{17}N_3O_3$ requires: C, 64.20; H, 5.72; N, 14.04.

Fractions containing 6-oxo isomer were combined and triturated with EtOAc to yield the pure product as a white solid (1.24 g, 30%).

4.41. *tert*-Butyl 4-[*N*-((6*RS*)-2-(2,2-dimethylpropionylamino)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)amino]benzoate (38)

4.41.1. Method 1. 2-(2,2-Dimethylpropionylamino)-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4,6-dione (0.65 g, 2.19 mmol), *tert*-butyl *p*-aminobenzoate (0.59 g, 3.32 mmol), *p*-toluenesulfonic acid monohydrate (0.026 g, 0.13 mmol) and anhydrous 1,2-dimethoxyethane (15 ml) were heated, in a round-bottomed flask fitted with a pressure-equalising dropping funnel, containing activated molecular sieves, and a condenser, to 120 °C under argon for 24 h. The suspension was cooled to room temperature and sodium cyanoborohydride (0.22 mg, 3.42 mmol) in anhydrous MeOH (6.5 ml) was added dropwise followed immediately by AcOH (0.65 ml). The resulting suspension was stirred at room temperature under argon for 18 h. The solvents were removed in vacuo and the residue partitioned between EtOAc (50 ml) and saturated aqueous $NaHCO_3$ (50 ml). The aqueous layer was extracted with EtOAc (3× 20 ml). The combined organic extracts were washed with brine (50 ml), dried (Na_2SO_4) and the solvent removed in vacuo. The residue was suspended in Et_2O (50 ml) and filtered to remove remaining ketone, the solid was washed with EtOAc and the mother liquor was concentrated in vacuo. The residue was purified by column chromatography eluting with a gradient of 0–15% EtOAc in CH_2Cl_2 to yield the desired product as a yellow oil (0.36 g, 35%).

4.41.2. Method 2. A suspension of 2-(2,2-dimethylpropionylamino)-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4,6-dione (0.30 g, 1.00 mmol) in anhydrous MeOH (15 ml) and anhydrous CH_2Cl_2 (18 ml) was treated with *tert*-butyl *p*-aminobenzoate (0.23 g, 1.26 mmol) followed by decaborane (0.048 g, 0.40 mmol) and the mixture stirred at room temperature under argon for 24 h. The solvent was removed in vacuo and the residue purified by column chromatography (40 g of silica gel) eluting with $CHCl_3$ to yield the desired product as a white solid (0.38 g, 79%); mp 251–253 °C; ^{1}H NMR (DMSO-d_6) δ 1.25 (s, 9H, CMe_3), 1.52 (s, 9H, CO_2CMe_3), 1.87 (m, 1H, 7-H), 2.54 (m, 1H, 7-H), 3.05 (m, 2H, 8-H), 5.17 (q, 1H, 6-H), 6.77 (d, J = 8.67 Hz, 2H, 3′-H, 5′-H), 6.83 (d, J = 8.42 Hz, 1H, 6-NH), 7.39 (s, 1H, 9-H), 7.67 (d, J = 8.60 Hz, 2H, 2′-H, 6′-H), 7.87 (s, 1H, 5-H), 11.00 (br s, 1H, CONH), 12.13 (br s, 1H, CONH); MS (FAB, *m*/*z*): 476 [M^+, 10%], 421 [$(M-C(CH_3)_3)^+$, 45%], 284 [(M−$HNC_6H_4COO^tBu$), 100%]; HRMS: measured 476.2419; calculated for $C_{27}H_{32}N_4O_4$ [M^+]: 476.2424. Found: C, 67.96; H, 6.91; N, 11.52. $C_{27}H_{32}N_4O_4$ requires: C, 68.05; H, 6.77; N, 11.76.

4.41.3. *tert*-Butyl 4-[*N*-((6*RS*)-2-(2,2-dimethylpropionylamino)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]benzoate (39). *tert*-Butyl 4-[*N*-((6*RS*)-2-(2,2-dimethylpropionylamino)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)amino]benzoate (0.30 g, 0.63 mmol) was dissolved in THF (27 ml). AcOH (14 ml) was added followed by 37% aqueous formaldehyde (0.56 ml, excess) and the resulting solution was stirred at room temperature for 5 h. After this time, sodium cyanoborohydride (0.090 g, 1.42 mmol) was slowly added and the solution stirred at room temperature for 17 h. The solvent was removed in vacuo and the residue partitioned between EtOAc (40 ml) and H_2O (40 ml) and the aqueous layer was extracted with EtOAc (30 ml). The combined organic extracts were washed with saturated aqueous $NaHCO_3$ (40 ml), H_2O (40 ml), dried (Na_2SO_4) and the solvent removed in vacuo. The residue was triturated with Et_2O (50 ml) to yield the product as an off-white solid (0.25 g, 82%); mp 252 °C; ^{1}H NMR (DMSO-d_6) δ 1.26 (s, 9H, CMe_3), 1.53 (s, 9H, CO_2CMe_3), 2.06, 2.45 (2× m, 2H, 7-H), 2.69 (s, 3H, N^{10}–CH_3), 3.00–3.12 (m, 2H, 8-H), 5.78 (t, J = 8.20 Hz, 1H, 6-H), 6.99 (d, J = 9.02 Hz, 2H, 3′-H, 5′-H), 7.42 (s, 1H, 9-H), 7.66 (s, 1H, 5-H), 7.76 (d, J = 8.88 Hz, 2H, 2′-H, 6′-H), 11.10 (br s, 1H, CONH), 12.10 (br s, 1H, CONH); MS (FAB, *m*/*z*): 490 [$(M+H)^+$, 40%], 435 [$(M+H-C(CH_3)_3)^+$, 90%]; HRMS: measured 490.2599; calculated for $C_{28}H_{34}N_4O_4$ (M^+): 490.2580. Found: C, 68.49; H, 6.99; N, 11.37. $C_{28}H_{34}N_4O_4$ requires C, 68.55; H, 6.99; N, 11.42.

4.42. 4-[*N*-((6*RS*)-2-(2,2-Dimethylpropionylamino)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]benzoic acid

A suspension of *tert*-butyl 4-[*N*-((6*RS*)-2-(2,2-dimethylpropionylamino)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]benzoate (0.24 g, 0.49 mmol) in TFA (10 ml) was stirred at room temperature with protection from the light for 1.5 h. The solvent was removed in vacuo and the residue was triturated with Et_2O to yield the product as a TFA salt, an off-white solid (0.31 g, TFA salt); ^{1}H NMR (DMSO-d_6) δ 1.25 (s, 9H, CMe_3), 2.04, 2.43 (2× m, 2H, 7-H), 2.68 (s, 3H, N–CH_3), 3.02 (m, 2H, 8-H), 5.80 (t, J = 8.03 Hz, 1H, 6-H), 7.00 (d, J = 8.97 Hz, 2H, 3′-H, 5′-H), 7.43 (s, 1H, 9-H), 7.66 (s, 1H, 5-H), 7.80 (d, J = 8.93 Hz, 2H, 2′-H, 6′-H), 11.05 (br s, 1H, CONH).

4.43. 4-[*N*-((6*RS*)-2-Amino-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]benzoic acid

4-[*N*-((6*RS*)-2-(2,2-Dimethylpropionylamino)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]benzoic acid (0.32 g, TFA salt) was dissolved in a saturated solution of NH_3 in MeOH (30 ml) at 0 °C. The solution was allowed to warm to room temperature and stirred for 22 h. The solvent was removed in vacuo and the residue triturated with Et_2O (15 ml). The precipitate was dissolved in 0.5 M NaOH (6 ml) and 0.5 M HCl was added until pH 4 was reached. The suspension was centrifuged and the precipitate was washed well with

H_2O then dried in vacuo over P_2O_5 for 24 h to yield the desired product as a pale brown solid (0.14 g, 82%); mp 216–218 °C; ^{1}H NMR (DMSO-d_6) δ 1.99, 2.43 (2× m, 2H, 7-H), 2.66 (s, 3H, N–CH_3), 2.94, 3.04 (2× m, 2H, 8-H), 5.71 (t, 1H, 6-H), 6.98 (d, J = 9.01 Hz, 2H, 3′-H, 5′-H), 7.34 (s, 1H, 9-H), 7.54 (s, 1H, 5-H), 7.78 (d, J = 8.94 Hz, 2H, 2′-H, 6′-H), 12.05 (br s, 1H, N^3–H); MS (FAB, m/z): 351 [$(M+H)^+$, 70%]; HRMS: measured 351.1462; calculated for $C_{19}H_{19}N_4O_3$ $(M+H)^+$: 351.1457.

4.44. Tri-*tert*-butyl *N*-{4-[*N*-((6*RS*)-2-amino-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*] quinazolin-6-yl)-*N*-methylamino]benzoyl}-L-γ-glutamyl-D-glutamate

4-[*N*-((6*RS*)-2-Amino-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]benzoic acid (0.11 g, 0.31 mmol) and tri-*tert*-butyl L-γ-glutamyl-D-glutamate (0.21 g, 0.47 mmol) were dissolved in anhydrous DMF (8 ml) under argon and with protection from the light. DEPC (0.11 ml, 0.68 mmol) was added followed by triethylamine (0.10 ml, 0.68 mmol) and the solution was stirred at room temperature under argon with protection from the light for 2 h then diluted with EtOAc (50 ml) and H_2O (50 ml). The aqueous layer was extracted with EtOAc (2× 50 ml). The combined organic extracts were washed with 10% aqueous citric acid (2× 25 ml), saturated aqueous $NaHCO_3$ (50 ml) and dilute brine (50 ml), dried (Na_2SO_4) and the solvent removed in vacuo. The residue was purified by column chromatography eluting with a gradient of 0–5% MeOH in CH_2Cl_2 to yield the product as a white solid after trituration with hexanes (0.10 g, 41%); mp 170 °C; ^{1}H NMR (DMSO-d_6) δ 1.39 (2× s, 27H, 3× CO_2CMe_3), 1.55–2.10 (m, 5H, 2× Glu β-CH_2, 7-CH), 2.10–2.50 (m, 5H, 2× Glu γ-CH_2, 7-H), 2.65 (s, 3H, N^{10}–CH_3), 2.80–3.15 (m, 2H, 8-CH_2), 4.15, 4.35 (2× m, 2H, 2× Glu α-CH), 5.69 (t, J = 12.14 Hz, 1H, 6-H), 6.28 (br s, 2H, 2-NH_2), 6.97 (d, J = 8.83 Hz, 2H, 3′-H, 5′-H), 7.10 (s, 1H, 9-H), 7.53 (s, 1H, 5-H), 7.79 (d, J = 8.70 Hz, 2H, 2′-H, 6′-H), 8.12 (d, J = 8.19 Hz, 1H), 8.27 (d, J = 7.42 Hz, 1H), (2× CONH), 10.95 (br s, 1H, N^3–H); MS (FAB, m/z): 777 [$(M+H)^+$, 77%], 799 [$(M+Na)^+$, 85%]; HRMS: measured 777.4194; calculated for $C_{41}H_{57}N_6O_9$ $(M+H)^+$: 777.4187. Found: C, 62.48; H, 7.18; N, 10.68. $C_{41}H_{56}N_6O_9{\cdot}1/2H_2O$ requires C, 62.66; H, 7.31; N, 10.69.

4.45. *N*-{4-[*N*-((6*RS*)-2-Amino-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]benzoyl}-L-γ-glutamyl-D-glutamic acid (3a)

A solution of tri-*tert*-butyl *N*-{4-[*N*-((6*RS*)-2-amino-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-methylamino]benzoyl}-L-γ-glutamyl-D-glutamate (0.085 g, 0.11 mmol) in TFA (5 ml) was stirred at room temperature with protection from the light for 1.5 h. The solvent was removed in vacuo and the residue dissolved in 0.5 M NaOH (5 ml). The pH was adjusted to pH 5 with 0.5 M HCl and the precipitate was collected by centrifugation, washed well with H_2O and dried in vacuo over P_2O_5 for 24 h to yield the desired product as a brown solid (0.045 g, 68%); mp 193 °C; ^{1}H NMR (DMSO-d_6) δ 1.70–2.22 (m, 5H, 2× Glu β-CH_2, 7-CH), 2.27–2.53 (m, 5H, 2× Glu γ-CH_2, 7-H), 2.65 (s, 3H, N^{10}–CH_3), 2.70–3.15 (m, 2H, 8-CH_2), 4.11–4.45 (2× m, 2H, 2× Glu α-CH), 5.70 (t, 1H, 6-H), 6.30 (br s, 2H, 2-NH_2), 6.97 (d, J = 8.99 Hz, 2H, 3′-H, 5′-H), 7.10 (s, 1H, 9-H), 7.53 (s, 1H, 5-H), 7.80 (d, J = 8.84 Hz, 2H, 2′-H, 6′-H), 8.11 (d, J = 7.50 Hz, 1H), 8.28 (d, 1 H) (2× CONH); MS (ESI, m/z): 609 [$(M+H)^+$, 70%], 631 [$(M+Na)^+$, 100%]; HPLC: Cyclobond I column, eluting with 88% phosphate buffer/12% CH_3CN. Retention times: 637 and 658 s, purity (6R + 6S) = 99.4.

4.46. *tert*-Butyl 4-[*N*-((6*RS*)-2-(2,2-dimethylpropionylamino)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoate (40)

A suspension of (propargyl)$Co_2(CO)_6^+BF_4^-$ (0.33 g, 0.79 mmol) in anhydrous CH_2Cl_2 (30 ml, distilled over CaH_2) was treated with *tert*-butyl 4-[*N*-((6*RS*)-2-(2,2-dimethylpropionylamino)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)amino]benzoate (0.30 g, 0.63 mmol) and the red solution stirred at room temperature under argon for 30 min. Diisopropylethylamine (0.23 ml, 1.32 mmol) was added and the mixture was stirred at room temperature under argon for 1.5 h then partitioned between EtOAc (70 ml) and brine (60 ml). The organic extract was dried (Na_2SO_4) and the solvent removed in vacuo. The residue was purified by column chromatography (20 g of silica gel) eluting with a gradient of 0–10% EtOAc in CH_2Cl_2 to yield the cobalt complex as a red oil (0.24 g, 48%); ^{1}H NMR ($CDCl_3$) δ 1.33 (s, 9H, CMe_3), 1.59 (s, 9H, CO_2CMe_3), 2.26 (m, 1H, 7-H), 2.61 (m, 1H, 7-H), 3.02, 3.15 (2× m, 2H, 8-H), 4.54 (AB system, J = 16.82 Hz, 2H, propargyl CH_2), 5.60 (t, J = 8.11 Hz, 1H, 6-H), 5.96 (s, 1H, propargyl CH), 6.92 (d, J = 8.87 Hz, 2H, 3′-H, 5′-H), 7.33 (s, 1H, 9-H), 7.90 (d, J = 8.84 Hz, 2H, 2′-H, 6′-H), 7.98 (s, 1H, 5-H), 8.22 (br s, 1H, N^3–H).

A solution of the cobalt complex (0.24 g, 0.30 mmol) in EtOH (30 ml) was treated with $Fe(NO_3)_3{\cdot}9H_2O$ (1.50 g, excess) and the solution stirred at room temperature for 1 h then partitioned between EtOAc (100 ml) and H_2O (70 ml). The organic extract was washed with brine (50 ml), dried (Na_2SO_4) and the solvent removed in vacuo. The residue was purified by column chromatography (50 g of silica gel) eluting with 20% EtOAc in CH_2Cl_2 to yield the desired product as an off-white solid (0.10 g, 67%); mp 226–228 °C; ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 9H, CMe_3), 1.58 (s, 9H, CO_2CMe_3), 2.18 (t, J = 2.34 Hz, 1H, propargyl CH), 2.33 (m, 1H, 7-H), 2.56 (m, 1H, 7-H), 3.00 (m, 1H, 8-H), 3.16 (m, 1H, 8-H), 3.91 (AB system, J = 18.45 Hz, 2H, propargyl CH_2), 5.62 (t, J = 8.02 Hz, 1H, 6-H), 6.95 (d, J = 9.10 Hz, 2H, 3′-H, 5′-H), 7.33 (s, 1H, 9-H), 7.92 (d, J = 9.01 Hz, 2H, 2′-H, 6′-H), 8.06 (s, 1H, 5-H); MS (ESI, m/z): 514 [$(M+H)^+$, 40%], 537 [$(M+Na)^+$, 100%]. Found: C, 69.94; H, 6.70; N, 10.77. $C_{30}H_{34}N_4O_4$ requires: C, 70.02; H, 6.66; N, 10.89.

4.47. 4-[*N*-((6*RS*)-2-Amino-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoic acid

A suspension of *tert*-butyl 4-[*N*-((6*RS*)-2-(2,2-dimethylpropionylamino)-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclo-

penta[*g*]quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoate (0.090 g, 0.18 mmol) in TFA (5 ml) was stirred at room temperature with protection from the light for 2.5 h. The solvent was removed in vacuo and the residue was dissolved in a saturated solution of NH_3 in MeOH (15 ml) at 0 °C. The solution was allowed to warm to room temperature and stirred for 24 h then the solvent was removed in vacuo and the residue was dissolved in 0.5 M NaOH (5 ml). HCl (0.5 M) was added until pH 4.5 was reached and the suspension was centrifuged, washed well with H_2O and the product was dried in vacuo over P_2O_5 for 24 h to yield a pale brown solid (0.053 g, 93%); mp 202 °C; ^{1}H NMR (DMSO-d_6) δ 2.14, 2.43 (2× m, 2H, 7-H), 2.90, 3.06 (2× m, 2H, 8-H), 3.12 (s, 1H, propargyl CH), 3.92 (AB system, J = 18.84 Hz, 2H, propargyl CH_2), 5.67 (t, J = 8.09 Hz, 1H, 6-H), 6.70 (br s, 2H, 2-NH_2), 7.01 (d, J = 9.11 Hz, 2H, 3′-H, 5′-H), 7.14 (s, 1H, 9-H), 7.62 (s, 1H, 5-H), 7.80 (d, J = 8.93 Hz, 2H, 2′-H, 6′-H); MS (ESI, *m*/*z*): 375 [(M+H)$^+$, 100%].

4.48. Tri-*tert*-butyl *N*-{4-[*N*-((6*RS*)-2-amino-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

A solution of 4-[*N*-((6*RS*)-2-amino-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*] quinazolin-6-yl)-*N*-(prop-2-ynyl)amino]benzoic acid (0.042 g, 0.11 mmol) in anhydrous DMF (5 ml) was treated with tri-*tert*-butyl L-γ-glutamyl-D-glutamate (0.076 g, 0.17 mmol), DEPC (0.041 ml, 0.27 mmol) and triethylamine (0.039 ml, 0.27 mmol). The solution was stirred at room temperature under argon with protection from the light for 3 h then partitioned between EtOAc (25 ml) and H_2O (25 ml). The aqueous layer was extracted with EtOAc (2× 15 ml). The combined organic extracts were washed with 10% aqueous citric acid (2× 20 ml), saturated aqueous $NaHCO_3$ (20 ml) and dilute brine (20 ml), dried (Na_2SO_4) and the solvent removed in vacuo. The residue was purified by column chromatography (20 g of silica gel) eluting with a gradient of 3–10% MeOH in CH_2Cl_2 to yield the desired product as a white solid (0.074 g, 82%); mp 150 °C; ^{1}H NMR (DMSO-d_6) δ 1.37 (s, 9H, $COOCMe_3$), 1.38 (s, 9H, $COOCMe_3$), 1.40 (s, 9H, $COOCMe_3$), 1.60–2.05 (m, 5H, 2× Glu β-CH_2, 7-CH), 2.22–2.35 (m, 4H, 2× Glu γ-CH_2), 2.42 (m, 1H, 7-H), 2.93 (m, 1H, 8-H), 3.03 (m, 1H, 8-H), 3.09 (s, 1H, propargyl CH), 3.92 (AB system, J = 19.37 Hz, 2H, propargyl CH_2), 4.12, 4.27 (2× m, 2H, 2× Glu α-CH), 5.65 (t, J = 7.75 Hz, 1H, 6-H), 6.30 (br s, 2H, NH_2), 7.00 (d, J = 8.98 Hz, 2H, 3′-H, 5′-H), 7.11 (s, 1H, 9-H), 7.62 (s, 1H, 5-H), 7.79 (d, J = 8.91 Hz, 2H, 2′-H, 6′-H), 8.14 (d, J = 7.42 Hz), 8.32 (d, J = 7.21Hz) (2H, 2× CONH); MS (ESI, *m*/*z*): 801 [(M+H)$^+$, 100%], 823 [(M+Na)$^+$, 10%]. Found: C, 63.15; H, 7.10; N, 10.09. $C_{43}H_{56}N_6O_9 \cdot 1H_2O$ requires: C, 63.07; H, 7.14; N, 10.26.

4.49. *N*-{4-[*N*-((6*RS*)-2-Amino-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 (3b)

Tri-*tert*-butyl *N*-{4-[*N*-((6*RS*)-2-amino-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 (0.063 g, 0.081 mmol) was dissolved in TFA (5 ml) and stirred at room temperature with protection from the light for 2 h. The solvent was removed in vacuo and the residue dissolved in 0.5 M NaOH solution (5 ml). The pH of the solution was adjusted to pH 4.5 with 0.5 M HCl and cooled to 0 °C. The precipitate was collected by filtration, washed with H_2O and dried in vacuo over P_2O_5 to yield the desired product as a pale yellow solid (0.023 g, 47%); mp 192 °C; ^{1}H NMR (DMSO-d_6) δ 1.70–2.00 (m, 5H, 2× Glu β-CH_2, 7-CH), 2.05–2.40 (m, 5H, 2× Glu γ-CH_2, 7-H), 2.90 (m, 1H, 8-H), 3.08 (s, 1H, propargyl CH), 3.10 (m, 1H, 8-H), 3.92 (AB system, J = 19.49 Hz, 2H, propargyl CH_2), 4.18, 4.33 (2× m, 2H, 2× Glu α-CH), 5.65 (t, J = 7.72 Hz, 1H, 6-H), 6.46 (br s, 2H, 2-NH_2), 7.00 (d, J = 9.01 Hz, 2H, 3′-H, 5′-H), 7.12 (s, 1H, 9-H), 7.63 (s, 1H, 5-H), 7.80 (d, J = 8.89 Hz, 2H, 2′-H, 6′-H), 8.14 (d, J = 7.81 Hz, 1H), 8.32 (d, J = 7.50 Hz, 1H) (2× CONH); MS (ESI, *m*/*z*): 633 [(M+H)$^+$, 100%], HRMS: measured 633.2315; calculated for $C_{31}H_{33}N_6O_9$ (M+H)$^+$: 633.2309. HPLC: Cyclobond I column, eluting with 83% phosphate buffer/17% CH_3CN. Retention times: 815 and 927 s, purity (6*R* + 6*S*) = 88.3.

4.50. 2-Acetoxymethyl-4-(2,2-dimethylpropionyloxymethyloxy)-7,8-dihydro-6*H*-cyclopenta[*g*]quinazolin-6-one (41) and 2-acetoxymethyl-3-(2,2-dimethylpropionyloxymethyl)-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4,6-dione

To a stirred solution of 2-acetoxymethyl-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4,6-dione (0.050 g, 0.18 mmol) in anhydrous DMA (5 ml) under argon was added K_2CO_3 (0.027 g, 0.19 mmol) and the suspension stirred at room temperature for 30 min. Chloromethyl pivalate (0.030 ml, 0.21 mmol) was added and the suspension stirred at room temperature under argon for 24 h then partitioned between EtOAC (30 ml) and half-saturated brine (30 ml). The aqueous layer was extracted with EtOAc (20 ml). The combined organic extracts were washed with H_2O (30 ml), saturated brine (30 ml), dried (Na_2SO_4) and the solvent removed in vacuo. The residue was purified by column chromatography (20 g silica gel) eluting with 5% MeOH and 40% Et_2O in hexanes to yield, in order of elution:

(a) 2-Acetoxymethyl-4-(2,2-dimethylpropionyloxymethyloxy)-7,8-dihydro-6*H*-cyclopenta[*g*]quinazolin-6-one (**41**), a clear oil (0.017 g, 24%): ^{1}H NMR ($CDCl_3$) δ 1.22 (s, 9H, POM-CMe_3), 2.27 (s, 3H, $COCH_3$), 2.83 (m, 2H, 7-H), 3.37 (m, 2H, 8-H), 5.32 (s, 2H, 2-CH_2), 6.29 (s, 2H, POM-CH_2), 7.98 (s, 1H, 9-H), 8.61 (s, 1H, 5-H).

(b) 2-Acetoxymethyl-3-(2,2-dimethylpropionyloxymethyl)-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4,6-dione, a white solid (0.021 g, 30%): mp 185 °C; ^{1}H NMR ($CDCl_3$) δ 1.21 (s, 9H, POM-CMe_3), 2.23 (s, 3H, $COCH_3$), 2.80 (m, 2H, 7-H), 3.30 (m, 2H, 8-H), 5.23 (s, 2H, 2-CH_2), 6.12 (s, 2H, POM-CH_2), 7.74 (s, 1H, 9-H), 8.71 (s, 1H, 5-H); MS (ESI, *m*/*z*): 387 [(M+H)$^+$, 20%], 409 [(M+Na)$^+$, 100%]. Found: C, 62.01; H, 5.72; N, 7.21. $C_{20}H_{22}N_2O_6$ requires: C, 62.17; H, 5.74; N, 7.25.

4.51. 2-(2,2-Dimethylpropionyloxymethyl)-3-(2,2-dimethylpropionyloxymethyl)-3,4,7,8-tetrahydro-6*H*- cyclopenta[*g*]quinazolin-4,6-dione, and 2-(2,2-dimethylpropionyloxymethyl)-4-(2,2-dimethylpropionyloxymethyloxy)-7,8-dihydro-6*H*-cyclopenta[*g*]quinazolin-6-one (42)

To a solution of 2-(2,2-dimethylpropionyloxymethyl)-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4,6-dione (0.10 g, 0.32 mmol) in anhydrous DMA (5 ml) under argon was added anhydrous K_2CO_3 (0.053 g, 0.38 mmol). The suspension was stirred at room temperature for 30 min then chloromethyl pivalate (0.070 ml, 0.49 mmol) was added and the suspension stirred under argon for 24 h. The mixture was partitioned between EtOAc (40 ml) and half-saturated brine (40 ml). The aqueous layer was extracted with EtOAc (30 ml). The combined organic extracts were washed with H_2O (40 ml), brine (40 ml), dried (Na_2SO_4) and the solvent removed in vacuo. The residue was purified by column chromatography (20 g of silica gel) eluting with $CHCl_3$ to yield, in order of elution:

(a) 2-(2,2-Dimethylpropionyloxymethyl)-4-(2,2-dimethylpropionyloxymethyloxy)-7,8-dihydro-6*H*-cyclopenta[*g*]quinazolin-6-one (**42**), a white solid (0.035 g, 26%); mp 152–154 °C; ^{1}H NMR (DMSO-d_6) δ 1.14 (s, 9H, POM-CMe_3),1.28 (s, 9H, CMe_3), 2.78 (m, 2H, 7-H), 3.34 (m, 2H, 6-H), 5.29 (s, 2H, 2-CH_2), 6.27 (s, 2H, POM-CH_2), 7.99 (s, 1H, 9-H), 8.29 (s, 1H, 5-H); MS (FAB, *m*/*z*): 429 [$(M+H)^+$, 42%]; HRMS: measured 429.2043; calculated for $C_{23}H_{29}N_2O_6$ $(M+H)^+$: 429.2026. Found: C, 63.86; H, 6.40; N, 6.34. $C_{23}H_{28}N_2O_6{\cdot}1/4H_2O$ requires: C, 63.80; H, 6.63; N, 6.47.

(b) 2-(2,2-Dimethylpropionyloxymethyl)-3-(2,2-dimethylpropionyloxymethyl)-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4,6-dione; mp 168–170 °C; ^{1}H NMR (DMSO-d_6) δ 1.14 (s, 9H, POM-CMe_3),1.29 (s, 9H, CMe_3), 2.73 (m, 2H, 7-H), 3.28 (m, 2H, 6-H), 5.35 (s, 2H, 2-CH_2), 6.06 (s, 2H, POM-CH_2), 7.70 (s, 1H, 9-H), 8.32 (s, 1H, 5-H); MS (FAB, *m*/*z*): 429 [$(M+H)^+$, 100%]; HRMS: measured 429.2043; calculated for $C_{23}H_{29}N_2O_6$ $(M+H)^+$: 429.2026. Found: C, 63.76; H, 6.50; N, 6.39. $C_{23}H_{28}N_2O_6{\cdot}1/4H_2O$ requires: C, 63.80; H, 6.63; N, 6.47.

4.52. 2-(2,2-Dimethylpropionylamino)-4-(2,2-dimethylpropionyloxymethyloxy)-7,8-dihydro-6*H*-cyclopenta[*g*]quinazolin-6-one (43)

To a stirred solution of 2-(2,2-dimethylpropionylamino)-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-4,6-dione (0.060 g, 0.20 mmol) in anhydrous DMA (2.5 ml) under argon was added K_2CO_3 (0.034 g, 0.23 mmol) and the suspension stirred at room temperature for 30 min. Chloromethyl pivalate (0.05 ml, 0.35 mmol) was added and the suspension stirred at room temperature under argon for 18 h then partitioned between EtOAc (20 ml) and half-saturated brine (20 ml). The aqueous layer was extracted with EtOAc (15 ml). The combined organic extracts were washed with H_2O (20 ml), brine (20 ml), dried (Na_2SO_4) and the solvent was removed in vacuo. The residue was purified by column chromatography (15 g silica gel) eluting with 30% Et_2O in hexanes to yield the desired product as a white solid (0.024 g, 29%); mp 254–255 °C; ^{1}H NMR ($CDCl_3$) δ 1.20 (s, 9H, POM-CMe_3), 1.24 (s, 9H, CMe_3), 2.79 (m, 2H, 7-H), 3.26 (m, 2H, 8-H), 6.26 (s, 2H, POM-CH_2), 7.21 (s, 1H, 9-H), 8.62 (s, 1H, 5-H); MS (ESI, *m*/*z*): 414 [$(M+H)^+$, 5%], 436 [$(M+Na)^+$, 25%]. Found: C, 63.88; H, 6.67; N, 9.94. $C_{22}H_{27}N_3O_5$ requires C, 63.91; H, 6.58; N, 10.16.

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