

Antifolate chemistry: synthesis of 4- $\{N-[(6RS)-2\text{-methyl-4-oxo-3,4,7,8-tetrahydro-6}H\text{-cyclopenta}[g]\text{quinazolin-6-yl}]-N\text{-}(\text{prop-2-ynyl})\text{amino}\}$ benzoic acid *via* a $(\text{propargyl})\text{Co}_2(\text{CO})_6^+$ complex

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A new route to compound **3** (4- $\{N-[(6RS)-2\text{-methyl-4-oxo-3,4,7,8-tetrahydro-6}H\text{-cyclopenta}[g]\text{quinazolin-6-yl}]-N\text{-}(\text{prop-2-ynyl})\text{amino}\}$ benzoic acid), a crucial intermediate for the synthesis of potent inhibitors of thymidylate synthase (TS), is described. In this sequence the C^6-N^{10} bond was constructed first, by the reductive amination of 5-acetamido-6-bromoindan-1-one **6** with *tert*-butyl 4-aminobenzoate, then the cyclopenta[*g*]quinazolinone ring was formed and the propargyl group was introduced on the N^{10} -position using the $(\text{propargyl})\text{Co}_2(\text{CO})_6^+$ complex as the electrophilic propargyl reagent.

Introduction

Thymidylate synthase (TS) catalyses the conversion of 2'-deoxyuridine 5'-monophosphate (dUMP) to thymidine 5'-monophosphate (TMP) and this enzyme proved to be an attractive target in anticancer drug design.¹ The prototype folate-based inhibitor of TS is CB3717 **1**, a compound that was first synthesised at the Institute of Cancer Research, UK and showed activity in clinical trials.^{2,3} Over the last two decades a large amount of research in the antifolate area has been directed towards the development of inhibitors of thymidylate synthase (TS).⁴ As a result a number of molecules have reached the stage of clinical trials, and Raltitrexed **2**^{5,6} has been approved in various countries for the treatment of colorectal cancer.

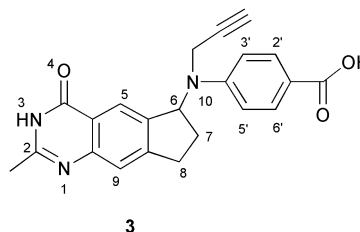
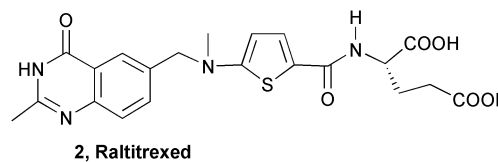
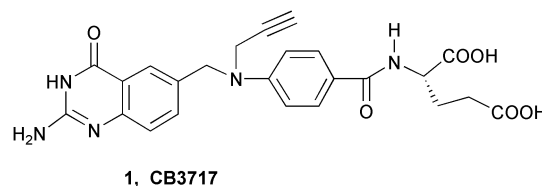
We have recently reported the synthesis of cyclopenta[*g*]quinazoline-based antifolates, a novel class of TS inhibitor.⁷ 4- $\{N-[(6RS)-2\text{-Methyl-4-oxo-3,4,7,8-tetrahydro-6}H\text{-cyclopenta}[g]\text{quinazolin-6-yl}]-N\text{-}(\text{prop-2-ynyl})\text{amino}\}$ benzoic acid **3**, the key intermediate for the synthesis of this class of compounds, was prepared from *N*-(4- $\{N-[(6RS)-2\text{-methyl-4-oxo-3,4,7,8-tetrahydro-6}H\text{-cyclopenta}[g]\text{quinazolin-6-yl}]-N\text{-}(\text{prop-2-ynyl})\text{amino}\}$ benzoyl)-*L*-glutamic acid by the enzymatic cleavage of its glutamyl residue.^{7,8} We now report an improved route to this key intermediate **3** in which the propargyl group was introduced in the penultimate step using the $(\text{propargyl})\text{Co}_2(\text{CO})_6^+$ complex as the electrophilic propargyl reagent.

Results and discussion

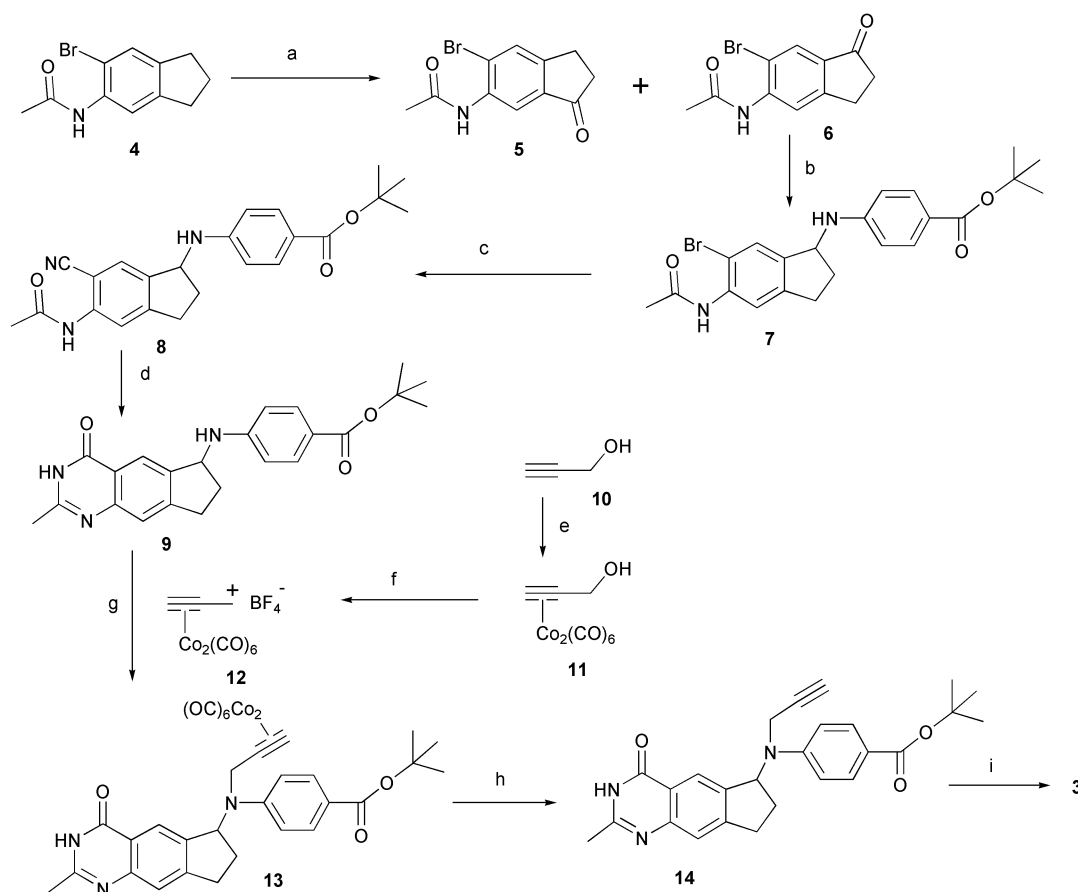
The route to compound **3** is outlined in Scheme 1. This methodology differs significantly from the published route to **3**.^{7,8}

The oxo-functionality, required for the formation of the C^6-N^{10} bond *via* a reductive amination reaction, was introduced in the initial steps of the synthesis whereas the cyclopenta[*g*]quinazolinone ring was constructed after the formation of the C^6-N^{10} bond making this route simpler and more flexible. Most significantly, the propargyl group was introduced in the penultimate step under mild conditions utilising the $(\text{propargyl})\text{Co}_2(\text{CO})_6^+$ complex as the electrophilic propargyl synthon.

In this synthetic sequence (Scheme 1) 5-acetamido-6-bromoindane **4** was used as the starting material. Oxidation of **4** with CrO_3 in acetic acid⁹ gave a mixture of two regioisomers **5** and **6**; the desired ketone **6** was isolated by column chromatography in 43% yield. Reductive amination of the ketone **6** with *tert*-butyl



4-aminobenzoate under classical conditions (*i.e.* TsOH , DME, reflux/ NaBH_3CN , MeOH, AcOH, room temperature) resulted in the formation of **7** in 35% yield. This yield was increased to 81% when the reductive amination was performed using decaborane in methanol.¹⁰ Cyanation of the aryl bromide **7** was effected utilising the Rosenmund–von Braun reaction¹¹ by heating **7** with copper(I) cyanide in 1-methyl-2-pyrrolidinone (NMP) at 140 °C. In the next step, the indane derivative **8** was cyclised to the cyclopenta[*g*]quinazolin-4-one **9** in 83% yield by treatment with H_2O_2 , NaOH in EtOH– H_2O . It was envisaged that the introduction of the N^{10} -propargyl substituent could be achieved by using a cobalt-complexed propargyl cation as the electrophilic propargyl synthon. It is known that cobalt-complexed propargyl cations react with a variety of nucleophiles¹² including primary or secondary amines.¹³ Indeed the tetrafluoroborate salt **12**, prepared by treatment of the dicobalt hexacarbonyl complex **11** with propionic acid and HBF_4 ¹⁴ at -20 °C, reacted smoothly with **9** at room temperature to afford **13** in high yield (76%) without the need to protect the N^3 -H. Decomplexation of **13** with $\text{Fe}(\text{NO}_3)_3$ in EtOH afforded the N^{10} -propargylated derivative **14**. In the final step the *tert*-butyl group was removed with TFA to afford the desired product **3** as



Scheme 1 Reagents and conditions: (a), CrO₃, AcOH, 55 °C; (b), *tert*-butyl 4-aminobenzoate, TsOH, DME, molecular sieves, reflux/NaBH₃CN, AcOH, MeOH, room temp. or *tert*-butyl 4-aminobenzoate, decaborane, MeOH, room temp.; (c) CuCN, NMP, 140 °C; (d), H₂O₂, NaOH, EtOH–H₂O, 55 °C; (e), Co₂(CO)₈, CH₂Cl₂, room temp.; (f), propionic acid, HBF₄, –20 °C; (g), DIEA, CH₂Cl₂, room temp.; (h), Fe(NO₃)₃, EtOH, room temp.; (i), CH₂Cl₂/TFA.

a racemic mixture. Chiral HPLC (column: Astec cyclobond BETA; mobile phase: 83% 25 mM Na₂HPO₄/25 mM NaH₂PO₄–17% CH₃CN; flow 1 ml min⁻¹; λ = 230 nm) indicated two peaks for compound **3** in a ratio of 1 : 1.

In summary, a new, simpler, and more flexible route to compound **3**, a crucial intermediate for the synthesis of novel inhibitors of TS, has been developed. In this route the *N*¹⁰-propargyl substituent was introduced under mild conditions utilising the (propargyl)Co₂(CO)₆⁺ complex as the electrophilic propargyl synthon. It should be noted that an *N*¹⁰ propargyl substituent provides optimum TS binding in a variety of classical quinazoline-based inhibitors of TS. Therefore, this methodology may be applied for the introduction of an *N*¹⁰-propargyl substituent in other antifolates.

Experimental

Thin layer chromatography (TLC) was performed on pre-coated sheets of silica 60F₂₅₄ (Merck Art 5735) visualised under UV light. Merck silica 60 (Art 15111) was used in low-pressure column chromatography. Petrol refers to light petroleum (bp 60–80 °C). Electrospray ionisation (ESI) mass spectra were recorded using a TSQ 700 triple quadrupole mass spectrometer (Finnigan MAT) fitted with an electrospray ionisation source (Analytica). Proton NMR spectra were recorded using a Bruker AC250 spectrometer at 250 MHz. Field strengths are expressed in units of δ (ppm) relative to tetramethylsilane, and peak multiplicities are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; dm, doublet of multiplets; t, triplet; q, quartet; br s, broad singlet; m, multiplet. Melting points were determined on a Kofler block and are uncorrected. Elemental analyses were determined by C.H.N. Analysis Ltd., Leicester, UK.

Synthesis

5-Acetamido-6-bromoindan-1-one 6. This compound was prepared as described in ref. 9: Purification of the crude product by column chromatography using a gradient of ethyl acetate in dichloromethane (10 to 20%) afforded in order of elution:

a. 5-Acetamido-6-bromoindan-1-one as a white solid which was further purified by trituration with ethyl acetate–hexane (1 : 4, v/v): 2.60 g (43%), mp 162–164 °C (Found: C, 49.18; H, 3.65; N, 5.13; Br, 29.89; C₁₁H₁₀BrNO₂ requires C, 49.28; H, 3.76; N, 5.22; Br, 29.80%); ν_{max} (film)/cm⁻¹ 3234 (w), 1702 (s), 1662 (s), 1526 (s); δ_H (CDCl₃) 2.30 (1 H, s, Me), 2.71 (2 H, m, 2-H), 3.11 (2 H, t, *J* 5.6, 3-H), 7.93 (1 H, br s, CONH), 7.94, 8.60 (each 1 H, s, 4-H, 7-H); *m/z* (ESI) 268, 270 {(M + H)⁺, 100%, 95% respectively, bromine isotopic pattern}, 226 (25).

b. 5-Acetamido-6-bromoindan-3-one as a white solid which was further purified by trituration with ethyl acetate–hexane (1 : 4, v/v): 0.45 g, (8%), mp 219–220 °C (Found: C, 49.24; H, 3.67; N, 5.12; Br, 29.73; C₁₁H₁₀BrNO₂ requires C, 49.28; H, 3.76; N, 5.22; Br, 29.80%); ν_{max} (film)/cm⁻¹ 3276 (w), 1700 (s), 1663 (s), 1605 (s); δ_H (CDCl₃) 2.26 (3 H, s, Me), 2.71 (2 H, m, 2-H), 3.10 (2 H, t, *J* 5.6, 1-H), 7.60 (1 H, br s, CONH), 7.72, 8.62 (each 1 H, s, 4-H, 7-H); *m/z* (ESI) 268, 270 {(M + H)⁺, 100%, 95% respectively, bromine isotopic pattern}, 188 (90).

***Tert*-butyl 4-[*N*-(5-acetamido-6-bromoindan-1-yl)amino]benzoate 7.** *Method A.* To a flask containing 5-acetamido-6-bromoindan-1-one **6** (0.900 g, 3.36 mmol), 4-toluenesulfonic acid monohydrate (0.045 g), and *tert*-butyl 4-aminobenzoate (0.972 g, 5.04 mmol) was added 1,2-dimethoxyethane (dried by distillation over CaH₂; 48 cm³). An azeotropic distillation apparatus (Aldrich)¹⁵ containing molecular sieves (3 Å) was fitted to the reaction flask that was placed in an oil bath

preheated to 60 °C. The temperature was raised to 110 °C and stirring was continued at this temperature for 7 h under argon. The reaction mixture was then allowed to cool to room temperature, then a solution of sodium cyanoborohydride (0.336 g) in anhydrous methanol (11 cm³) was added followed immediately by acetic acid (0.6 cm³). The black reaction mixture was stirred at room temperature for 24 h under argon; then partitioned between ethyl acetate (170 cm³) and saturated aqueous sodium bicarbonate (100 cm³). The aqueous layer was extracted with more ethyl acetate (2 × 100 cm³); the organic extracts were combined, dried (MgSO₄) and concentrated *in vacuo* to leave a dark oily residue. Purification by column chromatography on elution with ethyl acetate–hexane (1 : 1, v/v) afforded the title compound **7** as a white solid: 0.520 g (35%).

Method B. To a nearly clear solution of 5-acetamido-6-bromoindan-1-one **6** (0.964 g, 3.60 mmol) in anhydrous methanol (70 cm³) was added *tert*-butyl 4-aminobenzoate (0.733 g, 3.8 mmol) followed by decaborane (0.130 g, 1.08 mmol); a clear solution was obtained after stirring for approximately 0.5 h. The reaction mixture was stirred at room temperature overnight before being concentrated *in vacuo*. Purification by column chromatography and elution with a gradient of ethyl acetate in hexane (35 to 40%), afforded a gummy residue which was further purified by trituration with dichloromethane–hexane (1 : 4, v/v). The title compound **7** was obtained as a white solid: 1.32 g (81%) mp 153 °C (Found: C, 59.36; H, 5.62; N, 6.31; Br, 17.96; C₂₂H₂₅BrN₂O₃ requires C, 59.33; H, 5.66; N, 6.29; Br, 17.94%; ν_{\max} (film)/cm⁻¹ 1700 (s), 1684 (s), 1654 (s), 1604 (s); δ_{H} (CDCl₃) 1.57 (9 H, s, C(CH₃)₃), 2.25 (3 H, s, Me), 1.91, 2.63 (each 1 H, m, 2-H), 2.95 (2 H, m, 3-H), 5.03 (1 H, t, *J* 6.60, 1-H), 6.64 (2 H, d, *J* 8.75, 3', 5'-H), 7.60 (1 H, br s, CONH), 7.49, 8.25 (each 1 H, s, 4-H, 7-H), 7.85 (2 H, d, *J* 8.75, 2', 6'-H); *m/z* (ESI) 467, 469 {(M + Na)⁺, 100%, 95% respectively, bromine isotopic pattern}, 252, 254 (35).

***Tert*-butyl 4-[*N*-(5-acetamido-6-cyanoindan-1-yl)amino]benzoate **8**.** To a solution of **7** (1.170 g, 2.62 mmol) in 1-methyl-2-pyrrolidinone (NMP) (13 cm³) was added copper(I) cyanide (0.400 g, 4.70 mmol). The reaction mixture was placed in an oil-bath preheated to 140 °C and stirred at this temperature for 1 h and 40 min. The reaction mixture was allowed to cool to room temperature, then poured into a mixture of aqueous ammonia (*d* = 0.88 g cm⁻³, 12 cm³) and ice (~34 cm³) and the resulting brown mixture was stirred at room temperature for ~10 min. The brown solid was collected by filtration washed with plenty of water, then dissolved in dichloromethane (100 cm³). The mixture was stirred at room temperature for 10 min, dried (MgSO₄), and concentrated *in vacuo*. Purification by column chromatography and elution with 35% ethyl acetate in hexane, afforded a crispy solid that was reprecipitated from ethyl acetate–hexane. The title compound **8** was obtained as a white solid: 0.714 g, (70%) mp 173–174 °C (Found: C, 70.35; H, 6.44; N, 10.62; C₂₃H₂₅N₃O₃ requires C, 70.57; H, 6.44; N, 10.73%; ν_{\max} (film)/cm⁻¹ 2224 (w), 1700 (s), 1684 (s), 1653 (s), 1604 (s); δ_{H} (CDCl₃) 1.58 (9 H, s, C(CH₃)₃), 2.28 (3 H, s, Me), 1.96, 2.67 (each 1 H, m, 2-H), 3.04 (2 H, m, 3-H), 4.23 (1 H, d, *J* 8.31, N–H), 5.06 (1 H, q, *J* 7.50, 1-H), 6.65 (2 H, d, *J* 8.75, 3', 5'-H), 7.60 (1 H, br s, CONH), 7.54, 8.32 (each 1 H, s, 4-H, 7-H), 7.87 (2 H, d, *J* 8.75, 2', 6'-H); *m/z* (ESI) 783 {(2M + H)⁺, 100%,}, 414 {(M + Na)⁺, 55%}, 199 (15).

***Tert*-butyl 4-[*N*-[(6*RS*)-2-methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]amino]benzoate **9**.** A mixture of **8** (1.33 g, 3.40 mmol), ethanol (15 cm³), and water (3.1 cm³) was cooled in an ice-bath, then 30% aqueous H₂O₂ solution (2.8 cm³) was added followed by granulated sodium hydroxide pellets (0.230 g, 5.78 mmol). The reaction mixture was stirred at ~0 °C for 10 min, then it was placed in an oil bath preheated to 55 °C and stirred at this temperature for 40 min. The reaction mixture was allowed to cool to room temperature, then the

solvents were removed *in vacuo* and the residue was suspended in water (~40 cm³). The pH of this mixture was adjusted to ~5 with 1 N hydrochloric acid. The white precipitate was collected by filtration, washed with water, dried *in vacuo* over P₂O₅, then it was triturated with ether, collected by filtration and dried *in vacuo*. The title compound **9** was obtained as a white solid 1.11 g (83%), mp 277–281 °C (it melts with decomposition), (Found: C, 70.22; H, 6.43; N, 10.65; C₂₃H₂₅N₃O₃ requires C, 70.57; H, 6.44; N, 10.73%); δ_{H} (DMSO-*d*₆) 1.50 (9 H, s, C(CH₃)₃), 2.31 (3 H, s, Me), 1.87, 2.55 (each 1 H, m, 7-H), 2.97 (2 H, m, 8-H), 5.15 (1 H, m, 6-H), 6.77 (2 H, d, *J* 8.6, 3', 5'-H), 6.91 (1 H, d, *J* 8.70, N¹⁰-H), 7.44, 7.87 (each 1 H, s, 5-H, 9-H), 7.66 (2 H, d, *J* 8.75, 2', 6'-H); *m/z* (ESI) 783 {(2M + H)⁺, 100%}, 392 {(M + H)⁺, 30%}, 199 (90).

Dicobalt hexacarbonyl propargyl alcohol complex **11.** This is a known compound¹³ and in this study was prepared according to Nicholas' methodology:¹⁴ To a round bottom flask charged with Co₂(CO)₈ (5.12 g, 15.0 mmol) under argon in a well ventilated hood was added anhydrous dichloromethane (170 cm³) followed by a solution of propargyl alcohol (0.840 g, 15.0 mmol) in anhydrous dichloromethane (20 cm³). The deep red reaction mixture was stirred at room temperature for 7 h under argon, then it was filtered through a thin layer of neutral alumina. The filtrate was concentrated *in vacuo* to give a red residue. Purification by column chromatography, on elution with 40% diethyl ether in hexane, afforded the title compound **11** as a red solid 4.10 g (80%); δ_{H} (CDCl₃) 1.83 (1 H, t, *J* 6.0, OH, exchanges with D₂O), 4.80 (2 H, d, *J* 6.0, CH₂), 6.08 (1 H, s, C–H).

(Propargyl)Co₂(CO)₆⁺BF₄⁻ **12.** This is a known compound¹³ and in this study was prepared according to Nicholas' methodology:¹⁴ To a round bottom flask charged with the dicobalt hexacarbonyl complex **11** (1.60 g, 4.7 mmol) under argon was added (synged *via* a septum) propionic acid (2.2 cm³). The reaction mixture was cooled to –20 °C and then a solution of HBF₄ in diethyl ether (54% w/w, 2.05 cm³) was slowly synged into the reaction mixture *via* a septum. The reaction mixture was stirred at –20 °C for 40 min, then pre-cooled diethyl ether (50 cm³) was added. Trituration afforded a red precipitate that was collected by filtration, washed with plenty of dry diethyl ether and dried *in vacuo* over P₂O₅: 1.71 g (90%). This was immediately used in the next reaction without any further purification.

Dicobalt hexacarbonyl complex **13.** To a round bottom flask containing the tetrafluoroborate salt **12** (1.44 g, 3.5 mmol) was added anhydrous dichloromethane (dried by distillation over P₂O₅; 100 cm³). The nearly clear red dark solution was stirred at room temperature for a few minutes under argon, then **9** (1.04 g, 2.66 mmol) was added in one portion; a clear solution was obtained after approximately 2 min. Stirring was continued at this temperature for 5 min then diisopropylethylamine (0.92 cm³, 5.32 mmol) was added and the reaction mixture was stirred at room temperature for 45 min under argon. The reaction mixture was partitioned between ethyl acetate (300 cm³) and brine (120 cm³). The organic layer was washed with 10% aqueous citric acid (100 cm³), brine (100 cm³), dried (Na₂SO₄), and concentrated *in vacuo*. Purification by column chromatography and elution with ethyl acetate in dichloromethane (60 to 70%) gave **13** as a red solid 1.45 g (76%), mp >150 °C (with decomposition, red crystals turn black); δ_{H} (CDCl₃) 1.58 (9 H, s, C(CH₃)₃), 2.53 (3 H, s, 2-Me), 2.32, 2.60 (each 1 H, m, 7-H), 3.05, 3.20 (each 1 H, m, 8-H), 4.55 (2 H, AB system, *J* 17.2, N¹⁰-CH₂), 5.63 (1 H, t, *J* 8.6, 6-H), 5.96 (1 H, s, propargyl complex C–H), 6.93 (2 H, d, *J* 8.9, 3', 5'-H), 7.58, 8.00 (each 1 H, s, 5-H, 9-H), 7.90 (2 H, d, *J* 8.9, 2', 6'-H), 10.58 (1 H, s, N³-H); *m/z* (ESI) 716 {(M + H)⁺, 80%}, 231 (100), 199 (70).

Tert-Butyl 4-*N*-[(6*RS*)-2-methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta[*g*]quinazolin-6-yl]-*N*-(prop-2-ynyl)amino}benzoate **14.** To a solution of the complex **13** (1.40 g, 1.96 mmol) in ethanol (200 cm³) was added Fe(NO₃)₃ (26 g). The clear solution was stirred at room temperature for 10 min then a second portion of Fe(NO₃)₃ (~15 g) was added. The reaction mixture was stirred at room temperature for a further 10 min then a final portion of Fe(NO₃)₃ (~10 g) was added; the nearly clear solution turned dark red. Stirring was continued at room temperature for an extra 15 min, the reaction mixture was then partitioned between ethyl acetate (700 cm³) and dilute brine (200 cm³). The organic (not clear) layer was washed with more brine (3 × 150 cm³), dried (Na₂SO₄), and concentrated *in vacuo* to leave a brown solid. Purification by column chromatography and elution with 5% methanol in chloroform, afforded a solid. Trituration with dichloromethane–hexane gave the title compound **14** as an off white solid, 0.565 g (67%), mp 244–246 °C; (Found: C, 72.60; H, 6.37; N, 9.67; C₂₆H₂₇N₃O₃ requires C, 72.71; H, 6.34; N, 9.78%); δ_H (DMSO-*d*₆) 1.50 (9 H, s, C(CH₃)₃), 2.32 (3 H, s, 2-Me), 2.18, 2.55 (obscured by the DMSO peak) (each 1 H, m, 7-H), 2.97 (1 H, m, 8-H), 3.14 (2 H, m, C≡CH, 8-H), 3.96 (2 H, AB system, *J* 18.1, CH₂C≡C), 5.75 (1 H, t, *J* 8.2, 6-H), 6.98 (2 H, d, *J* 9.5, 3',5'-H), 7.48, 7.76 (each 1 H, s, 5-H, 9-H), 7.74 (2 H, d, *J* 8.7, 2',6'-H), 12.11 (1 H, s, N³-H); *m/z* (ESI) 430 {(M + H)⁺, 90%}, 374 (55%), 231 (100), 199 (60).

***N*-[(6*RS*)-2-Methyl-4-oxo-3,4,7,8-tetrahydro-6*H*-cyclopenta-*g*]quinazolin-6-yl]-*N*-(prop-2-ynyl)amino}benzoic acid **3**.** A solution of **14** (0.245 g, 0.57 mmol) in dichloromethane (2 cm³) and trifluoroacetic acid (10 cm³) was stirred at room temperature for 1.5 hours, then the solvents were removed *in vacuo*. The residue was triturated with diethyl ether and the precipitate was collected by filtration, washed with diethyl ether and dried *in vacuo* over P₂O₅ to afford the title compound as the trifluoroacetate salt (0.255 g). Part of this material (0.067 g) was suspended in water (6 cm³), and the pH was adjusted to ~12 with 1 N aqueous sodium hydroxide. The pH of the clear solution was adjusted to ~4 with 1 N aqueous hydrochloric acid. The white precipitate was collected by filtration, washed with water and dried *in vacuo* over P₂O₅ to afford the title compound **3** as a white solid (0.040 g) mp 305–307 °C (ref. 7: >325 °C); (Found: C, 68.65; H, 4.97; N, 10.87; C₂₂H₁₉N₃O₃·0.6H₂O requires C, 68.78; H, 5.29; N, 10.93%); δ_H (DMSO-*d*₆) 2.33 (3 H, s, 2-Me), 2.24, 2.50 (obscured by the DMSO peak) (each 1 H, m, 7-H), 2.97 (1 H, m, 8-H), 3.20 (1 H, m (obscured by H₂O peak), 8-H), 3.13 (1 H, s, C≡CH), 3.96 (2 H, AB system, *J* 19.0, CH₂C≡C), 5.75 (1 H, t, *J* 8.3, 6-H), 7.01 (2 H, d, *J* 9.0, 3',5'-H), 7.48, 7.78 (each 1 H, s, 5-H, 9-H), 7.80 (2 H, d, *J* 9.4, 2',6'-H), 12.11 (1 H,

s, N³-H); *m/z* (ESI) 747 {(2M + H)⁺, 100%}, 374 {(M + H)⁺, 70%}, 199 (20%). Chiral HPLC (column: Astec cyclobond BETA; mobile phase: 83% 25mM Na₂HPO₄–25 mM NaH₂PO₄–17% CH₃CN; flow 1 ml min⁻¹; λ = 230 nm): two peaks in a ratio of approximately 1 : 1; retention times: 621, 680 seconds.

Acknowledgements

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