Quinazoline Antifolate Thymidylate Synthase Inhibitors: Bridge Modifications and Conformationally Restricted Analogues in the C2-Methyl Series

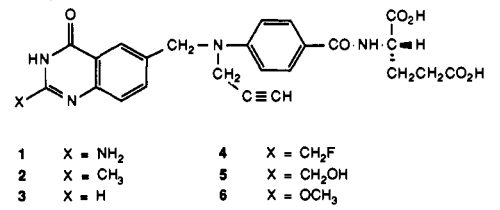
Peter R. Marsham,*,† Ann L. Jackman,‡ Anthony J. Hayter,† Melanie R. Daw,† Jayne L. Snowden,† Brigid M. O'Connor,‡ Joel A. M. Bishop,‡ A. Hilary Calvert,‡,§ and Leslie R. Hughes†

ICI Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, England, and Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey SM2 5NG, England. *Received October 10, 1990*

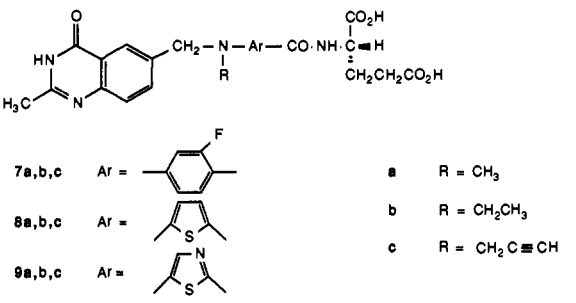
Several C2-methylquinazoline-based antifolates (14–26) have been prepared in which the C9,N10 bridge has been replaced by the reversed N9,C10 unit. This series was extensively studied by incorporating further substituents at N9 and C10 as well as by modifications to the *p*-aminobenzoate ring. The C2-methylquinazoline analogues 29, 30, and 31 containing the methyleneoxa, methylenethia, and thia bridge units were also synthesized. In general these isosteric replacements of the bridge unit in the parent C2-methyl-N10-propargylquinazoline antifolate 2 were much less potent as inhibitors of isolated thymidylate synthase (TS) but several were at least as potent as inhibitors of L1210 cell growth in culture. The fusion of the *p*-aminobenzoate ring into the bicyclic systems 75 and 76 also reduced activity against TS but again gave highly cytotoxic compounds. The cytotoxicities were largely prevented by thymidine, confirming that TS is the major locus.

Introduction

Several recent investigations have demonstrated that quite diverse structural modifications to the folic acid molecule can produce potent antitumor agents that act through inhibition of thymidylate synthase (TS, EC 2.1.1.45) alone with no effect on other folate-dependent enzymes. One of these agents, the quinazoline-based antifolate N10-propargyl-5,8-dideazafolic acid (1)[1,2] is a potent inhibitor of mouse[1] and human[3] TS which has shown potent antitumor activity in vitro,[1,3] in animal models,[4] and in the clinic.[5–7] However its renal toxicity and hepatotoxicity[8] cause its eventual withdrawal from the clinic and stimulated the search for TS inhibitors that would be as effective as 1 as antitumor agents but without the unacceptable toxic effects which result from its poor aqueous solubility.[9,10] We have recently reported[11–16] a number of modifications to the structure of 1 that have given potent antitumor agents that are considerably more water soluble than 1 and have the inhibition of TS as their sole locus of action, as confirmed by protection experiments with thymidine. The C2-methyl analogue 2[13] was the most potent antitumor agent of a series where the C2-amino group of 1 was replaced by small lipophilic substituents, although hydrogen (3),[11] fluoromethyl (4),[13] hydroxymethyl (5),[13] and methoxy (6)[12] C2 substituents also gave potent compounds. Studies[17] with 2–5 in mice have confirmed our hypothesis that more soluble TS inhibitors would lack the hepatic and renal toxicity of 1. More recently we have discovered[14–16] that the antitumor activity in the C2-methyl series is enhanced even further when the *p*-aminobenzoate moiety either contains fluorine substituents or is replaced by certain heterocyclic rings. Indeed the best compounds **7a–c**,[15] **8a–c**,[16] and **9a–c**[16] showed enhancements of 50–500-fold in potency over 1 as inhibitors of L1210 cell growth. Of particular interest from a structure–activity point of view is the observation that in these benzene ring variants the N10-propargyl group no longer gives significantly better inhibitory activity than N10-methyl or N10-ethyl against isolated L1210 TS or against L1210 cell growth. Indeed in the thiophene (8) and thiazole (9) containing molecules the smaller N10 substituents confer enhanced potency over propargyl as inhibitors of L1210 cell growth in culture.[16] These observations suggested that it would be of interest to investigate alternative bridge



1 X = NH_2 4 X = CH_2F
2 X = CH_3 5 X = CH_2OH
3 X = H 6 X = OCH_3



7a,b,c Ar = 8a,b,c Ar = 9a,b,c Ar =

a R = CH_3
b R = CH_2CH_3
c R = $CH_2C{\equiv}CH$

units between the 2-methylquinazolinone and the benzoylglutamate moiety. A number of isosteric replacements

(1) Jones, T. R.; Calvert, A. H.; Jackman, A. L.; Brown, S. J.; Jones, M.; Harrap, K. R. *Eur. J. Cancer* 1981, *17*, 11.
(2) Synonyms: ICI 155387, CB 3717, NSC 327182, *N*-[4-[*N*-[(2-amino-3,4-dihydro-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-glutamic acid.
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(6) Calvert, A. H.; Newell, D. R.; Jackman, A. L.; Gumbrell, L. A.; Sikora, E.; Grzelakowska-Sztabert, B.; Bishop, J. A. M.; Judson, I. R.; Harland, S. J.; Harrap, K. R. *NCI Monogr.* 1987, *5*, 213.
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(11) Jones, T. R.; Thornton, T. J.; Flinn, A.; Jackman, A. L.; Newell, D. R.; Calvert, A. H. *J. Med. Chem.* 1989, *32*, 847.

† ICI Pharmaceuticals.
‡ Institute of Cancer Research.
§ Present address: Cancer Research Unit, Medical School, Framlington Place, Newcastle upon Tyne NE2 4HH, England.

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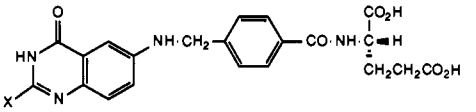
of the CH_2N bridge have been synthesized by Hynes and his colleagues in the C2-amino series[18-20] and more recently in the C2-desamino series.[21] The prototype in this class IAHQ (13)[18,22] has the reversed N9,C10 bridge between the two aromatic rings rather than the normal C9,N10 configuration. IAHQ is a potent cytotoxic agent in a number of cell lines[23,24] despite having only 1% of the potency of 1 as an inhibitor of TS.[21] As with 1 and its C2-methyl analogues, thymidine alone protects against this cytotoxicity and there is now considerable evidence[23,24] that IAHQ is extensively metabolized intracellularly to poly-γ-glutamate derivatives that are the potent TS inhibitors responsible for the cytotoxic activity. The isosteric 10-oxa (27) and 10-thia (28) analogues were also highly cytotoxic to L1210 cells and effective substrates for mammalian folylpolyglutamate synthetase (FPGS).[21] In general the C2-desamino derivatives of these C9,N10 isosteres are 3–6-fold more cytotoxic than their C2-amino counterparts despite being less potent against TS itself.[21] We were encouraged by these reports to incorporate isosteric replacements for the bridge region into some of our recent quinazoline antifolate structures where C2-methyl and benzoyl ring modifications have given extremely potent cytotoxic agents.

We have also studied the effect of incorporating the *p*-aminobenzoate moiety into bicyclic ring systems. This would have the effect of restricting the conformations available to whichever of the two substituents on this central benzene ring is incorporated into the bicycle. It may even have the effect of forcing a substituent into a conformation which would normally be unfavorable but which may be advantageous for interaction with the TS enzyme. For instance the incorporation of a chlorine substituent into the 3′-position (i.e. ortho to the propargylamino substituent) reduces the affinity for TS by approximately 150-fold.[25] This bulky 3′-Cl atom would strongly disfavor the ability of the tertiary amine to adopt a conformation in which it eclipses the benzene ring. Such an eclipsed conformation may be sterically preferred for interaction with the enzyme, but it also has the subsidiary effect of allowing the lone pair of electrons on N10 to delocalize into the ring. A similarly favorable effect could apply if the glutamate nitrogen atom were locked into an eclipsed conformation. In the 2′-F series[14,15] there is NMR evidence that such an eclipsed conformation is favored and these molecules show enhanced inhibition of TS.

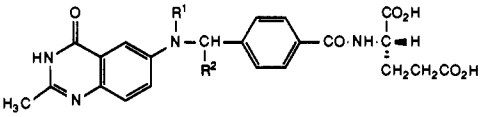
Chemistry

The overall strategy for the synthesis of the reversed bridge compounds 14–26 was the sequential alkylation of 6-amino-2-methylquinazolinone (33)[26] with the appropriate alkyl halides in *N,N*-dimethylformamide (DMF) using $CaCO_3$ to scavenge HBr (Scheme I). The reduction of the nitro compound 32 by hydrogen over 10% Pd–C gave 33



13 X = NH_2

14 X = CH_3

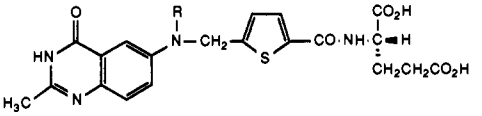


15 R^1 = CH_3 R^2 = H

16 R^1 = $CH_2C{\equiv}CH$ R^2 = H

25 R^1 = H R^2 = CH_3

26 R^1 = CH_3 R^2 = CH_3



17 R = H

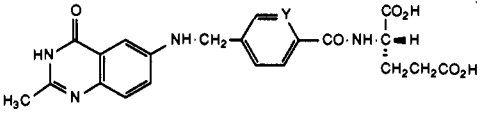
18 R = CH_3

19 R = CH_2CH_3

20 R = CH_2CH_2F

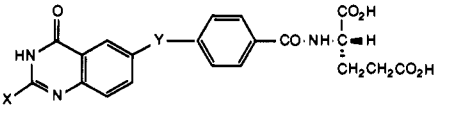
21 R = CH_2CH_2OH

22 R = $CH_2CH{=}CH_2$



23 Y = N

24 Y = CF



27 X = NH_2 Y = CH_2O

28 X = NH_2 Y = CH_2S

29 X = CH_3 Y = CH_2O

30 X = CH_3 Y = CH_2S

31 X = CH_3 Y = S

in high yield and was more convenient than the literature procedure.[26] The second component required for the synthesis of 14 was the [(chloromethyl)benzoyl]glutamate diester 35, which was isolated when 4-(bromomethyl)-

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(22) Synonyms: H-338, NSC 289517, *N*-[4-[[*N*-(2-amino-3,4-dihydro-4-oxo-6-quinazolinyl)amino]methyl]benzoyl]-L-glutamic acid.
(23) Fernandes, D. J.; Bertino, J. R.; Hynes, J. B. *Cancer Res.* **1983**, *43*, 1117.
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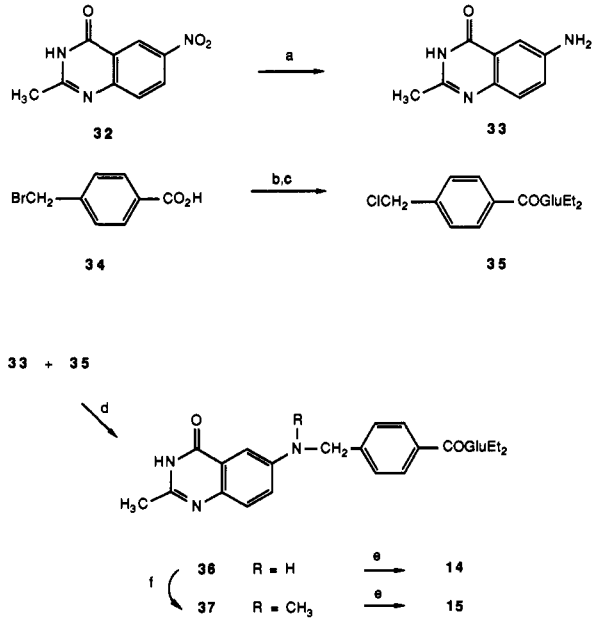
Table I. Preparation of the Quinazoline Glutamate Esters

compd[a]	bridge unit	aromatic ring	method	temp, °C	% yield
36	$-NHCH_2-$	1,4-benzene	A	86	48
37	$-N(CH_3)CH_2-$	1,4-benzene	C	65	11
	$-N(CH_2C{\equiv}CH)CH_2-$	1,4-benzene	A	100	28
42	$-NHCH_2-$	2,5-thiophene	G	0→20	*b*
45	$-N(CH_3)CH_2-$	2,5-thiophene	G	0→20	55
	$-N(CH_2CH_3)CH_2-$	2,5-thiophene	G	0→20	78
	$-N(CH_2CH_2F)CH_2-$	2,5-thiophene	G	0→20	95
	$-N(CH_2CH_2OH)CH_2-$	2,5-thiophene	G	0→20	60
48	$-N(CH_2CH{=}CH_2)CH_2-$	2,5-thiophene	G	0→20	51
	$-NHCH_2-$	2,5-pyridine	G	0→20	90
	$-NHCH_2-$	2-F-1,4-benzene	G	0→20	*b*
	$-NHCH(CH_3)-$	1,4-benzene	G	0→20	48
	$-N(CH_3)CH(CH_3)-$	1,4-benzene	G	0→20	72
	$-CH_2O-$	1,4-benzene	G	0→20	72
67	$-CH_2S-$	1,4-benzene	I	20	46
	$-S-$	1,4-benzene	G	0→20	31

[a] Compounds not numbered were prepared by the same methods as those shown in the appropriate schemes. All the antifolate diesters had NMR spectra consistent with the assigned structures. [b] The pure diesters were not isolated.

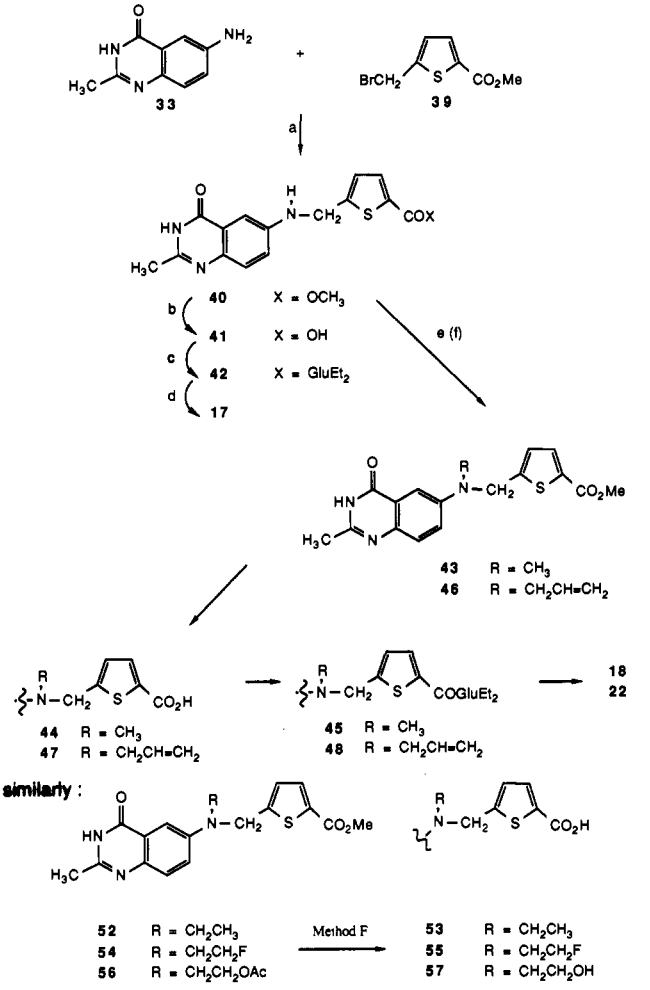
Scheme I[a]



[a] (a) H_2, 10% Pd–C, MeOH; (b) $(COCl)_2$, DMF, CH_2Cl_2; (c) diethyl glutamate·HCl, 2,6-lutidine, CH_2Cl_2; (d) $CaCO_3$, DMF (method A); (e) 1 N aqueous NaOH, EtOH (method B); (f) MeI, $CaCO_3$, DMF (method C).

benzoyl chloride was condensed with diethyl glutamate. Condensation of **33** and **35** (method A; Table I) afforded the diethyl ester **36**, which on saponification with 1 N aqueous NaOH in EtOH (method B) yielded the target 2-methyl-5,8-dideazaisofolate **14**. Direct methylation of **36** to the precursor **37** of the N9-methyl analogue **15** could be achieved with MeI–$CaCO_3$ (method C) but only in 11% yield. For the N9-propargyl compound **16** the preferred method was to make the monopropargylated derivative **38** (method D, 84%) and to react this with **35** according to method A. This approach failed for **15** as the direct methylation of **33** with MeI gave only the *N,N*-dimethyl derivative. The thiophene analogues **17–22** were all prepared with use of the (bromomethyl)thiophene ester **39**[27] as the alkylating agent (Scheme II). Condensation of **39** and **33** (method E) and saponification (method F) of the resulting ester **40** gave the thiophenecarboxylic acid derivative **41** in high yield. This was coupled via the in situ generated

Scheme II[a]

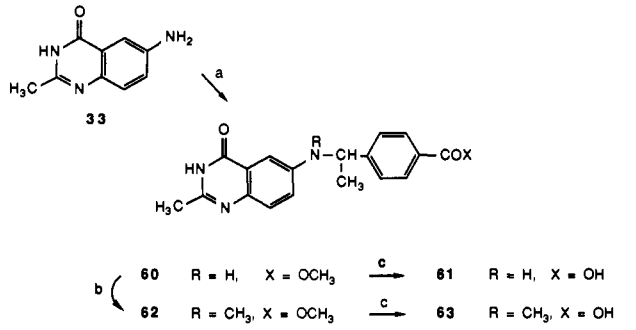


[a] (a) $CaCO_3$, DMF (method E); (b) 1 N aqueous NaOH, EtOH (method F); (c) diethyl glutamate·HCl, diphenyl phosphorazidate, Et_3N, DMF (method G); (d) 1 N aqueous NaOH, EtOH (method B); (e) CH_3I (method C); (f) allyl bromide (method C).

azide (method G) with diethyl glutamate to yield the diethyl ester (**42**) of **17**. All of the N9-alkyl analogues **18–22** were made with the glutamate incorporation as the penultimate step but the precursor monomethyl esters were assembled by two basic sequences: either direct alkylation of **40** (N9-methyl, N9-allyl; method C) or alkylation of **33** to **49–51** (method D) followed by condensation with **39** (N9-ethyl, N9-fluoroethyl, N9-acetoxyethyl; method A).

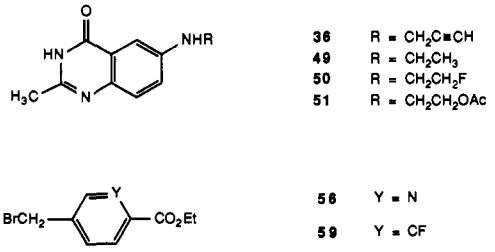
(27) Fujirebio Kabushiki Kaisha, Eur. Patent Appl. 214 823 A2, 1987.

Scheme III[a]

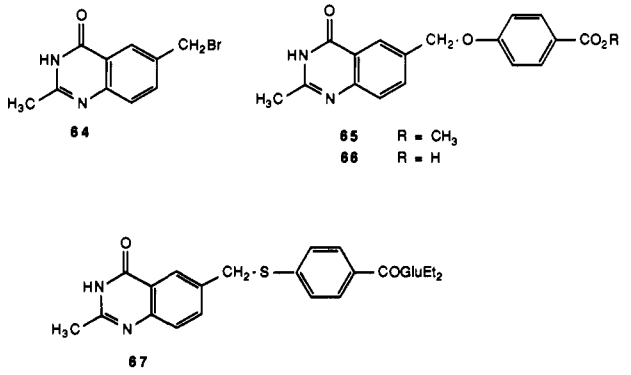


[a] (a) Methyl 4-(1-bromoethyl)benzoate, $CaCO_3$, DMF; (b) HCHO, $NaCNBH_3$, HOAc, MeCN, DMF; (c) 1 N aqueous NaOH, EtOH (method F).

The pyridine isostere **23** and the 2′-fluoro analogue **24** were synthesized exactly as for **17** starting from the bromomethyl compounds **58**[28] and **59**.[29] Two examples, **25** and **26**, of C10-methyl analogues were prepared as 10(*R*,*S*) mixtures essentially by the standard route (Scheme III). However the N9-methyl group was incorporated via the reductive methylation (aqueous HCHO, $NaCNBH_3$; method H) of **60** to afford **62**.

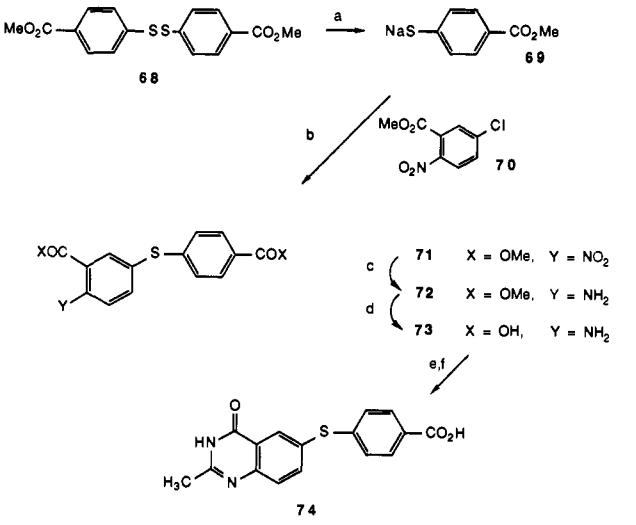


The 10-oxa (**29**) and 10-thia (**30**) isosteres of **2** were synthesized from the (bromomethyl)quinazolinone **64**[13] by approaches similar to those used by Hynes et al.[21] for the C2-desamino analogues. Condensation of **64** with methyl 4-hydroxybenzoate using $CaCO_3$ as base yielded **65**. For this reaction $CaCO_3$ is obviously inferior to the $CsHCO_3$ used by Hynes et al. as this latter reagent gives much higher yields of the ether derivative. Coupling of the derived carboxylic acid **66** to diethyl glutamate (method G) and saponification of the product yielded **29**. The sodium salt of diethyl (4-mercaptobenzoyl)-L-glutamate was generated and reacted with **64** according to the literature procedure[21] to give a modest (46%) yield of the diester **67**



of the 2-methyl-10-thia analogue **30** (method I). A de novo synthesis of the 2-methylquinazolinone system was required in the route to the direct thia-linked analogue **31** (Scheme IV). The sodium salt **69** of methyl 4-

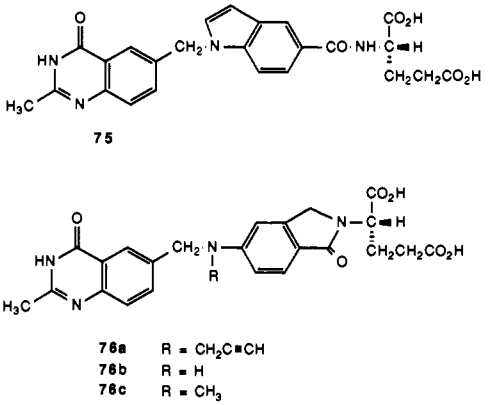
Scheme IV[a]



[a] (a) $NaBH_4$, DMA; (b) DMA; (c) Fe, $FeSO_4 \cdot 7H_2O$, MeOH; (d) 1 N aqueous NaOH, EtOH; (e) Ac_2O, reflux; (f) aqueous NH_3, 60 °C.

mercaptobenzoate was generated in situ in *N*,*N*-dimethylacetamide (DMA) from the disulfide diester **68** ($NaBH_4$) and reacted with methyl 5-chloro-2-nitrobenzoate (**70**) to afford the thioether **71**. Reduction of the nitro group of **71** (Fe, $FeSO_4$, MeOH) followed by saponification gave the amino diacid **73**. The anthranilic acid moiety of **73** readily underwent ring closure to the quinazoline **74** via the corresponding 2-methylbenzoxazinone on refluxing with Ac_2O followed by treatment with aqueous NH_3.[30] Final elaboration of **74** to **31** was achieved by using methods G and B.

The antifolate diacid **75** in which the nitrogen atom in the 10-position is incorporated into an indole ring system was prepared by the standard procedures (methods G and B) from the indole 5-carboxylic acid derivative **81**. The



synthesis of this key intermediate was achieved by N-alkylation of the sodium salt of methyl indole-5-carboxylate with the (bromomethyl)quinazolinone **79**[31] followed by saponification (Scheme V). The (pivaloyloxy)methyl group was used here to protect the quinazolinone N3-atom from alkylation under the anionic conditions of the coupling and was then conveniently removed during the hydrolysis of the methyl ester **80**. The location of the quinazolinylmethyl moiety on N1 of the indole in **81** was confirmed by the presence of a pair of doublets at δ 6.67 and 7.65 (J = 3.4 Hz, indole 3-H and 2-H, respec-

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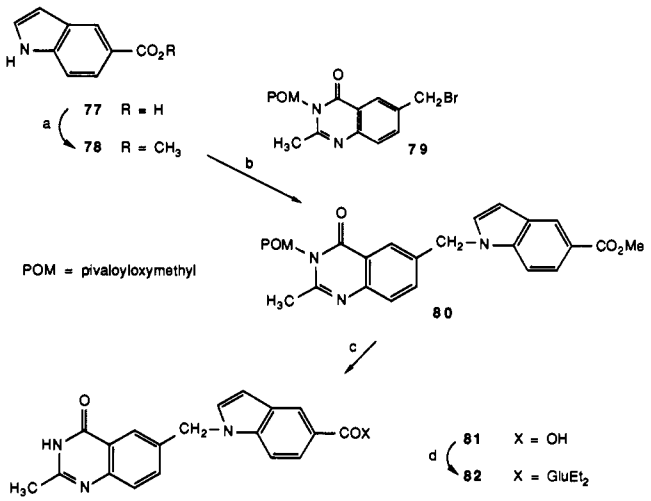
(31) Hughes, L. R. Eur. Patent Appl. 239 362 A2, 1987.

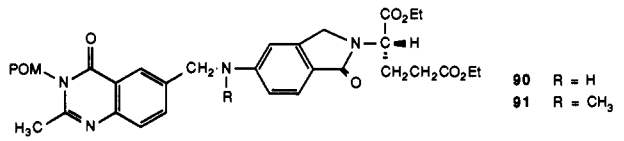
Scheme V[a]



[a] (a) MeI, $NaHCO_3$, DMF; (b) NaH, DMF; (c) 1 N aqueous NaOH, EtOH; (d) diethyl glutamate·HCl, diphenyl phosphorazidate, Et_3N, DMF (method G).

tively) in the 400-MHz NMR spectrum.

Three antifolates analogues **76a–c** were synthesized with the benzoate ring and the glutamic acid nitrogen atom incorporated into the phthalimidine system (Scheme VI). The required nucleus **85** was achieved in 50% overall yield when methyl 2-methyl-4-nitrobenzoate (**83**)[32] was brominated (NBS, CCl_4) and the product **84** treated with diethyl glutamate hydrochloride in the presence of K_2CO_3. The reduction of **85** to the aniline **86** was effected in almost quantitative yield by catalytic hydrogenation over 10% Pd–C. The N-propargylation of **86** (propargyl bromide, 2,6-lutidine) actually gave a 3:1 mixture of the required amine **87** and its *N,N*-dipropargyl derivative, but this mixture could be reacted with the bromomethyl compound **64** (method E) to afford **88**. Similarly, **86** was coupled with **64** to obtain **89**. The N10-methyl analogue **76c** in this series utilized the 3-(pivaloyloxy)methyl derivative **90**, which was efficiently converted to **91** under reductive methylation conditions (method H).



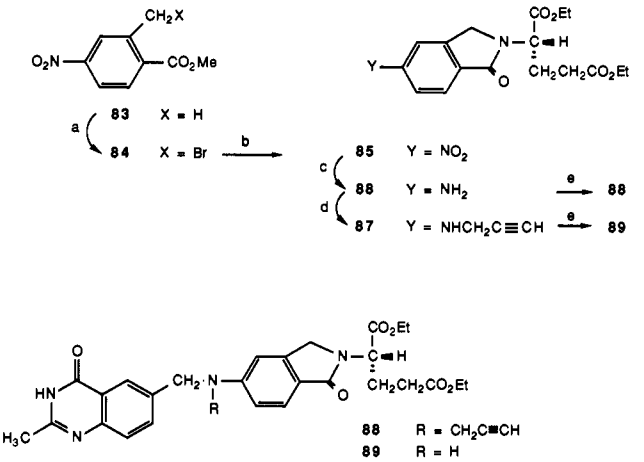
Results and Discussion

The IC_{50} values of compounds **14–26**, **29–31**, **75**, and **76a–c** for the inhibition of partially purified L1210 TS and for the growth inhibition of L1210 cells were obtained as described previously[14] and are shown in Tables II and III. In the C2-methyl isofolate series the unsubstituted analogue **14** has comparable potencies to its folate isomer **10** as an inhibitor of the isolated TS enzyme and of L1210 cell growth. However in the pair of *N*-methyl compounds **15** and **11** reversal of the bridge causes a 10-fold reduction in inhibition of TS despite the same level of potency of both compounds against L1210 cells. Reversing the bridge of the highly potent N10-propargyl compound **2** to give **16** causes an even more dramatic (>200-fold) decrease in TS affinity, which is accompanied by a 26-fold drop in potency against L1210 cells. The effects of these bridge modifications in the C2-methyl series on TS inhibition are qualitatively similar to those resulting from the corresponding modifications in the C2-amino series.[19,24] Also, as for IAHQ (**13**),[23] TS alone is the cytotoxic locus of action of these three C2-methyl reversed bridge analogues (**14–16**) since thymidine (10 μM) protects against L1210 growth inhibition at high concentrations (10 × IC_{50}) of each antifolate. The derivatives of **17** and **18** where the central benzene ring is replaced by a thiophene nucleus show comparable activity to **14** and **15** against TS but are ~2.5-fold more potent inhibitors of L1210 cell growth. Four further examples (**19–22**) were synthesized in the N9-alkyl reversed bridge thiophene series. Here there appears to be a steady drop in the affinity for TS as the size of the substituent on N9 increases although only the hydrophilic N9-hydroxyethyl group in **21** markedly diminishes the potency against L1210 cells. Replacement of the benzene ring of **14** with either a pyridine (**23**) or a 2′-fluorobenzene (**24**) has no significant effect on either property. Because of the well-established[13,33] preference of TS for small alkyl groups at N10 of the quinazoline antifolate we prepared two examples, **25** and **26**, of C10-methyl reversed bridge antifolates. Although this modification produces a slight (~2-fold) enhancement of affinity for TS, there is no improvement against the L1210 cells, indeed the C10-methylation (**25**) of the parent compound **24** decreases potency here by 10-fold.

The 10-oxa (**29**) and 10-thia (**30**) isosteres of **2** are also poor inhibitors of isolated TS but do show significant inhibition of cell growth. The high level of potency of the 10-oxa analogue **29** against L1210 cells (IC_{50} 0.04 μM) appears to be mainly due to a TS locus since thymidine will protect the cells from this growth inhibition. In contrast the analogue **31** with the direct sulfur link between the two aromatic rings has very poor cytotoxicity.

The above observations suggest that the inhibition of TS by these bridge-modified analogues is not the only factor in the observed cytotoxicities. Efficient transport into cells by the reduced folate/methotrexate carrier mechanism (RFC) and conversion of folylpolyglutamate synthetase to highly cytotoxic polyglutamylated species are probably involved and, in this class of antifolates, are particularly important. These factors are known to markedly affect the cytotoxic potencies of similar com-

Scheme VI[a]



[a] (a) NBS, $(PhCOO)_2$, CCl_4; (b) diethyl glutamate·HCl, K_2CO_3, DMA; (c) H_2, 10% Pd–C, EtOAc; (d) propargyl bromide, 2,6-lutidine, DMF, 80 °C; (e) bromomethyl compound **64** (method E).

(32) Peltier, D. *Bull. Soc. Sci. Bretagne* **1956**, *31*, 7; *Chem. Abstr.* **1958**, *52*, 9016h.

(33) Jones, T. R.; Calvert, A. H.; Jackman, A. L.; Eakin, M. A.; Smithers, M. J.; Betteridge, R. F.; Newell, D. R.; Hayter, A. J.; Stocker, A.; Harland, S. J.; Davies, L. C.; Harrap, K. R. *J. Med. Chem.* **1985**, *28*, 1468.

Table II. Preparation[a] and in Vitro Activities of Antifolate Diacids 14–26 and 29–31

compd	bridge unit	aromatic ring	% yield	mp, °C	formula[b]	mass spectra (FAB), m/z	inhibn of TS: IC_{50}, μM	inhibn of L1210 cell growth in culture: IC_{50}, μM	L1210 cell growth in the presence of dThd (% of control)
2[c]	$-CH_2N(CH_2C{\equiv}CH)-$	1,4-benzene		165[d]	$C_{25}H_{24}N_4O_6{\cdot}2H_2O$		0.04	0.09	
10[c]	$-CH_2NH-$	1,4-benzene		197–201	$C_{22}H_{22}N_4O_6{\cdot}H_2O$		4.5	0.07	
11[c]	$-CH_2N(CH_3)-$	1,4-benzene		254–257	$C_{23}H_{24}N_4O_6{\cdot}0.75H_2O$		0.25	0.11	
12[e]	$-CH_2NH-$	2,5-thiophene		183–189[d]	$C_{20}H_{20}N_4O_6S{\cdot}3H_2O$		24.8	3.00	
8a[e]	$-CH_2N(CH_3)-$	2,5-thiophene		180–184	$C_{21}H_{22}N_4O_6S{\cdot}H_2O$		0.67	0.007	
14	$-NHCH_2-$	1,4-benzene	70	193–196	$C_{22}H_{22}N_4O_6{\cdot}H_2O$	437[f]	8.28	0.07	93
15	$-N(CH_3)CH_2-$	1,4-benzene	44	167–170	$C_{23}H_{24}N_4O_6{\cdot}2H_2O$	451[f]	2.62	0.7	93
16	$-N(CH_2C{\equiv}CH)CH_2-$	1,4-benzene	64	172–176	$C_{25}H_{24}N_4O_6{\cdot}2H_2O$	475[f]	9.52	2.4	89
17	$-NHCH_2-$	2,5-thiophene	~20[h]	168–172	$C_{20}H_{24}N_4O_6S{\cdot}1.5H_2O$	443[f]	6.98	0.026	88
18	$-N(CH_3)CH_2-$	2,5-thiophene	81	158–161	$C_{21}H_{22}N_4O_6S{\cdot}1.8H_2O$	457[f]	3.82	0.3	–
19	$-N(CH_2CH_3)CH_2-$	2,5-thiophene	57	140–150[d]	$C_{22}H_{24}N_4O_6S{\cdot}0.75H_2O$	473[g]	8.42	0.66	94
20	$-N(CH_2CH_2F)CH_2-$	2,5-thiophene	75	147–150[d,i]	$C_{22}H_{23}FN_4O_6S{\cdot}2H_2O$	491[g]	9.90	0.38	95
21	$-N(CH_2CH_2OH)CH_2-$	2,5-thiophene	66	224–227	$C_{22}H_{24}N_4O_7S{\cdot}H_2O$[j]	489[g]	13.28	6.20	91
22	$-N(CH_2CH{=}CH_2)CH_2-$	2,5-thiophene	~55[h]	145–148	$C_{23}H_{24}N_4O_6S{\cdot}0.8NH_4HCO_3$	485[g]	14.12	0.54	95
23	$-NHCH_2-$	2,5-pyridine	14	182–185[k]	$C_{21}H_{21}N_5O_6{\cdot}2H_2O$	438[f]	16.66	0.06	–
24	$-NHCH_2-$	2-F-1,4-benzene	~30[h]	213–216	$C_{22}H_{21}FN_4O_6{\cdot}1.3H_2O$	455[f]	6.73	0.053	94
25	$-NHCH(CH_3)-$	1,4-benzene	82	227–229[d]	$C_{23}H_{24}N_4O_6{\cdot}1.3H_2O$	451[f]	4.30	0.80	93
26	$-N(CH_3)CH(CH_3)-$	1,4-benzene	59	165–175[l]	$C_{24}H_{26}N_4O_6{\cdot}H_2O$	465[f]	1.07	0.35	–
29	$-CH_2O-$	1,4-benzene	82	247–250[d]	$C_{22}H_{21}N_3O_7{\cdot}0.25H_2O$	438[f]	21.48	0.04	75
30	$-CH_2S-$	1,4-benzene	65	160–162	$C_{22}H_{21}N_3O_6S{\cdot}0.75H_2O$	454[f]	4.66	0.42	99
31	$-S-$	1,4-benzene	83	247–248	$C_{21}H_{19}N_3O_6S{\cdot}1.5H_2O$	440[f]	10.3	>20.	–

[a] All compounds were prepared by method B. [b] Anal. C, H, N except where stated otherwise. [c] See ref 13. [d] Decomposes at this temperature. [e] See ref 16. [f] $[M-H]^-$. [g] $[MH]^+$. [h] Prepared from the impure diethyl ester and purified by chromatography on DE52 DEAE-cellulose. [i] Softens >130 °C. [j] N: calcd, 11.0; found, 10.4. [k] Softens >158 °C. [l] Softens >160 °C.

Table III. Preparation[a] and in Vitro Activities of Antifolate Diacids 75 and 76a–c

compd	% yield	mp, °C	formula[b]	mass spectra (FAB), m/z $[M-H]^-$	inhibn of TS: IC_{50}, μM	inhibn of L1210 cell growth in culture: IC_{50}, μM	L1210 cell growth in the presence of dThd (% of control)
75	92	176–180	$C_{24}H_{22}N_4O_6{\cdot}1.67H_2O$	461	3.27	0.036	90
76a	92	201–205	$C_{26}H_{24}N_4O_6{\cdot}2.25H_2O$	487	0.15	0.17	96
76b	62	211–214	$C_{23}H_{22}N_4O_6{\cdot}1.5H_2O$	449	8.27	3.00	–
76c	80[c]	186–189	$C_{24}H_{24}N_4O_6{\cdot}1.25H_2O$	463	0.72	1.30	90

[a] All compounds were prepared by method B. [b] Anal. C, H, N. [c] Prepared from the 3-pivaloyloxy-protected diethyl ester 91.

Table IV. Comparison of Representative Antifolates in the L1210 and L1210:1565 Cell Lines

compd	bridge unit	aromatic ring	inhibn of TS: IC_{50}, μM	inhibn of cell growth in culture: IC_{50}, μM		L1210:1565 IC_{50}/ L1210 IC_{50}
				L1210	L1210:1565	
15	$-N(CH_3)CH_2-$	1,4-benzene	2.62	0.7	7.7	11
18	$-N(CH_3)CH_2-$	2,5-thiophene	3.82	0.3	2.1	7
11	$-CH_2N(CH_3)-$	1,4-benzene	0.25	0.11	4.2	38
8a	$-CH_2N(CH_3)-$	2,5-thiophene	0.67	0.007	0.96	137

pounds as illustrated by a number of 10-alkyl-2-methyl-5,8-dideazafolic acid analogues where the benzene ring has been replaced by heterocyclic isosteres (thiophene, thiazole, and pyridine).[16,34] Although compound availability has limited such a comprehensive study of the compounds described here, experiments performed with the L1210:1565 cell line[35] (impaired uptake of compounds that require the RFC) have demonstrated that N9,C10 compounds such as 15 and 18 use the RFC, but less efficiently than their C9,N10 isosteres 11 and 8a (Table IV).

The incorporation of the nitrogen atom at N10 into an indole ring to give the analogue 75 causes an 80-fold drop in potency against TS compared to the N10-propargyl compound 2. On the other hand 75 is 2.5-fold more potent against L1210 cell growth. Since thymidine effectively prevents this growth inhibition in the presence of 0.36 μM 75, it can be concluded that the locus of action is predominantly against TS and that the compound is transported and/or polyglutamylated very efficiently by the cells. The poor potency of 75 against TS was a disappointment since the C≡C substituent on N10 is sterically less bulky than propargyl. Presumably the fusion of this substituent onto the benzene ring forces it into a conformation which is less favorable for interaction with TS but which may be highly favored for substrate activity for FPGS. The glutamate nitrogen atom has been incorporated into a phthalimidine ring, thereby locking it into a conformation where it eclipses the aminobenzoate ring. The resulting N10-propargyl (76a) and N10-methyl (76c) analogues are only slightly (2–4-fold) less active against TS, supporting our earlier view[15] that such an eclipsed conformation is highly favored for enzyme binding. The poor inhibition of cell growth by 76c was surprising in this light but underlines the interplay of other factors in the observed cytotoxicity of these antifolates. In parallel with our earlier observations in related series[13,15,16] a hydrogen substituent on N10, i.e. 76b, gives very poor inhibition of TS.

In conclusion we have shown that in the series of 2-methylquinazolinone TS inhibitors isosteric modifications of the C9,N10 bridge and restriction of conformations around the *p*-aminobenzoate moiety in general give analogues with decreased affinity for the TS enzyme. However a number of these analogues are highly potent cytotoxic agents, and moreover this cytotoxicity has TS as its major locus.

Experimental Section

The general procedures used were described in the earlier paper[12] in this series. All evaporations of solutions were carried out in vacuo with a Büchi Rotavapor. The 400-MHz NMR spectrum was obtained on a Bruker AM400. All NMR spectra were run in Me_2SO-d_6.

6-Amino-3,4-dihydro-2-methyl-4-oxoquinazoline (33). A solution of **32**[26] (19.67 g, 0.096 mol) in MeOH (350 mL) was stirred for 6.5 h with 10% Pd–C (2.0 g) in an atmosphere of H_2. The reaction mixture was filtered through Celite and the filter cake was washed sequentially with MeOH and DMF. The combined filtrates were evaporated to dryness: 16.0 g (95%); MS (EI) *m/z* 175 $[M]^+$.

Diethyl *N*-[4-(Chloromethyl)benzoyl]-L-glutamate (35). Oxalyl chloride (6.07 mL, 0.07 mol) was added over 20 min to a stirred mixture of 4-(bromomethyl)benzoic acid (**34**) (10.0 g, 0.047 mol) and DMF (4 mL) in CH_2Cl_2 (70 mL). After stirring for a further 30 min the solution was evaporated to dryness below 30 °C and the crude acid chloride, a sticky solid, was dried over 20 min under vacuum (0.1 mmHg).

This acid chloride was dissolved in CH_2Cl_2 (70 mL) and the solution was added over 30 min to a stirred solution of diethyl glutamate hydrochloride (11.13 g, 0.046 mol) and 2,6-lutidine (14.6 mL, 0.125 mol) in CH_2Cl_2 (90 mL) at 0–5 °C. Stirring was continued for 2 h below 5 °C and at room temperature overnight. The reaction mixture was washed with 0.5 M aqueous H_2SO_4 and brine (50 mL of each), dried, and evaporated. The product was recrystallized from EtOAc–hexane: 12.70 g (78%); mp 109–110 °C; NMR δ 1.15, 1.2 (2 t, 6 H, 2 OCH_2CH_3), 2.05 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.45 (t, 2 H, $CHCH_2CH_2CO_2Et$), 4.05, 4.1 (2 q, 4 H, 2 OCH_2CH_3), 4.45 (m, 1 H, CH), 4.8 (s, 2 H, CH_2Cl), 7.55 (d, 2 H, Ar 3-H and 5-H), 7.9 (d, 2 H, Ar 2-H and 6-H), 8.7 (d, 1 H, CONH). Anal. ($C_{17}H_{22}ClNO_5$) C, H, N; Cl: calcd, 10.0; found, 9.5.

Diethyl *N*-[4-[[*N*-(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)amino]methyl]benzoyl]-L-glutamate (36). Method A. A mixture of the amine **33** (2.36 g, 13.5 mmol), the chloromethyl compound **35** (4.77 g, 13.41 mmol) and powdered $CaCO_3$ (2.01 g, 20.1 mmol) in DMF (16 mL) was stirred for 4.5 h at 86 °C under argon. The cooled reaction mixture was filtered and the filtrate was evaporated to dryness below 40 °C. The residue was purified by chromatography using a gradient of 0–5% v/v CH_2Cl_2 as eluent to give a gum: 3.18 g (48%); NMR δ 1.15, 1.2 (2 t, 6 H, 2 OCH_2CH_3), 2.0 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.25 (s, 3 H, CH_3), 2.4 (t, 2 H, $CHCH_2CH_2CO_2Et$), 4.05, 4.1 (2 q, 4 H, 2 OCH_2CH_3), 4.4 (d and m, 3 H, NCH_2 and CH), 6.75 (t, 1 H, NH), 7.0 (d, 1 H, quinazoline 5-H), 7.15 (dd, 1 H, quinazoline 7-H), 7.3 (d, 1 H, quinazoline 8-H), 7.45 (d, 2 H, 3′-H and 5′-H), 7.8 (d, 2 H, 2′-H and 6′-H), 8.6 (d, 1 H, CONH), 11.8 (s, 1 H, quinazoline 3-H).

***N*-[4-[[*N*-(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)amino]methyl]benzoyl]-L-glutamic Acid (14). Method B.** The diester **36** (372 mg, 0.75 mmol) was stirred for 2 h under argon in a mixture of 1 N aqueous NaOH (4.5 mL, 4.5 mmol), EtOH (8 mL), and H_2O (8 mL). The resulting solution was evaporated below 30 °C to ca. 8 mL, filtered into a centrifuge tube, and brought to pH 3.0 with 2 N aqueous HCl. The precipitate was isolated by centrifugation and freed from inorganic ions by repeated cycles of aqueous suspension–centrifugation–decantation until the supernatant was free of chloride ions ($AgNO_3$ test). The damp product was freeze-dried to give a pale yellow amorphous solid: 239 mg (70%); mp 193–196 °C; NMR δ 2.05 (m, 2 H, $CHCH_2CH_2CO_2H$), 2.3 (s, 3 H, CH_3), 2.35 (t, 2 H, $CHCH_2CH_2CO_2H$), 4.4 (m, 1 H, CH), 4.4 (br s, 2 H, NCH_2), 6.85 (t, 1 H, NH), 7.0 (d, 1 H, quinazoline 5-H), 7.15 (dd, 1 H, quinazoline 7-H), 7.35 (d, 1 H, quinazoline 8-H), 7.45 (d, 2 H, 3′-H and 5′-H), 7.85 (d, 2 H, 2′-H and 6′-H), 8.5 (d, 1 H, CONH); MS (FAB) *m/z* 437 $[M - H]^-$. Anal. ($C_{22}H_{22}N_4O_6 \cdot H_2O$) C, H, N.

Diethyl *N*-[4-[[*N*-(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)-*N*-methylamino]methyl]benzoyl]-L-glutamate (37). Method C. A mixture of **36** (2.52 g, 5.1 mmol), MeI (0.64 mL, 10.2 mmol), and powdered $CaCO_3$ (1.02 g, 10.2 mmol) in DMF (8 mL) in a stoppered flask was stirred for 2 h at 65 °C. The cooled

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(35) Fry, D. W.; Besserer, J. A.; Borizki, T. J. *Cancer Res.* **1984**, *44*, 3366. The L1212:1565 cell line was the generous gift of Dr. D. W. Fry, Warner-Lambert, Ann Arbor, MI.

mixture was filtered and the filter cake was washed with DMF (4 mL). The filtrate was evaporated to dryness and the residue was chromatographed with a gradient of 0–8% v/v EtOH in CH_2Cl_2 as eluent to give **37** as a gum: 275 mg (11%); NMR δ 1.15, 1.2 (2 t, 6 H, 2 OCH_2CH_3), 2.05 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.3 (s, 3 H, CH_3), 2.4 (t, 2 H, $CHCH_2CH_2CO_2Et$), 3.15 (s, 3 H, NCH_3), 4.05, 4.1 (2 q, 4 H, 2 OCH_2CH_3), 4.4 (m, 1 H, CH), 4.7 (br s, 2 H, NCH_2), 7.15 (d, 1 H, quinazoline 5-H), 7.25 (dd, 1 H, quinazoline 7-H), 7.3 (d, 2 H, 3′-H and 5′-H), 7.4 (d, 1 H, quinazoline 8-H), 7.8 (d, 2 H, 2′-H and 6′-H), 8.6 (d, 1 H, CONH), 11.9 (s, 1 H, quinazoline 3-H).

3,4-Dihydro-2-methyl-4-oxo-6-(*N*-prop-2-ynylamino)quinazoline (38). Method D. A mixture of the amine **33** (500 mg, 2.86 mmol), propargyl bromide (0.64 mL of an 80% w/w solution in toluene, 5.75 mmol), and powdered $CaCO_3$ (580 mg, 5.8 mmol) in DMF (4 mL) was stirred for 3 h at 70 °C under argon. The cooled reaction mixture was filtered and the filtrate was evaporated to dryness. Chromatography of the crude product using a gradient of 0–10% v/v EtOH in CH_2Cl_2 as eluent afforded a gum: 513 mg (84%); NMR δ 2.3 (s, 3 H, CH_3), 3.05 (t, 1 H, C≡CH), 4.25 (d, 2 H, $CH_2C{\equiv}C$), 6.45 (br, 1 H, NH), 7.15 (d and dd, 2 H, quinazoline 5-H and 7-H), 7.45 (d, 1 H, quinazoline 8-H).

The procedure was repeated using EtI, $BrCH_2CH_2F$, and $BrCH_2CH_2OAc$ as the alkylating agents to yield **49–51**.

Methyl 5-[[*N*-(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)amino]methyl]thiophene-2-carboxylate (40). Method E. A mixture of the amine **33** (1.18 g, 6.74 mmol), methyl 5-bromomethylthiophene-2-carboxylate (**39**)[27] (1.59 g, 6.76 mmol), and powdered $CaCO_3$ (1.02 g, 10.2 mmol) in DMF (6 mL) was stirred for 1 h at 80 °C under argon. The cooled reaction mixture was filtered and the filtrate was evaporated to dryness below 40 °C. The crude product was purified by chromatography using a gradient of 0–8% v/v EtOH in CH_2Cl_2 as eluent: 1.31 g (59%); NMR δ 2.3 (s, 3 H, CH_3), 3.8 (s, 3 H, CO_2CH_3), 4.6 (d, 2 H, NCH_2), 6.85 (t, 1 H, NH), 7.1 (d, 1 H, quinazoline 5-H), 7.15 (dd, 1 H, quinazoline 7-H), 7.15 (d, 1 H, thiophene 4-H), 7.35 (d, 1 H, quinazoline 8-H), 7.7 (d, 1 H, thiophene 3-H).

The procedure was repeated using the amines **49–51** in the reaction with **39** to give **52**, **54**, and **56** and using the bromomethyl compounds **58**[28] and **59**[29] in the reaction with **33**.

5-[[*N*-(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)amino]methyl]thiophene-2-carboxylic Acid (41). Method F. The methyl ester **40** (1.24 g, 3.78 mmol) was stirred in a mixture of 1 N aqueous NaOH (11.3 mL, 11.3 mmol) and EtOH (15 mL) for 30 min at 52 °C under argon. The resulting solution was cooled, evaporated to ca. 10 mL, and brought to pH 3.0 with 2 N aqueous HCl. The precipitate was isolated by centrifugation, washed with H_2O, and vacuum dried: 951 mg (78%); 285–287 °C dec. Anal. ($C_{15}H_{13}N_3O_3S{\cdot}0.5H_2O$) C, H, N.

***N*-[5-[[*N*-(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)amino]methyl]-2-thenoyl]-L-glutamic Acid (17). Method G.** A solution of the acid **41** hemihydrate (945 mg, 2.92 mmol) and diethyl glutamate hydrochloride (2.27 g, 9.46 mmol) in DMF (14 mL) was stirred at 0 °C during the dropwise addition over 15 min of diphenyl phosphorazidate (DPPA) (2.04 mL, 9.46 mmol) followed by Et_3N (3.30 mL, 24 mmol) again over 15 min. The reaction mixture was stirred at room temperature for 16 h, poured into ice–H_2O (100 mL), and extracted with CH_2Cl_2 (2 × 100 mL). The combined CH_2Cl_2 solutions were washed with H_2O, dried, and evaporated to dryness. The crude product was chromatographed with a gradient of 0–8% v/v EtOH in CH_2Cl_2 as eluent to give a gum (1.52 g) which by NMR was the required product **42** contaminated with diphenyl phosphate impurities.

A portion (727 mg) of this impure **42** was hydrolyzed according to method B. The crude diacid (318 mg) was dissolved in 0.1 M aqueous NH_4HCO_3 (5 mL) and applied to a column of DE 52 DEAE-cellulose (30 mL) which had been preequilibrated with the same solvent. The column was eluted with a gradient of 0.1–0.4 M aqueous NH_4HCO_3. Fractions containing pure product by HPLC were pooled and lyophilized to a white solid (199 mg). A portion (147 mg) of this solid was redissolved in the minimum volume of H_2O. The solution was brought to pH 3.0 with 2 N aqueous HCl. The precipitated solid was isolated by centrifugation, washed (4×) with H_2O, and vacuum dried: 103 mg (~ 20%); mp 168–172 °C; NMR δ 1.9 (m, 2 H, $CHCH_2CH_2CO_2H$), 2.2 (t, 2 H, $CHCH_2CH_2CO_2H$), 2.25 (s, 3 H, CH_3), 4.2 (m, 1 H, CH), 4.5 (d, 2 H, NCH_2), 6.8 (t, 1 H, NH), 7.05 (d, 1 H, thiophene 4-H), 7.1 (d, 1 H, quinazoline 5-H), 7.15 (dd, 1 H, quinazoline 7-H), 7.35 (d, 1 H, quinazoline 8-H), 7.6 (d, 1 H, thiophene 3-H), 8.05 (br s, 1 H, CONH); MS (FAB) *m/z* 443 $[M - H]^-$. Anal. ($C_{20}H_{20}N_4O_6S{\cdot}1.5H_2O$) C, H, N.

Methyl 5-[[*N*-(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)-*N*-methylamino]methyl]thiophene-2-carboxylate (43). The secondary amine **40** (11.0 g, 33.4 mmol) was treated with MeI (12.5 mL, 0.20 mol) at 50 °C according to method C to yield **43** as a gum: 9.67 g (84%); NMR δ 2.3 (s, 3 H, CH_3), 3.05 (s, 3 H, NCH_3), 3.75 (s, 3 H, CO_2CH_3), 4.9 (br s, 2 H, NCH_2), 7.1 (d, 1 H, thiophene 4-H), 7.2 (d, 1 H, quinazoline 5-H), 7.4 (dd, 1 H, quinazoline 7-H), 7.4 (d, 1 H, quinazoline 8-H), 7.65 (d, 1 H, thiophene 3-H).

Methyl 5-[[*N*-(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)-*N*-allylamino]methyl]thiophene-2-carboxylate (46). **40** (1.00 g, 3.04 mmol) was treated with allyl bromide (1.32 mL, 15.2 mmol) at 70 °C according to method C to yield **46** as a gum: 473 mg (42%); NMR δ 2.3 (s, 3 H, CH_3), 3.75 (s, 3 H, CO_2CH_3), 4.1 (d, 2 H, $NCH_2CH{=}CH_2$), 4.85 (br s, 2 H, NCH_2), 5.2 (m, 2 H, $CH{=}CH_2$), 5.9 (m, 1 H, $CH{=}CH_2$), 7.1 (d, 1 H, thiophene 4-H), 7.15 (d, 1 H, quinazoline 5-H), 7.4 (dd, 1 H, quinazoline 7-H), 7.4 (d, 1 H, quinazoline 8-H), 7.65 (d, 1 H, thiophene 3-H).

(*RS*)-4-[1-[*N*-(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)amino]ethyl]benzoic Acid (61). A solution of methyl 4-ethylbenzoate (3.00 g, 18.3 mmol) in CCl_4 (30 mL) was stirred under reflux for 3.5 h with NBS (3.59 g, 20.2 mmol) and benzoyl peroxide (480 mg, 2.0 mmol). The warm reaction mixture was filtered and the precipitate was washed with CCl_4. The combined filtrates were evaporated to afford crude methyl 4-(1-bromoethyl)benzoate (4.83 g) as a pale yellow oil: NMR δ 2.0 (d, 3 H, $CH_3CBr<$), 3.85 (s, 3 H, CO_2CH_3), 5.55 (q, 1 H, CHBr), 7.65 (d, 2 H, 3′-H and 5′-H), 7.95 (d, 2 H, 2′-H and 6′-H).

This crude bromoethyl compound was reacted with amine **33** (3.85 g, 22.0 mmol) at 95 °C according to method E to give 11.8 g of **60** as a brown gum which was used without purification.

A portion (9.14 g) of this sample of **60** was hydrolyzed according to method F to afford **61** (3.83 g); NMR δ 1.45 (d, 3 H, $CHCH_3$), 2.3 (s, 3 H, CH_3), 4.65 (m, 1 H, CH), 6.8 (br d, 1 H, NH), 6.9 (d, 1 H, quinazoline 5-H), 7.15 (dd, 1 H, quinazoline 7-H), 7.35 (d, 1 H, quinazoline 8-H), 7.5 (d, 2 H, 3′-H and 5′-H), 7.9 (d, 2 H, 2′-H and 6′-H). Anal. ($C_{18}H_{17}N_3O_3{\cdot}NaCl$) C, H; N: calcd, 11.0; found, 10.5; Na: calcd, 6.0, found, 6.5.

(*RS*)-4-[1-[*N*-(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)-*N*-methylamino]ethyl]benzoic Acid (63). Method H. $NaCNBH_3$ (900 mg, 14.3 mmol) was added to a stirred solution of **60** (2.17 g of the above material) in a mixture of MeCN (90 mL), DMF (3.6 mL), and 37% aqueous HCHO (3.6 mL). Glacial HOAc (3.6 mL) was then added dropwise over 15 min while the temperature was kept below 20 °C (ice bath). The reaction mixture was stirred for a further 40 h at room temperature with additional portions of aqueous HCHO (3.6 mL), $NaCNBH_3$ (900 mg), and HOAc (3.6 mL) being added at 16 h. The reaction mixture was filtered and the filtrate was evaporated to dryness. The residue was partitioned between EtOAc and H_2O. The EtOAc solution was dried and evaporated. Purification of the crude product by chromatography using a gradient of 0–100% v/v EtOAc in CH_2Cl_2 afforded **62** as a golden gum: 550 mg. This was hydrolyzed to **63** by using method F: 447 mg (70%); mp 190–194 °C; NMR δ 1.55 (d, 3 H, $CHCH_3$), 2.3 (s, 3 H, CH_3), 2.75 (s, 3 H, $>NCH_3$), 5.3 (br q, 1 H, CH), 7.25 (2 d, 1 H, quinazoline 5-H), 7.4 (m, 1 H, quinazoline 7-H and 8-H), 7.4 (d, 2 H, 3′-H and 5′-H), 7.9 (d, 2 H, 2′-H and 6′-H). Anal. ($C_{19}H_{19}N_3O_3{\cdot}NaCl{\cdot}H_2O$) C, H, N.

4-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methoxy]benzoic Acid (66). A mixture of **64**[31] (8.31 g, 32.85 mmol), methyl 4-hydroxybenzoate (5.00 g, 32.85 mmol), and powdered $CaCO_3$ (4.93 g, 49.3 mmol) in DMA (30 mL) was stirred for 2 h at 60 °C and 1 h at 100 °C under argon. HPLC indicated only 13% conversion of **64** to **65**. Powdered K_2CO_3 (6.81 g, 49.3 mmol) was therefore added. After a further 4 h at 100 °C, HPLC indicated that all of **64** had been consumed. The cooled reaction mixture was filtered and the filtrate was evaporated to dryness. The residue was triturated with EtOAc to a buff solid. Purification by chromatography using a gradient of 0–8% v/v EtOH in CH_2Cl_2

as eluent yielded **65**: 524 mg (5%); mp 281–284 °C.

Hydrolysis of **65** (506 mg, 1.56 mmol) according to method F afforded the acid **66**: 426 mg (81%); mp >300 °C; NMR δ 2.45 (s, 3 H, CH_3), 5.35 (s, 2 H, $ArCH_2O$), 7.1 (d, 2 H, 3′-H and 5′-H), 7.7 (d, 1 H, quinazoline 8-H), 7.9 (dd, 1 H, quinazoline 7-H), 7.9 (d, 2 H, 2′-H and 6′-H), 8.2 (d, 1 H, quinazoline 5-H). Anal. ($C_{17}H_{14}N_2O_4 \cdot 1.5H_2O$) C, H, N.

Diethyl *N*-[4-[[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]thio]benzoyl]-L-glutamate (67). Method I. $NaBH_4$ (380 mg, 10 mmol) was added to a stirred solution of tetraethyl 4,4′-dithiobis(*N*-benzoyl-L-glutamate)[36] (2.27 g, 3.36 mmol) in EtOH (30 mL). After 10 min the EtOH was evaporated below 40 °C and the residue was suspended in DMF (10 mL). The (bromomethyl)quinazolinone **64** was added, and the mixture was stirred 16 h and partitioned between H_2O and EtOAc. A solid which remained in suspension between the two layers was filtered off and combined with the residue from evaporation of the dried EtOAc solution. Purification of this crude product by chromatography was achieved with use of a gradient of 0–8% EtOH in CH_2Cl_2: 1.58 g (46%); mp 179.5–185 °C; NMR δ 1.2, 1.3 (2 t, 6 H, 2 OCH_2CH_3), 2.05 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.35 (s, 3 H, CH_3), 2.4 (t, 2 H, $CHCH_2CH_2CO_2Et$), 4.1, 4.2 (2 q, 4 H, 2 OCH_2CH_3), 4.4 (m, 1 H, CH), 4.5 (s, 2 H, CH_2SAr), 7.4 (d, 2 H, 3′-H and 5′-H), 7.5 (d, 1 H, quinazoline 8-H), 7.7 (dd, 1 H, quinazoline 7-H), 7.7 (d, 2 H, 2′-H and 6′-H), 8.1 (d, 1 H, quinazoline 8-H), 8.65 (d, 1 H, CONH). Anal. ($C_{26}H_{29}N_3O_6S$) C, H, N.

Dimethyl 4,4′-Dithiobis(benzoate) (68). A mixture of 4,4′-dithiobis(benzoic acid)[37] (4.94 g, 16.1 mmol), MeI (8.7 mL, 0.14 mol), and powdered $NaHCO_3$ (8.7 g, 0.13 mol) in DMA (25 mL) was stirred for 5 days under argon. The reaction mixture was diluted with H_2O (50 mL) and extracted with EtOAc (3 × 50 mL). The combined EtOAc extracts were washed with H_2O, dried, and evaporated. Chromatography of the resulting dark brown oil using CH_2Cl_2 as eluent gave a tan solid: 4.77 g (89%); mp 105–107 °C. Anal. ($C_{16}H_{14}O_4S_2$) C, H.

Methyl 4-[[3-(Methoxycarbonyl)-4-nitrophenyl]thio]benzoate (71). $NaBH_4$ (960 mg, 25.3 mmol) was added to a stirred suspension of **68** (4.26 g, 12.75 mmol) in DMA (50 mL) at 0 °C under argon. A brief vigorous effervescence was observed. After 25 min at 0 °C powdered methyl 5-chloro-2-nitrobenzoate (**70**) (5.51 g, 25.5 mmol) was added. The ice bath was removed and the reaction mixture was stirred for 40 h at room temperature. The DMA was removed by rotary evaporation below 40 °C, the residue was dissolved in EtOAc (100 mL), and this solution was washed with H_2O, brine, dried, and evaporated to dryness. Chromatography of the crude product using 25% v/v CH_2Cl_2 in toluene as eluent afforded an oil: 3.82 g (43%); NMR δ 3.85, 3.9 (2 s, 6 H, OCH_3), 7.6 (dd, 1 H, Ar 6-H), 7.65 (d, 2 H, Ar 3′-H and 5′-H), 7.68 (d, 1 H, Ar 2-H), 8.02 (d, 2 H, Ar 2′-H and 6′-H), 8.07 (d, 1 H, Ar 5-H).

Methyl 4-[[4-Amino-3-(methoxycarbonyl)phenyl]thio]benzoate (72). A mixture of **71** (3.82 g, 11.0 mmol), Fe powder (activated by sequential washing with 2 N aqueous HCl, H_2O, and acetone; 6.15 g, 0.22 g-atom), $FeSO_4 \cdot 7H_2O$ (3.06 g, 11.0 mmol), and H_2O (5 mL) in MeOH (300 mL) was stirred for 4 h under reflux with the addition of a second portion of Fe powder (6.15 g) at 2.5 h. The reaction mixture was filtered through Celite and the filtrate was evaporated to dryness to give a brown oil. Traces of MeOH and H_2O were removed by azeotropic rotary evaporation in the presence of added toluene and the resulting product was purified by chromatography (0–2% v/v EtOAc in CH_2Cl_2): 2.42 g (69%); mp 97–100 °C. Anal. ($C_{16}H_{15}NO_4S$) C, H, N.

4-[(4-Amino-3-carboxyphenyl)thio]benzoic Acid (73). The diester **72** (1.76 g, 5.54 mmol) was stirred in a mixture of 1 N aqueous NaOH (28 mL, 28 mmol) and EtOH (50 mL) for 40 h under argon. The resulting solution was evaporated to dryness. The residue was dissolved in H_2O (10 mL), and the solution was filtered into a centrifuge tube and brought to pH 3.0 with 2 N aqueous HCl. The precipitated solid was isolated by centrifugation, washed with H_2O, and vacuum dried: 1.48 g (93%); 261–263 °C dec. Anal. ($C_{14}H_{11}NO_4S \cdot 0.2H_2O$) C, H, N.

4-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)thio]benzoic Acid (74). A suspension of **73** (1.46 g, 5.0 mmol) in Ac_2O (25 mL) was stirred under reflux for 40 min to give a clear solution. The Ac_2O was removed by distillation at atmospheric pressure followed by the distillation of added xylene (2 × 25 mL). The resulting solid was vacuum dried and stirred for 1 h in aqueous NH_3 (sp gr 0.9; 50 mL) at 60 °C (bath temperature). The solution was cooled, filtered to remove a small amount of insoluble solid, and brought to pH 2.0 with 2 N aqueous HCl. The precipitate was filtered off, washed with H_2O, and vacuum dried: 1.04 g (67%); mp >300 °C; NMR δ 2.6 (s, 3 H, CH_3), 7.4 (d, 4 H, Ar 3,5-H_2 and quinazoline 8-H), 7.9 (d, 2 H, Ar 2,6-H_2), 7.9 (dd, 1 H, quinazoline 7-H), 8.05 (d, 1 H, quinazoline 5-H).

Methyl 1-[[3,4-Dihydro-2-methyl-4-oxo-3-[(pivaloyloxy)methyl]-6-quinazolinyl]methyl]indole-5-carboxylate (80). A mixture of indole-5-carboxylic acid (3.57 g, 22.17 mmol), powdered $NaHCO_3$ (5.95 g, 87 mmol), and MeI (5.95 mL, 96 mmol) in DMF (25 mL) was stirred for 72 h under argon. The reaction mixture was diluted with H_2O (200 mL) and extracted with EtOAc (2 × 200 mL). The combined EtOAc solutions were washed with aqueous $NaHCO_3$, dried, and evaporated to give methyl indole-5-carboxylate (**78**): 3.67 g (94%); mp 127–128 °C (lit.[38] mp 127–128 °C).

NaH (51 mg of a 55% dispersion in oil, 1.17 mmol) was added to a stirred cooled (ice bath) solution of **78** (178 mg, 1.02 mmol) in DMF (2 mL) under argon. After 1 h the bromomethyl compound **79** (424 mg, 1.15 mmol) was added in one portion and the ice bath was removed. The reaction mixture was stirred for 16 h, diluted with H_2O, and extracted with EtOAc. The EtOAc solution was washed with H_2O, dried, and evaporated to an oil which was purified by chromatography using a gradient of 0–5% v/v EtOAc in CH_2Cl_2 as eluent: 131 mg (28%); mp 137–140 °C; NMR δ 1.1 (s, 9 H, *t*-Bu), 2.6 (s, 3 H, CH_3), 3.85 (s, 3 H, CO_2CH_3), 5.65 (br s, 2 H, $ArCH_2N<$), 6.0 (s, 2 H, OCH_2N), 6.7 (d, 1 H, indole 3-H), 7.55 (d, 1 H, indole 7-H), 7.6 (d, 1 H, quinazoline 8-H), 7.65 (dd, 1 H, indole 6-H), 7.7 (d, H, indole 2-H), 7.75 (dd, 1 H, quinazoline 7-H), 7.9 (d, 1 H, indole 4-H), 8.3 (d, 1 H, quinazoline 5-H). Anal. ($C_{26}H_{27}N_3O_5 \cdot 0.5H_2O$) C; H: calcd, 6.0; found, 6.5; N: calcd, 8.9; found, 8.4.

1-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]indole-5-carboxylic Acid (81). The methyl ester **80** (538 mg, 1.17 mmol) was stirred for 48 h under argon in a mixture of 1 N aqueous NaOH (5.85 mL, 5.85 mmol) and EtOH (10 mL). The resulting solution was evaporated below 30 °C to ca. 2 mL, brought to pH 3.0 with 2 N aqueous HCl, and cooled to 0 °C. The precipitate was filtered off, washed with H_2O, and vacuum dried: 346 mg (83%); mp >300 °C; 400-MHz NMR δ 2.30 (s, 3 H, CH_3), 5.60 (br s, 2 H, $ArCH_2N<$), 6.67 (d, 1 H, indole 3-H), 7.52 (d, 1 H, indole 7-H), 7.53 (d, 1 H, quinazoline 8-H), 7.62 (dd, 1 H, indole 6-H), 7.65 (d, H, indole 2-H), 7.70 (dd, 1 H, quinazoline 7-H), 7.81 (d, 1 H, indole 4-H), 8.24 (d, 1 H, quinazoline 5-H); MS (FAB) *m/z* 334 $[MH]^+$. Anal. ($C_{19}H_{15}N_3O_3 \cdot 1.4H_2O$) C, H, N.

Diethyl *N*-[1-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]indol-5-ylcarbonyl]-L-glutamate (82). The acid **81** (317 mg of the 1.4 hydrate, 0.89 mmol) was condensed with diethyl glutamate hydrochloride according to method G to yield **82**: 300 mg (61%); mp 192–197 °C; NMR δ 1.15, 1.2 (2 t, 6 H, 2 OCH_2CH_3), 2.1 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.3 (s, 3 H, CH_3), 2.45 (t, 2 H, $CHCH_2CH_2CO_2Et$), 4.05, 4.1 (2 q, 4 H, 2 OCH_2CH_3), 4.45 (m, 1 H, CH), 5.6 (br s, 2 H, $ArCH_2N<$), 6.65 (d, 1 H, indole 3-H), 7.52 (d, 1 H, indole 7-H), 7.54 (d, 1 H, quinazoline 8-H), 7.6 (dd, 1 H, indole 6-H), 7.62 (d, H, indole 2-H), 7.65 (dd, 1 H, quinazoline 7-H), 7.85 (d, 1 H, indole 4-H), 8.2 (d, 1 H, quinazoline 5-H), 8.65 (d, 1 H, CONH); MS (EI) *m/z* 518 $[M]^+$. Anal. ($C_{28}H_{30}N_4O_6 \cdot 1.75H_2O$) C, H, N.

Diethyl (*S*)-2-(2,3-Dihydro-5-nitro-1-oxo-2-1*H*-isoindolyl)glutarate (85). A mixture of methyl 2-methyl-4-nitrobenzoate[32] (9.37 g, 48 mmol), NBS (8.55 g, 48 mmol), and benzoyl peroxide (350 mg, 1.5 mmol) in CCl_4 (200 mL) was stirred under reflux for 16 h, cooled, and filtered. The filtrate was evaporated to give **84** as an oil: 13.5 g.

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Without purification, this oil was stirred for 16 h with diethyl glutamate hydrochloride (23.6 g, 98.5 mmol) and powdered K_2CO_3 (27.2 g, 0.02 mol) in DMA (50 mL) under argon. The reaction mixture was diluted with H_2O (200 mL) and extracted with EtOAc (3 × 200 mL). The combined EtOAc solutions were washed twice with brine, dried, and evaporated to an oil. This oil was chromatographed with 2:1 hexane–EtOAc as eluent to yield **85** as an orange waxy solid: 9.36 g (50%); mp 73–74 °C; NMR δ 1.15, 1.2 (2 t, 6 H, 2 OCH_2CH_3), 2.25 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.4 (t, 2 H, $CHCH_2CH_2CO_2Et$), 4.0, 4.15 (2 q, 4 H, 2 OCH_2CH_3), 4.65 (s, 2 H, isoindolinone CH_2), 4.95 (m, 1 H, CH), 7.95 (d, 1 H, isoindolinone 7-H), 8.35 (dd, 1 H, isoindolinone 6-H), 8.5 (d, 1 H, isoindolinone 4-H); MS (CI) m/z 365 $[MH]^+$. Anal. ($C_{17}H_{20}N_2O_7 \cdot 0.75H_2O$) C, H, N.

Diethyl (*S*)-2-(5-Amino-2,3-dihydro-1-oxo-2-1*H*-isoindolyl)glutarate (86). A solution of **85** (9.36 g of 0.75 hydrate, 24.8 mmol) in EtOAc (50 mL) was stirred with 10% Pd–C in an atmosphere of H_2 until complete reduction was indicated by HPLC (ca. 10 h). The mixture was filtered through Celite and the filtrate was evaporated to an orange oil: 8.10 g (94%). Anal. ($C_{17}H_{22}N_2O_5 \cdot 0.67H_2O$) C, H, N.

Diethyl (*S*)-2-[2,3-Dihydro-5-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-1-oxo-2-1*H*-isoindolyl]glutarate (88). A mixture of **86** (1.00 g, 2.9 mmol), 2,6-lutidine (0.70 mL, 6.0 mmol), and propargyl bromide (0.67 mL of an 80% v/v solution in toluene, 6.0 mmol) in DMF (10 mL) was stirred for 4 h at 80 °C under argon. The cooled reaction mixture was evaporated to dryness and the residue was partitioned between H_2O (20 mL) and EtOAc (3 × 25 mL). The combined EtOAc solutions were washed with H_2O, dried, and evaporated. The crude product was purified by chromatography using a gradient of 0–10% v/v EtOAc in CH_2Cl_2 as eluent to yield a golden oil (718 mg) which was shown by NMR and HPLC to be a mixture of 75% of **87** and 25% of the corresponding *N,N*-dipropargyl derivative.

The bromomethyl compound **64** (354 mg, 1.40 mmol) and the above impure sample of **87** (700 mg) were reacted at 90 °C according to method E: 270 mg (35%); mp 90.5–92.5 °C; NMR δ 1.1, 1.15 (2 t, 6 H, 2 OCH_2CH_3), 2.15 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.3 (t, 2 H, $CHCH_2CH_2CO_2Et$), 2.35 (s, 3 H, CH_3), 3.2 (t, 1 H, C≡CH), 3.95, 4.1 (2 q, 4 H, 2 OCH_2CH_3), 4.3 (s, 2 H, isoindolinone CH_2), 4.35 (t, 2 H, $CH_2C{\equiv}C$), 4.8 (br s, 2 H, $ArCH_2N<$), 4.8 (m, 1 H, CH), 6.9 (dd, 1 H, isoindolinone 6-H), 6.95 (d, 1 H, isoindolinone 4-H), 7.5 (d, 1 H, isoindolinone 7-H), 7.55 (d, 1 H, quinazoline 8-H), 7.7 (dd, 1 H, quinazoline 7-H), 8.0 (d, 1 H, quinazoline 5-H); MS (CI) m/z 545 $[MH]^+$. Anal. ($C_{30}H_{32}N_4O_6 \cdot 1.5H_2O$) C, H, N.

Diethyl (*S*)-2-[2,3-Dihydro-5-[*N*-[(3,4-dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]amino]-1-oxo-2-1*H*-isoindolyl]glutarate (89). The bromomethyl compound **64** (760 mg, 3.0 mmol) and the above amine **86** (1.00 g) were reacted according to method E: 429 mg (27%); mp 74–78 °C; NMR δ 1.1, 1.15 (2 t, 6 H, 2 OCH_2CH_3), 2.1 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.25 (t, 2 H, $CHCH_2CH_2CO_2Et$), 2.35 (s, 3 H, CH_3), 4.05, 4.1 (2 q, 4 H, 2 OCH_2CH_3), 4.25 (s, 2 H, isoindolinone CH_2), 4.5 (br s, 2 H, $ArCH_2N<$), 4.8 (m, 1 H, CH), 6.65 (d, 1 H, isoindolinone 4-H), 6.7 (dd, 1 H, isoindolinone 6-H), 7.1 (br s, 1 H, NH), 7.35 (d, 1 H, isoindolinone 7-H), 7.55 (d, 1 H, quinazoline 8-H), 7.75 (dd, 1 H, quinazoline 7-H), 8.05 (d, 1 H, quinazoline 5-H); MS (CI) m/z 507 $[MH]^+$. Anal. ($C_{27}H_{30}N_4O_6 \cdot H_2O$) C, H; N: calcd, 10.7; found, 9.9.

Diethyl (*S*)-2-[2,3-Dihydro-5-[*N*-[[3,4-dihydro-2-methyl-4-oxo-3-[(pivaloyloxy)methyl]-6-quinazolinyl]methyl]-*N*-methylamino]-1*H*-1-oxo-2-isoindolyl]glutarate (91). A mixture of **86** (1.11 g, 3.20 mmol), the bromomethyl compound **79** (1.22 g, 3.32 mmol), and powdered $CaCO_3$ (660 mg, 6.6 mmol) in DMA (10 mL) was stirred for 3 h at 65 °C under argon. The cooled reaction mixture was filtered and the filtrate was evaporated to dryness below 40 °C in the presence of added xylene. The crude dark brown oily **90** was purified by chromatography using a gradient of 0–60% v/v EtOAc in CH_2Cl_2 as eluent: 1.032 g (51%); mp 136–138 °C. Anal. ($C_{33}H_{40}N_4O_8 \cdot 0.5H_2O$) C, H, N.

Methylation of **90** hemihydrate (223 mg, 0.35 mmol) with aqueous HCHO and $NaCNBH_3$ according to method H afforded **91**, a pale yellow gum: 191 mg (83%); NMR δ 1.1 (s, 9 H, *t*-Bu), 1.15, 1.2 (2 t, 6 H, 2 OCH_2CH_3), 2.15 (m, 2 H, $CHCH_2CH_2CO_2Et$), 2.3 (t, 2 H, $CHCH_2CH_2CO_2Et$), 2.6 (s, 3 H, CH_3), 3.2 (s, 3 H, NCH_3), 4.0, 4.1 (2 q, 4 H, 2 OCH_2CH_3), 4.3 (s, 2 H, isoindolinone CH_2), 4.78 (m, 1 H, CH), 4.82 (br s, 2 H, $ArCH_2N<$), 6.05 (s, 2 H, OCH_2N), 6.8 (dd, 1 H, isoindolinone 6-H), 6.85 (d, 1 H, isoindolinone 4-H), 7.45 (d, 1 H, isoindolinone 7-H), 7.55 (d, 1 H, quinazoline 8-H), 7.65 (dd, 1 H, quinazoline 7-H), 7.9 (d, 1 H, quinazoline 5-H); MS (CI) m/z 635 $[MH]^+$. Anal. ($C_{34}H_{42}N_4O_8 \cdot H_2O$) C, H, N.

Registry No. **14**, 133446-45-4; **15**, 133446-46-5; **16**, 133446-47-6; **16** (diethyl ester), 133447-10-6; **17**, 133446-48-7; **18**, 133446-49-8; **19**, 133446-50-1; **19** (diethyl ester), 133447-07-1; **20**, 133446-51-2; **20** (diethyl ester), 133447-08-2; **21**, 133446-52-3; **21** (diethyl ester), 133447-09-3; **22**, 133446-43-2; **22**·NH_3·H_2CO_3, 133446-44-3; **23**, 133446-53-4; **23** (diethyl ester), 133447-11-7; **24**, 133446-54-5; **24** (diethyl ester), 133447-12-8; **25**, 133446-55-6; **25** (diethyl ester), 133447-13-9; **26**, 133446-56-7; **26** (diethyl ester), 133447-14-0; **29**, 133446-57-8; **29** (diethyl ester), 133447-15-1; **30**, 133446-58-9; **31**, 133446-59-0; **31** (diethyl ester), 133447-16-2; **32**, 24688-36-6; **33**, 17329-24-7; **34**, 6232-88-8; **35**, 133446-60-3; **36**, 133446-61-4; **37**, 133446-62-5; **38**, 133446-63-6; **39**, 108499-32-7; **40**, 133446-64-7; **41**, 133446-65-8; **42**, 133446-66-9; **43**, 133446-67-0; **44**, 133446-68-1; **45**, 133446-69-2; **46**, 133446-70-5; **47**, 133446-71-6; **48**, 133446-72-7; **49**, 133446-73-8; **50**, 133446-74-9; **51**, 133446-75-0; **52**, 133446-76-1; **53**, 133446-77-2; **54**, 133446-78-3; **55**, 133446-79-4; **56**, 133446-80-7; **57**, 133446-81-8; **58**, 55876-86-3; **59**, 133446-82-9; **60**, 133446-83-0; **61**, 133446-84-1; **62**, 133446-85-2; **63**, 133446-86-3; **64**, 112888-43-4; **65**, 133446-87-4; **66**, 133446-88-5; **67**, 133446-89-6; **68**, 35190-68-2; **70**, 51282-49-6; **71**, 133446-90-9; **72**, 133446-91-0; **73**, 133446-92-1; **74**, 133446-93-2; **75**, 133446-94-3; **76a**, 133446-95-4; **76b**, 133446-41-0; **76c**, 133446-42-1; **77**, 1670-81-1; **78**, 1011-65-0; **79**, 112888-39-8; **80**, 133446-96-5; **81**, 133446-97-6; **82**, 133446-98-7; **83**, 62621-09-4; **84**, 133446-99-8; **85**, 133447-00-4; **86**, 133447-01-5; **87**, 133447-02-6; **88**, 133447-03-7; **89**, 133447-04-8; **90**, 133447-05-9; **91**, 133447-06-0; TS, 9031-61-2; H-Glu(OEt)-OEt·HCl, 1118-89-4; $BrCH_2C{\equiv}Ch$, 106-96-7; EtI, 75-03-6; $BrCH_2CH_2F$, 762-49-2; $BrCH_2CH_2OAc$, 927-68-4; 4-EtC_6H_4COOMe, 7364-20-7; (±)-4-(CH_3CHBr)C_6H_4COOMe, 133446-40-9; 4-HOC_6H_4COOMe, 99-76-3; (*S*-*p*-C_6H_4CO-Glu(OEt)-OEt), 56527-28-7; (S-*p*-$C_6H_4COOH)_2$, 1155-51-7; thymidine, 50-89-5.