# Synthesis and Biological Properties of 3-Methyl-10-propargyl-5,8-dideazafolic Acid



Terence R. Jones* [1], Richard F. Betteridge, David R. Newell and Ann L. Jackman

Drug Development Section, Institute of Cancer Research,
Sutton, Surrey SM2 5NG, England

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The synthesis of $N^3$-methyl-10-propargyl-5,8-dideazafolic acid (**1b**) is described. Ring closure of methyl-5-methylanthranilate with chloroformamidine hydrochloride gave a high yield of pure 2-amino-4-hydroxy-6-methylquinazoline treatment of which with iodomethane/sodium hydroxide provided the corresponding 3-methylquinazoline (**6**) which was converted to its 2-pivaloylamino derivative. This synthetic approach, next involving functionalisation of the 6-methyl group, was not further pursued because of difficulty encountered in removing the pivaloyl group. Methyl 5-methylanthranilate was treated with *p*-toluenesulfonyl chloride and the product then *N*-methylated. The tosyl group was cleaved with hydrogen bromide/phenol and the resulting methylamine ring-closed with chloroformamidine hydrochloride to provide 2-amino-1,4-dihydro-1,6-dimethyl-4-oxoquinazoline (**11**). The 2-pivaloylamino derivative of **11** was prone to hydrolytic deamination when attempts were made to remove the pivaloyl group and further elaboration of this heterocycle, with the intention of obtaining $N^1$-methyl-10-propargyl-5,8-dideazafolic acid was, too, not attempted. Di-*t*-butyl *N*-(4-propargylamino)benzoyl)-L-glutamate was therefore prepared and coupled with 2-amino-6-bromomethyl-4-hydroxyquinazoline hydrobromide. The resulting antifolate diester was *N*-monomethylated. Removal of the *t*-butyl groups with trifluoracetic acid afforded the target compound **1b** and its structure was proved by degradation to the quinazoline **6**. Its $IC_{50}$ for L1210 thymidylate synthase (TS) was 26 $\mu M$; the control value for 10-propargyl-5,8-dideazafolic acid (**1a**) was 0.02 $\mu M$. Thus the substitution of the lactam hydrogen in **1a** by a methyl group reduced the TS inhibition by 1300-fold. Compound **1b** was poorly cytotoxic to L1210 cells in culture ($ID_{50} > 100$ $\mu M$). An unperturbed lactam group in this class of antifolate is important for binding to TS.

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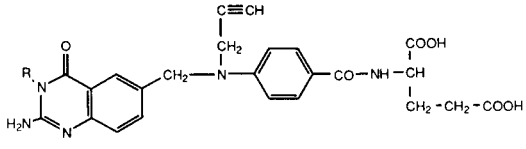
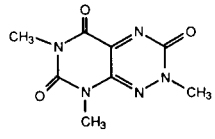
In a preceding paper [2] we set out reasons for the belief that the heteroatoms which comprise the 2-amino-3,4-dihydro-4-oxopyrimidine moiety of the antifolate $N^{10}$-propargyl-5,8-dideazafolic acid **1a** [3] (Chart I) cause strong intermolecular hydrogen bonding and hence insolubility. We showed that removal of the 2-amino group dramatically improved the solubility and conferred many improved biochemical and pharmacological properties upon the compound. In a parallel study we noted that the lactam NH in **1a** is weakly acidic which enhances its hydrogen bonding capability [4]. Is it necessary for binding to the target enzyme thymidylate synthase? In general, tampering with a hydrogen bonding group in heterocyclic drugs of this sort causes a loss of activity. It is, however, interesting to note that the trislactam 2-methylfervenulone **2** [5] is an antibiotic in spite of having no NH group. We therefore undertook the synthesis and biological testing of 3-methyl-10-propargyl-5,8-dideazafolic acid **1b** (Chart I) in order to answer the question posed. At the outset we thought it desirable to procure both the 3-methyl derivative **1b**, in which the lactam hydrogen is removed by direct substitution, and also the 1-methyl derivative **3** in which it is removed by remote substitution with bond rearrangement. To this end our first approach was to synthesise the appropriate dimethylquinazolines **6** and **11** (Scheme I) and to functionalise the 6-methyl group in each case to enable coupling of the heterocycle to an appropriate derivative of *N*-((4-propargylamino)benzoyl)-L-glutamic acid. In the event this did not succeed although the isomeric heterocyclic bases **6** and **11** were unequivocally synthesised. Resort was then made to direct methylation of a diester derivative of **1a** which gave a mixture of products the predominant compound of which was isolated and proved to have structure **1b**.

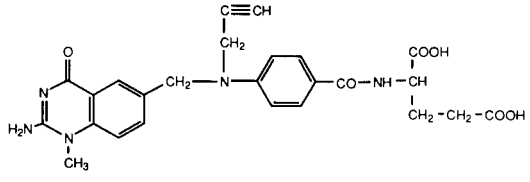
Chart I



**1a**, R = H
**b**, R = $CH_3$

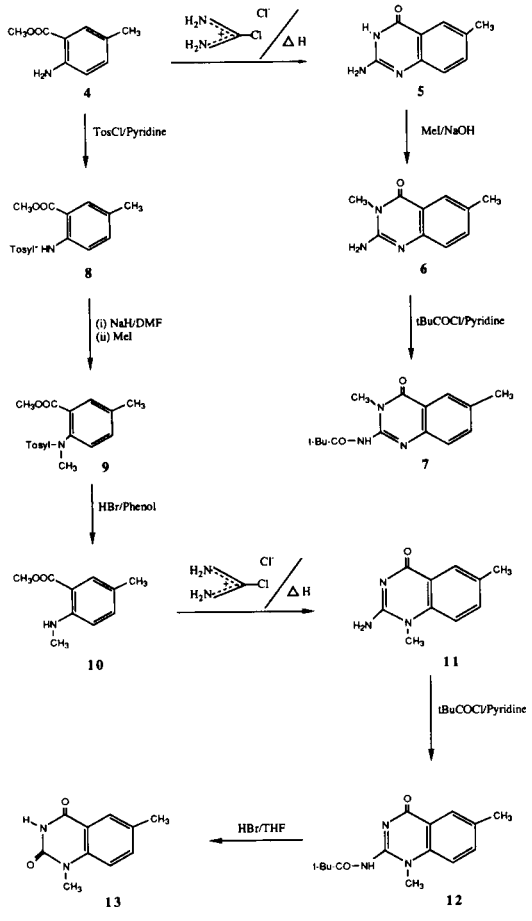


**2**



**3**

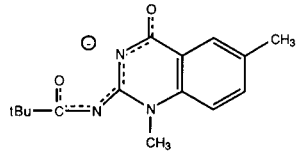
Scheme I




Chemistry.

The direct methylation of the aminohydroxyquinazoline **5** was first investigated in the expectation of obtaining the 3-methyl compound **6** (Scheme I). The base **5** has been previously described [6,7] and the most recent, improved, synthesis [8] involved fusion of a homologue (the ethyl ester) of the anthranilate **4** with guanidine. In our hands, we suspected that this reaction gave contaminating polymers of guanidine (melamines) which are difficult to remove. Since fusion with chlorformamidine hydrochloride had closed an anthranilonitrile to a diaminoquinazoline [9] it seemed reasonable to expect that this reagent would close an anthranilate ester to an aminohydroxyquinazoline and this we explored. The anthranilate ester **4**, prepared from the corresponding acid by the use of thionyl chloride and methanol [10], reacted speedily, vigorously, and cleanly with chlorformamidine hydrochloride at 200° to give a pure preparation of the quinazoline **5**. In a related application the use of chlorformamidine hydrochloride for guanine synthesis has been advocated [11]. The lactam **5**, dissolved in base, was treated with iodomethane to give a mixture of products. For the chromatography of this material acetone was found to be a peculiarly effective solvent and the major component was isolated in 44% yield. The nmr spectrum showed signals from an intact $NH_2$ and an *N*-methyl; a carbonyl frequency was observed in the ir. These facts together with the fact that the compound was an isomer of the $N^1$-methyl derivative **11** which was synthesised unequivocally (*vide infra*) enabled assignment of the structure **6**. The anthranilate **4** was homologated to the methylamine **10** with the intermediacy of the *p*-toluenesulfonamide derivatives **8** and **9**. A clean ring closure of **10** with chlorformamidine hydrochloride yielded the 1,6-dimethylquinazoline **11**. The bases **6** and **11** were noticeably different in polarity. The isomer **11**, having aligned C=N and C=O dipoles, had a higher mp and ran slower on tlc. The 400 MHz nmr spectra of these bases, taken in DMSO solution, were similar; however, the $NH_2$ signals were of noticeably different chemical shift. That for **6** was $\delta$ 6.90 and that for **11** $\delta$ 7.20. These values are understood if **11** is seen as a vinylogous amide with the electron pair on the amino group delocalised by C=N and C=O in series whereas **6** is seen as a cyclc amidine with less delocalisation from the amino group. Neither **6** nor **11** was particularly soluble and solubilisation and protection of the amino nitrogen in each was achieved by converting them to the pivaloyl derivatives **7** and **12**, respectively. In a preliminary attempt at brominating the 6-methyl group of **12** the use of *N*-bromosuccinimide in dichloromethane under nitrogen with illumination from a sun lamp gave substantial formation of the desired product (tlc and Epstein [12] spray). However, refinement of this bromination was discontinued when we found that the pivaloyl protecting group could not be removed without concurrent hydrolytic deamination. Thus treatment of the amide **12** in THF with hydrogen bromide [8] gave the dioxo compound **13** in 68% yield (not optimised). Other attempts at hydrolysing the amide bond in **12** e.g. Amberlyst 15 resin suspended in refluxing methanolic THF, *p*-toluenesulphonic acid in refuxing methanol, hydrogen bromide in refluxing methanol or TFA in refluxing methanol with all four acids in catalytic amount (0.1 eq) failed to deliver the desired amino compound **11**. An attempt at alkaline hydrolysis using cold 0.2*N* sodium hydroxide in aqueous

Chart II

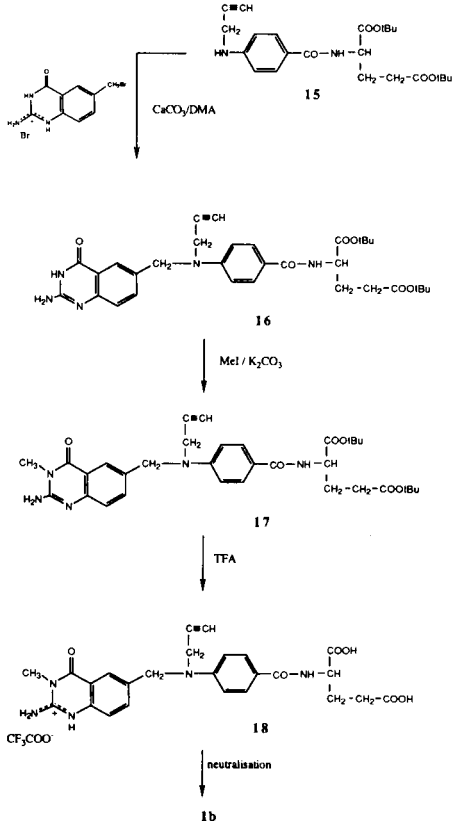


**14**

methanol for 72 hours gave only slight conversion to the amine, a result probably attributable to the first-formed delocalised anion **14** (Chart II) resisting further attack by hydroxide ion (*pace* the reactivity of a similar but less sterically hindered benzoylcytidine derivative) [13]. Warming the reaction to 50° then gave a mixture of the amino compound **11** and the dioxo compound **13**. The use of the pivaloyl group in the $N^1$-methylated series was therefore abandoned. Experiments to test the hydrolytic stability of the isomeric amide **7** showed first that it was completely stable to 0.4*N* sodium hydroxide in 20% ethanol in water for 22 days at 25° and second that, although in 1*N* hydrobromic acid in 14% water in THF for 18.5 hours at 25° it did convert to the desired amino compound **6**, it also converted to a roughly equal amount of a second unknown species.

At this juncture elaboration of the bases **6** and **11** was abandoned in favour of direct methylation of a performed antifolate. Compound **1a** [14] was thus methylated with MeI in ethanolic sodium hydroxide to give two major products by hplc. However, attempts to separate and isolate these on Whatman Partisil 10SAX anion exchange resin, on DEAE cellulose, or on reversed phase silica (Merck RP18 failed. Therefore methylation of an antifolate diester was undertaken and the substrate chosen was di-*t*-butyl 10-propargyl-5,8-dideazafolate **16**. In view of the unwanted deaminations which we had earlier observed it seemed prudent to prove the stability of the methylated systems to TFA, which reagent would be used at the final stage. Complete stability of the bases **6** and **11** to neat TFA/3 hours was thus proved by tlc (silica gel with either 10% water in acetonitrile or with acetone). Di-*t*-butyl *N*-(4-aminobenzoyl)-L-glutamate [15,16] was *N*-alkylated with propargyl bromide to provide the secondary amine **15** which, put with 2-amino-6-bromomethyl-4-hydroxyquinazoline hydrobromide [17], provided the antifolate diester **16** (Scheme II). The lactam function in **16** was *N*-methylated with iodomethane under basic conditions to give a mixture from which the major product was isolated by chromatography in 33% yield and easily characterised as a monomethyl derivative upon nitrogen. The chemical shift of the amino group, $\delta$ 6.93, enabled the $N^3$-methyl structure **17** to be assigned. Treatment of this diester with neat TFA gave the trifluoroacetate salt **18**, which was characterised and neutralisation of which finally yielded the desired $N^3$-methyl derivative **1b**. Finally, reductive cleavage of the benzylic $C^9$-$N^{10}$ bond in this derivative by catalytic transfer hydrogenation [18] was seen to give the quinazoline **6** to put the structure **1b** beyond doubt.

Scheme II




## Biological Evaluation.

The assays for the inhibition of L1210 TS and for the inhibition of the growth of L1210 cells in culture were performed as previously described [2]. The $N^3$-methyl compound **1b** inhibited L1210 TS with an $IC^{50}$ of 26 $\mu M$ in an assay wherein the value for the parent drug **1a** as a

Table I

Ultraviolet Spectra of Quinazoline Bases [a]

| Compound | λ/max,nm | ε | λ/min,nm | ε |
|---|---|---|---|---|
| **5** | 227 | 34,300 | 248 | 8,000 |
| | 233 [b] | 29,000 | 287 | 850 |
| | 264 | 12,100 | | |
| | 269 [b] | 11,100 | | |
| | 324 | 3,400 | | |
| **6** [c] | 228 | 34,700 | 248 | 6,500 |
| | 233 [b] | 30,400 | 292 | 730 |
| | 267.5 | 14,500 | | |
| | 272 [b] | 13,500 | | |
| | 335 | 3,900 | | |
| **11** [d] | 228.5 | 40,000 | 280 | 1,300 |
| | 254 [e] | 7,500 | | |
| | 300 [b] | 3,200 | | |
| | 310.5 | 4,500 | | |
| | 321 [e] | 3,700 | | |

[a] Spectra determined from solutions in absolute ethanol. [b] Inflection. [c] Agrees with spectrum of compound lacking 6-Me group [20]. [d] Agrees with spectrum of compound lacking 6-Me group [21]. However, the upper legend on page 414 should read "X, R = Me, $R^1$ = H" (K. Lempert,personal communication. [e] Shoulder.

positive control was 0.02 $\mu M$. Thus substitution of the lactam hydrogen by a methyl group reduced the TS inhibition by 1300-fold. The compound was poorly cytotoxic since the $ID^{50}$ for the growth of L1210 cells in culture was found to be > 100 $\mu M$; the control value for **1a** was 5 $\mu M$. These results were a disappointment and we draw the conclusion that an unperturbed lactam group in the amino-hydroxy heterocycle is an important part of the molecule for binding to thymidylate synthase.

## EXPERIMENTAL

General procedures are as given in ref 15 except as follows. Melting points were determined on a Reichert-Jung Thermovar hot stage and are corrected. Extracts were dried using magnesium sulfate. Potassium carbonate and calcium carbonate were dried at 100°. Elemental analyses were determined by Butterworth Laboratories, Teddington, Middlesex, England. TFA is trifluoroacetic acid. The chemical ionisation mass spectrum was determined with a VG 7070H spectrometer and VG 2235 data system at an ionising voltage of 50eV with an ion source temperature of 200° and with methane as the reagent gas using the direct insertion method.

Methyl 5-methylanthranilate (**4**).

Methanol (450 ml) in a 2 ℓ 3-necked flask fitted with an overhead stirrer, thermometer, and dropping funnel was cooled to -10 to -15° and to it thionyl chloride (117 mℓ) was added dropwise during 30 minutes keeping the temperature between -10 and -15°. The mixture was stirred at -10° for 10 minutes and then 2-amino-5-methylbenzoic acid (68.0 g) was added during 10 minutes with no attempt at cooling. The resulting slurry was stirred whilst it warmed to room temperature during 2 hours and then it was brought to reflux in a mantle with overhead stirring. After 30 minutes a dark brown solution was obtained which was heated under reflux for 20.5 hours. The mixture was taken to near dryness on a rotary evaporator between 30 and 60°. The residue was dissolved in water (500 mℓ) and ether (250 mℓ) was added followed by solid sodium bicarbonate (160 g) cautiously in portions until *p*H 8 was reached. The mixture was transferred to a 5 ℓ separating funnel, making the aqueous layer up to 2 ℓ and the ether layer to 2.5 ℓ and extracted. The ether layer was washed with saturated sodium bicarbonate (1 ℓ) and then with brine (2 ℓ). The extract was placed in a 5 ℓ beaker and stirred for 30 minutes with solid potassium carbonate (60 g, to remove last unchanged acid) and magnesium sulfate. Filtration through celite gave a clear solution which was taken to dryness on the rotary evaporator to give a brown oil (61.7 g, 83%) pure by tlc (acetonitrile:water, 3:1) and nmr.

2-Amino-3,4-dihydro-6-methyl-4-oxoquinazoline (**5**).

A mixture of the anthranilate **4** (61.7 g), chlorformamidine hydrochloride [19] (53.7 g, 1.25 molar equivalents), and diglyme (180 mℓ) was made into a homogeneous slurry in a 1 ℓ flanged neck flask fitted with a hand-powered overhead stirrer and an air-condensed distillation set up. The flask was dropped into a large oil bath preheated to 225° on a large hotplate. After 1.5 minutes vigorous reaction ensued and judicious rotation of the stirring rod between the hands was necessary to control the frothing as large amounts of hydrogen chloride gas were released together with a distillate (15 mℓ). After 10 minutes the oil bath temperature had dropped to 185° in spite of the hot plate and there was solid product which was very difficult to stir. The reaction was cooled to room temperature and treated with ethanol (150 mℓ) and the resulting thick paste was filtered on a sintered glass funnel. The beige-colored product (a hydrochloride salt) was resuspended in ethanol (150 mℓ) and filtered off and washed with ether and dried (74.6 g). This was placed in a 5 ℓ beaker equipped with a motorised paddle and dissolved in a mixture of 1.0 *N* aqueous hydrochloric acid (1 ℓ), DMF (1.2 ℓ) and water (1.2 ℓ). The brown solution was carbon treated and filtered through a glass fibre paper to give a yellow solution to which was added concentrated aqueous ammonium hydroxide (85 mℓ) to give *p*H 8 and a flocculent gelatinous precipate. This was coagulated by heating the mixture to 55° and cooling. The precipitate was then filtered off on a large sintered glass funnel and washed with water (2 x 800 mℓ), rinsed with ethanol, (100 mℓ) followed by ether (100 mℓ), and dried over phosphorus pentoxide *in vacuo* (58.0 g, 87%). This snow-white product was pure by tlc (chlorform:methanol:acetic acid, 75:20:5), uv, Table I.

*Anal.* Calcd. for $C_9H_9N_3O$: C, 61.70; H, 5.18; N, 23.99. Found: C, 61.74; H, 5.32; N, 24.10.

2-Amino-3,4-dihydro-3,6-dimethyl-4-oxoquinazoline (**6**).

The lactam **5** (17.52 g, 0.1 mole) was dissolved in a mixture of methanol (275 mℓ) and 0.1 *N* aqueous sodium hydroxide (120 mℓ) and treated with iodomethane (37.5 mℓ, 0.6 mole). The clear, homogeneous solution was kept at 4° for 7 days during which time a precipitate formed. This was filtered off and washed with 0.1 *N* aqueous sodium hydroxide (20 mℓ) followed by water (3 x 100 mℓ) and dried over phosphorus pentoxide *in vacuo* (14.65 g). This crude product was dissolved in DMA (195 mℓ) at 90° and silica (Merck Art 7734, 55 g) was added and the resulting slurry evaporated to dryness at 90°/0.6 mm on a rotary evaporator. Final drying was performed over phosphorus pentoxide *in vacuo*. The coated silica was applied to a column of silica (Merck Art 7734, 1040 g, 105 x 4.5 cm) made up in acetone. The column was eluted with acetone. The first 2.3 ℓ of eluant was discarded and the product was collected in the next 6.5 ℓ evaporation of which gave an off-white solid which was dried over phosphorus pentoxide *in vacuo* (8.34 g, 44%). The analytical sample was recrystallized from ethanol, mp 267°, $^1$H nmr (DMSO-$d_6$): 400 MHz $\delta$ 2.35 (s, 3H, 6-$CH_3$), 3.41 (s, 3H, 3-$CH_3$), 6.90 (s, 2H, $NH_2$), 7.11 (d, J = 8.5 Hz, 1H, $H^8$), 7.39 (dd, J = 8.5, 2.0 Hz, 1H, $H^7$), 7.71 (d, J = 2.0 Hz, 1H, $H^5$), ir: $\nu$ C=O 1646 $cm^{-1}$; uv, Table I.

*Anal.* Calcd. for $C_{10}H_{11}N_3O$: C, 63.48; H, 5.86; N, 22.21. Found: C, 63.28; H, 5.97; N, 22.33.

2-Methoxycarbonyl-4-Methyl-*N*-(*p*-toluenesulfonyl)aniline (**8**).

*p*-Toluenesulfonyl chloride (20.02 g, 0.105 mole) was added during 3 minutes to a mixture of the amine **4** (16.52 g, 0.1 mole) and pyridine (50 mℓ). The mixture was stirred at 25° for 2 hours and then poured into water (1500 mℓ). The resulting oil eventually solidified and it was filtered off, washed with water, partially dried and recrystallized from methanol (26.35 g, 83%), mp 106-106.5°; $^1$H nmr (250 MHz, deuteriochloroform): $\delta$ 2.26 (s, 3H, anthranilate methyl), 2.35 (s, 3H, tosyl methyl), 3.84 (s, 3H, *O*-methyl), 7.20 (d, 2H, J = 8.4 Hz, tosyl aromatic protons), 7.25 (dd, 1H, J = 8.0 & 2.2 Hz, H-5), 7.59 (d, 1H, J = 8.0 Hz, H-6), 7.70 (hidden, 1H, H-3), 7.70 (d, 2H, J = 8.4 Hz, tosyl aromatic protons), 10.40 (s, 1H, NH).

*Anal.* Calcd. for $C_{16}H_{17}NO_4S$: C, 60.17; H, 5.37; N, 4.39. Found: C, 60.11; H, 5.35; N, 4.30.

*N*,4-Dimethyl-2-methoxycarbonyl-*N*-(*p*-toluenesulfonyl)aniline (**9**).

The sulfonamide **8** (24.91 g, 78 mmoles) was added during 5 minutes to a suspension of sodium hydride (1.87 g, 78 mmoles) in DMF (100 mℓ) keeping the temperature below 20°. The mixture was stirred for 30 minutes to obtain a solution of the sodium salt. This was cooled to 10° and to it iodomethane (9.73 mℓ, 156 mmoles) was added during 7 minutes and the resulting mixture was then stirred for 2 hours at 25°. The solvent was removed at 80° *in vacuo* and the residue partitioned between toluene (1 ℓ) and water (750 mℓ). The organic phase was washed sequentially with 0.1 *N* sodium hydroxide (200 mℓ), water (2 x 1 ℓ), and brine (1 ℓ) and dried. Evaporation gave a stiff oil (26.00 g, 100%), pure by tlc (dichloromethane). A small sample was triturated with petrol to give white crystals mp 69-70°; $^1$H nmr (250 MHz, deuteriochloroform): δ 2.37 (s, 3H, anthranilate methyl), 2.42 (s, 3H, tosyl methyl), 3.24 (s, 3H, *N*-methyl), 3.82 (s, 3H, *O*-methyl), 6.79 (d, 1H, J = 8.0 Hz, H-6), 7.20 (dd, 1H, J = 8.0 & 2.2 Hz, H-5), 7.25 (d, 2H, J = 8.4 Hz, tosyl aromatic protons), 7.52 (d, 2H, J = 8.4 Hz, tosyl aromatic protons), 7.64 (d, 1H, J = 2.2 Hz, H-3).

*Anal.* Calcd. for $C_{17}H_{19}NO_4S$: C, 61.24; H, 5.74; N, 4.20. Found: C, 61.05; H, 5.84; N, 3.97.

*N*,4-Dimethyl-2-(methoxycarbonyl)aniline (**10**).

A mixture of the sulfonamide **9** (25.67 g, 77 mmoles), phenol (15.98 g), and 45% w/v hydrogen bromide in acetic acid (160 mℓ) was shaken vigorously by hand for 2 hours to give a dark red solution which was kept at 25° for 14.5 hours. The solution was partitioned between iced 5*N* hydrochloric acid (250 mℓ) and ether (1 ℓ). The aqueous layer was separated and washed with ether (500 mℓ) and then made alkaline (*p*H 10.5) with solid potassium carbonate (350 g). The resulting oil was extracted into ether (2 ℓ). The extract was washed with brine (1 ℓ) and dried and evaporated to dryness to give an oil (13.12 g, 95%). $^1$H nmr (deuteriochloroform): 250 MHz δ 2.23 (s, 3H, C-Me), 2.89 (s, 3H, N-Me), 3.84 (s, 3H, O-Me), 6.61 (d, J = 8.5 Hz, 1H, $H^6$), 7.21 (dd, J = 8.5, 2.0 Hz, 1H, $H^5$), 7.5 (br, 1H, NH), 7.70 (d, J = 2.0 Hz, 1H, $H^3$).

*Anal.* Calcd. for $C_{10}H_{13}NO_2$: C, 67.02; H, 7.31; N, 7.82. Found: C, 67.00; H, 7.27; N, 7.80. The picrate had mp 123-127°.

*Anal.* Calcd. for $C_{16}H_{16}N_4O_9$: C, 47.06; H, 3.95; N, 13.72. Found: C, 47.16; H, 3.91; N, 13.83.

2-Amino-1,4-dihydro-1,6-dimethyl-4-oxoquinazoline (**11**).

The methylamine **10** (10.36 g) and chlorformamidine hydrochloride (8.31 g, 1.25 molar equivalents in diglyme (30 mℓ) were condensed as described for **5** but in a smaller apparatus. The resulting mixture was stirred with 0.25 *N* hydrochloric acid for 30 minutes to give a solution which was carbon treated and filtered. Basification with concentrated ammonium hydroxide (13 mℓ) gave an off-white precipitate which was filtered off and washed with water (2 x 200 mℓ) and pulled nearly dry. It was then washed with ether (100 mℓ) to remove some starting amine and dried at 100° *in vacuo* (8.05 g, 73%), mp 306-311° (immersed at 265°). The analytical sample was recrystallised from ethanol mp 309-314° (immersed at 265°). The mp is dependent upon immersion temp and rate of heating and can be anywhere between 297 and 314°; $^1$H nmr (DMSO-$d_6$): 400 MHz δ 2.38 (s, 3H, 6-Me), 3.50 (s, 3H, 1-Me), 7.20 (s, 2H, $NH_2$), 7.36 (d, J = 8.5 Hz, 1H, $H^8$), 7.46 (dd, J = 8.5, 2.0 Hz, 1H, $H^7$), 7.75 (d, J = 2.0 Hz, 1H, $H^5$); ir: ν C=O 1633 cm$^{-1}$; uv Table I.

*Anal.* Calcd. for $C_{10}H_{11}N_3O$: C, 63.48; H, 5.86; N, 22.21. Found: C, 63.28; H, 5.89; N, 22.08.

1,4-Dihydro-1,6-dimethyl-4-oxo-2-trimethylacetamidoquinazoline (**12**).

The quinazolone **11** (0.946 g, 5 mmoles) was suspended in a mixture of DMA (10 mℓ) and pyridine (0.81 mℓ, 10 mmoles) and treated with pivaloyl chloride (0.80 mℓ, 6.5 mmoles). The mixture was stirred at 110° for 2.5 hours (becoming a solution after 50 mintes) and then poured into water (750 mℓ). The resulting white solid was filtered off, washed twice with water and dried over phosphorus pentoxide *in vacuo* (0.861 g, 63%, pure by tlc). Recrystallization from ethanol afforded white crystals, mp 221-221.5°; $^1$H nmr (deuteriochloroform): 250 MHz δ 1.25 (s, 9H, *t*-Bu), 2.42 (s, 3H, C-Me), 3.76 (s, 3H, N-Me), 7.25 (d, J = 8.6 Hz, 1H, $H^8$), 7.54 (dd, J = 8.6, 2.0 Hz, 1H, $H^7$), 8.00 (d, J = 2.0 Hz, 1H, $H^5$), 13.47 (br s, 1H, NH).

*Anal.* Calcd. for $C_{15}H_{19}N_3O_2$: C, 65.91; H, 7.01; N, 15.37. Found: C, 65.83; H, 7.11; N, 15.54.

3,4-Dihydro-3,6-dimethyl-4-oxo-2-trimethylacetamidoquinazoline (**7**).

The quinazolone **6** (2.65 g, 14 mmoles), DMA (20 mℓ), pyridine (2.26 mℓ, 28 mmoles), and pivaloyl chloride (2.42 mℓ, 19.6 mmoles) were heated at 100° for 6.5 hours. Work up as for **12** gave the product (3.36 g, 87%). Recrystallisation from ethanol afforded white crystals, mp 142-143°; $^1$H nmr (deuteriochloroform): 60 MHz δ 1.27 (s, 9H, *t*-Bu), 2.41 (s, 3H, 6-Me), 3.59 (s, 3H, 3-Me), 7.02 (d, J = 8 Hz, 1H, $H^8$), 7.45 (dd, J = 8, 2 Hz, 1H, $H^7$), 7.95 (d, J = 2 Hz, 1H, $H^5$), 14.0 (br s, 1H, NH).

*Anal.* Calcd. for $C_{15}H_{19}N_3O_2$: C, 65.91; H, 7.01; N, 15.37. Found: C, 66.03; H, 7.07; N, 15.30.

1,6-Dimethyl-2,4-dioxo-1,2,3,4-tetrahydroquinazoline (**13**).

The pivaloyl derivative **12** (1.367 g, 5 mmoles) dissolved in boiling THF (30 mℓ) was treated with a mixture of 60% w/w aqueous hydrobromic acid (1.82 mℓ, 22.5 mmoles) and water (1.08 mℓ, 60 mmoles). A white precipitate appeared immediately but redissolved in the refluxing mixture during the next 40 minutes. After 1.5 hours the mixture was cooled to 2°. The resulting white crystalline solid was filtered off (0.646 g, 68%) and recrystallised from ethanol to give white needles, mp 254-255.5°; (0.43 g); $^1$H nmr (DMSO-$d_6$): 250 MHz δ 2.36 (s, 3H, 6-Me), 3.42 (s, 3H, 1-Me), 7.31 (d, J = 8.5 Hz, 1H, $H_8$), 7.57 (dd, J = 8.5, 2.0 Hz, 1H, $H^7$), 7.79 (d, J = 2.0 Hz, 1H, $H^5$), 11.48 (br s, 1H, NH); ms: (ci) m/z 191 (M + 1)$^+$, 148 (M-NCO).

*Anal.* Calcd. for $C_{10}H_{10}N_2O_2$: C, 63.15; H, 5.30; N, 14.73. Found: C, 63.02; H, 5.31; N, 14.86.

Di-*t*-butyl (*N*-butyl *N*-(4-propargylamino)benzoyl)-L-glutamate (**15**).

A mixture of di-*t*-butyl *N*-(4-aminobenzoyl)-L-glutamate [15,16] (7.57 g, 20 mmoles), propargyl bromide (80% in toluene, 11.14 mℓ, 100 mmoles), potassium carbonate (2.76 g, 20 mmoles) and ethanol (100 mℓ) was stirred under reflux for 3 hours. The solvent was removed and the residue partitioned between chloroform (1 ℓ) and water (1 ℓ). The organic layer was washed with brine (1 ℓ), dried and evaporated to dryness to give a gum (9.83 g). Chromatography (silica gel, Merck Art 15111, 250 g) with dichloromethane as eluant gave an essentially pure product (3.89 g, 47%) suitable for further use plus a less pure fraction (0.83 g).

Rechromatography of the latter (silica, Merck Art 9385, 220 g) with ether as eluant gave the analytical sample as a yellow gum (0.45 g, 5%) which could not be induced to crystallise; $^1$H nmr (deuteriochloroform): 250 MHz δ 1.41 (s, 9H, *t*-Bu), 1.48 (s, 9H, *t*-Bu), 2.15 (m, 2H, $CH_2\beta$), 2.24 (t, J = 2.4 Hz, 1H, ≡CH), 2.38 (m, 2H, $CH_2\gamma$), 3.97 (dd, J = 2.4, 5.9 Hz, 2H, $CH_2$-C≡), 4.41 (t, J = 5.9 Hz, 1H, aniline NH), 4.68 (m, 1H, $CH^{\alpha}$), 6.66 (d, J = 8.7 Hz, 2H, H$^3$ & H$^5$), 6.81 (d, J = 7.6 Hz, 1H, amidic NH), 7.71 (d, J = 8.7 Hz, 2H, H$^2$ & H$^6$).

*Anal.* Calcd. for $C_{23}H_{32}N_2O_5$: C, 66.32; H, 7.74; N, 6.73. Found: C, 66.21; H, 8.00; N, 6.78.

Di-*t*-butyl *N*-(4-(*N*-((2-Amino-4-hydroxy-6-quinazolinyl)-methyl)prop-2-ynylamino)benzoyl)-L-glutamate (**16**).

The amine **15** (3.85 g, 9.24 mmoles) and 2-amino-6-bromomethyl-4-hydroxyquinazoline hydrobromide [17] (3.41 g, 10.18 mmoles) in DMA solution (35 mℓ) was stirred over calcium carbonate (1.85 g, 18.48 mmoles) for 96 hours at 25°. The mixture was filtered through celite and the solids washed with DMA. The filtrate was evaporated to dryness at 70°/0.3 mm to give a stiff gum (11.3 g). Chromatography on silica (Art 15111, 240 g) with 2.5% ethanol in chloroform as the eluant gave the pure product which was dried (phosphorus pentoxide *in vacuo*) to an off-white, friable solid (4.12 g, 76%) mp 150-151°; $^1$H nmr (250 MHz, dimethyl sulfoxide-$d_6$): δ 1.38 (s, 9H, *t*-Bu), 1.40 (s, 9H, *t*-Bu), 1.95 (m, 2H, $CH_2\beta$), 2.31 (m, 2H $CH_2\gamma$), 3.21 (t, 1H, J = 2.0 Hz, ≡CH), 4.29 (bs, 2H, $CH_2$-C≡), 4.30 (m, 1H, $CH^{\alpha}$), 4.67 (s, 2H, $CH_2^9$), 6.40 (bs, 2H, $NH_2$), 6.85 (d, 2H, J = 8.9 Hz, H$^{3'}$ & H$^{5'}$), 7.17 (d, 1H, J = 8.4 Hz, H-8), 7.50 (dd, 1H, J = 8.4 & 1.9 Hz, H-7), 7.74 (d, 2H, J = 8.9 Hz, H$^{2'}$ & H$^{6'}$), 7.80 (d, 1H, J = 1.9 Hz, H-5), 8.26 (d, 1H, amidic NH), 11.02 (bs, 1H, lactam NH).

*Anal.* Calcd. for $C_{32}H_{39}N_5O_6$: C, 65.18; H, 6.67; N, 11.88. Found: C, 64.95; H, 6.84; N, 11.87.

Di-*t*-butyl *N*-(4-(*N*-((2-Amino-3,4-dihydro-3-methyl-4-oxo-6-quinazolinyl)methyl)prop-2-ynylamino)benzoyl)-L-glutamate (**17**).

A solution of the lactam **16** (1.18 g, 2 mmoles), and iodomethane (12.45 mℓ, 200 mmoles) is dry methanol (20 mℓ)was stirred over potassium carbonate (1.382 g, 10 mmoles) for 45 hours at 25° with the exclusion of light. Analysis (tlc) (silica gel, 10% ethanol in chloroform) showed a strong product spot in advance of **16**. The mixture was partitioned between chloroform (500 mℓ) and water (500 mℓ) and the aqueous layer thereof (*p*H 9.6) was further extracted with chloroform (3 x 100 mℓ). The original chloroform extract was washed with water (500 mℓ) and this wash was then reextracted with chloroform (3 x 100 mℓ). The combined chloroform extracts were dried and the solvent removed *in vacuo* to give a yellow solid (1.34 g). This was chromatographed on silica (Art 9385, 200 g) eluting with 66% acetone in dichloromethane to give the pure product as a pale yellow, friable solid (0.400 g, 33%) of indeterminate mp; $^1$H nmr (DMSO-$d_6$): 400 MHz δ 1.38 (s, 9H, *t*-Bu), 1.39 (s, 9H, *t*-Bu), 1.95 (m, 2H, $CH_2\beta$), 2.30 (t, J = 7.5 Hz, 2H, $CH_2\gamma$), 3.20 (t, J = 2.2 Hz, 1H, ≡CH), 3.36 (s, 3H, $CH_3$), 4.28 (m, 1H, $CH^{\alpha}$), 4.28 (d, J = 2.2 Hz, 2H, $CH_2$-C≡), 4.67 (s, 2H, $CH_2^9$), 6.84 (d, J = 8.9 Hz, 2H, H$^{3'}$ & H$^{5'}$), 6.93 (s, 2H, $NH_2$), 7.15 (d, J = 8.4 Hz, 1H, H$^8$), 7.49 (dd, J = 8.4, 1.9 Hz, 1H, H$^7$), 7.72 (d, J = 8.9 Hz, 2H, H$^{2'}$ & H$^{6'}$), 7.82 (d, J = 1.9 Hz, 1H, H$^5$), 8.22 (d, J = 7.6 Hz, 1H, amidic NH). The acetone and water were seen in the $^1$H nmr spectrum.

*Anal.* Calcd. for $C_{33}H_{41}N_5O_6 \cdot 0.5\ C_3H_6O \cdot H_2O$: C, 63.67; H, 7.12; N, 10.76. Found: C, 63.28, 63.38; H, 7.09, 6.94; N, 10.50, 10.49.

*N*-(4-(*N*-((2-Amino-3,4-dihydro-3-methyl-4-oxo-6-quinazolinyl)-methyl) prop-2-ynylamino)benzoyl)-L-glutamic acid (**1b**).

The solvated diester **17** (0.366 g) was dissolved in TFA (3.7 mℓ) and the solution was kept at 25° for 1 hour. It was then added to ether (40 mℓ) contained in a glass centrifuge tube whereup an off-white precipitate formed. Centrifugation at 2,500 g for 15 minutes gave a pellet which was resuspended in ether (40 mℓ) and centrifuged once more and the fluid decanted. The precipitate was dried *in vacuo* over phosphorus pentoxide/potassium hydroxide pellets to give the trifluoracetate salt **18** of the product (0.249 g, 72%), mp 155-170°; $^1$H (DMSO-$d_6$): 250 MHz δ 3.38 (s, 3H, $CH_3$), 7.36 (d, 8.5 Hz, 1H, H$^8$), 7.74 (part of m, 1H, H$^7$), 7.95 (d, J = 1.7 Hz, 1H, H$^5$), 8.88 (br s, 2H, $NH_2$), plus all other signals as expected.

*Anal.* Calcd. for $C_{25}H_{25}N_5O_6 \cdot CF_3COOH \cdot 0.5\ H_2O$: C, 52.77; H, 4.43; N, 11.40; F, 9.27. Found: C, 52.73, 52.16; H, 4.33, 4.03; N, 11.86, 11.55; F, 8.35, 8.55.

This salt (0.205 g) was stirred with 0.1 *N* sodium hydroxide (13.4 mℓ, 4 molar equivalents) for 10 minutes to give a solution which was filtered and then acidified with 1 *N* hydrochloric acid to *p*H 2.5. A white gelatinous precipitate resulted which was centrifuged at 2,500 g, 30 minutes give an off-white solid which was washed three times by cycles of resuspension (water, 40 mℓ)- centrifugation - decantation and then dried over phosphorus pentoxide *in vacuo*, first at 25° and then in a glass vial at 60°, (0.124 g, 73%) mp 202-205°; $^1$H nmr (DMSO-$d_6$): 250 MHz δ 2.00 (m, 2H, $CH_2\beta$), 2.33 (m, 2H, $CH_2\gamma$), 3.20 (t, J = 1.9 Hz, 1H, ≡CH), 3.37 (s, 3H, $CH_3$), 4.29 (d, J = 1.9 Hz, 2H, $CH_2$-C≡), 4.36 (m, 1H, $CH^{\alpha}$), 4.68 (s, 2H, $CH_2^9$), 6.85 (d, J = 8.8 Hz, 2H, H$^{3'}$ & H$^{5'}$), 6.98 (br, s, 2H, $NH_2$), 7.16 (d, J = 8.4 Hz, 1H, H$^8$), 7.49 (dd, J = 8.4, 2.0 Hz, 1H, H$^7$), 7.74 (d, J = 8.8 Hz, 2H, H$^{2'}$ & H$^{6'}$), 7.83 (d, J = 2.0 Hz, 1H, H$^5$), 8.25 (d, J = 7.7 Hz, 1H, amidic NH), 12.3 (br, 2H, COOH's).

*Anal.* Calcd. for $C_{25}H_{25}N_5O_6 \cdot H_2O$: C, 58.93; H, 5.34; N, 13.75. Found: C, 59.19, 58.50; H, 5.25, 4.97; N, 13.66, 13.58.

Degradation of **1b** to the Quinazoline **6** [18].

Compound **1b** (4 mg) was dissolved in 98% formic acid (0.2 mℓ) and 10% palladium on carbon (6 mg) added. After 1 hour, the mixture was filtered and the solvent removed *in vacuo*. The residue was dissolved in 10% aqueous concentrated ammonium hydroxide in *N,N*-dimethylacetamide (5 drops) and applied to a 20 x 5 cm silica tlc plate. Samples of the bases **6** and **11** were similarly prepared and applied as controls. The plate was dried for 10 minutes at 1 mm over phosphorus pentoxide and then eluted with 10% water in acetonitrile. Apart from a base spot, the only spot in the chromatogram ran identically with the base **6** to $R^f$ 0.45 and also appeared identical under 254 nm and 366 nm uv lights. The base **11** had $R^f$ 0.18.

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