The Synthesis and Thymidylate Synthase Inhibitory Activity of L-γ-L-Linked Dipeptide and L-γ-Amide Analogues of 2-Desamino-2-methyl-N^{10}-propargyl-5,8-dideazafolic Acid (ICI 198583)†

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Sixteen γ-linked dipeptide and four L-Glu-γ-amide analogues of 2-desamino-2-methyl-N^{10}-propargyl-5,8-dideazafolic acid (ICI 198583) have been synthesized and evaluated as inhibitors of thymidylate synthase (TS). Z-blocked L-Glu-γ-L-linked dipeptides and L-Glu-γ-amides were prepared by condensing α-*tert*-butyl-*N*-(benzyloxycarbonyl)-L-glutamic acid with the appropriate *tert*-butyl-protected L-amino acid or amine. The Z group was removed by catalytic hydrogenolysis, and the resulting dipeptides or L-Glu-γ-amides were condensed with the appropriate pteroic acid analogue trifluoroacetate salt using diethyl cyanophosphoridate as coupling reagent. Deprotection with trifluoroacetic acid in the final step gave the desired quinazoline γ-linked dipeptides and L-Glu-γ-amides as their trifluoroacetate salts. Nearly all the dipeptide analogues were potent inhibitors of TS, the best being ICI 198583-γ-L-2-aminoadipate (IC_{50} = 2 nM). Several of these dipeptides were found to be susceptible to enzymatic hydrolysis in mice. The quinazoline monocarboxylate L-Glu-γ-amides, lacking an α′-carboxyl group, are less active against TS and L1210 cell growth but are also not susceptible to enzymatic hydrolysis in mice.

Introduction

The design and synthesis of N^{10}-propargyl-5,8-dideazafolic acid (**1**, CB 3717) in our laboratories represented a major breakthrough in the search for a folate-based thymidylate synthase (TS) inhibitor.[1-3] CB 3717[4] was a potent TS inhibitor (K_i = 3 nM), although its clinical usefulness was limited because of its poor solubility at physiological pH which gave rise to dose-limiting renal toxicity.[5-7] An extensive search for a more suitable candidate for clinical evaluation has led to a second generation of quinazoline compounds, more water-soluble and more cytotoxic than CB 3717.[8-12] This necessitated replacing the 2-amino group with a 2-methyl,[10,13] which improved aqueous solubility and cellular uptake via the reduced folate/methotrexate carrier (RFC). Further modifications were made to the quinazoline nucleus,[10] the methyleneamino bridge, the N^{10}-substituent, and the benzoyl ring.[11,12] One of these compounds, ZD1694[14] (**2**), is currently in phase III clinical investigation. Although significantly less potent than CB 3717 against isolated TS (20-fold), rapid and almost complete metabolism to polyglutamate forms (mainly tetra- and pentaglutamates) renders the drug 500-fold more cytotoxic.[15] Poly-γ-glutamyl metabolites are intracellularly synthesized through the action of the enzyme folylpolyglutamyl synthetase (FPGS)[16,17] and are more potent inhibitors of TS than the monoglutamate forms, and in addition, their polyionic nature leads to prolonged retention within the cells.[18] However, drugs such as ZD1694, which are dependent on polyglutamation for their antitumor activity, may have some disadvantages such as (a) lack of activity in tumors expressing low levels of, or an altered expression of, FPGS[19-21] or (b) prolonged normal tissue toxicities caused by poly-γ-glutamate retention. For these reasons, we were interested in designing and synthesizing potent TS inhibitors which would *not* depend on polyglutamation for antitumor activity. ZD1694 monoglutamate (K_i = 60 nM) was not considered as a model for the above class of compounds precisely because of its dependency on metabolism to the tetra- and pentaglutamates (K_i = 1 nM) for tight binding to TS.[15] A better model compound was thought to be the 2-desamino-2-methyl-N^{10}-propargyl-5,8-dideazafolic acid (**3**, ICI 198583[22]) with a K_i for TS of 10 nM. Addition of one L-glutamate residue on the γ-carboxyl group of ICI 198583, *i.e.*, dipeptide **4**, resulted in better inhibition of TS by approximately 30-fold.[23] Although potency was enhanced by the addition of yet further glutamate residues, growth inhibition was compromised, probably because of the increased negative charge impeding cellular uptake. Thus **4** became the starting point in our search, since we envisioned that replacement of the second glutamate by other L-amino acids could lead to potent TS inhibitors that should not be substrates for FPGS. By changing the terminal amino acid or replacing it with simple amines, we also hoped to learn more about the role and relative importance of the α′- and γ′-carboxyls in the binding of **4** to TS.

† Abbreviations: TS, thymidylate synthase; FPGS, folylpolyglutamyl synthetase; MTX, methotrexate; DHFR, dihydrofolate reductase; DEPC, diethyl cyanophosphoridate; pg, propargyl; Glu, glutamic acid; Gly, glycine; Ala, Alanine; abu, α-aminobutyric acid; Nva, norvaline; Val, valine; Ile, isoleucine; Phg, phenylglycine; Phe, phenylalanine; Gln, glutamine; Ser, serine; βAla, β-alanine; Asp, aspartic acid; aad, α-aminoadipic acid; gaba, γ-aminobutyric acid.

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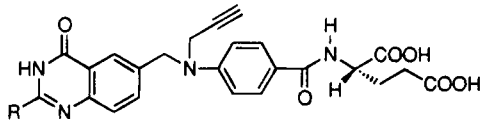
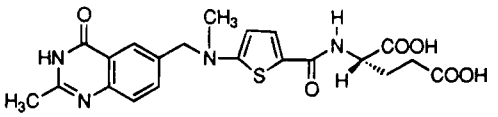
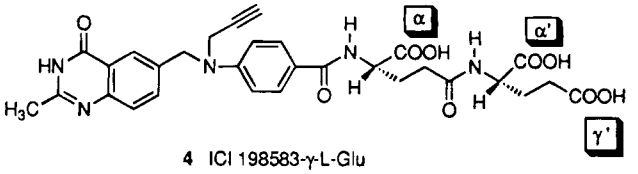


1 R = NH_2 CB 3717
3 R = CH_3 ICI 198583



2 ZD1694



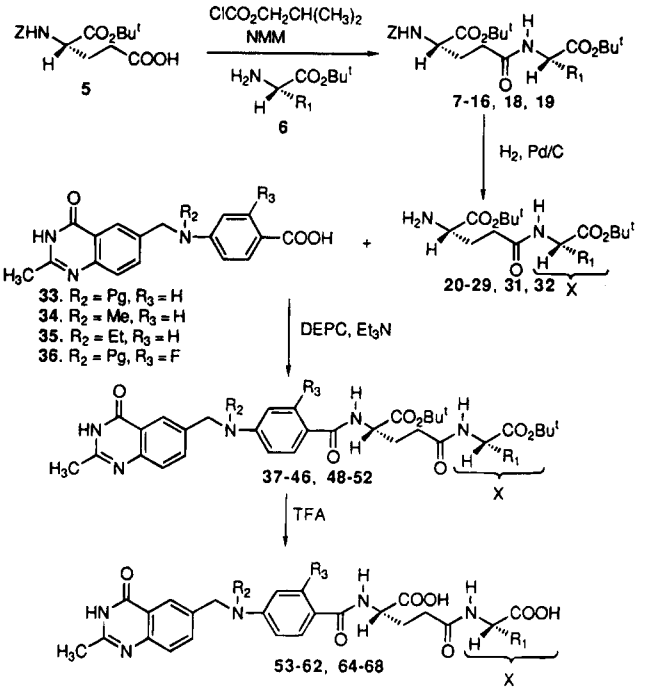
4 ICI 198583-γ-L-Glu

Several synthetic folate γ-linked peptides and amides have been reported in the literature. MTX-γ-glucine, -γ-aspartate, and -γ-glutamate have been synthesized and were found to inhibit L1210 cell DHFR as effectively as MTX.[24] More importantly, the MTX-γ-aspartate and -γ-glutamate compounds were shown to enhance binding to human TS by 5-fold (K_i = 6 μM) and 8-fold (K_i = 4 μM), respectively, when compared with the parent monoglutamate MTX.[25] Baugh *et al.*[26] prepared several pteroyl γ-linked tripeptides in order to characterize the human liver γ-glutamyl carboxypeptidase (conjugase). A number of groups have synthesized MTX-γ-amides for evaluation as antifolates.[24,25,27-29] The MTX-γ-methylamide, -γ-*n*-butylamide, and -γ-benzylamide all inhibited L1210 cell DHFR as effectively as MTX,[24,28] while MTX-γ-alkylamides such as γ-methyl and γ-pentyl were reported to be equipotent to MTX (K_i = 30 μM) against human TS.[25] McGuire *et al.* had reported that replacement of the glutamate of various folate analogues with aspartate, adipate, pimelate, or D-glutamate leads to compounds that are not substrates for mammalian FPGS.[30] γ-Fluoromethotrexate (FMTX), a closely related analogue of MTX, was found to be a poor substrate for FPGS, if at all.[31,32] Pteroylglutamyl-γ-4-fluoroglutamate (PteGluFGlu) and 4-NH_2-10-CH_3PteGluFGlu were also reported to be very poor substrates for FPGS.[33] By way of contrast, FMTX-γ-Glu was proved to be a substrate for both FPGS and γ-glutamyl hydrolase.[34] We report here the synthesis of 16 quinazoline antifolate dipeptides and 4 γ-amide analogues of ICI 198583-γ-glutamate (**4**).

Chemistry

Piper *et al.* prepared γ-amide and peptide analogues of methotrexate by alkylation of the appropriate *N*-[4-(methylamino)benzoyl]-L-γ-glutamyl precursor with 6-(bromomethyl)-2,4-diaminopteridine.[24] Rosowsky synthesized diglutamate derivatives of MTX by either coupling diethyl γ-L-glutamyl-L-glutamate to 4-amino-4-deoxy-N^{10}-methylpteroic acid employing the isobutyl

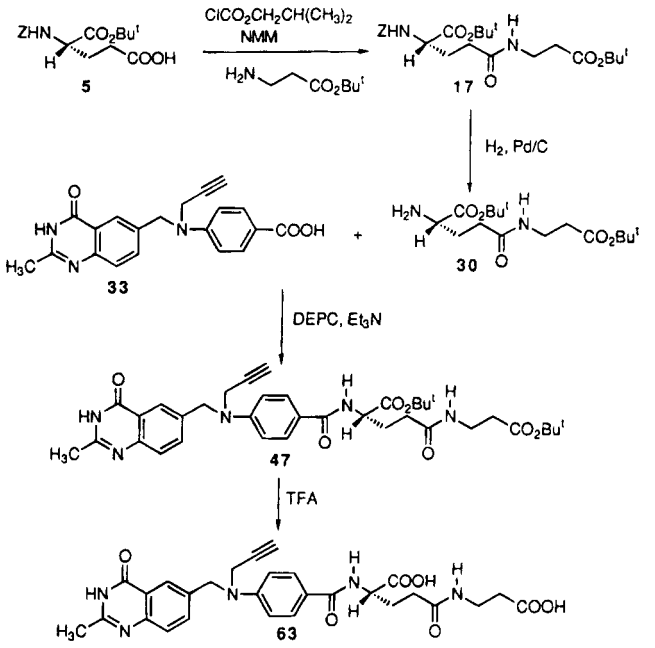
Scheme 1



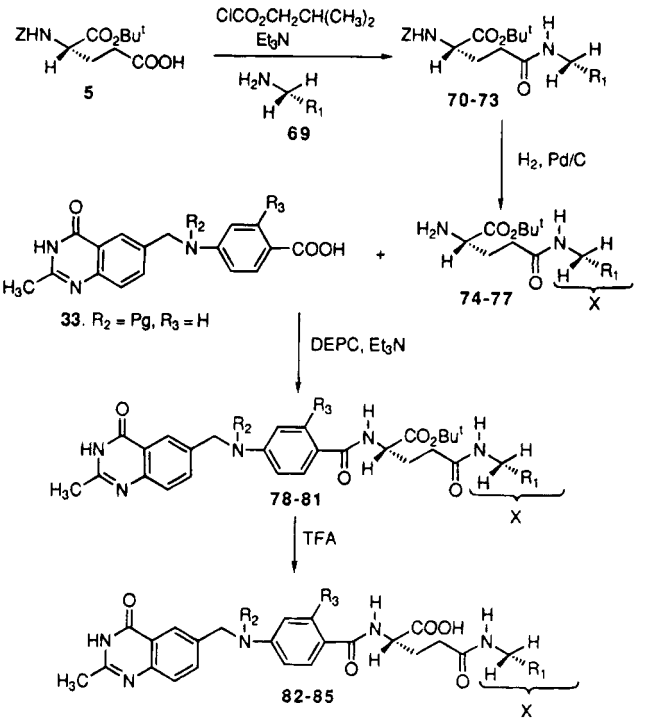
mixed anhydride coupling method or condensing diethyl L-glutamate hydrochloride salt with methotrexate α-monomethyl ester via diphenyl phosphorazidate coupling.[35] γ-Amide derivatives of methotrexate were synthesized from 4-amino-4-deoxy-N^{10}-methylpteroic acid and the appropriate L-glutamic acid γ-amide precursor using diethyl cyanophosphoridate (DEPC),[28,29] or by alkylating the appropriate *N*-[4-(methylamino)benzoyl]-L-Glu-γ-amides with 6-(bromomethyl)-2,4-diaminopteridine.[27] Lipophilic γ-amide derivatives of aminopterin (AMT) were synthesized from 4-amino-4-deoxy-N^{10}-formylpteroic acid and the appropriate L-glutamate γ-amide precursor via mixed carbonic anhydride coupling.[29] Baugh *et al.* used solid phase procedures for the preparation of a number of analogues of pteroylglutamyl-γ-glutamyl-γ-glutamic acid in which the terminal glutamate was replaced by other amino acids.[26] In addition, several synthetic strategies have been developed for the synthesis of poly-γ-glutamyl forms of folates and antifolates employing either conventional solution or solid peptide chemistry.[23]

Our approach to the synthesis of γ-linked dipeptides and amide analogues of ICI 198583 is summarized in Scheme 1. It involves the strategy applied for the synthesis of pteroyloligo-γ-glutamates[36] and the poly-γ-glutamates of ICI 198583.[23] Z-blocked dipeptides **7**−**16**, **18**, and **19** were prepared by condensing α-*tert*-butyl-*N*-(benzyloxycarbonyl)-L-glutamic acid (**5**)[37] with the appropriate L-amino acid **6** using the mixed carbonic anhydride method.[38] Carboxyl groups were masked as their *tert*-butyl esters to avoid the possibility of γ→α transpeptidation associated with alkaline carboxyl deprotection.[39] The serine hydroxyl group in **16** was also protected as a *tert*-butyl ether. Amino acids **6** were obtainable commercially or, in the case of L-norvaline, L-α-amino-*n*-butyric acid, and L-α-aminoadipic acid, by direct transesterification[40,41] using *tert*-butyl acetate and perchloric acid. Catalytic hydrogenolysis of **7**−**16**, **18**, and **19** using 10% palladium on charcoal afforded the dipeptide free bases **20**−**29**, **31**, and **32** in high yields. These free bases were characterized by ^{1}H-NMR and

Scheme 2



Scheme 3



then taken forward into the next reaction. Quinazoline L-γ-L dipeptide *tert*-butyl esters **37**–**46**, **48**, and **49** were synthesized by condensing the trifluoroacetate salt of 2-desamino-2-methyl-N^{10}-propargyl-5,8-dideazapteroic acid (**33**)[23] with the required dipeptide, **20**–**29**, **31**, and **32**, using DEPC as coupling reagent.[28] Quinazoline L-γ-L dipeptide esters **50**–**52** were prepared by condensing Glu-Ala **21** with the appropriate pteroic acid analogue, *e.g.*, **34**[42] (prepared by coupling 4-(methylamino)-benzoic acid to 6-(bromomethyl)-3,4-dihydro-2-methyl-4-oxoquinazoline[10]), **35**,[42,43] and **36**[23] (both prepared by an analogous route to the one used to synthesize **33**[23]). Esters **37**–**46** and **48**–**52** were purified by column chromatography. Removal of the *tert*-butyl protecting groups in the last step was accomplished with TFA to give the quinazoline L-γ-L dipeptides **53**–**62** and **64**–**68** as their trifluoroacetate salts in good overall yields. A longer time was required for the deprotection of **46** (Glu-Ser), necessitating the use of HPLC to monitor the completion of the reaction. Compound **63** (ICI 198583-γ-βAla) was prepared by a route identical to the one described for L-γ-L dipeptides **53**–**62** and **64**–**68** (Scheme 2).

Quinazoline L-Glu-γ-amide *tert*-butyl esters **78**–**81** were prepared by a similar route to the one described above, except that primary amines **69** (R_1 = H, methyl, propyl, and phenyl) were used in the coupling to **5** in place of amino acids (Scheme 3). TFA deprotection afforded the quinazoline L-Glu-γ-amides **82**–**85**, which, with the exception of **84**, were isolated as their corresponding free bases. The structure and purity of all new compounds were established by elemental microanalysis (Tables 1–3) and ^{1}H-NMR spectroscopy. Independent evidence for the structure of all compounds was also obtained by FAB mass spectrometry. All compounds were shown to be homogeneous by analytical HPLC.

Biological Evaluation

The antifolates listed in Table 3 were tested as inhibitors of TS partially purified from L1210 mouse leukemia cells that overproduce TS due to amplification of the TS gene.[44] The partial purification and the assay method used in this study were as previously described and used (±)-5,10-methylenetetrahydrofolic acid at a concentration of 200 μM.[18,44] Inhibition of L1210 and L1210:1565 cell growth was also as previously described.[44] L1210:1565 is a L1210 mutant cell line with impaired reduced folate/MTX transport carrier. This cell line was made resistant to CI-90, a compound that uses the RFC transport system, and hence is cross-resistant to MTX.[45]

Results and Discussion

The quinazoline antifolate dipeptide and γ-amide analogues were tested as inhibitors of thymidylate synthase and L1210 cell growth. The results are shown in Table 4.

All N^{10}-propargyl dipeptide analogues were potent inhibitors of TS (IC_{50} 2–24 nM) and inhibited growth of L1210 cells in the range 0.1–10 μM. In an attempt to probe for alternative binding sites around the γ'-carboxyl in **4**, and at the same time block the possibility of poly-γ-glutamation, the propionate side chain of the terminal amino acid was substituted by hydrogen, alkyl groups, or branched alkyl groups, giving a series of dicarboxylates (**53**–**58**) with activity against TS in the range 10–24 nM. Replacing the propionate chain with aromatic (**59**, **60**) or polar groups (**61**, **62**) again produced no substantial increase in binding affinity for TS. Next, the effect on TS of repositioning the γ'-carboxyl was studied. Deleting a methylene from the propionate side chain of **4** gave the Glu-Asp tricarboxylate **64** with TS activity (~10 nM) comparable to that of the dicarboxylates above but still approximately 5-fold less potent than the parent diGlu **4**. Lengthening the propionate by one methylene gave our best dipeptide inhibitor, Glu-aad **65**, with a binding affinity for TS equal to that of **4** (2 nM). Although preserving the γ'-carboxyl did not preclude the possibility of poly-γ-glutamation, it had

Table 1. Preparation of Z-Protected Dipeptides and L-Glu-γ-Amides

compd	X	yield, %	mp, °C	mass spectra m/z, $(M + H)^+$	formula	analyses
7	Gly(O^{t}Bu)	100	oil	451	$C_{23}H_{34}N_2O_7 \cdot 0.25H_2O$	C, H, N
8	Ala(O^{t}Bu)	97	oil	465	$C_{24}H_{36}N_2O_7$	C, H, N
9	abu(O^{t}Bu)	31	67–68	479	$C_{25}H_{38}N_2O_7$	C, H, N
10	Nva(O^{t}Bu)	92	74	493	$C_{26}H_{40}N_2O_7$	C, H, N
11	Val(O^{t}Bu)	83	108–109	493	$C_{26}H_{40}N_2O_7$	C, H, N
12	Ile(O^{t}Bu)	84	86	507	$C_{27}H_{42}N_2O_7$	C, H, N
13	Phg(O^{t}Bu)	61	65	527	$C_{29}H_{38}N_2O_7$	C, H, N
14	Phe(O^{t}Bu)	53	oil	541	$C_{30}H_{40}N_2O_7$	C, H, N
15	Gln(O^{t}Bu)	62	110–111	521[a]	$C_{26}H_{39}N_3O_8$	C, H, N
16	Ser(O^{t}Bu)$_2$	47	95–96	537	$C_{28}H_{44}N_2O_8$	C, H, N
17	βAla(O^{t}Bu)	100	oil	465	$C_{24}H_{36}N_2O_7 \cdot 0.25H_2O$	C, H, N
18	Asp(O^{t}Bu)$_2$	91	87–88	565	$C_{29}H_{44}N_2O_9$	C, H, N
19	aad(O^{t}Bu)$_2$	65	59–60	593	$C_{31}H_{48}N_2O_9 \cdot 0.25H_2O$	C, H, N
70	NHMe	83	104–105[b]			
71	NHEt	73				
72	NHnBu	90	86–87[c]	393	$C_{21}H_{32}N_2O_5$	C, H, N
73	NHBz	78	76–78[d]			

[a] FAB. [b] lit.[27] mp 106–107 °C. [c] lit.[27] mp 86–87 °C. [d] lit.[27] mp 78–80 °C.

Table 2. Preparation of Quinazoline γ-Linked Dipeptide and L-Glu-γ-Amide *tert*-Butyl Esters

compd	X	R_2	R_3	yield, %	mp, °C	mass spectra m/z, $(M + H)^+$	HPLC purity, %	formula	analyses
37	Gly(O^{t}Bu)	pg	H	57	123–124	646	99	$C_{35}H_{43}N_5O_7 \cdot 0.5H_2O$	C, H, N
38	Ala(O^{t}Bu)	pg	H	77	110–111	660	100	$C_{36}H_{45}N_5O_7 \cdot 0.5H_2O$	C, H, N
39	abu(O^{t}Bu)	pg	H	79	115–116	674	100	$C_{37}H_{47}N_5O_7 \cdot 0.5H_2O$	C, H, N
40	Nva(O^{t}Bu)	pg	H	62	112–114	688[a]	99	$C_{38}H_{49}N_5O_7 \cdot 0.5H_2O$	C, H, N
41	Val(O^{t}Bu)	pg	H	53	115–116	688	99	$C_{38}H_{49}N_5O_7 \cdot 0.75H_2O$	C, H, N
42	Ile(O^{t}Bu)	pg	H	71	114–115	702	99	$C_{39}H_{51}N_5O_7 \cdot 0.25H_2O$	C, H, N
43	Phg(O^{t}Bu)	pg	H	59	120–121	722	99	$C_{41}H_{47}N_5O_7 \cdot 0.5H_2O$	C, H, N
44	Phe(O^{t}Bu)	pg	H	80	109–110	736	100	$C_{42}H_{49}N_5O_7 \cdot 0.25H_2O$	C, H, N
45	Gln(O^{t}Bu)	pg	H	63	123–124	717	100	$C_{38}H_{48}N_6O_8 \cdot 1.1H_2O$	C, H, N
46	Ser(O^{t}Bu)$_2$	pg	H	60	113–114	730	100	$C_{40}H_{53}N_5O_8 \cdot 0.5H_2O$	C, H, N
47	βAla(O^{t}Bu)	pg	H	82	145–146	660	99	$C_{36}H_{45}N_5O_7 \cdot 0.5H_2O$	C, H, N
48	Asp(O^{t}Bu)$_2$	pg	H	78	114–115	760	99	$C_{41}H_{53}N_5O_9 \cdot 0.25H_2O$	C, H, N
49	aad(O^{t}Bu)$_2$	pg	H	73	104–105	810[b]	100	$C_{43}H_{57}N_5O_9 \cdot 0.75H_2O$	C, H, N
50	Ala(O^{t}Bu)	Me	H	72	118–119	636	99	$C_{34}H_{45}N_5O_7 \cdot 0.5H_2O$	C, H, N
51	Ala(O^{t}Bu)	Et	H	74	137–140	672	99	$C_{35}H_{47}N_5O_7$	C, H, N
52	Ala(O^{t}Bu)	pg	F	60	111–112	678	100	$C_{36}H_{44}FN_5O_7 \cdot 0.6H_2O$	C, H, N, F
78	NHMe	pg	H	60	134–138			$C_{30}H_{35}N_5O_5 \cdot 0.5H_2O$	C, H, N
79	NHEt	pg	H	79	135–139			$C_{31}H_{37}N_5O_5 \cdot 0.5H_2O$	C, H, N
80	NHnBu	pg	H	80	139–140	588	100	$C_{33}H_{41}N_5O_5 \cdot 0.4H_2O$	C, H, N
81	NHBz	pg	H	53	162–164			$C_{36}H_{39}N_5O_5 \cdot 0.5H_2O$	C, H, N

[a] ESI. [b] $(M + Na)^+$.

Table 3. Preparation of Deprotected Quinazoline γ-Linked Dipeptides and L-Glu-γ-Amides

compd	X	R_2	R_3	yield, %	mp, °C	mass spectra m/z, $(M + H)^+$	HPLC purity, %	formula	analyses
53	Gly	pg	H	90	145–146	534	98	$C_{27}H_{27}N_5O_7 \cdot 0.4CF_3CO_2H \cdot 1.5H_2O$	C, H, N, F
54	Ala	pg	H	97	173–175	548	99	$C_{28}H_{29}N_5O_7 \cdot 0.5CF_3CO_2H \cdot 1.3H_2O$	C, H, N, F
55	abu	pg	H	98	145–147	562	99	$C_{29}H_{31}N_5O_7 \cdot CF_3CO_2H \cdot 0.5H_2O$	C, H, N
56	Nva	pg	H	95	147 dec	576[a]	98	$C_{30}H_{33}N_5O_7 \cdot 0.6CF_3CO_2H$	C, H, N
57	Val	pg	H	46	152–153	576	98	$C_{30}H_{33}N_5O_7 \cdot 0.2CF_3CO_2H \cdot 1.5H_2O$	C, H, N, F
58	Ile	pg	H	94	148–149	590	99	$C_{31}H_{35}N_5O_7 \cdot 0.6CF_3CO_2H \cdot H_2O$	C, H, N, F
59	Phg	pg	H	96	159–161	610	99	$C_{33}H_{31}N_5O_7 \cdot 0.6CF_3CO_2H \cdot 2H_2O$	C, H, N, F
60	Phe	pg	H	88	139–141	624	100	$C_{34}H_{33}N_5O_7 \cdot CF_3CO_2H \cdot 0.75H_2O$	C, H, N
61	Gln	pg	H	90	150–151	605	99	$C_{30}H_{32}N_6O_8 \cdot 0.8CF_3CO_2H \cdot 2.1H_2O$	C, H, N, F
62	Ser	pg	H	97[b]	147–149	564	97	$C_{28}H_{29}N_5O_8 \cdot CF_3CO_2H \cdot 1.5H_2O$	C, H, N
63	βAla	pg	H	96	130–131	548	99	$C_{28}H_{29}N_5O_7 \cdot 0.7CF_3CO_2H \cdot 0.7H_2O$	C, H, N, F
64	Asp	pg	H	94	156–157	592	99	$C_{29}H_{29}N_5O_9 \cdot 0.75CF_3CO_2H \cdot 2H_2O$	C, H, N, F
65	aad	pg	H	86	132–133	620	98	$C_{31}H_{33}N_5O_9 \cdot 0.9CF_3CO_2H \cdot 1H_2O \cdot 1Et_2O$	C, H, N, F
66	Ala	Me	H	93	153–154	524	99	$C_{26}H_{29}N_5O_7 \cdot 0.9CF_3CO_2H \cdot 1.5H_2O \cdot 0.3Et_2O$	C, H, N, F
67	Ala	Et	H	81	155 dec	537	98	$C_{27}H_{31}N_5O_7 \cdot CF_3CO_2H \cdot H_2O$	C, H, N, F
68	Ala	pg	F	93	141–142	566	99	$C_{28}H_{28}FN_5O_7 \cdot 0.9CF_3CO_2H \cdot 2H_2O$	C, H, N, F
82	NHMe	pg	H	75	150–154	490[a]	99	$C_{26}H_{27}N_5O_5 \cdot 1.5H_2O$	C, H, N
83	NHEt	pg	H	77	145–147	504[a]	99	$C_{27}H_{29}N_5O_5 \cdot 1.5H_2O$	C, H, N
84	NHnBu	pg	H	85	124–125	532	99	$C_{29}H_{33}N_5O_5 \cdot 0.7CF_3CO_2H \cdot H_2O$	C, H, N, F
85	NHBz	pg	H	82	145–149	566[a]	100	$C_{32}H_{31}N_5O_5 \cdot 1.5H_2O$	C, H, N

[a] ESI. [b] TFA deprotection time = 2.25 h.

been anticipated that **64** and **65** would be poor or nonsubstrates for FPGS, since Moran *et al.* had previously reported that MTX analogues in which Glu had been replaced by Asp or aad were also poor substrates for FPGS.[46] Clearly there is a large amount of tolerance regarding the nature of the second, terminal amino acid.

Table 4. Inhibition Data for Quinazoline γ-Linked Dipeptides and L-Glu-γ-Amides

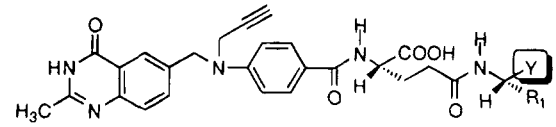
				IC_{50}			
compd	X	R_2	R_3	inhibn of TS, nM	inhibn of L1210 cell growth in culture, μM	inhibn of L1210:1565 in culture, μM	1565/L1210 ratio
4	Glu	pg	H	2	0.12	4.6	38
53	Gly	pg	H	11	0.11	10	91
54	Ala	pg	H	18	0.56	22	39
55	abu	pg	H	17	0.3		
56	Nva	pg	H	16	1.2	27	23
57	Val	pg	H	20	2.5	~65	~26
58	Ile	pg	H	24	3.2		
59	Phg	pg	H	14	0.6	22	37
60	Phe	pg	H	18	10	~56	~6
61	Gln	pg	H	16	0.6	19	32
62	Ser	pg	H	22	1.2		
63	βAla	pg	H	14	0.9		
64	Asp	pg	H	10	2.4	17	7
65	aad	pg	H	2	0.33	6	18
66	Ala	Me	H	152	1.8		
67	Ala	Et	H	49	5.6		
68	Ala	pg	F	4.3	0.4		
82	NHMe	pg	H	64	2.1		
83	NHEt	pg	H	70	5.2	45	9
84	NHnBu	pg	H	118	20		
85	NHBz	pg	H	170	22		
86	gaba	pg	H	14	0.33	16	48

We have used a computerized model of the humanized *Escherichia coli* active site of TS and demonstrated that the α′-carboxyl probably interacts with an arginine residue (via a salt bridge) but the terminal γ′-carboxyl is free in space. This is thought to be why the nature of the second amino acid is not particularly important for TS inhibition. L1210 growth inhibition was more affected by the nature of the terminal acid, which may relate, in part, to the rate of transport across the cell membrane. Three compounds had poorer L1210 growth inhibition than might be expected for their corresponding TS inhibition. These were the two branched chain dipeptides **57** and **58** and the Glu-Asp **64**. Two were better, the Glu-Gly **53** and the Glu-abu **55**.

In an attempt to study how other structural modifications affect TS and L1210 cell growth inhibition, compounds **66**–**68** were synthesized, all being analogues of the Glu-Ala derivative **54**. Replacement of the N^{10}-propargyl substituent in compound **54** by a methyl or ethyl resulted in much poorer inhibitors of TS and L1210 cell growth. However, introduction of a 2′-F group, compound **68**, enhanced TS inhibition by approximately 4-fold when compared with the parent Glu-Ala analogue **54**. Similar trends were observed for CB 3717, ICI 198583, and their corresponding analogues.[10,11,47,48]

Deletion of the α′-carboxyl from **53**, **54**, **56**, and **59** gave a series of monocarboxylate γ-amides (**82**–**85**) which were clearly less active than the corresponding parent dipeptides against TS (Table 5). Ethylamide **83** is a weaker inhibitor of TS by about 5-fold compared with the parent Glu-Ala dipeptide derivative **54**, while benzylamide **85** inhibits TS approximately 12 times less than the corresponding parent dipeptide Glu-Phg **59**. This loss in activity is expected since the α′-carboxyl interaction with the hypothesized arginine residue cannot occur. The poorer cell growth inhibition displayed by these monocarboxylate γ-amides against L1210 cultured cells may in part be explained by the poor potency of these compounds as inhibitors of TS but also by their inability to use the RFC transport system efficiently (*vide infra*). Deletion of the α′-carboxyl from diglutamate **4** gave the Glu-gaba compound **86**[49] which was 7-fold less active than its parent **4** against TS yet only marginally less active against L1210 cell growth. In this case, however, the reduction in activity against TS is probably offset by the ability of **86** to get into cells more efficiently using the reduced folate carrier than monocarboxylate γ-amides **82**–**85**.

Table 5. Effect of Removing the α′-COOH from the Terminal Amino Acid



compd	Y	R_1	TS IC_{50}, nM	L1210 IC_{50}, μM	stable *in vivo*?
53	COOH	H	11	0.11	no
82	H	H	64	2.1	
54	COOH	CH_3	18	0.56	no
83	H	CH_3	70	5.2	yes
56	COOH	$CH_2CH_2CH_3$	16	1.2	
84	H	$CH_2CH_2CH_3$	118	20	
59	COOH	Ph	14	0.6	no
85	H	Ph	170	22	yes
4	COOH	CH_2CH_2COOH	2	0.12	no
86	H	CH_2CH_2COOH	14	0.33	yes
64	COOH	CH_2COOH	10	2.4	no
63	H	CH_2COOH	14	0.9	no

By way of contrast to the preceding pairs of compounds, deletion of the α′-carboxyl from Glu-Asp **64** gave the Glu-βAla **63** without significant loss in activity against TS. A possible explanation for this anomaly may be that the remaining β′-carboxyl lies sufficiently close to the γ-amidic bond that it can mimic the role of the deleted α′-carboxyl and hence maintain binding affinity for TS.

Several compounds were tested as inhibitors of L1210:1565 cell growth (Table 4). Poor activity in this cell line relative to the parental L1210 line suggests that the L-γ-L dicarboxylate dipeptides use the reduced folate carrier quite efficiently for cell entry with one possible exception, that of the Glu-Phe dipeptide (compound **60**).

However, the monocarboxylate γ-ethylamide **83** and the tricarboxylate Glu-Asp **64** appear to utilize the reduced folate carrier less efficiently, demonstrating again this carrier's apparent preference for the transport of dianions.[50] Piper *et al.* had previously reported the reduced influx into L1210 cells of γ-amide derivatives such as MTX-γ-methylamide (K_m = 27.6 μM) and particularly the L-γ-L dipeptide MTX-γ-aspartate (K_m = >300 μM).[24]

Studies on the stability of the quinazoline γ-linked dipeptides and L-Glu-γ-amides revealed some interesting findings. Although very little breakdown was observed with these compounds *in vitro* (data not shown), when L-γ-L dipeptides **53**, **54**, **59**, **64**, and **4** were administered to mice, they were partially degraded to their monoglutamate forms[51,52] by γ-glutamyl hydrolases, a group of enzymes which act by cleaving the γ-glutamyl amide bond.[53] By way of contrast, removal of the α′-carboxyl from **54**, **59**, and **4** gave L-Glu-γ-amides **83**, **85**, and **86**,[42] all of which were stable in mice (Table 5). MTX-γ-benzylamide was similarly reported to be stable in mice.[29] Deletion of the α′-carboxyl from the Glu-Asp **64** gave the Glu-βAla **63**, *both* of which were partially degraded to their monoglutamate forms in mice. These observations indicate that there is an apparent requirement for a free carboxyl group (*e.g.*, an α′- or β′-carboxyl) in close proximity to the γ-amidic bond for hydrolysis by γ-hydrolases to occur.[51,52]

In conclusion, nearly all dipeptide derivatives were potent inhibitors of TS, the best example being the Glu-aad **65** with equal activity to the Glu-Glu **4**. Polyglutamation is believed not to occur since activity was retained against the L1210:R^{D1694} (L1210:MB3) cell line, a line unable to polyglutamate antifolates.[19] Compound **65** is ~30-fold more active as a TS inhibitor than ICI 198583 (**3**) with very little loss in L1210 growth inhibition, despite the fact that activation through polyglutamation cannot occur. The L-Glu-γ-amides, lacking an α′-carboxyl group, are less active against TS and L1210 cell growth but are also not susceptible to enzymatic hydrolysis *in vivo* by γ-hydrolases.

Experimental Section

N,*N*-Dimethylformamide (DMF) and *N*,*N*-dimethylacetamide (DMA) (Aldrich HPLC grades) were dried over 3 Å molecular sieves. Anhydrous tetrahydrofuran (THF) was purchased from Aldrich. TLC was performed on precoated sheets of silica $60F_{254}$ (Merck Art. 5735). Spots were visualized with chlorine–tolidine reagent. Merck silica 60 (Art. 15111) was used in low-pressure column chromatography. HPLC analyses were performed using a Waters Model 510 solvent delivery system, Model 680 automated gradient controller, Model U6K injector, and Model 490 programmable wavelength detector set to monitor at 230 and 280 nm. Retention times were determined on a Trivector Trilab 3000 multichannel chromatography system. Separations were performed on a 15 cm × 0.46 cm column packed with 5 μM Spherisorb C6 (Phase Separations Ltd., U.K.) and eluted isocratically with different ratios of MeOH/H_2O containing 1% HOAc. Electron impact mass spectra were determined with a VG 7070H spectrometer and a VG 2235 data system using the direct-insertion method, an ionizing voltage of 70 eV, a trap current of 100 μA, and an ion source temperature of 160 °C. Fast atom bombardment (FAB) mass spectra were determined with a VG ZAB-SE spectrometer, operating at 20 kV Cs^+ at 8 kV accelerating voltage in the source. Electrospray ionization mass spectra were determined using a TSQ 700 triple quadrapole mass spectrometer (Finnigan MAT) fitted with an electrospray ionization source (Analytica). Samples were dissolved in methanol:water (50:50 v/v) containing 1% acetic acid and infused into the mass spectrometer using a Harvard infusion pump (Cambridge) at 1 μL/min. Masses were scanned from 200 to 800 amu at a scanning speed of 3 s/scan. NMR spectra were determined on a Bruker WM250 spectrometer using tetramethylsilane as internal standard. Melting points were determined on a Kofler block and are uncorrected. Elemental analyses were determined by C.H.N. Analysis Ltd., Leicester.

The syntheses of compounds **4** and **86** have been reported previously.[23,49]

Preparation of *tert*-Butyl α-Amino Esters. Di-*tert*-butyl α-Aminoadipate. A mixture of L-α-aminoadipate hydrochloride (1 g, 6.2 mmol) and 70% perchloric acid (0.99 g, 6.8 mmol) in *tert*-butyl acetate (85 mL) was stirred at room temperature for 2 days. The solution was then extracted with cold 0.5 N HCl (2 × 50 mL), the aqueous layer was carefully neutralized with small portions of powdered $NaHCO_3$, and the product was extracted into Et_2O (2 × 150 mL). The Et_2O extracts were combined, dried, and reduced in volume to ~50 mL. The ethereal solution was acidified using anhydrous HCl in Et_2O and then cooled, whereupon the product crystallized out as the hydrochloride salt. The salt was filtered off, washed well with Et_2O (3 × 10 mL), and dried *in vacuo* to give the required product as white needles: 0.763 g (40%); mp 132–133 °C; NMR (Me_2SO-d_6) δ 1.40, 1.47 (2 × s, each 9H, $C(CH_3)_3$), 1.54, 1.64 (2 × m, 2H, CH_2^β), 1.74 (m, 2H, CH_2^γ), 2.24 (t, J = 7.2 Hz, 2H, CH_2^δ), 3.87 (t, J = 5.8 Hz, 1H, aad α-CH), 8.24 (s, 3H, NH_3^+).

The procedure was repeated with L-α-amino-*n*-butyric acid and L-norvaline. However, the *tert*-butyl esters of these amino acids were isolated as their free bases and then taken forward into the next step without further purification.

Preparation of Z-Blocked Dipeptide *tert*-Butyl Esters. Di-*tert*-butyl *N*-[*N*-(Benzyloxycarbonyl)-L-γ-glutamyl]-L-alaninate (8). To a stirred solution of **5**[31] (1.011 g, 3 mmol) and 4-methylmorpholine (0.303 g, 3 mmol) in THF (3 mL) cooled to −20 °C was added isobutyl chloroformate (0.408 g, 3 mmol). After 10 min, a suspension of α-*tert*-butyl alanine hydrochloride (0.545 g, 3 mmol) in THF (3 mL) containing 4-methylmorpholine (0.303 g, 3 mmol) was added. Stirring was continued for 10 min at −20 °C, and the mixture was then allowed to warm to room temperature. 4-Methylmorpholine hydrochloride was filtered off and the filtrate evaporated *in vacuo*. The resulting crude oil was purified by chromatography on a silica gel column (Merck 15111) using 5% EtOAc in CH_2Cl_2 as the eluent. Product-containing fractions were combined and evaporated *in vacuo*, affording **8** as a colorless oil: 1.348 g (97%), which did not crystallize; NMR (Me_2SO-d_6) δ 1.21 (d, J = 6.8 Hz, 3H, Ala CH_3), 1.38, 1.39 (2 × s, each 9H, $C(CH_3)_3$), 1.74, 1.91, (2 × m, each 1H, CH_2^β), 2.19 (t, J = 7.5 Hz, 2H, CH_2^γ), 3.89 (m, 1H, Glu α-CH), 4.08 (m, 1H, Ala α-CH), 5.03, 5.04 (ABq, J_{AB} = 14.1 Hz, 2H, $C_6H_5CH_2$), 7.36 (m, 5H, C_6H_5-CH_2), 7.64 (d, J = 6.8 Hz, 1H, Glu NH), 8.15 (d, J = 6.3 Hz, 1H, Ala NH); MS *m/z* 465 $(M + H)^+$. Anal. ($C_{24}H_{36}N_2O_7$) C, H, N.

Z-protected dipeptides **7** and **9**–**19** were synthesized by the method described above. Purifications were effected by chromatography on silica gel columns (Merck 15111) using CH_2Cl_2/EtOAc (2:1) as eluent (**7**, **11**, **12**), 1% MeOH in CH_2Cl_2 as eluent (**15**), or 2% MeOH in CH_2Cl_2 as eluent (**9**, **10**, **13**, **14**, **16**–**19**). Those products that crystallized were triturated in either hexane (**9**, **11**, **12**, **18**, **19**) or petroleum ether (**16**) or were precipitated from either CH_2Cl_2/petroleum ether (**10**, **13**) or CH_2Cl_2/Et_2O (**15**) and then filtered off and dried *in vacuo*. Yields and mass spectral and analytical data of these products are given in Table 1. The ^1H NMR spectra of these compounds were consistent with the assigned structures.

Preparation of Z-Blocked L-Glu-γ-amide *tert*-Butyl Esters. α-*tert*-Butyl *N*-[*N*-(Benzyloxycarbonyl)-L-γ-glutamyl]-*n*-butylamide (72). *n*-Butylamine and **5** were coupled together as described for the preparation of **8** above, except that only 1 equiv of 4-methylmorpholine was used. Purification was effected by column chromatography (2% MeOH in CH_2Cl_2) and subsequent precipitation (CH_2Cl_2/petroleum ether), affording **72** as a white powder in 90% yield: mp 86–87 °C (lit.[27] mp 86–87 °C); NMR (Me_2SO-d_6) δ 0.85 (t, J = 6.9 Hz, 3H, $(CH_2)_3CH_3$), 1.29 (m, 4H, $CH_2CH_2CH_2CH_3$), 1.39 (s, 9H,

$C(CH_3)_3$), 1.73, 1.90 (2 × m, 2H, Glu CH_2^{β}), 2.14 (t, J = 7.4 Hz, 2H, Glu CH_2^{γ}), 3.01 (q, J = 6.5 Hz, 2H, $NHCH_2(CH_2)_2CH_3$), 3.86 (m, 1H, Glu α-CH), 5.02, 5.04 (ABq, J_{AB} = 12.2 Hz, 2H, $C_6H_5CH_2$), 7.36 (m, 5H, $C_6H_5CH_2$), 7.63 (d, J = 6.8 Hz, 1H, Glu NH), 7.78 (t, J = 6.5 Hz, 1H, $NHCH_2(CH_2)_2CH_3$); MS m/z 393 $(M + H)^+$. Anal. ($C_{21}H_{32}N_2O_5$) C, H, N.

Z-blocked γ-amides **70**, **71**, and **73** were also prepared by the method described above and had ^1H NMR spectra consistent with the assigned structures.

Hydrogenolysis of Z-Blocked Dipeptide and L-Glu-γ-amide *tert*-Butyl Esters. Di-*tert*-butyl L-γ-Glutamyl-L-alaninate (21). A solution of di-*tert*-butyl *N*-[*N*-(benzyloxycarbonyl)-L-γ-glutamyl]-L-alaninate (**8**) (1.348 g, 2.9 mmol) in THF (50 mL) containing 10% Pd/C (0.14 g) in suspension was stirred under hydrogen at atmospheric pressure for 4 h, whereupon TLC showed the absence of starting material. The catalyst was filtered off and the filtrate concentrated *in vacuo* to give an oil (0.92 g, 96%), which was used without further purification: NMR (Me_2SO-d_6) δ 1.21 (d, J = 6.7 Hz, 3H, Ala CH_3), 1.38, 1.41 (2 × s, each 9H, $C(CH_3)_3$), 1.56, 1.76 (2 × m, each 1H, CH_2^{β}), 2.18 (t, J = 7.5 Hz, 2H, CH_2^{γ}), 3.14 (dd, J = 8.0, 4.5 Hz, 1H, Glu α-CH), 4.07 (m, 1H, Ala α-CH), 8.14 (d, J = 6.7 Hz, 1H, Ala NH).

The procedure was repeated with the appropriate Z-blocked dipeptide and L-Glu-γ-amide *tert*-butyl esters **7**, **9**–**19**, and **70**–**73** to yield the dipeptide and L-Glu-γ-amide *tert*-butyl esters **20**, **22**–**32**, and **74**–**77**. These compounds had ^1H NMR spectra consistent with the assigned structures.

Preparation of Pteroic Acid Analogues. *tert*-Butyl 4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)methyl]-*N*-ethylamino]benzoate (87). A solution of *tert*-butyl 4-(ethylamino)benzoate (7.29 g, 33 mmol, prepared by alkylating *tert*-butyl 4-aminobenzoate with ethyl iodide as described for the preparation of *tert*-butyl 4-(prop-2-ynylamino)benzoate[23]), 6-(bromomethyl)-3,4-dihydro-2-methyl-4-oxoquinazoline[10] (7.97 g, 32 mmol), and dry $CaCO_3$ (4 g, 40 mmol) in DMA (50 mL) was stirred at 50 °C in the dark. After 20 h, the $CaCO_3$ was filtered off and the filtrate concentrated *in vacuo* to give a brown oil. The oil was partitioned between EtOAc (500 mL) and dilute ammonium hydroxide solution (H_2O/18 N NH_3, 10:1) (250 mL) and the EtOAc layer separated and washed with more dilute NH_4OH (2 × 250 mL) and then H_2O (250 mL). The EtOAc layer was separated, dried (Na_2SO_4), and reduced in volume to 200 mL *in vacuo*. After cooling in ice, the precipitate was collected by filtration, washed with cold EtOAc (2 × 25 mL), and dried *in vacuo* to give a white solid. Two further crystallizations from EtOAc/petroleum ether, 60/80, yielded the product as a white powder: 5.81 g (47%); mp 156 °C; NMR (Me_2SO-d_6) δ 1.16 (t, J = 7.0 Hz, 3H, CH_2CH_3), 1.48 (s, 9H, $CO_2C(CH_3)_3$), 2.33 (s, 3H, C^2-CH_3), 3.57 (q, J = 7.0 Hz, 2H, CH_2CH_3), 4.74 (s, 2H, quinazoline 6-CH_2N), 6.70 (d, J = 9.0 Hz, 2H, benzene 3′,5′-H), 7.55 (d, J = 8.4 Hz, 1H, quinazoline 8-H), 7.63 (dd, J = 8.3, 1.9 Hz, 1H, quinazoline 7-H), 7.66 (d, J = 8.9 Hz, 2H, benzene 2′,6′-H), 7.84 (d, J = 1.8 Hz, 1H, quinazoline 5-H), 12.21 (s, 1H, quinazoline 3-H); MS m/z 394 $(M + H)^+$. Anal. ($C_{23}H_{27}N_3O_3$·0.1H_2O) C, H, N.

4-[*N*-[(3,4-Dihydro-2-methyl-4-oxo-6-quinazolinyl)-methyl]-*N*-ethylamino]benzoic Acid, Trifluoroacetate Salt (35). The foregoing *tert*-butyl ester **87** (2 g, 5.09 mmol) was dissolved in CF_3COOH (20 mL). After the reaction mixture was stirred under N_2 at ambient temperature and in the dark for 20 min, the solution was concentrated under reduced pressure. The colorless oily residue was triturated with EtOAc (80 mL), and the precipitate was filtered off and washed well with petroleum ether to give a white powder: 2.23 g (97%); mp >270 °C; NMR (Me_2SO-d_6) δ 1.18 (t, J = 6.9 Hz, 3H, CH_2CH_3), 2.45 (s, 3H, C^2-CH_3), 3.59 (q, J = 6.9 Hz, 2H, CH_2CH_3), 4.77 (s, 2H, CH_2N), 6.72 (d, J = 9.1 Hz, 2H, benzene 3′,5′-H), 7.61 (d, J = 8.4 Hz, 1H, quinazoline 8-H), 7.73 (d, J = 9.0 Hz, 3H, benzene 2′,6′-H, quinazoline 7-H), 7.88 (d, J = 1.7 Hz, 1H, quinazoline 5-H); MS m/z 338 $(M + H)^+$. Anal. ($C_{19}H_{19}N_3O_3$·1.05CF_3CO_2H) C, H, N, F.

Pteroic acid analogues **33** and **36** were prepared in a similar way.[23] Pteroic acid analogue **34** was prepared as described in ref 42.

Preparation of Quinazoline γ-Linked Dipeptide *tert*-Butyl Esters. Di-*tert*-butyl *N*-[*N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxoquinazolin-6-yl)methyl]-*N*-prop-2-ynylamino]-benzoyl]-L-γ-glutamyl]-L-alaninate (38). The pteroic acid analogue, trifluoroacetate salt, **33** (0.461 g, 1 mmol) and di-*tert*-butyl L-γ-glutamyl-L-alaninate (**21**) (0.417 g, 1.3 mmol) were dissolved in dry DMF (15 mL) at room temperature, and to this solution was added diethyl cyanophosphoridate (0.359 g, 2.2 mmol) and then Et_3N (0.222 g, 2.2 mmol). The mixture was stirred under nitrogen and in the dark for 2 h and then diluted with EtOAc (100 mL) and H_2O (100 mL). The water layer was separated and extracted with EtOAc (2 × 100 mL). The combined EtOAc extracts were washed with 10% aqueous citric acid (2 × 50 mL), saturated $NaHCO_3$ (100 mL), and dilute NaCl (100 mL) and then dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by chromatography on a silica gel column (Merck 15111) using EtOAc and then 2% MeOH in EtOAc as the eluent. Product containing fractions were combined and evaporated *in vacuo* to give a foam. The foam was dissolved in CH_2Cl_2 (10 mL) and petroleum ether (50 mL) added, giving a white gelatinous precipitate which was filtered off and dried *in vacuo*. The required product **38** was obtained as a white powder: 0.505 g (77%); mp 110–111 °C; NMR (Me_2SO-d_6) δ 1.20 (d, J = 7.3 Hz, 3H, Ala CH_3), 1.37, 1.40 (2 × m, 18H, $C(CH_3)_3$), 1.90, 2.00 (2 × m, 2H, CH_2^{β}), 2.22 (t, J = 6.0 Hz, 2H, CH_2^{γ}), 2.34 (s, 3H, quinazoline 2-CH_3), 3.24 (s, 1H, C≡CH), 4.07 (m, 1H, Ala α-CH), 4.23 (m, 1H, Glu α-CH), 4.34 (s, 2H, $CH_2C{\equiv}C$), 4.79 (s, 2H, quinazoline 6-CH_2N), 6.83 (d, J = 8.8 Hz, 2H, benzene 3′,5′-H), 7.55 (d, J = 8.4 Hz, 1H, quinazoline 8-H), 7.71 (dd, J = 9.1, 1.5 Hz, 1H, quinazoline 7-H), 7.74 (d, J = 8.6 Hz, 2H, benzene 2′,6′-H), 7.97 (s, 1H, quinazoline 5-H), 8.18 (d, J = 6.9 Hz, 1H, Ala NH), 8.34 (d, J = 7.5 Hz, 1H, Glu NH), 12.26 (s, 1H, quinazoline 3-H); MS m/z 660 $(M + H)^+$. Anal. ($C_{36}H_{45}N_5O_7$·0.5H_2O) C, H, N.

The procedure was repeated with the appropriate primary amines **20**–**32** and the appropriate pteroic acid analogues **33**–**36** to give the coupled quinazoline γ-linked dipeptide *tert*-butyl esters **37** and **39**–**52**. Yields and mass spectral and analytical data of these products are given in Table 2. The ^1H NMR spectra of these compounds were consistent with the assigned structures.

Preparation of Quinazoline γ-Linked Dipeptides. *N*-[*N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxoquinazolin-6-yl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-γ-glutamyl]-L-alanine, Trifluoroacetate Salt (54). A solution of **38** (0.348 g, 0.53 mmol) in TFA (10 mL) was stirred at room temperature for 1 h in the dark and under a nitrogen atmosphere. The solution was then concentrated *in vacuo* and the residue triturated with anhydrous Et_2O (30 mL). The solid was isolated by filtration, washed with Et_2O (4 × 10 mL), and dried *in vacuo* over P_2O_5, giving a white powder: 0.339 g (97%); mp 173–175 °C; NMR (Me_2SO-d_6) δ 1.22 (d, J = 7.3 Hz, 3H, Ala CH_3), 1.90, 2.05 (2 × m, each 1H, CH_2^{β}), 2.23 (t, J = 6.8 Hz, 2H, CH_2^{γ}), 2.38 (s, 3H, quinazoline 2-CH_3), 3.24 (s, 1H, C≡CH), 4.17 (m, 1H, Ala α-CH), 4.30 (m, 1H, Glu α-CH), 4.35 (s, 2H, $CH_2C{\equiv}C$), 4.80 (s, 2H, quinazoline 6-CH_2N), 6.83 (d, J = 9.0 Hz, 2H, benzene 3′,5′-H), 7.57 (d, J = 8.4 Hz, 1H, quinazoline 8-H), 7.75 (d, J = 8.9 Hz, 3H, quinazoline 7-H and benzene 2′,6′-H), 7.99 (s, 1H, quinazoline 5-H), 8.16 (d, J = 7.1 Hz, 1H, Ala NH), 8.33 (d, J = 7.5 Hz, 1H, Glu NH), 12.45 (b s, 1H, quinazoline 3-H); MS m/z 548 $(M + H)^+$. Anal. ($C_{28}H_{29}N_5O_7$·0.5CF_3-$COOH$·1.3H_2O) C, H, N, F.

The procedure was repeated with the appropriate quinazoline γ-linked dipeptide *tert*-butyl esters **37** and **39**–**52** to yield the quinazoline γ-linked dipeptides **53** and **55**–**68**, all of which had ^1H NMR spectra consistent with the assigned structures. Yields and analytical data are gathered in Table 3.

Some of the trifluoroacetate salts were found to be both hygroscopic and light-sensitive, and appropriate steps were taken to prevent decomposition of these during storage.

Preparation of Quinazoline L-Glu-γ-Linked Amide *tert*-Butyl Esters. *tert*-Butyl *N*-[*N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxoquinazolin-6-yl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-γ-glutamyl]-*n*-butylamide (80). Compound **80** was prepared by coupling pteroic acid analogue **33** to amine

76 as described for the preparation of **38**. The crude product was purified by chromatography on a silica gel column (Merck 15111) using EtOAc and then 2% MeOH in EtOAc as the eluent. Product-containing fractions were combined and evaporated *in vacuo* to give a solid. The solid was dissolved in $CHCl_3$ (10 mL) and petroleum ether (50 mL) added, giving a white gelatinous precipitate which was filtered off and dried *in vacuo*. The required product **80** was obtained as a white powder in 80% yield: mp 139–140 °C; NMR (Me_2SO-d_6) δ 0.83 (t, J = 6.2 Hz, 3H, $(CH_2)_3CH_3$), 1.30 (m, 4H, $CH_2CH_2CH_2CH_3$), 1.39 (s, 9H, $C(CH_3)_3$), 1.88, 2.00 (2 × m, 2H, Glu CH_2^{β}), 2.18 (t, J = 7.0 Hz, 2H, Glu CH_2^{γ}), 2.33 (s, 3H, quinazoline 2-CH_3), 3.00 (q, J = 5.9 Hz, 2H, $NHCH_2(CH_2)_2CH_3$), 3.24 (d, J = 1.5 Hz, 1H, C≡CH), 4.21 (m, 1H, Glu α-CH), 4.34 (s, 2H, $CH_2C{\equiv}C$), 4.78 (s, 2H, quinazoline 6-CH_2N), 6.83 (d, J = 8.1 Hz, 2H, benzene 3′,5′-H), 7.54 (d, J = 8.3 Hz, 1H, quinazoline 8-H), 7.69 (d, 1H, quinazoline 7-H), 7.73 (d, J = 7.9 Hz, 2H, benzene 2′,6′-H), 7.81 (t, J = 5.4 Hz, 1H, $NH(CH_2)_3CH_3$), 7.96 (s, 1H, quinazoline 5-H), 8.32 (d, J = 7.1 Hz, 1H, Glu NH), 12.19 (s, 1H, quinazoline 3-H); MS m/z 588 $(M + H)^+$. Anal. ($C_{33}H_{41}N_5O_5 \cdot 0.4H_2O$) C, H, N.

The procedure was repeated with the appropriate amines **74**, **75**, and **77** and the pteroic acid analogue **33** to give the coupled quinazoline L-γ-linked amide *tert*-butyl esters **78**, **79**, and **81**. Yields and mass spectral and analytical data of these products are given in Table 2. The ^1H NMR spectra of these compounds were consistent with the assigned structures.

Preparation of Quinazoline L-Glu-γ-Linked Amides. *N*-[*N*-[4-[*N*-[(3,4-Dihydro-2-methyl-4-oxoquinazolin-6-yl)methyl]-*N*-prop-2-ynylamino]benzoyl]-L-γ-glutamyl]-*n*-butylamide, Trifluoroacetate Salt (84). Compound **80** was deprotected with TFA as described for the synthesis of **54**, affording **84** as a white powder in 85% yield: mp 124–125 °C; NMR (Me_2SO-d_6) δ 0.83 (t, J = 6.8 Hz, 3H, $(CH_2)_3CH_3$), 1.29 (m, 4H, $CH_2CH_2CH_2CH_3$), 1.90, 2.04 (2 × m, 2H, Glu CH_2^{β}), 2.18 (t, J = 6.7 Hz, 2H, Glu CH_2^{γ}), 2.38 (s, 3H, quinazoline 2-CH_3), 3.00 (q, J = 6.3 Hz, 2H, $NHCH_2(CH_2)_2CH_3$), 3.24 (s, 1H, C≡CH), 4.28 (m, 1H, Glu α-CH), 4.35 (s, 2H, $CH_2C{\equiv}C$), 4.80 (s, 2H, quinazoline 6-CH_2N), 6.83 (d, J = 7.7 Hz, 2H, benzene 3′,5′-H), 7.57 (d, J = 8.4 Hz, 1H, quinazoline 8-H), 7.73 (d, J = 7.4 Hz, 3H, benzene 2′,6′-H and quinazoline 7-H), 7.82 (t, J = 4.9 Hz, 1H, $NH(CH_2)_3CH_3$), 7.98 (s, 1H, quinazoline 5-H), 8.33 (d, J = 7.4 Hz, 1H, Glu NH), 12.50 (b s, 1H, quinazoline 3-H); MS m/z 532 $(M + H)^+$. Anal. ($C_{29}H_{33}N_5O_5 \cdot 0.7CF_3COOH \cdot H_2O$) C, H, N, F.

The procedure was repeated with the appropriate quinazoline L-γ-linked amide *tert*-butyl esters **78**, **79**, and **81** to yield the quinazoline L-γ-linked amides **82**, **83**, and **85** (isolated as their free bases), all of which had ^1H NMR spectra consistent with the assigned structures. Yields and analytical data are gathered in Table 3.

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Supplementary Material Available: ^1H NMR spectral data of Z-protected dipeptides **7** and **9–19**, Z-protected L-Glu-γ-amides **70** and **71–73**, quinazoline γ-linked dipeptide *tert*-butyl esters **37–52**, quinazoline γ-linked dipeptides **53–68**, quinazoline L-Glu-γ-linked amide *tert*-butyl esters **78–81**, and quinazoline L-Glu-γ-linked amides **82–85** (6 pages). Ordering information is given on any current masthead page.

References

(1) Jones, T. R.; Calvert, A. H.; Jackman, A. L.; Brown, S. J.; Jones, M.; Harrap, K. R. A Potent Antitumour Quinazoline Inhibitor of Thymidylate Synthetase: Synthesis, Biological Properties and Therapeutic Results in Mice. *Eur. J. Cancer* **1981**, *17*, 11–19.

(2) Jackson, R. C.; Jackman, A. L.; Calvert, A. H. Biochemical Effects of a Quinazoline Inhibitor of Thymidylate Synthetase, CB 3717, on Human Lymphoblastoid Cells. *Biochem. Pharmacol.* **1983**, *32*, 3783–3790.

(3) Calvert, A. H.; Alison, D. L.; Harland, S. J.; Robinson, B. A.; Jackman, A. L.; Jones, T. R.; Newell, D. R.; Siddick, Z. H.; Wiltshaw, E.; McElwain, T. J.; Smith, I. E.; Harrap, K. R. A Phase I Evaluation of the Quinazoline Antifolate Thymidylate Synthase Inhibitor, N^{10}-Propargyl-5,8-dideazafolic Acid, CB 3717. *J. Clin. Oncol.* **1986**, *4*, 1245–1252.

(4) Synonyms: PDDF; Z 155,387; NSC 327182; *N*-[4-[*N*-[(2-amino-3,4-dihydro-4-oxo-6-quinazolinyl)methyl]prop-2-ynylamino]benzoyl]-L-glutamic acid.

(5) Newell, D. R.; Siddik, Z. H.; Calvert, A. H.; Jackman, A. L.; Alison, D. L.; McGhee, K. G.; Harrap, K. R. Pharmacokinetics and Toxicity Studies With CB 3717. *Proc. Am. Assoc. Cancer Res.* **1982**, *23*, 181.

(6) Alison, D. L.; Newell, D. R.; Sessa, C.; Harland, S. J.; Hart, L. I.; Harrap, K. R.; Calvert, A. H. The Clinical Pharmacokinetics of the Novel Antifolate N^{10}-Propargyl-5,8-dideazafolic Acid (CB 3717). *Cancer Chemother. Pharmacol.* **1985**, *14*, 265–271.

(7) Newell, D. R.; Alison, D. L.; Calvert, A. H.; Harrap, K. R.; Jarman, M.; Jones, T. R.; Manteuffel-Cymborowska, M.; O'Connor, B. Pharmacokinetics of the Thymidylate Synthase Inhibitor N^{10}-Propargyl-5,8-dideazafolic Acid (CB 3717) in the Mouse. *Cancer Treat. Rep.* **1986**, *70*, 971–979.

(8) Jones, T. R.; Thornton, T. J.; Flinn, A.; Jackman, A. L.; Newell, D. R.; Calvert, A. H. Quinazoline Antifolates Inhibiting Thymidylate Synthase: 2-Desamino Derivatives with Enhanced Solubility and Potency. *J. Med. Chem.* **1989**, *32*, 847–852.

(9) Jackman, A. L.; Taylor, G. A.; O'Connor, B. M.; Bishop, J. A.; Moran, R. G.; Calvert, A. H. Activity of the Thymidylate Synthase Inhibitor 2-Desamino-N^{10}-propargyl-5,8-dideazafolic Acid and Related Compounds in Murine (L1210) and Human (W1L2) systems in Vitro and in L1210 in Vivo. *Cancer Res.* **1990**, *50*, 5212–5218.

(10) Hughes, L. R.; Jackman, A. L.; Oldfield, J.; Smith, R. C.; Burrows, K. D.; Marsham, P. R.; Bishop, J. A. M.; Jones, T. R.; O'Connor, B. M.; Calvert, A. H. Quinazoline Antifolate Thymidylate Synthase Inhibitors: Alkyl, Substituted Alkyl, and Aryl Substituents in the C2 position. *J. Med. Chem.* **1990**, *33*, 3060–3067.

(11) Marsham, P. R.; Jackman, A. L.; Oldfield, J.; Hughes, L. R.; Thornton, T. J.; Bisset, G. M. F.; O'Connor, B. M.; Bishop, J. A. M.; Calvert, A. H. Quinazoline Antifolate Thymidylate Synthase Inhibitors: Benzoyl Ring Modifications in the C2-Methyl Series. *J. Med. Chem.* **1990**, *33*, 3072–3078.

(12) Marsham, P. R.; Hughes, L. R.; Jackman, A. L.; Hayter, A. J.; Oldfield, J.; Wardleworth, J. M.; Bishop, J. A. M.; O'Connor, B. M.; Calvert, A. H. Quinazoline Antifolate Thymidylate Synthase Inhibitors: Heterocyclic Benzoyl Ring Modifications. *J. Med. Chem.* **1991**, *34*, 1594–1605.

(13) Jackman, A. L.; Newell, D. R.; Gibson, W.; Jodrell, D. I.; Taylor, G. A.; Bishop, J. A.; Hughes, L. R.; Calvert, A. H. The Biochemical Pharmacology of the Thymidylate Synthase Inhibitor, 2-Desamino-2-methyl-N^{10}-propargyl-5,8-dideazafolic Acid (ICI 198583). *Biochem. Pharmacol.* **1991**, *42*, 1885–1895.

(14) Synonym: *N*-[5-[*N*-(3,4-dihydro-2-methyl-4-oxoquinazolin-6-ylmethyl)-*N*-methylamino]-2-thenoyl]-L-glutamic acid.

(15) Jackman, A. L.; Taylor, G. A.; Gibson, W.; Kimbell, R.; Brown, M.; Calvert, A. H.; Judson, I. R.; Hughes, L. R. ICI D1694, A Quinazoline Antifolate Thymidylate Synthase Inhibitor That is a Potent Inhibitor of L1210 Tumour Cell Growth *In Vitro* and *In Vivo*; A New Agent for Clinical Study. *Cancer Res.* **1991**, *51*, 5579–5586.

(16) Shane, B. Folypolyglutamate Synthesis and Role in the Regulation of one-carbon metabolism. *Vitamin. Horm. (N. Y.)* **1989**, *45*, 263–335 and references cited therein.

(17) Kisliuk, R. L. The Biochemistry of Folates. In *Folate Antagonists as Therapeutic Agents*; Sirotnak, F., Burchall, J. J., Ensminger, W. D., Montgomery, J. A., Eds.; Academic Press: New York, 1984; pp 1–68.

(18) Sikora, E.; Jackman, A. L.; Newell, D. R.; Calvert, A. H. Formation and Retention and Biological Activity of N^{10}-Propargyl-5,8-dideazafolic Acid (CB 3717) Polyglutamates in L1210 Cells in Vitro. *Biochem. Pharmacol.* **1988**, *37*, 4047–4054.

(19) Jackman, A. L.; Kelland, L. R.; Brown, M.; Gibson, W.; Kimbell, R.; Aherne, W.; Judson, I. R. ICI D1694 Resistant Cell Lines. *Proc. Am. Assoc. Cancer Res.* **1992**, *33*, 406.

(20) Pizzorno, G.; Chang, Y.-M.; McGuire, J. J.; Bertino, J. R. Inherent Resistance of Squamous Carcinoma Cell Lines to Methotrexate as a Result of Decreased Polyglutamation of This Drug. *Cancer Res.* **1989**, *49*, 5275–5280.

(21) Cowan, K. H.; Jolivet, J. A. A Methotrexate Resistant Human Breast Cancer Cell Line with Multiple Defects Including Diminished Formation of MTX Polyglutamates. *J. Biol. Chem.* **1984**, *259*, 10793–10800.

(22) Synonyms: MPDDF; ICI 198,583; *N*-[4-[*N*-[(2-methyl-3,4-dihydro-4-oxo-6-quinazolinyl)methyl]prop-2-ynylamino]benzoyl]-L-glutamic acid.

(23) Bisset, G. M. F.; Pawelczak, K.; Jackman, A. L.; Calvert, A. H.; Hughes, L. R. Syntheses and Thymidylate Synthase Inhibitory Activity of the Poly-γ-glutamyl Conjugates of *N*-[5-[*N*-(3,4-

Dihydro-2-methyl-4-oxoquinazolin-6-ylmethyl)-*N*-methylamino]-2-thenoyl]-L-glutamic Acid (ICI D1694) and Other Quinazoline Antifolates. *J. Med. Chem.* **1992**, *35*, 859–866 and references cited therein.

(24) Piper, J. R.; Montgomery, J. A.; Sirotnak, F. M.; Chello, P. L. Synthesis of α- and γ-Substituted Amides, Peptides, and Esters of Methotrexate and Their Evaluation as Inhibitors of Folate Metabolism. *J. Med. Chem.* **1982**, *25*, 182–187.

(25) Szeto, D. W.; Cheng, Y.-C.; Rosowsky, A.; Yu, C.-S.; Modest, E. J.; Piper, J. R.; Temple, C., Jr.; Elliott, R. D.; Rose, J. D.; Montgomery, J. A. Human Thymidylate Synthetase-III. Effects of Methotrexate and Folate Analogs. *Biochem. Pharmacol.* **1979**, *28*, 2633–2637.

(26) Baugh, C. M.; Stevens, J. C.; Krumdieck, C. L. Studies on γ-Glutamyl Carboxypeptidase. I. The Solid Phase Synthesis of Analogues of Polyglutamates of Folic Acid and Their Effects on Human Liver γ-Glutamyl Carboxypeptidase. *Biochim. Biophys. Acta* **1970**, *212*, 116–125.

(27) Antonjuk, D. J.; Boadle, D. K.; Cheung, H. T. A.; Tran, T. Q. Synthesis of Monoamides of Methotrexate from L-Glutamic Acid Monoamide *t*-Butyl Esters. *J. Chem. Soc., Perkin Trans. 1* **1984**, 1989–2003.

(28) Rosowsky, A.; Forsch, R.; Uren, J.; Wick, M. Methotrexate Analogues. 14. Synthesis of New γ-Substituted Derivatives As Dihydrofolate Reductase Inhibitors and Potential Anticancer Agents. *J. Med. Chem.* **1981**, *24*, 1450–1455.

(29) Rosowsky, A.; Bader, H.; Radike-Smith, M.; Cucchi, C. A.; Wick, M. M.; Freisheim, J. H. Methotrexate Analogues. 28. Synthesis and Biological Evaluation of New γ-Monoamides of Aminopterin and Methotrexate. *J. Med. Chem.* **1986**, *29*, 1703–1709.

(30) McGuire, J. J.; Hsieh, P.; Bertino, J. R.; Coward, J. K. Studies on the Substrate Specificity of Mammalian Folylpolyglutamate Synthetase. In *Chemistry and Biology of Pteridines*; Walter de Gruyter & Co.: Berlin, 1983; pp 615–619.

(31) Galivan, J.; Inglese, J.; McGuire, J. J.; Nimec, Z.; Coward, J. K. γ-Fluoromethotrexate: Synthesis and Biological Activity of a Potent Inhibitor of Dihydrofolate Reductase With Greatly Diminished Ability to Form Poly-γ-glutamates. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 2598–2602.

(32) McGuire, J. J.; Graber, M.; Licato, N.; Vincenz, C.; Coward, J. K.; Nimec, Z.; Galivan, J. Biochemical and Growth Inhibitory Effects of the *erythro* and *threo* Isomers of γ-Fluoromethotrexate, a Methotrexate Analogue Defective in Polyglutamylation. *Cancer Res.* **1989**, *49*, 4517–4525.

(33) McGuire, J. J.; Coward, J. K. DL-*threo*-4-Fluoroglutamic Acid, a Chain-Terminating Inhibitor of Folylpolyglutamate Synthesis. *J. Biol. Chem.* **1985**, *260*, 6747–6754.

(34) Licato, N. J.; Coward, J. K.; Nimec, Z.; Galivan, J.; Bolanowska, W. E.; McGuire, J. J. Synthesis of *N*-[*N*-(4-Deoxy-4-amino-10-methylpteroyl)-4-fluoroglutamyl]-γ-glutamate, an Unusual Substrate for Folylpoly-γ-glutamate Synthetase and γ-Glutamyl Hydrolase. *J. Med. Chem.* **1990**, *33*, 1022–1027.

(35) Rosowsky, A.; Yu, C.-S. Methotrexate Analogues. 10. Direct Coupling of Methotrexate and Diethyl L-Glutamate in the Presence of Peptide Bond-Forming Reagents. *J. Med. Chem.* **1978**, *21*, 170–175.

(36) (a) Godwin, H. A.; Rosenberg, I. H.; Ferenz, C. R.; Jacobs, P. M.; Meienhofer, J. The Synthesis of Biologically Active Pteroyl-oligo-L-γ-Glutamates (Folic Acid Conjugates). *J. Biol. Chem.* **1972**, *247*, 2266–2271. (b) Meienhofer, J.; Jacobs, P. M.; Godwin, H. A.; Rosenberg, I. H. Synthesis of Hepta-γ-L-glutamic Acid by Conventional and Solid-Phase Techniques. *J. Org. Chem.* **1970**, *35*, 4137–4140.

(37) Pawelczak, K.; Krzyzanowski, L.; Rzeszotarska, B. Improved Synthesis of Some α-Esters of *N*-protected L-Glutamic Acid. *Org. Prep. Proced. Int.* **1985**, *17*, 416–419.

(38) Meienhofer, J. The Mixed Carbonic Anhydride Method of Peptide Synthesis. In *The Peptides, Analysis, Synthesis, Biology*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1985; Vol. 1, pp 264–309.

(39) Kovacs, J.; Giannotti, R.; Kapoor, A. Polypeptides with Known Repeating Sequence of Amino Acids. Synthesis of Poly-L-glutamyl-L-alanyl-L-glutamic Acid and Polyglycylglycyl-L-phenylalanine through Pentachlorophenyl Active Esters. *J. Am. Chem. Soc.* **1966**, *88*, 2282–2292.

(40) Rosowsky, A.; Forsch, R.; Uren, J.; Wick, M.; Kumar, A. A.; Freisheim, J. H. Methotrexate Analogues. 20. Replacement of Glutamate by Longer-Chain Amino Diacids: Effects on Dihydrofolate Reductase Inhibition, Cytotoxicity, and In Vivo Antitumour Activity. *J. Med. Chem.* **1983**, *26*, 1719–1724.

(41) (a) Taschner, E.; Chimiak, A.; Bator, B.; Sokolowska, T. Preparation of *tert*-Butyl Esters of Free Amino Acids. *Justus Liebigs Ann. Chem.* **1961**, *646*, 133–136. (b) *The Practice of Peptide Synthesis*; Bodanszky, M., Bodanszky, A., Eds.; Springer-Verlag: Berlin, 1984; pp 48–49.

(42) Hughes, L. R.; Oldfield, J.; Pegg, S. J.; Barker, A. J.; Marsham, P. R. Quinazoline Derivatives as Antitumour Agents. U.K. Pat. Appl. GB 2 227 016A, 1990 (EP Pat. Appl. 373891A2, 1990); *Chem. Abstr.* **1988**, *114*, 23978m.

(43) Bisset, G. M. F.; Jackman, A. L.; Jodrell, D. I. Preparation of Dipeptidyl Quinazolones as Neoplasm Inhibitors. U.K. Pat. Appl. GB 2 253 849A1, 1992; *Chem. Abstr.* **1992**, *118*, 102484h.

(44) Jackman, A. L.; Alison, D. L.; Calvert, A. H.; Harrap, K. R. Increased Thymidylate Synthase in L1210 Cells Possessing Acquired Resistance to N^{10}-Propargyl-5,8-dideazafolic Acid (CB 3717): Development, Characterisation and Cross-Resistance Studies. *Cancer Res.* **1986**, *46*, 2810–2815.

(45) Fry, D. W.; Besserer, J. A.; Boritzki, T. J. Transport of the Antitumour Antibiotic CI-90 in L1210 Leukemia Cells by the Reduced Folate Carrier System. *Cancer Res.* **1984**, *44*, 3366–3370.

(46) Moran, R. G.; Colman, P. D.; Rosowsky, A.; Forsch, R. A.; Chan, K. K. Structural Features of 4-Amino Antifolates Required for Substrate Activity with Mammalian Folylpolyglutamate Synthetase. *Mol. Pharmacol.* **1985**, *27*, 156–166.

(47) 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. H. Quinazoline Antifolates Inhibiting Thymidylate Synthase: Variations of the N^{10} Substituent. *J. Med. Chem.* **1985**, *28*, 1468–1476.

(48) Jackman, A. L.; Marsham, P. R.; Thornton, T. J.; Bishop, J. A. M.; O'Connor, B. M.; Hughes, L. R.; Calvert, A. H.; Jones, T. R. Quinazoline Antifolate Thymidylate Synthase Inhibitors: 2'-Fluoro-N^{10}-propargyl-5,8-dideazafolic Acid and Derivatives with Modifications in the C2 Position. *J. Med. Chem.* **1990**, *33*, 3067–3071.

(49) Pawelczak, K.; Kempny, M.; Bisset, G. M. F.; Jackman, A. L. Antifolate Conjugates Containing γ-Aminobutyric Acid in the Poly-γ-glutamyl Chain. Synthesis, Enzyme Binding and Cell Growth Inhibition. In *Pteridines And Related Biogenic Amines and Folates*; Blau, N., Curtius, H. Ch., Levine, R., Yim, J., Ed.; Hanrim Publishing Co.: Korea, 1992; pp 293–304.

(50) Henderson, G. B.; Zevely, E. M. *Arch. Biochem. Biophys.* **1983**, *221*, 438–446.

(51) Jackman, A. L.; Bisset, G. M. F.; Jodrell, D. I.; Gibson, W.; Kimbell, R.; Bavetsias, V.; Calvert, A. H.; Harrap, K. R.; Stephens, T. C.; Smith, M. N.; Boyle, F. T. γ-Linked Dipeptide Analogues of 2-Desamino-2-methyl-N^{10}-propargyl-5,8-dideazafolate As Antitumour Agents. In *Chemistry and Biology of Pteridines and Folates*; Ayling, J. E., Nair, M. G., Baugh, C. M., Eds.; Plenum Press: New York, 1993; pp 579–584.

(52) Jodrell, D. I.; Gibson, W.; Bisset, G. M. F.; Boyle, F. T.; Judson, I. R.; Jackman, A. L. The *In Vivo* Metabolic Stability of Dipeptide Analogues of the Quinazoline Antifolate, ICI 198583, In Mice. *Biochem. Pharmacol.* **1993**, *46*, 2229–2234.

(53) McGuire, J. J.; Coward, J. K. Pteroylpolyglutamates: Biosynthesis, Degradation and Function. In *Folates and Pterins*; Blakley, R. L., Benkovic, S. J., Eds.; John Wiley & Sons: New York, 1984; Vol. 1, pp 135–190 and references cited therein.