methyl)-3,4-dihydro-4-oxo-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamate (**43**) as a gum: 4.2 g (53%); NMR δ 1.15, (2 t, 6 H, *J* = 6.5 Hz, 2 OCH_2CH_3), 2.04 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.40 (t, 2 H, *J* = 7 Hz, $CHCH_2CH_2CO_2Et$), 3.18 (t, 1 H, *J* = 1.5 Hz, C≡CH), 4.04, 4.08 (2 q, 4 H, *J* = 6.5 Hz, 2 OCH_2CH_3), 4.39 (d, 2 H, *J* = 1.5 Hz, $CH_2C{\equiv}C$), 4.40 (m, 1 H, CH), 4.56 (br s, 2 H, CH_2Cl), 4.82 (br s, 2 H, $ArCH_2N<$), 6.85 (d, 2 H, *J* = 9 Hz, 3′-H and 5′-H), 7.61 (d, 1 H, *J* = 8 Hz, quinazoline 8-H), 7.75 (d, 2 H, *J* = 9 Hz, 2′-H and 6′-H), 7.75 (dd, 1 H, *J* = 8 and 2 Hz, quinazoline 7-H), 8.03 (d, 1 H, *J* = 2 Hz, quinazoline 5-H), 8.34 (d, 1 H, *J* = 6.5 Hz, CONH).

Powdered 2-mercaptopyrimidine (110 mg, 0.98 mmol) was added to a stirred suspension of NaH (47 mg of a 50% dispersion in oil, 0.98 mmol) in DMF (10 mL). After 30 min a solution of the chloromethyl compound **43** (560 mg, 0.98 mmol) in DMF (5 mL) was added and the mixture was stirred for a further 17 h. The standard workup with EtOAc followed by purification of the crude product by chromatography using EtOAc as eluent afforded 470 mg (75%) of the 2-pyrimidinylthio derivative **44** as a gum. This product was stirred for 2 h in a mixture of EtOH (5 mL) and 1 N aqueous NaOH (6.9 mL, 6.9 mmol). The reaction was worked up according to method C to give the off-white solid **11a**: 320 mg (87%); mp 143–147 °C; NMR δ 2.00 (m, 2 H, $CHCH_2CH_2CO_2H$), 2.35 (t, 2 H, *J* = 7 Hz, $CHCH_2CH_2CO_2H$), 3.18 (t, 1 H, *J* = 2 Hz, C≡CH), 4.35 (m, 1 H, CH), 4.35 (d, 2 H, *J* = 2 Hz, $CH_2C{\equiv}C$), 4.41 (s, 2 H, CH_2S), 4.78 (br s, 2 H, $ArCH_2N<$), 6.85 (d, 2 H, *J* = 9 Hz, 3′-H and 5′-H), 7.25 (t, 1 H, *J* = 5 Hz, pyrimidine 5-H), 7.53 (d, 1 H, *J* = 9 Hz, quinazoline 8-H), 7.70 (m, 3 H, 2′-H, 6′-H, and quinazoline 7-H), 8.00 (d, 1 H, *J* = 2 Hz, quinazoline 5-H), 8.34 (d, 1 H, *J* = 7 Hz, CONH), 8.65 (d, 2 H, *J* = 5 Hz, pyrimidine 4-H and 6-H); MS (FAB) *m/e* 587 $[MH]^+$. Anal. ($C_{29}H_{26}N_6O_6S \cdot H_2O$) C, H, N.

Quinazoline Antifolate Thymidylate Synthase Inhibitors: 2′-Fluoro-N^{10}-propargyl-5,8-dideazafolic Acid and Derivatives with Modifications in the C2 Position

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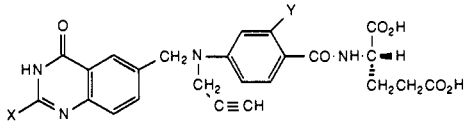
The synthesis of 2′-fluoro-10-propargyl-5,8-dideazafolic acid and its 2-desamino, 2-desamino-2-hydroxymethyl, and 2-desamino-2-methoxy analogues is described. In general the synthetic route involved the coupling of diethyl *N*-[2-fluoro-4-(prop-2-ynylamino)benzoyl]-L-glutamate (**15**) with the appropriate 6-(bromomethyl)quinazoline followed by deprotection with mild alkali. These four compounds together with the 2-desamino-2-methyl analogue were tested for their activity against L1210 thymidylate synthase (TS). They were also examined for their inhibition of the growth of the L1210 cell line and of two mutant L1210 cell lines, the L1210:R7A that overproduces dihydrofolate reductase (DHFR) and the L1210:1565 that has impaired uptake of reduced folates. Compared with their non-fluorinated parent compounds, the 2′-fluoro analogues were all ~2-fold more potent as TS inhibitors. Similarly, they also showed improved inhibition of L1210 cell growth (1.5–5-fold), and this activity was prevented by co-incubation with thymidine. All had retained or improved activity against both the L1210:R7A and L1210:1565 cell lines.

Introduction

The discovery that the quinazoline-based analogue of folic acid N^{10}-propargyl-5,8-dideazafolic acid (**1**)[1] was a



1	X = NH_2	Y = H
2	X = NH_2	Y = F
3	X = H	Y = H
4	X = H	Y = F
5	X = CH_3	Y = H
6	X = CH_3	Y = F
7	X = CH_2OH	Y = H
8	X = CH_2OH	Y = F
9	X = OCH_3	Y = H
10	X = OCH_3	Y = F

potent inhibitor of thymidylate synthase (TS, EC 2.1.1.45) and had experimental antitumor activity[1-4] led to several clinical studies in man.[5-10] Significant activity was observed in some tumor types, particularly ovarian, breast, and liver cancer. However, this drug was withdrawn from clinical study because of its nephrotoxicity and unpredictable hepatotoxicity. The relative insolubility of **1** at

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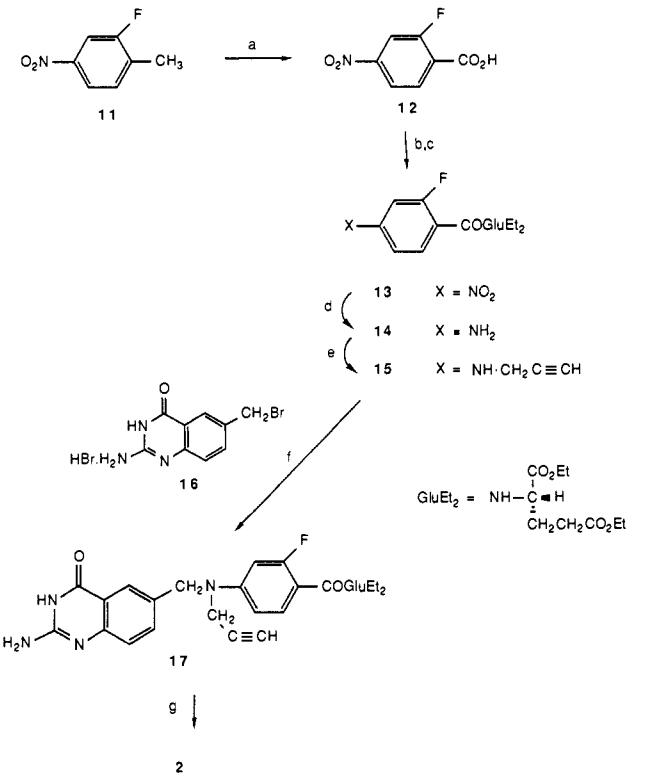
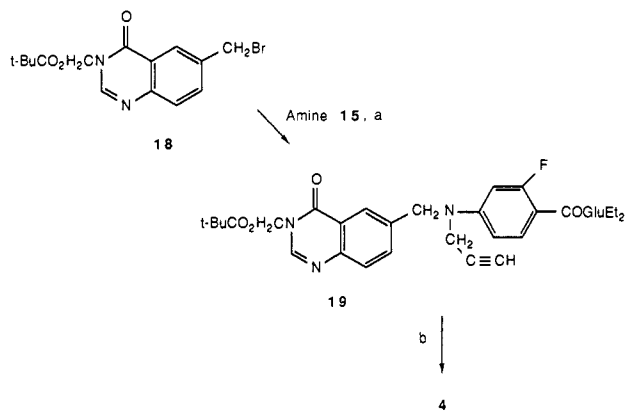
the acid pH of urine was identified as the probable cause of at least the nephrotoxicity,[11,12] and this led to the development of more water-soluble analogues. We first synthesized 2-desamino-N^{10}-propargyl-5,8-dideazafolic acid (3) on the hypothesis that removal of the amino group from the 2-position would reduce intermolecular hydrogen bonding and hence increase water solubility, which indeed it did.[13] A number of 2-desamino-2-substituted analogues of 1 followed, and some have interesting in vitro activities.[14-16] Two of these compounds, the 2-desamino (3)[13] and 2-desamino-2-methyl (5)[15] analogues of 1 were identified as nonnephrotoxic agents with increased antitumor potency both in vitro and in vivo.[17-19] This activity is exhibited despite their slightly reduced activity against isolated TS. Although the substrate activity of 1 and these analogues for folylpolyglutamate synthetase (FPGS) is the same (Km ~ 50 μM), improved cellular uptake and formation of intracellular polyglutamates was concluded to be the cause of the improved cytotoxic potency.[18,19] For example in L1210 cells analogues 3 and 5 appear to use the reduced folate transport mechanism unlike 1. The use of both tritiated 1 and 5 has demonstrated that the latter is far more readily taken up by L1210 cells and converted to polyglutamate metabolites.[18,20] Once formed these polyglutamates are not readily effluxed from the cells. Similarly, the analogues 3 and 5 were more potent in their ability to inhibit TS in whole cells after resuspension in drug-free medium, this being consistent with intracellular polyglutamate formation. As with a number of folate-based TS inhibitors, the polyglutamate derivatives of 1, 3, and 5 are substantially more potent as inhibitors of TS than their monoglutamate forms.[17,19-22] A previous report showed the effects of chlorine and methyl substitution on the benzoyl ring of 1.[23] Introduction of a 2′-chloro substituent essentially preserved the inhibition of TS, while

Scheme I[a]



[a] (a) $Na_2Cr_2O_7$, H_2SO_4, HOAc; (b) $SOCl_2$, DMF; (c) diethyl glutamate hydrochloride, Et_3N; (d) iron powder, HOAc or H_2, 10% Pd–C; (e) propargyl bromide, K_2CO_3, DMF; (f) $CaCO_3$, DMA; (g) 1 N aqueous NaOH, EtOH.

Scheme II[a]



[a] (a) $CaCO_3$, DMA; (b) 1 N aqueous NaOH, EtOH.

a 2′-methyl group reduced it by 21-fold. These two substituents are of similar size, indicating that there is little steric effect causing the observed difference. However the dipoles are in opposite directions, and it was hypothesized that this may account for this difference. A chlorine substituent in the 3′-position substantially worsened TS inhibition.[23] Therefore, working on the assumption that a substituent on the 2′-position with a negative dipole could at least be tolerated by the enzyme, we synthesized the 2′-fluoro-analogue 2 in an attempt to enhance enzyme inhibition. This would have the advantage of introducing a strongly electron withdrawing substituent, not greatly different in bulk from hydrogen. We also describe the synthesis and biological activities of the 2′-fluoro analogues of some of the more water soluble analogues of 1 modified in the 2-position, namely the 2-desamino,[13] 2-methyl,[14,15] 2-hydroxymethyl,[14,15] and 2-methoxy[16] analogues.

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Table I. In Vitro Activities of Antifolate Diacids 1–10

compd	C2 substituent	C2' substituent	inhibn of TS: IC_{50}, μM	inhibition of cell growth in culture: IC_{50} μM					L1210 cell growth in the presence of dThd (% of control)
				L1210	L1210:R7A	relative resistance	L1210:1565	relative resistance	
1	NH_2	H	0.02	3.5	41	12	3.6	1	96
2	NH_2	F	0.009	1.1	6.2	5.6	0.6	0.55	84
3	H	H	0.17	0.36	7.5	21	4.1	10	100
4	H	F	0.058	0.29	3.8	13.1	1.2	4	84
5	CH_3	H	0.05	0.085	0.39	4.6	8.3	98	100
6	CH_3	F	0.02	0.027	0.11	4	2.2	81	97
7	CH_2OH	H	0.098	5.0	11	2.2	21	4	100
8	CH_2OH	F	0.058	1.9			6	2	82
9	OCH_3	H	0.068	1.9	14	7.4	160	84	100
10	OCH_3	F	0.036	1.0			45	45	89

Chemistry

The 2'-fluoro-10-propargyl-5,8-dideazafolic acid analogues **2**, **4**, **6**, **8**, and **10** were prepared by the methods developed for the corresponding desfluoro compounds **1**[24] (Scheme I), **3**[13] (Scheme II), **5**,[15] **7**,[15] and **9**[16] but starting from the 2'-fluoro *N*-propargylamine **15**. This key intermediate was obtained in 60% yield on treatment of the amine **14** with propargyl bromide in the presence of K_2CO_3. The synthesis of **14** (Scheme I) followed a similar route to that developed by Henkin and Washtien[25] for the corresponding di-*tert*-butyl ester. However the poor yield (25%) of 2-fluoro-4-nitrobenzoic acid (**12**) in the literature route[26] prompted us to investigate this initial step. When the oxidation of 2-fluoro-4-nitrotoluene (**11**) was carried out with sodium dichromate in HOAc, an improved yield (57%) of **12** was obtained. The reduction of the diethyl (2-fluoro-4-nitrobenzoyl)glutamate (**13**) with hydrogen in the presence of Adams catalyst[25] in our hands gave a partial reduction of the C–F bond. Several alternative reducing conditions were tried, and two successful methods were found (H_2, 10% palladium on carbon in EtOAc at 1 atm pressure and iron powder in HOAc) which gave **14** in excellent yield (>95%) and free from the corresponding desfluoro derivative.

Biological Evaluation

The antifolate diacids listed in Table I were tested as inhibitors of TS partially purified from L1210 mouse leukemia cells that overproduce TS due to amplification of the TS gene.[27] The partial purification and assay method used was as previously described and used a (±)-5,10-methylenetetrahydrofolic acid concentration of 200 μM.[20,27] The results are expressed as IC_{50} values, that is the concentration of compound to inhibit the control reaction rate by 50%. The compounds were also tested for their inhibition of the growth of L1210 cells in culture, and the results are expressed as the concentration of compound required to inhibit cell growth by 50% (IC_{50}). The L1210:R7A is a subline of the L1210 that is 600-fold resistant to methotrexate (MTX) by virtue of elevated levels of dihydrofolate reductase (DHFR).[28] This line was routinely subcultured in 1 μM MTX, but MTX was omitted 2 weeks prior to the experiment. The L1210:1565 cell line[29] has acquired resistance to the antitumor antibiotic CI-920.[30] Evidence suggests that this agent penetrates cells via the reduced folate carrier mechanism and that the L1210:1565 line is resistant due to a very much reduced drug uptake and hence it is cross resistant to MTX (~200-fold).[31] Both cell lines were grown by suspension culture in RPMI 1640 medium without sodium bicarbonate but containing 20 mM HEPES[32] and supplemented with 10% horse serum (L1210 and L1210:R7A cells) or 10% fetal calf serum (L1210:1565). Incubation time for the 5-mL cultures was 48 h (72 h for the L1210:1565). For some of the compounds the L1210 cell line was also grown in 10% fetal calf serum for comparison with the L1210 cell line, and no significant difference was observed between the results obtained in the two sera. The initial cell concentration was 5×10^4 mL^{-1}. For the thymidine protection experiments the L1210 cells were coincubated with the compounds at concentrations of 10 times the IC_{50} values and 10 μM thymidine. All cell counts were performed with a Model ZM Coulter counter. The cell-doubling times for L1210, L1210:R7A, and L1210:1565 were 12, 14, and 24 h, respectively.

Results and Discussion

The IC_{50} values for the inhibition of partially purified L1210 TS and for growth inhibition of L1210 cells are shown in Table I. The introduction of fluorine into the 2'-position of **1** to give **2** resulted in a 2-fold improvement in TS inhibitory potency. This improvement was also observed upon fluorination of the 2-desamino (**3** vs **4**), 2-methyl (**5** vs **6**), 2-hydroxymethyl (**7** vs **8**), and 2-methoxy (**9** vs **10**) analogues of **1**. Compounds **3**–**6** were also tested against human TS, and a similar pattern was observed (data not shown). One possible explanation for the beneficial influence of the 2'-fluoro substituent on TS inhibition is that a hydrogen bond between the 2'-fluorine atom and the amide hydrogen atom holds this part of the molecule in a more favorable conformation for binding to TS. This was evidenced by ^1H NMR spectroscopy where a spin–spin coupling value of up to 6.4 Hz between these two atoms was observed. Fluorination in the 2'-position also improved the inhibition of L1210 cell growth for all the compounds tested (between 1.5- and 5-fold). The compounds were examined for their intracellular locus of action. The inhibition of L1210 cell growth was prevented by the co-incubation with thymidine, suggesting that TS remains the locus after the introduction of fluorine into

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(30) Synonyms: NSC 339638; 5,6-dihydro-6-(3,6,13-trihydroxy-3-methyl-4-phosphonoxy-1,7,9,11-tridecatetraenyl)-2*H*-pyran-2-one monosodium salt.
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(32) Flow Laboratories, Irvine, Scotland, U.K.

the 2′-position. Consistent with this was the fact that the 2′-fluoro compounds tested against the DHFR overproducing L1210:R7A cell line have retained or improved activity over their equivalent nonfluorinated parent compounds. The mutant human W1L2:C1 cell line with acquired resistance to **5** due to TS gene amplification[33] was cross resistant to all the compounds tested (**1**, **3**, **4**, **6**) (data not shown).

The activities of compounds **1–10** were determined in the L1210:1565 cell line (impaired reduced folate transport). Compounds **1–8** had IC_{50} values ranging between 1 and 21 μM. However the relative resistance values were very much determined by the substituent in the 2-position. As previously reported,[18,19] no cross resistance was observed to **1**, suggesting that this compound does not use the reduced folate carrier in L1210 cells. However the removal of the 2-amino group to give compound **3** or its replacement by a methyl group to give **5** resulted in a greater degree of resistance in the L1210:1565 cell line (10- and 100-fold, respectively).[18,19] We therefore concluded that the greater potency of **3** and **5** over **1** was due to their improved affinity for the reduced folate transport system. We have now extended this study to compounds **7**[15] and **9**.[16] The low degree of cross resistance observed for **7** suggests that the poor potency of this 2-hydroxymethyl analogue in L1210 culture is due to a poor affinity for the reduced folate carrier. The 2-methoxy compound **9** is interesting in that although the cross-resistance value is high (84-fold), the actual concentration of the compound required to give an IC_{50} value in the L1210:1565 cells is substantially higher than for the other compounds. We conclude that **9** is able to utilize the reduced folate carrier but is poorly transported via the second mechanism that must operate for the other compounds. Fluorination in the 2′-position gave a similar resistance pattern to the nonfluorinated compounds although, with the exception of **6**, the relative resistance was reduced by about half. This is attributed to a greater degree of improvement in cell growth inhibition for the L1210:1565 line when compared with a smaller degree of improvement with the L1210 line. This may suggest that the 2′-fluoro analogues are marginally better transported via the second uptake mechanism.

In summary, the fluorination of the 2′-position of N^{10}-propargyl-5,8-dideazafolic acid and of its 2-desamino and some of its 2-desamino-2-substituted analogues results in more active compounds both as inhibitors of TS and as inhibitors of cell growth. The intracellular locus of action remains as TS, and generally the 2′-fluoro analogues have enhanced activity against two mutant L1210 cell lines, one of which has impaired uptake of reduced folates.

Experimental Section

The general procedures used were described in earlier papers[16,24] in this series.

2-Fluoro-4-nitrobenzoic Acid (12). Powdered 2-fluoro-4-nitrotoluene (**11**) (31.03 g, 0.2 mol) was added in portions to a stirred solution of $Na_2Cr_2O_7$ (80.46 g, 0.26 mol) in HOAc (400 mL). Concentrated H_2SO_4 (200 g) was added over a period of 10 min. The initially bright orange solution became black and viscous, and an exotherm to 100 °C was observed. The resulting slurry was heated at 90 °C for 1 h. H_2O (236 mL) was then added and the resulting solution cooled to 0 °C to give pale green crystals, which were collected by filtration and washed sparingly with cold water. The product was recrystallized from H_2O (200 mL) to yield pale green microneedles: 21.16 g (57%); mp 175–178 °C (lit.[26] mp 176–177 °C).

Diethyl *N*-(2-Fluoro-4-nitrobenzoyl)-L-glutamate (13). A slurry of **12** (426 mg, 2.3 mmol) in dry toluene (10 mL) was stirred with $SOCl_2$ (410 mg, 3.45 mmol) under reflux for 1.5 h. The cooled solution was filtered through Celite and concentrated to give the acid chloride as a viscous brown oil which solidified on standing. A solution of this acid chloride in CH_2Cl_2 (50 mL) was added over 15 min to a stirred mixture of diethyl L-glutamate hydrochloride (550 mg, 2.3 mmol) and Et_3N (634 μL, 4.6 mmol) in CH_2Cl_2 (100 mL) below 25 °C under N_2. The resulting brown solution was stirred for 2 h, washed with H_2O (2 × 250 mL), treated with charcoal, dried, filtered, and evaporated to a solid which was recrystallized from toluene–cyclohexane to afford white needles: 730 mg (86%); mp 80–81 °C; NMR ($CDCl_3$) δ 1.25, 1.3 (2 t, 6 H, 2 OCH_2CH_3), 2.3 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.5 (t, 2 H, $CHCH_2CH_2CO_2Et$), 4.15, 4.25 (2 q, 4 H, 2 OCH_2CH_3), 4.85 (m, 1 H, CH), 7.5 (br t, 1 H, CONH), 8.05 (dd, 1 H, $J_{3,5}$ = 2.1 Hz, $J_{3,F}$ = 10.9 Hz, Ar 3-H), 8.15 (dd, 1 H, $J_{5,3}$ = 2.1 Hz, $J_{5,6}$ = 8.6 Hz, Ar 5-H), 8.25 (dd, 1 H, $J_{6,5}$ = 8.6 Hz, $J_{6,F}$ = 7.4 Hz, Ar 6-H). Anal. ($C_{16}H_{19}FN_2O_7$) C, H, N, F.

Diethyl *N*-(4-Amino-2-fluorobenzoyl)-L-glutamate (14). A solution of **13** (9.26 g, 25 mmol) in HOAc (50 mL) was stirred with iron powder (6.14 g, 0.11 g-atom) at 50 °C for 2 h. The mixture was cooled to 25 °C, partitioned between CH_2Cl_2 (250 mL) and H_2O (1 L), and filtered to remove iron residues. The CH_2Cl_2 solution was washed with a second portion of H_2O, treated with charcoal, dried, filtered, and evaporated to dryness. The residue was recrystallized from toluene–hexane to give fluffy off-white crystals: 8.12 g (95%); mp 129–131 °C; NMR ($CDCl_3$) δ 1.25, 1.3 (2 t, 6 H, 2 OCH_2CH_3), 2.2 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.45 (t, 2 H, $CHCH_2CH_2CO_2Et$), 4.1, 4.25 (2 q, 4 H, 2 OCH_2CH_3), 4.25 (br s, 2 H, NH_2), 4.85 (m, 1 H, CH), 6.35 (dd, 1 H, Ar 3-H), 6.45 (dd, 1 H, Ar 5-H), 7.2 (dd, 1 H, CONH), 7.8 (dd, 1 H, Ar 6-H); MS (EI) *m/z* 340 $[M]^+$. Anal. ($C_{16}H_{21}FN_2O_5$) C, H, N, F.

The alternative method for the reduction of **13** to **14** (H_2, 10% Pd–C) is described in ref 34.

Diethyl *N*-[2-Fluoro-4-(prop-2-ynylamino)benzoyl]-L-glutamate (15). A mixture of **14** (7.49 g, 22 mmol), propargyl bromide (4.9 mL of an 80% solution in toluene, 44 mmol), and K_2CO_3 (3.04 g, 22 mmol) in DMF (200 mL) was stirred for 3 h at 110–120 °C under argon. The cooled reaction mixture was evaporated to dryness. The residue was partitioned between EtOAc (250 mL) and H_2O (250 mL). The organic phase was dried and evaporated to dryness. The crude product was purified by chromatography using a gradient of 30–40% EtOAc in hexane as eluent. The product (5.03 g, 60%) was isolated as a pale yellow oil: NMR ($CDCl_3$) δ 1.25, 1.3 (2 t, 6 H, 2 OCH_2CH_3), 2.2 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.25 (t, 1 H, C≡CH), 2.45 (t, 2 H, $CHCH_2CH_2CO_2Et$), 3.95 (br s, 2 H, $CH_2C{\equiv}C$), 4.1, 4.2 (2 q, 4 H, 2 OCH_2CH_3), 4.55 (br s, 1 H, amine NH), 4.85 (m, 1 H, CH), 6.35 (dd, 1 H, Ar 3-H), 6.5 (dd, 1 H, Ar 5-H), 7.2 (dd, 1 H, CONH), 7.9 (dd, 1 H, Ar 6-H); MS (EI) *m/z* 378 $[M]^+$. Anal. ($C_{19}H_{23}FN_2O_5$) C, H, N, F.

Diethyl *N*-[4-[*N*-[(2-Amino-3,4-dihydro-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]-L-glutamate (17). A mixture of **15** (1.04 g, 2.75 mmol), the (bromomethyl)quinazolinone **16**[35] (838 mg, 2.5 mmol) and powdered $CaCO_3$ (500 mg, 5 mmol) in DMA (8 mL) was stirred for 48 h at 55 °C under argon. The cooled mixture was filtered through Celite and the filtrate was evaporated to dryness. The crude product was purified by chromatography using a 5% v/v EtOH in CH_2Cl_2 as eluent to give a pale yellow amorphous solid: 170 mg (51%); mp 133–136 °C; NMR (Me_2SO-d_6) δ 1.15, 1.2 (2 t, 6 H, 2 OCH_2CH_3), 2.0 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.4 (t, 2 H, $CHCH_2CH_2CO_2Et$), 3.25 (t, 1 H, C≡CH), 4.05, 4.1 (2 q, 4 H, 2 OCH_2CH_3), 4.3 (br s, 2 H, $CH_2C{\equiv}C$), 4.4 (m, 1 H, CH), 4.7 (s, 2 H, $ArCH_2N<$), 6.3 (br s, 2 H, NH_2), 6.65 (dd, 1 H, 3′-H), 6.7 (dd, 1 H, 5′-H), 7.15 (d, 1 H, quinazoline 8-H), 7.5 (dd, 1 H,

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quinazoline 7-H), 7.5 (dd, 1 H, 6′-H), 7.8 (d, 1 H, quinazoline 5-H), 8.1 (dd, 1 H, CONH), 10.95 (br s, 1 H, quinazoline 3-H). Anal. ($C_{28}H_{30}FN_5)_6 \cdot 0.5H_2O$) C, H, N, F.

***N*-[4-[*N*-[(2-Amino-3,4-dihydro-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]-L-glutamic Acid (2).** The diester **17** (629 mg, 1.1 mmol) was stirred for 13 h under argon in a mixture of 1 N aqueous NaOH (3.75 mL, 3.75 mmol), EtOH (19 mL), and H_2O (19 mL). The resulting solution was filtered through Celite into a centrifuge tube and brought to pH 3.5 with 2 N aqueous HCl. The white gelatinous precipitate was isolated by centrifugation at 2500 rpm for 30 min and freed from inorganic ions by three cycles of aqueous suspension–centrifugation–decantation. The damp product was vacuum dried to give an amorphous solid: 268 mg (48%); mp 233–236 °C; NMR (Me_2SO-d_6) δ 1.95 (m, 2 H, $CHCH_2CH_2CO_2H$), 2.3 (t, 2 H, $CHCH_2CH_2CO_2H$), 3.25 (t, 1 H, C≡CH), 4.3 (br s, 2 H, $CH_2C{\equiv}C$), 4.35 (m, 1 H, CH), 4.7 (s, 2 H, $ArCH_2N<$), 6.4 (br s, 2 H, NH_2), 6.65 (dd, 1 H, 3′-H), 6.7 (dd, 1 H, 5′-H), 7.15 (d, 1 H, quinazoline 8-H), 7.5 (dd, 1 H, quinazoline 7-H), 7.55 (dd, 1 H, 6′-H), 7.8 (d, 1 H, quinazoline 5-H), 7.95 (dd, 1 H, CONH), 11.1 (br s, 1 H, quinazoline 3-H). Anal. ($C_{24}H_{22}FN_5O_6 \cdot H_2O$) C, H, N, F.

Diethyl *N*-[4-[*N*-[[3,4-Dihydro-4-oxo-3-[(pivaloyloxy)methyl]-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]-L-glutamate (19). A mixture of **15** (2.00 g, 5.3 mmol), the bromomethyl compound **18**[13] (1.77 g, 5 mmol), and powdered $CaCO_3$ (1.06 g, 10.6 mmol) in DMA (17 mL) was stirred for 18 h at 60 °C under argon. The cooled mixture was filtered and the filtrate was evaporated to dryness. The crude product was purified by chromatography using 10% EtOAc in CH_2Cl_2 as eluent to give a white glass: 2.92 g (90%); NMR (Me_2SO-d_6) δ 1.1 (s, 9 H, *t*-Bu), 1.15, 1.2 (2 t, 6 H, 2 OCH_2CH_3), 2.0 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.4 (t, 2 H, $CHCH_2CH_2CO_2Et$), 3.25 (t, 1 H, C≡CH), 4.05, 4.1 (2 q, 4 H, 2 OCH_2CH_3), 4.4 (br s, 2 H, $CH_2C{\equiv}C$), 4.4 (m, 1 H, CH), 4.85 (br s, 2 H, $ArCH_2N<$), 5.9 (s, 2 H, OCH_2N), 6.65 (dd, 1 H, 3′-H), 6.67 (dd, 1 H, 5′-H), 7.5 (dd, 1 H, 6′-H), 7.7 (d, 1 H, quinazoline 8-H), 7.8 (dd, 1 H, quinazoline 7-H), 8.1 (d, 1 H, quinazoline 5-H), 8.15 (dd, 1 H, CONH), 8.45 (s, 1 H, quinazoline 2-H); MS (EI) *m/z* 650 $[M]^+$. Anal. ($C_{34}H_{39}FN_4O_8$) C, H, N, F.

***N*-[4-[*N*-[(3,4-Dihydro-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]-L-glutamic Acid (4).** The pivaloyloxy diester **19** (1.94 g, 3.0 mmol) was stirred for 24 h under argon in a mixture of 1 N aqueous NaOH (14.9 mL, 14.9 mmol), EtOH (53 mL), and H_2O (53 mL). The resulting solution was filtered through Celite and brought to pH 3.2 with 2 N aqueous HCl. The precipitate was filtered off, washed with H_2O, and vacuum dried to give a pale yellow amorphous solid: 1.24 g (82%); mp 185–188 °C; NMR (Me_2SO-d_6) δ 2.0 (m, 2 H, $CHCH_2CH_2CO_2H$), 2.3 (t, 2 H, $CHCH_2CH_2CO_2H$), 3.25 (t, 1 H, C≡CH), 4.35 (br s, 2 H, $CH_2C{\equiv}C$), 4.35 (m, 1 H, CH), 4.8 (s, 2 H, $ArCH_2N<$), 6.65 (dd, 1 H, 3′-H), 6.7 (dd, 1 H, 5′-H), 7.55 (dd, 1 H, 6′-H), 7.65 (d, 1 H, quinazoline 8-H), 7.75 (dd, 1 H, quinazoline 7-H), 7.95 (dd, 1 H, CONH), 8.0 (d, 1 H, quinazoline 5-H), 8.05 (s, 1 H, quinazoline 2-H), 12.25 (br s, 1 H, quinazoline 3-H). Anal. ($C_{24}H_{21}FN_4O_6 \cdot H_2O$) C, H, N, F.

***N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]-L-glutamic Acid (6).** The synthesis of **6** is described in detail in ref 34.

***N*-[4-[*N*-[[3,4-Dihydro-2-(hydroxymethyl)-4-oxo-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]-L-glutamic Acid (8).** A mixture of **15** (800 mg, 2.12 mmol), 2-(acetoxymethyl)-6-(bromomethyl)-3,4-dihydro-4-oxoquinazoline[15] (660 mg, 2.12 mmol), and 2,6-lutidine (500 μL, 4.3 mmol) in DMF (5 mL) was stirred for 24 h at 80 °C under argon. The cooled reaction mixture was partitioned between EtOAc (2 × 20 mL) and H_2O (8 mL) containing 5 M aqueous H_2SO_4 (500 μL). The EtOAc solution was washed repeatedly with H_2O until the washings had pH >6. The organic solution was dried and evaporated to dryness. The crude product was purified by chromatography using a gradient of 0–10% EtOAc in CH_2Cl_2 as eluent. The resulting triester, diethyl *N*-[4-[*N*-[2-(acetoxymethyl)-3,4-dihydro-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]-L-glutamate (150 mg, 12%), was stirred for 4 h under argon in 1 N aqueous NaOH (2.5 mL, 2.5 mmol). The resulting solution was filtered into a centrifuge tube and brought to pH 3.0 with 2 N aqueous HCl. The amorphous off-white precipitate was isolated by centrifugation, washed with H_2O, and vacuum dried: 60 mg (46%); softened above 110 °C and slowly decomposed between 110 and 150 °C; NMR (Me_2SO-d_6) δ 2.0 (m, 2 H, $CHCH_2CH_2CO_2H$), 2.35 (t, 2 H, $CHCH_2CH_2CO_2H$), 3.2 (t, 1 H, C≡CH), 4.35 (d, 2 H, $CH_2C{\equiv}C$), 4.4 (m, 1 H, CH), 4.45 (br s, 2 H, CH_2OH), 4.8 (br s, 2 H, $ArCH_2N<$), 6.65 (dd, 1 H, 3′-H), 6.7 (dd, 1 H, 5′-H), 7.55 (dd, 1 H, 6′-H), 7.55 (d, 1 H, quinazoline 8-H), 7.7 (dd, 1 H, quinazoline 7-H), 7.95 (d, 1 H, quinazoline 5-H), 8.0 (dd, 1 H, CONH); MS (FAB) *m/z* 509 $[M - H]^-$. Anal. ($C_{25}H_{23}FN_4O_7 \cdot 1.25H_2O$) C, H, N.

***N*-[4-[*N*-[(3,4-Dihydro-2-methoxy-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]-L-glutamic Acid (10).** Compound **10** was prepared by the method[16] described for the synthesis of the desfluoro analogue **9** from **15** (1.14 g, 3.04 mmol) and 6-(bromomethyl)-2,4-dimethoxyquinazoline[16] (2.55 g, 9.0 mmol): 132 mg (8.5%); mp 215–217 °C; NMR (Me_2SO-d_6) δ 2.0 (m, 2 H, $CHCH_2CH_2CO_2H$), 2.3 (t, 2 H, $CHCH_2CH_2CO_2H$), 3.2 (t, 1 H, C≡CH), 3.95 (s, 3 H, OCH_3), 4.3 (br s, 2 H, $CH_2C{\equiv}C$), 4.4 (m, 1 H, CH), 4.75 (br s, 2 H, $ArCH_2N<$), 6.65 (dd, 1 H, 3′-H), 6.7 (dd, 1 H, 5′-H), 7.45 (d, 1 H, quinazoline 8-H), 7.55 (dd, 1 H, 6′-H), 7.65 (dd, 1 H, quinazoline 7-H), 7.9 (br, 1 H, CONH), 7.92 (d, 1 H, quinazoline 5-H); MS (FAB) *m/z* 509 $[M - H]^-$. Anal. ($C_{25}H_{23}FN_4O_7$) C, H, N.