Articles

Design and Synthesis of Potent Non-Polyglutamatable Quinazoline Antifolate Thymidylate Synthase Inhibitors

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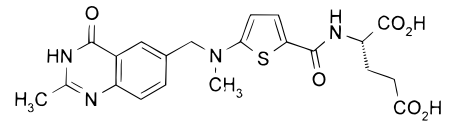
The synthesis is described of a series of analogues of the potent thymidylate synthase (TS) inhibitor, *N*-[4-[*N*-[(3,4-dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]-L-glutamic acid (**4**, ZM214888), in which the glutamic acid moiety is replaced by homologous amino acids and α-amino acids where the *ω*-carboxylate is replaced by acylsulfonamides and acidic heterocycles. In general these modifications when compared to **4** gave compounds with increased potency as inhibitors of isolated TS and as cytotoxic agents against murine tumor cell lines. The new compounds require transport by the reduced folate carrier for entry into cells but are not converted intracellularly into polyglutamated species. Agents with this profile are expected to show activity against tumors that are resistant to classical antifolates due to low expression of folylpolyglutamate synthetase. The analogue (*S*)-2-[4-[*N*-[(3,4-dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzamido]-4-(1*H*-1,2,3,4-tetrazol-5-yl)butyric acid (**35**, ZD9331) has been selected as a clinical development candidate and is currently undergoing phase I studies.

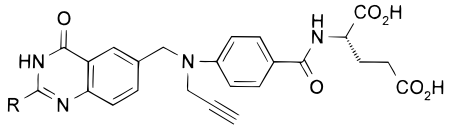
Introduction

Tomudex (ZD1694, **1**) (Chart 1),[1,2] a new quinazoline-based inhibitor of thymidylate synthase (TS), has recently been introduced in a number of European territories for the treatment of advanced colorectal cancer. It is a highly potent cytotoxic agent in vitro and shows in vivo antitumor activity in a range of preclinical models[3] without the unacceptable kidney toxicity[4] associated with its predecessor, CB 3717 (**2**). In international phase III studies in patients with previously untreated advanced colorectal cancer, the efficacy and acceptable safety profile of Tomudex was confirmed.[5] Response rates, time to progression, and survival are consistent with the published literature for 5-fluorouracil and leucovorin.[5] In addition there were reductions in the incidence of certain potentially serious adverse events and benefits in terms of improvements in quality of life, performance status, and weight gain. All the evidence suggests that the high cytotoxicity of Tomudex is due to rapid intracellular localization via the reduced folate carrier protein (RFC) and then extensive metabolism by folylpolyglutamate synthetase (FPGS) to polyglutamates which are retained within cells and are 60−70 times more potent as inhibitors of TS.[6] Tumor cells however may be resistant to classical folate-based antimetabolites through reduced expression of FPGS[7] or upregulation of the polyglutamate-hydrolyzing enzyme *γ*-glutamyl hydrolase.[8] This fact has prompted the search for a complementary class of agents which would not be substrates for FPGS and hence insensitive to *γ*-glutamyl hydrolase but would also still rely on the RFC for cellular uptake. This requirement for RFC

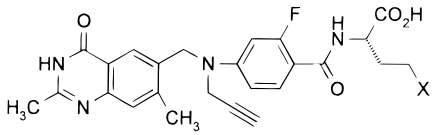
Chart 1



1 Tomudex



2 R = NH_2 CB 3717
3 R = CH_3 ICI198583



4 X = CO_2H ZM214888

35 X = ZD9331

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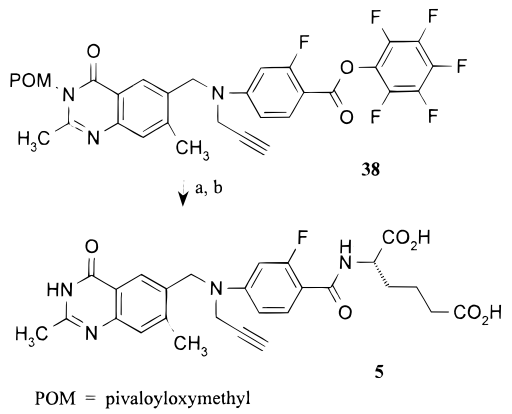
Scheme 1[a]



[a] (method A): (a) (*S*)-2-aminoadipic acid dimethyl ester, Et_3N, DMF; (b) 2 N aq NaOH, MeOH then 2 N HCl.

substrate affinity stemmed in part from the observation of the Sirotnak group that in murine tumor models[9] compounds with favorable kinetic parameters for the RFC may offer a tumor-selective advantage. Agents with this biochemical profile should therefore be active in tumors expressing (1) low levels or modified FPGS expression and (2) high levels of γ-glutamyl hydrolase and which are therefore resistant to folate-based antimetabolites that require polyglutamation for their cytotoxicity. The lack of prolonged drug retention through polyglutamation may also allow for greater control over the duration of TS inhibition.

A compound not subject to metabolic activation through polyglutamation needs to have a high intrinsic potency as a TS inhibitor. As a starting point modifications to the quinazoline antifolate ICI198583 (**3**)[10] (the more soluble, less toxic C2-methyl analogue of CB 3717) were undertaken. The combined effect of the incorporation of 7-methyl and 2′-fluoro substituents gave the analogue ZM214888 (**4**), a compound showing enhanced inhibition of TS and an overall retention of growth inhibition in cell lines.[11] Moreover, the cytotoxicity of **4** results entirely from the parent monoglutamate since 7-methyl-substituted N10-propargyl quinazoline antifolates are not substrates for FPGS.[12] Molecular modeling studies[13] based on an analysis of the X-ray structures of *E. coli* TS enzyme ternary complexes of CB 3717[14,15] and its tetraglutamate derivative[16] suggested that tighter binding inhibitors may be available in this class of compounds through extension of the glutamate moiety into the dipeptide region of the ternary complex. This paper describes the synthesis and biological activities of new analogues of ZM214888 that were prepared to explore these hypotheses. This program has resulted in compounds with improved TS and growth inhibitory properties leading to the next generation of antitumor TS inhibitors, which are not substrates for FPGS but which still require RFC-mediated uptake into cells.

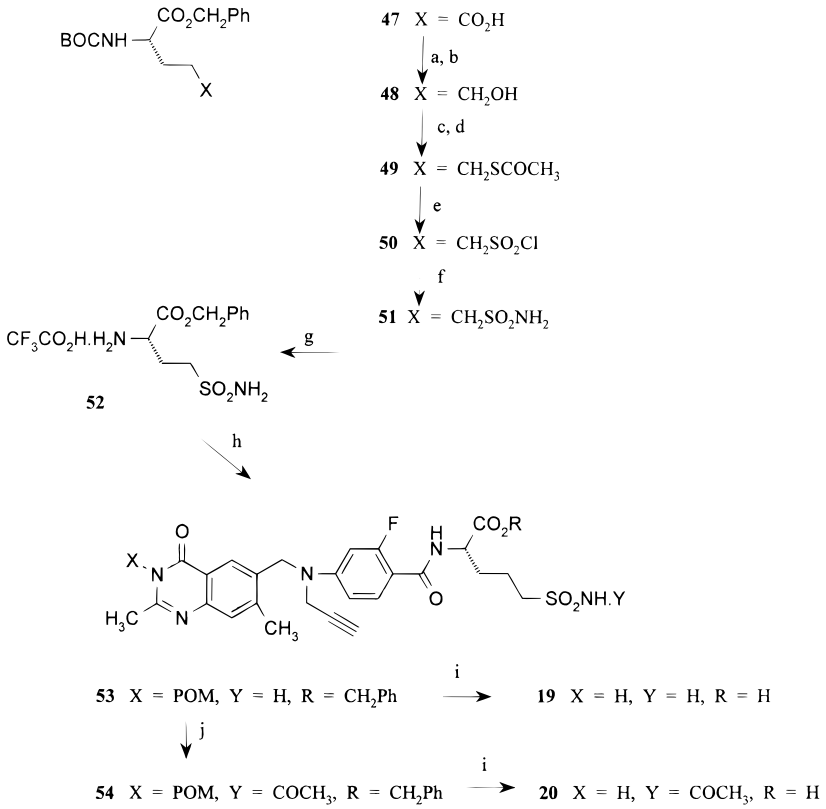
Chemistry

The general method for the synthesis of these new TS inhibitors containing chain-extended homologues of glutamic acid is outlined in Scheme 1. The pentafluorophenyl ester **38**[11] was condensed with dialkyl esters[17] of the appropriate α-aminodicarboxylic acids followed by alkaline hydrolysis of the ester function (method A). Schemes 2−5 illustrate variants of the general method, and the synthesis of the remaining examples is described in detail in the Experimental Section. The corresponding ω-acylsulfonamide analogues **8**−**16** were prepared from *N*-(benzyloxycarbonyl)-L-glutamic acid α-benzyl ester (**39**) and the similarly protected α-aminoadipic, α-aminopimelic, and α-aminosuberic acids. Reaction of **39** with methanesulfonamide in the presence of dicyclohexylcarbodiimide (DCCI) and 4-(dimethylamino)pyridine (DMAP) afforded the γ-acylsulfonamide α-ester **40** (method B). The Cbz and benzyl ester protecting groups were removed by catalytic hydrogenation, and the resulting amino acid **41** was condensed with **38**. The target γ-acylsulfonamide analogue was finally obtained by saponification of **42**. An alternative procedure[11] (method F), used to remove the POM protecting group from a number of these acylsulfonamide analogues (e.g. **11**), involved treatment with a saturated solution of ammonia in MeOH.

Reversal of the acylsulfonamide unit produced two sets of analogues having two (**17**, **18**) and three (**20**, **21**) methylene links, respectively. The racemic sulfonyl chloride, 2-(benzyloxycarbonyl)-4-(chlorosulfonyl)butanoic acid benzyl ester (**43**), readily prepared[18] from DL-homocystine, was elaborated into **17** and **18**. The pair of homologous reversed acylsulfonamides **20** and **21** was prepared (Scheme 2) as the pure *S*-enantiomers starting from *N*-BOC-L-glutamic acid α-benzyl ester (**47**) which was reduced by a modification of the method of Kokotos[19] to the alcohol **48**. Conversion of **48** to the pivotal intermediate sulfonamide **51** was achieved via chlorination of the thioacetate **49** to the sulfonyl chloride **50** followed by treatment with ammonia in $CHCl_3$. The BOC protecting group was removed (CF_3CO_2H) and the amino ester **52** condensed with **38** to give **53**. Saponification of **53** yielded the δ-sulfonamide analogue **19**. Acylation of **53** with CH_3COCl and PhCOCl (method I) gave the δ-acylsulfonamides **20** and **21**.

Incorporation of azole sulfides, sulfoxides, and sulfones as potential ω-acid mimics was achieved in four series with one to four methylene spacer units. Our initial attempt to prepare a system with a single methylene spacer unit involved the reaction of the disodium salt of 4-mercapto-1,2,3-triazole with either the tosylate **55**[20] of Cbz-L-serine or the acrylate **56**[20] (Scheme 3). Both reactions gave the same racemic Cbz-amino acid **57**, implying that the reaction of **55** occurred through the intermediacy of **56**. Removal of the Cbz protecting group with aqueous HBr was followed by coupling to **38** and hydrolysis to the triazolyl sulfide **22**. This underwent oxidation with peracetic acid (method J) to the sulfoxide **23** as the *RS*,*RS*-diastereomeric mixture. To avoid this racemization in the formation of S-linked azole analogues, we investigated the reaction of commercially available β-chloroalanine (**60**) with a sulfur nucleophile, 5-mercaptotetrazole[21] sodium salt (**61**). Although the yield of the required product **62** was only 21%, the product was the pure *R*-enantiomer, confirming that the chlorine is displaced via an S_N2 mechanism. The tetrazolyl sulfide analogue **26** with a dimethylene spacer and its sulfoxide **27** were synthesized as the pure *S*-enantiomers via the key intermediate **66** which was obtained by the nucleophilic displacement of 5-chloro-1-*tert*-butyl-1*H*-tetrazole[22] with the

Scheme 2[a]

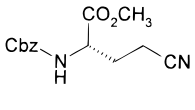


[a] (a) EtOCOCl, Et_3N, THF, −10 °C; (b) $NaBH_4$, MeOH; (c) MsCl, Et_3N, CH_2Cl_2; (d) $KSCOCH_3$, acetone; (e) Cl_2, HOAc, NaOAc, H_2O; (f) NH_3, $CHCl_3$; (g) CF_3CO_2H, CH_2Cl_2; (h) pentafluorophenyl ester **38**, HOBT, Et_3N, DMA; (i) aq NaOH, EtOH (method H); (j) CH_3COCl, Pr^i_2EtN, DMAP, DMF (method I).

sodium salt of L-homocysteine (prepared in situ from L-homocystine). Removal of the *tert*-butyl group using HCl/anisole was accompanied by some rearrangement to the 2-*tert*-butyl isomer **68**, but separation of the required amino acid **67** was readily achieved by preparative reverse-phase chromatography.

In the trimethylene series sulfur-linked tetrazole (**28**, **29**) and 1,2,4-triazolin-3-one (**30**−**32**) substituents were introduced as ω-acid mimics. The key intermediate mesylate **71** was prepared from (*S*)-2-phthalimidoglutaric acid 1-ethyl ester (**69**)[23] via the alcohol **70** (Scheme 4). Reaction of **71** with 5-mercaptotetrazole[21] and Et_3N followed by removal of the phthalimide ($N_2H_4 \cdot H_2O$) gave the amino ester **73** which afforded **74** on coupling with pentafluorophenyl ester **38** (method K). Oxidation of **74** with 3-chloroperbenzoic acid (method L) yielded the sulfoxide **75** as the *S*,*RS*-diastereomeric mixture. Esters **74** and **75** were finally saponified (method M) to **28** and **29**. The sulfur-linked triazolinone system was incorporated by treatment of mesylate **71** with the disodium salt of 5-mercapto-2*H*-1,2,4-triazol-3(4*H*)-one. The tetramethylene-linked tetrazolyl sulfide **33** and its sulfoxide **34** were prepared via the amino ester **82** from the ϵ-bromobutyl acetamidomalonate ester (**79**).[24]

For the synthesis of the γ-tetrazolylglutamic acid isostere ZD9331 (**35**), the pentafluorophenyl ester **38** was condensed with the γ-tetrazolyl amino ester **84**[25,26] and the product hydrolyzed with 2 N NaOH. To confirm the enantiomeric purity of **35**, the *R*-enantiomer **36** was also prepared, utilizing the *R*-amino ester **85** (derived from D-glutamine by the literature methods).[25,26] Chiral analytical HPLC of **35** and **36** using a Chirobiotic T



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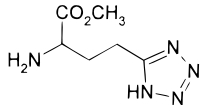
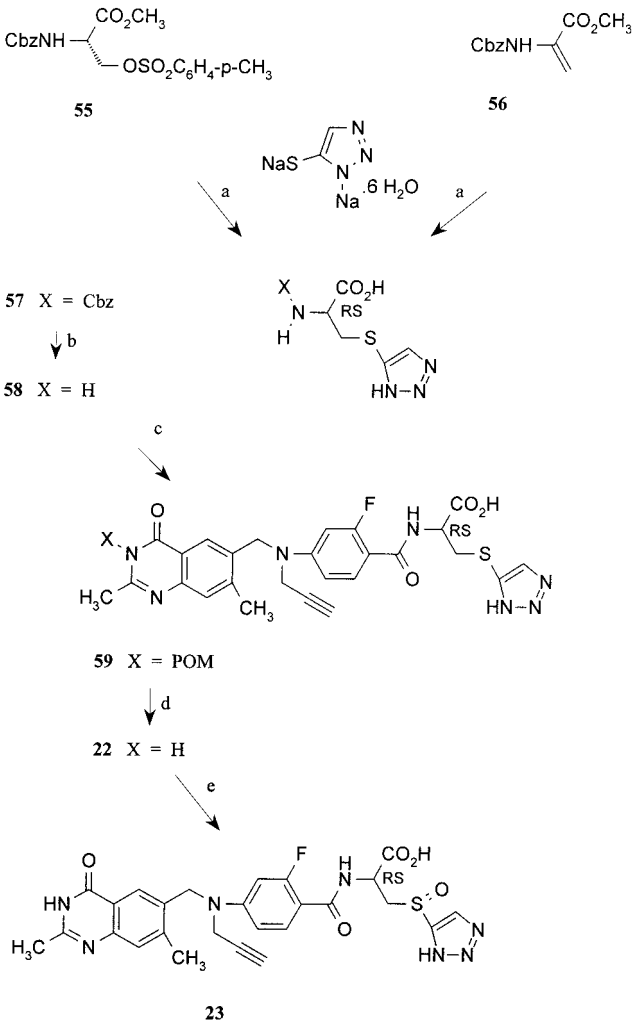


84 S-configuration

85 R-configuration

(Teichoplanin) column[27] demonstrated that both were >99.7% enantiomerically pure. The higher homologue **37** with a δ-tetrazole as the acid mimic was elaborated from the mesylate **86**, which on treatment with KCN gave the nitrile **87**. Formation of the tetrazole ring by heating **87** with NaN_3 in the presence of NH_4Cl in DMF caused almost complete racemization of the asymmetric center, since on completion of the synthesis (Scheme 5), via the amino ester **89**, the product **37** was shown by chiral HPLC to be a 53:47 mixture of the *S*- and *R*-enantiomers. Although the precise stage at which racemization occurred was not confirmed, it is likely that the conditions used for the nitrile to tetrazole conversion were responsible, since the same racemization occurred when the glutamine derivative **83** was reacted with NaN_3/NH_4Cl in DMF under the same conditions. Presumably the formation of traces of dimethylamine from DMF at the elevated temperature of the reaction causes a deprotonation in the α-position of the protected amino esters. In the event, the biological activity of **37** was insufficiently interesting to justify investigation of potential methods for the synthesis of the pure *S*-enantiomer.

Scheme 3[a]



[a] (a) MeOH, 45−50 °C; (b) 48% aq HBr; (c) pentafluorophenyl ester **38**, HOBT, Et_3N; (d) method E; (e) CH_3CO_3H, $CHCl_3$, MeOH (method J).

Biological Evaluation

The compounds prepared are listed in Table 1 and were tested as inhibitors of isolated TS partially purified from L1210 mouse leukemia cells that overproduce the enzyme.[28] The partial purification and assay methods used were as previously described and used a (±)-5,10-methylenetetrahydrofolate concentration of 200 μM.[28] Results are expressed as the IC_{50}, which is the concentration of compound that inhibits the control reaction rate by 50%. Compounds were also tested for their ability to inhibit the growth of L1210 cells in culture, and the results are expressed as the concentration of compound required to inhibit cell growth by 50% (IC_{50}). The L1210:1565 cell line[29] has acquired resistance tothe antitumor antibiotic CI-920.[30] Evidence suggests that this agent enters cells via the RFC and that the L1210:1565 line is resistant due to a very much reduced drug uptake; hence it is cross-resistant to MTX (~200-fold).[31] The L1210:R^{D1694} cell line has acquired resistance to Tomudex and is unable to polyglutamate this drug and related folate analogues.[32−34] These cell lines were grown by suspension culture in RPMI medium without sodium bicarbonate but containing 20 mM HEPES[35] and supplemented with 10% horse serum (L1210 and L1210:R^{D1694}) and 10% fetal calf serum (L1210:1565). Incubation times for the 5-mL cultures were 48 h (L1210 and L1210:R^{D1694}) and 72 h (L1210:1565). The initial cell concentration was 5×10^4 mL^{-1}. For the thymidine protection experiments on compounds **4**, **6**, and **7** the L1210 cells were co-incubated with the compound at a concentration 10 times the IC_{50} values and 10 μM thymidine. Under these conditions the cells had the same rates of growth to those obtained in a drug-free medium. In Table 1, the L1210:1565 and L1210:R^{D1694} relative resistance ratios are calculated as the ratio of the mutant line IC_{50} over the L1210 IC_{50}. All cell counts were performed with a model ZM Coulter counter. The cell doubling times were 12 h (L1210 and L1210:R^{D1694}) and 18−24 h (L1210:1565).

Results and Discussion

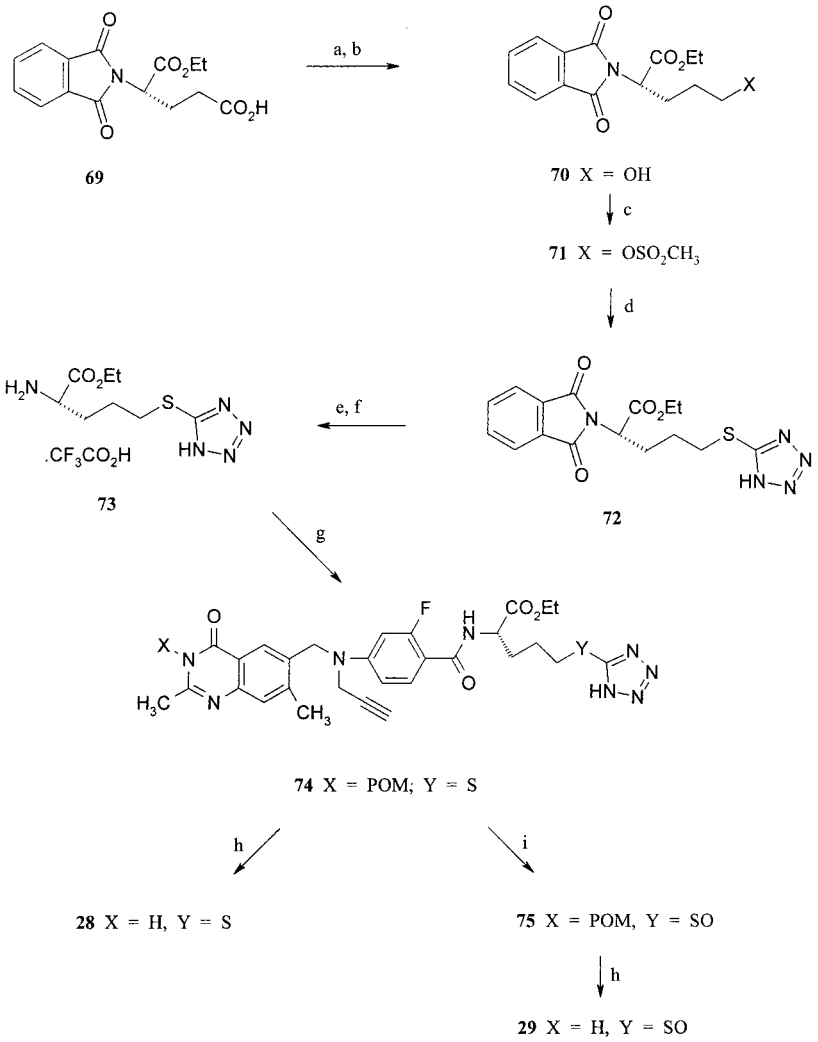
Extension of the chain length in a series of glutamate homologues of **4**[11] led to the α-aminoadipic acid (**5**), α-aminopimelic acid (**6**), and α-aminosuberic acid (**7**) containing homologues. These showed enhanced TS inhibitory potencies when compared to **4** and were particularly marked for **6** and **7** (6-fold). These results support our hypothesis that extension of the glutamic acid chain would give rise to enhanced binding to TS. Growth inhibitory potency is retained in these homologues, and the TS locus of this activity was confirmed for three examples, **4**, **6**, and **7**, by thymidine protection studies. The relatively low activity of the three homologues **5**−**7** in the L1210:1565 line supports the view that uptake by the RFC remains the predominant mode of cell entry. The low relative resistance ratios in the L1210:R^{D1694} line, determined for **5**−**7**, suggest that, as expected, these homologues are not substrates for FPGS and therefore do not require polyglutamation in order to exhibit potent growth inhibition.

Replacement of the ω-carboxylic acids in this series by acylsulfonamides also gives potent TS and growth inhibitors. This replacement gives pK_a values close to those of the carboxylic acids but renders this end of the molecule less liable to metabolism. These analogues (**8**−**16**) retain at least the TS and growth inhibitory potencies of the parent ω-carboxylic acids when the relatively small methanesulfonamide or trifluoromethanesulfonamide is incorporated, but there is in general a reduction in these potencies with the larger arylsulfonamides. Most potent of these analogues is the methanesulfonamide **15** which shows equivalent activity to the aminopimelate **6**. The high IC_{50} values of representative analogues **11** and **15** in the L1210:1565 line suggest that a high affinity for the RFC plays an important role in their cytotoxicities.

Reversal of the acylsulfonamide function in compounds **17**−**21** appears to retain the TS inhibitory potency. Again the small acetamido group gives better binding than the larger benzamide. However compounds in this series appear to have poorer cell penetration than the conventional acylsulfonamides since their growth inhibitory activities do not reflect their potencies against the enzyme. This conclusion is borne out by the fact that the IC_{50} value of **20** in the L1210:1565 line is only 10-fold higher than that for the parent.

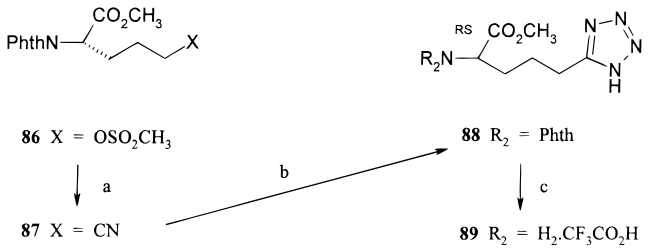
A series of analogues of **4**−**7** has also been prepared in which the ω-carboxylate has been replaced by sulfur-linked heterocycles. Precedent for this approach was

Scheme 4[a]



[a] (a) EtOCOCl, Et_3N, CH_2Cl_2; (b) $NaBH_4$, MeOH; (c) MsCl, Et_3N, CH_2Cl_2; (d) 5-mercapto-1*H*-1,2,3,4-tetrazole, Et_3N, EtOH, DMF; (e) $N_2H_4 \cdot H_2O$, EtOH; (f) CF_3CO_2H; (g) pentafluorophenyl ester **38** (method K); (h) 2 N NaOH, MeOH (method M); (i) 3-Cl-$C_6H_4CO_3H$, CH_2Cl_2 (method L).

Scheme 5[a]

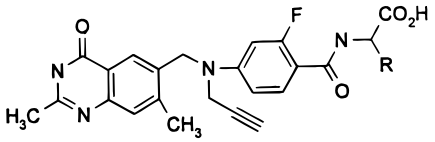


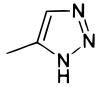
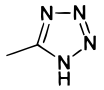
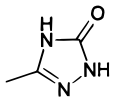
[a] (a) KCN, DMSO; (b) NaN_3, NH_4Cl, DMF, 90 °C; (c) $N_2H_4 \cdot H_2O$; (d) CF_3CO_2H.

established by Chenard et al.[24] who investigated benzylthiotriazoles, benzylthiotriazolones, and their corresponding sulfoxides and sulfones to give compounds of equivalent activity to the corresponding carboxylic acids as *N*-methyl-D-aspartic acid inhibitors. They clearly established that not only were these functional groups effective surrogates of carboxylic acids but also a wide range of pK_a values can be achieved through the choice of combination of heterocycle and oxidation level of the sulfur link. For a given heterocycle, the pK_a can be varied by up to 3 units between the sulfide and the sulfone. We elected to evaluate whether such an approach was useful in our series of TS inhibitors. The incorporation of sulfur-linked heterocycles of this type as ω-acid isosteres (compounds **22**−**34**) has in general produced potent inhibitors of TS regardless of the nature of the acid isostere or its position relative to the α-carboxylate. No clear structure−activity relationship has emerged for compounds in this series as there appears to be a complex interplay of the spatial and electronic parameters. However the most potent TS inhibitors (**28**, **29**, **31**, and **32**) have a trimethylene link, but the precise pK_a value of the heterocycle apparently causes little variation in binding as measured by enzyme inhibition. The TS inhibition of these compounds translates well into growth inhibitory activity although the L1210:1565 values suggest variable reliance on the RFC for uptake into cells. For example the thiotetrazole analogue **24** has a higher growth inhibitory potency and a relative resistance ratio against the L1210:1565 line than the corresponding thiotriazole **22** suggesting that it can be better internalized by the RFC.

It is also well-known that tetrazole analogues of amino acids have pK_a values that agree closely with those of the corresponding amino acids.[36] We found that replacement of the γ-carboxylate in **4** with a tetrazole

Table 1



TRZ = 
TET = 
TZO = 

compd	R	config	formula[a]	method	yield (%)	inhibn of TS, IC_{50} (nM)	inhibition of cell growth in culture, IC_{50} (μM): L1210	protection at 10×IC_{50} (% of control)	L1210: 1565	L1210: 1565 relative resistance	L1210: R^{D1694}	L1210: R^{D1694} relative resistance
1	(Tomudex)	*S*	$C_{21}H_{22}N_4O_6S·H_2O$			670	0.009		0.76	90	95	11000
2	(CB3717)	*S*	$C_{24}H_{23}N_5O_6$			20[m]	5.5		5.5	1	13.	2.36
3	(ICI 198583)	*S*	$C_{25}H_{24}N_4O_6·2H_2O$			40[n]	0.13		8.2	62	2.7	21.
4	$(CH_2)_2CO_2H$	*S*	$C_{26}H_{25}FN_4O_6·1.7H_2O$	*h*	80	9.0[o]	0.08		5.92	74	0.43	5.4
5	$(CH_2)_3CO_2H$	*S*	$C_{27}H_{27}FN_4O_6·2.5H_2O$	A	61	3.2	0.21		3.0	14	0.6	3.5
6	$(CH_2)_4CO_2H$	*S*	$C_{28}H_{29}FN_4O_6·7NaCl·2H_2O$	A	78	1.4	0.10	97	2.7	27	0.31	3.1
7	$(CH_2)_5CO_2H$	*RS*	$C_{29}H_{31}FN_4O_6·0.5NaCl·2H_2O$	A	55	1.6	0.23	103	10.0	43	0.93	4.0
8	$(CH_2)_2CONHSO_2CH_3$	*S*	$C_{27}H_{28}FN_5O_7S·3NaCl·H_2O$	B, C, D, E	60	4.4	0.19		4.4		0.32	1.7
9	$(CH_2)_2CONHSO_2CF_3$	*S*	$C_{27}H_{25}F_4N_5O_7S·3.5H_2O·0.5NaCl$[b]	B, C, D, E	46	5.2	0.15					
10	$(CH_2)_2CONHSO_2C_6H_4$-p-CH_3	*S*	$C_{33}H_{32}FN_5O_7S·2.5NaCl·2H_2O$	B, C, D, E	64	9.0	0.7					
11	$(CH_2)_3CONHSO_2CH_3$	*S*	$C_{28}H_{30}FN_5O_7S·0.75NH_4Cl·1.75H_2O$	B, C, D, F	82	1.9	0.19		4.8	25	0.45	2.4
11a	$(CH_2)_3CONHSO_2CH_3$	*RS*	$C_{28}H_{30}FN_5O_7S·0.5NH_4Cl$	B, C, D, F	94	5.4	0.29		8.5	29		
12	$(CH_2)_3CONHSO_2Ph$	*RS*	$C_{33}H_{32}FN_5O_7S·0.75NaCl·3H_2O$	B, C, D, F	77	6.8	0.30					
13	$(CH_2)_3CONHSO_2C_6H_4$-*p*-CH_3	*RS*	$C_{34}H_{34}FN_5O_7S·1.75NaCl$[c]	B, C, D, F	74	14.2	0.47					
14	$(CH_2)_3CONHSO_2C_6H_4$-*m*-NO_2	*RS*	$C_{33}H_{31}FN_6O_9S·NaCl·3H_2O$	B, C, D, F	71	15.4	1.4					
15	$(CH_2)_4CONHSO_2CH_3$	*S*	$C_{29}H_{32}FN_5O_7S·NH_3·2.5H_2O$	B, C, D, F	79	2.4	0.05		3.1	62	0.14	2.8
16	$(CH_2)_5CONHSO_2CH_3$	*RS*	$C_{30}H_{34}FN_5O_7S·NH_3·0.5NH_4Cl$[d]	B, C, D, F	68	3.2	0.22		6.6	30		
17	$(CH_2)_2SO_2NHCOCH_3$	*RS*	$C_{27}H_{28}FN_5O_7S·1.2NaCl·0.5NH_4Cl$	G, C, D, F	76	13.4	3.4					
18	$(CH_2)_2SO_2NHCOPh$	*RS*	$C_{32}H_{30}FN_5O_7S·8H_2O$	G, C, D, F	61	64	5.4					
19	$(CH_2)_3SO_2NH_2$	*S*	$C_{26}H_{28}FN_5O_6S$	H	72	5.6	6.3					
20	$(CH_2)_3SO_2NHCOCH_3$	*S*	$C_{28}H_{30}FN_5O_7S·1.75H_2O$	I, H	76	2.2	0.32		3.3	10.3		
21	$(CH_2)_3SO_2NHCOPh$	*S*	$C_{33}H_{32}FN_5O_7S$	I, H	80	7.2	6.0					
22	CH_2S-TRZ	*RS*	$C_{26}H_{24}FN_7O_4S·0.9Me_3CO_2H·1.75H_2O$	D, E	50	13	1.8		5.0	2.8		
23	CH_2SO-TRZ	*RS*	$C_{26}H_{24}FN_7O_5S·CHCl_3·CH_3OH$[e]	J	70	5.4	0.75		2.4	3.2		
24	CH_2S-TET	*R*	$C_{25}H_{23}FN_8O_4S·1.75H_2O$	D, E	69	2.0	0.11		5.6	51	0.39	3.5
25	CH_2SO-TET	*R*	$C_{25}H_{23}FN_8O_5S·0.5CHCl_3·CH_3OH$	J	83	1.8	0.28		0.8	2.9		
26	$(CH_2)_2S$-TET	*S*	$C_{26}H_{25}FN_8O_4S·CF_3CO_2H$	D, E[i]	68	5.8	0.25		5.2	20.8		
27	$(CH_2)_2SO$-TET	*S*	$C_{26}H_{25}FN_8O_5S·CHCl_3·CH_3OH$[f]	J	45	2.0	0.32		3.1	9.7		
28	$(CH_2)_3S$-TET	*S*	$C_{27}H_{27}FN_8O_4S·0.6H_2O$	K, M	36	0.6	0.07		1.3	19.7		
29	$(CH_2)_3SO$-TET	*S*	$C_{27}H_{27}FN_8O_5S·0.5NaCl·1.2H_2O$	K, L,[j] M	67	0.52	0.06		0.75	12.5		
30	$(CH_2)_3S$-TZO	*S*	$C_{28}H_{28}FN_7O_5S·0.5NaCl·0.5H_2O$	K, M	96	1.62	0.58		2.0	3.4		
31	$(CH_2)_3SO$-TZO	*S*	$C_{28}H_{28}FN_7O_6S·0.3NaCl·0.5H_2O$	L,[j] M	67	0.86	0.15		1.3	8.7		
32	$(CH_2)_3SO_2$-TZO	*S*	$C_{28}H_{28}FN_7O_7S·0.4NaCl·0.5H_2O$	L,[k] M	69	0.62	0.73		0.94	1.3		
33	$(CH_2)_4S$-TET	*RS*	$C_{28}H_{29}FN_8O_4S·1.5H_2O$	K, M	86	3.4	0.16		3.8	23.7		
34	$(CH_2)_4SO$-TET	*RS*	$C_{28}H_{29}FN_8O_5S·0.5NaCl·H_2O$	K, L, M	55	1.4	0.18		1.8	10	0.24	1.3
35	$(CH_2)_2$-TET	*S*	$C_{26}H_{25}FN_8O_4$	K,[l] M	62	1.4[p]	0.024		1.3	54	0.076	3.2
36	$(CH_2)_2$-TET	*R*	$C_{26}H_{25}FN_8O_4·1.5H_2O$[g]	K,[l] M	77	15	0.96		23	24		
37	$(CH_2)_3$-TET	*RS*	$C_{27}H_{27}FN_8O_4·NaCl·0.5H_2O$	K, M	58	2.4	0.14		5.3	39.2		

[a] Anal. C, H, N except when stated otherwise. [b] N: calcd, 9.6; found, 9.0. [c] N: calcd, 9.0; found, 9.5. [d] H: calcd, 5.9; found, 6.5. [e] C: calcd, 46.8; found, 47.3. [f] C: calcd, 45.9; found, 46.4. [g] N: calcd, 20.0; found, 19.2. [h] See ref 10. [i] Purified by chromatography on a Dynamax ODS preparative column, eluting with 1:1 CH_3OH/H_2O containing 0.2% CF_3CO_2H. [j] Method O, 1 equiv of MCPBA. [k] Method O, 2 equiv of MCPBA. [l] Condensation performed in the absence of Et_3N. [m] K_i = 60 nM. [n] K_i = 10 nM. [o] K_i = 7.5 nM. [p] K_i = 0.44 nM.

to give **35** surprisingly resulted in an analogue that had a 6-fold enhancement of TS and a 3-fold enhancement of growth inhibition. The activity of **35** is highly stereospecific since its enantiomer **36** is at least an order of magnitude less potent against both parameters. Interestingly in this case the synthesis of the higher homologue **37** of **35** resulted in the retention of TS inhibitory potency and a small drop in potency against L1210 cell growth, allowing for the fact that **37** is a racemic mixture.

More extensive in vitro studies[37,38] on **35** (Zeneca ZD9331) have shown that it has a 17-fold improvement in K_i (0.44 nM vs 7.5 nM) against TS compared to its parent glutamate **4** (ZM214888). The improved cellular growth inhibition carries over into other cell lines (e.g. the human lymphoblastoid W1L2).[38] Relative resistance ratios for ZD9331 in the L1210 variants 1565 and R^{D1694} confirm that it relies predominantly on RFC transport into cells but not on polyglutamation to exhibit its growth inhibition. Further support for affinity for the RFC with ZD9331 is derived from transport competition studies. In these ZD9331 inhibits the transport of [^{3}H]-methotrexate into L1210 and W1L2 cells with a K_i of ~1 μM.[37,38] That ZD9331 is not retained in cells is confirmed in short exposure whole cell TS assay,[37,38] and it therefore only exerts its effects while it is present in the extracellular medium. This feature, combined with a rapid plasma clearance in mice,[39] gives a greater degree of control over inhibition of DNA synthesis in preclinical models. ZD9331 was therefore chosen as the most promising compound to emerge from the current series with activity against a number of experimental tumors.[40−42] It is currently undergoing phase II clinical studies to define more closely the toxicology and optimum administration protocols.

Experimental Section

General Procedures. All experiments were carried out under an inert atmosphere and at room temperature unless otherwise stated. *N*,*N*-Dimethylformamide (DMF) and *N*,*N*-dimethylacetamide (DMA) were purified by azeotropic distillation at 10 mmHg. Flash chromatography was carried out on Merck Kieselgel 60 (Art. 9385). The purities of compounds for test were assessed by analytical HPLC on a Hichrom S5ODS1 Spherisorb column system set to run isocratically with 60−70% MeOH + 0.2% CF_3CO_2H in H_2O as eluent. TLC was performed on precoated silica gel plates (Merck Art. 5715), and the resulting chromatograms were visualized under UV light at 254 nm. Melting points were determined with a Büchi melting point apparatus and are uncorrected. Most of the final products did not give discrete melting points and softened with decomposition over a wide temperature range. However spectral and microanalytical data of all final products were consistent with the proposed structures and formulas. The ^{1}H NMR spectra were determined in Me_2SO-d_6 solution (unless stated otherwise) on a Bruker AM 200 (200 MHz) spectrometer. Chemical shifts are expressed in units of δ (ppm), and peak multiplicities are expressed as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; br s, broad singlet; m, multiplet. Fast atom bombardment (FAB) mass spectra were determined with a VG MS9 spectrometer and Finnigan Incos data system using Me_2SO or MeOH as the solvent and glycerol as the matrix. With the appropriate mode either positive or negative ion data could be collected. NMR and mass spectra were run on all isolated intermediates and final products and are consistent with the proposed structures.

(*S*)-2-[*N*-[4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]amino]adipic Acid (5). Method A. A mixture of the pentafluorophenyl ester **38** (350 mg, 0.536 mmol), (*S*)-2-aminoadipic acid dimethyl ester hydrochloride (131 mg, 0.58 mmol), and Et_3N (800 μL, 5.8 mmol) in DMF (10 mL) was stirred for 16 h. The resulting slightly cloudy solution was evaporated to a gum which was purified by chromatography, eluting with 50−80% v/v EtOAc in isohexane. The pure product fractions were evaporated to a gum which on trituration with Et_2O yielded the white solid pivaloyloxymethyl dimethyl ester derivative of **4**: 310 mg (84%).

This dimethyl ester (300 mg, 0.451 mmol) was stirred for 16 h in a mixture of 2 N aq NaOH (2.0 mL, 4.0 mmol) and MeOH (5 mL). The solvent was evaporated, the residue was dissolved in H_2O (ca. 5 mL), and the solution was filtered into a centrifuge tube. The filtrate was acidified (2 N HCl) to pH 5.0. The resulting white precipitate was isolated by centrifugation, washed (4×) with H_2O, and vacuum-dried: 157 mg (61%); NMR δ 1.57 (m, 2 H, $CH_2CH_2CH_2$), 1.77 (m, 2 H, $CHCH_2CH_2$), 2.21 (t, 2 H, CH_2CO_2H), 2.30 (s, 3 H, CH_3), 2.43 (s, 3 H, CH_3), 3.20 (t, 1 H, C≡CH), 4.28 (m, 3 H, CH_2C≡C & CH), 4.68 (br s, 2 H, $ArCH_2N$<), 6.65 (m, 2 H, 3′-H & 5′-H), 7.43 (s, 1 H, quinazoline 8-H), 7.57 (dd, 1 H, 6′-H), 7.70 (s, 1 H, quinazoline 5-H), 7.85 (m, 1 H, CONH); MS *m*/*z* 567 $[MH]^+$. Anal. ($C_{27}H_{27}FN_4O_6 \cdot 2.5H_2O$) C, H, N.

***N*^{2}-(Benzyloxycarbonyl)-*N*^{5}-(methanesulfonyl)-L-glutamine Benzyl Ester (40). Method B.** A solution of DCCI (1.44 g, 7.0 mmol) in CH_2Cl_2 (5 mL) was added over 1 min to a stirred mixture of Cbz-L-glutamic acid α-benzyl ester (2.0 g, 5.4 mmol), DMAP (860 mg, 7.0 mmol), and methanesulfonamide (660 mg, 7.0 mmol) in CH_2Cl_2 (20 mL) at 0 °C. The mixture was then stirred for 24 h and filtered to remove a white precipitate and the filtrate was evaporated. The crude product was purified by chromatography, eluting sequentially with 1:1 EtOAc/isohexane, EtOAc, and 5−10% MeOH in CH_2Cl_2 to give a colorless gum: 1.40 g (58%); NMR δ 1.90 (m, 2 H, $CHCH_2CH_2CO$), 2.20 (t, 2 H, $CHCH_2CH_2CO$), 2.95 (s, 3 H, SO_2CH_3), 4.10 (m, 1 H, CH), 4.15 (br s, 1 H, $CONHSO_2$), 5.05 (s, 2 H, OCH_2Ph), 5.15 (s, 2 H, OCH_2Ph), 7.32 (m, 10 H, 2 × C_6H_5), 7.82 (d, 1 H, OCONH).

***N*^{5}-(Methanesulfonyl)-L-glutamine (41). Method C.** A solution of **40** (1.40 g, 3.13 mmol) in HOAc (60 mL) was stirred for 2 h with 10% Pd/C (50 mg) in an atmosphere of H_2. The catalyst was filtered off through Celite. The filtrate was evaporated to a gum which was triturated with 1:1 CH_2Cl_2/Et_2O to yield a pale brown solid: 200 mg (29%); NMR (D_2O) δ 2.32 (m, 2 H, $CHCH_2CH_2CO_2H$), 2.60 (t, 2 H, $CHCH_2CH_2CO_2H$), 3.25 (s, 3 H, SO_2CH_3), 4.0 (m, 1 H, CH).

***N*^{2}-[4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-3-[(pivaloyloxy)methyl]-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]-*N*^{5}-(methanesulfonyl)-L-glutamine (42). Method D.** A mixture of the amino acid **41** (150 mg, 0.67 mmol), the pentafluorophenyl ester **38** (300 mg, 0.45 mmol), HOBT (10 mg), and Et_3N (700 μL, 5.0 mmol) in DMF (10 mL) and DMSO (20 mL) was stirred for 45 min to give a clear yellow solution. This was concentrated by rotary evaporation at 0.1 mmHg to a gum which was chromatographed using 5−50% MeOH in CH_2Cl_2 as eluent to yield a yellow solid: 150 mg (48%); NMR δ 1.10 (s, 9 H, Bu^t), 2.00 (m, 4 H, $CH_2CH_2CO_2H$), 2.45 (s, 3 H, CH_3), 2.55 (s, 3 H, CH_3), 2.70 (s, 3 H, SO_2CH_3), 3.20 (t, 1 H, C≡CH), 4.15 (m, 1 H, CH), 4.30 (br s, 2 H, CH_2C≡C), 4.70 (br s, 2 H, $ArCH_2N$<), 6.00 (s, 2 H, OCH_2N), 6.65 (dd, 1 H, 3′-H), 6.68 (dd, 1 H, 5′-H),7.50 (s, 1 H, quinazoline 8-H), 7.65 (dd, 1 H, 6′-H), 7.85 (s, 1 H, quinazoline 5-H).

***N*^{2}-[4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]-*N*^{5}-(methanesulfonyl)-L-glutamine (8). Method E.** A solution of **42** (150 mg, 0.214 mmol) in EtOH (15 mL) was stirred with 2 N aqueous NaOH (8 mL, 16 mmol) for 30 min. The resulting clear solution was evaporated to dryness and the residue was dissolved in H_2O (ca. 5 mL). The solution was filtered into a centrifuge tube and acidified (2 N HCl) to pH 3.0. The pale yellow precipitate was isolated by centrifugation, washed (4×) with H_2O, and vacuum-dried: 100 mg (60%); NMR δ 2.00 (m, 2 H, CH_2CH_2CONH), 2.30 (m, 2 H, CH_2CH_2CONH), 2.32 (s, 3

H, CH_3), 2.43 (s, 3 H, CH_3), 3.05 (s, 3 H, SO_2CH_3), 3.20 (t, 1 H, C≡CH), 4.30 (m, 3 H, CH & CH_2C≡C), 4.68 (br s, 2 H, $ArCH_2N$<), 6.60 (m, 2 H, 3′-H & 5′-H), 7.60 (s, 1 H, quinazoline 8-H), 7.70 (dd, 1 H, 6′-H), 7.92 (s, 1 H, quinazoline 5-H); MS *m*/*z* 584 $[M - H]^-$. Anal. ($C_{27}H_{28}FN_5O_7S\cdot 3NaCl\cdot 2.5H_2O$) C, H, N.

(*S*)-2-[[4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]amino]-5-[*N*-(methanesulfonyl)amino]carboxypentanoic acid (11). Method F. (*S*)-2-[[4-[*N*-[[3,4-Dihydro-2,7-dimethyl-4-oxo-3-[(pivaloyloxy)methyl]-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]amino]-5-[*N*-(methanesulfonyl)amino]carboxypentanoic acid (720 mg, 1.0 mmol) was dissolved in a saturated solution of ammonia in MeOH (200 mL). After 16 h the solution was evaporated to dryness and the residue was triturated with EtOAc. The white solid product was dissolved in 1 N aqueous NaOH (5 mL). This solution was filtered into a centrifuge tube and acidified (2 N HCl) to pH 3.0. The white precipitate was isolated by centrifugation, washed (3×) with H_2O, and vacuum-dried: 560 mg (82%); NMR δ 1.50 (m, 2 H, $CH_2CH_2CH_2CONH$), 1.70 (m, 2 H, $CH_2CH_2CH_2CONH$), 2.05 (t, 2 H, $CH_2CH_2CH_2CONH$), 2.32 (s, 3 H, CH_3), 2.42 (s, 3 H, CH_3), 2.85 (s, 3 H, SO_2CH_3), 3.21 (t, 1 H, C≡CH), 4.15 (m, 1 H, CH), 4.27 (br s, 2 H, CH_2C≡C), 4.69 (br s, 2 H, $ArCH_2N$<), 6.64 (m, 2 H, 3′-H & 5′-H), 7.41 (s, 1 H, quinazoline 8-H), 7.61 (dd, 1 H, 6′-H), 7.69 (s, 1 H, quinazoline 5-H), 7.84 (dd, 1 H, CONH); MS *m*/*z* 600 $[MH]^+$. Anal. ($C_{28}H_{30}FN_5O_7S\cdot 0.75NH_4Cl\cdot 1.75H_2O$) C, H, N.

(*RS*)-2-(Benzyloxycarbonylamino)-4-(aminosulfonyl)-butanoic Acid Benzyl Ester (44). A solution of the sulfonyl chloride **43**[18] (11.0 g, 25.8 mmol) in $CHCl_3$ (110 mL) was added dropwise to a saturated solution of ammonia in $CHCl_3$ (110 mL) below 10 °C. A white precipitate rapidly formed. After a further 30 min at room temperature the reaction mixture was evaporated to dryness and the residue was partitioned between EtOAc (2 × 250 mL) and H_2O (250 mL). The EtOAc solution was dried and evaporated to a yellow solid. The solid was stirred for 1 h in isohexane (250 mL), filtered off, washed with isohexane, and vacuum-dried to an orange solid: 9.95 g (93%); NMR δ 2.14 (m, 2 H, $CH_2CH_2SO_2NH_2$), 3.04 (m, 2 H, $CH_2CH_2SO_2NH_2$), 4.30 (m, 1 H, CH), 5.05 (s, 2 H, CH_2Ph), 5.15 (s, 2 H, CH_2Ph), 6.85 (s, 2 H, SO_2NH_2), 7.35 (m, 10 H, C_6H_5); MS *m*/*z* 407 $[MH]^+$. Anal. ($C_{19}H_{22}N_2O_6S\cdot 0.5H_2O$) C, H; N: calcd, 6.7; found, 6.2.

(*RS*)-2-(Benzyloxycarbonylamino)-4-(*N*-acetylaminosulfonyl)butanoic Acid Benzyl Ester (45). Method G. A solution of **44** (2.03 g, 5.0 mmol) in DMF (30 mL) was stirred for 16 h with acetyl chloride (470 μL, 6.6 mmol), DMAP (810 mg, 6.6 mmol), and Pr^i_2EtN (1.15 mL, 6.6 mmol). The DMF was removed by rotary evaporation at 0.1 mmHg followed by azeotropic removal of traces of DMF by rotary evaporation of added xylene. The residue was stirred for 10 min with 2 N HCl (50 mL) and extracted with EtOAc (2 × 75 mL). The EtOAc solutions were washed with brine, dried, and evaporated. The crude oily product was purified by chromatography, eluting with 3:1 EtOAc/isohexane to yield a viscous yellow oil: 1.30 g (58%); NMR δ 1.98 (s, 3 H, $NHCOCH_3$), 2.13 (m, 2 H, $CH_2CH_2SO_2NH_2$), 3.46 (m, 2 H, $CH_2CH_2SO_2NH_2$), 4.31 (m, 1 H, CH), 5.05 (s, 2 H, CH_2Ph), 5.15 (s, 2 H, CH_2Ph), 6.85 (s, 2 H, SO_2NH_2), 7.35 (m, 10 H, C_6H_5); MS *m*/*z* 449 $[MH]^+$.

(*RS*)-2-[[4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]amino]-4-(*N*-acetylaminosulfonyl)butanoic Acid (17). The benzyl ester **45** (1.30 g, 2.90 mmol) was hydrogenated according to method C to give (*RS*)-2-amino-4-(*N*-acetylaminosulfonyl)butanoic acid which was condensed (method D) with **38** to yield (*RS*)-2-[[4-[*N*-[[3,4-dihydro-2,7-dimethyl-4-oxo-3-[(pivaloyloxy)methyl]-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]amino]-4-(*N*-acetylaminosulfonyl)butanoic acid: 680 mg (33%). This was hydrolyzed (method F) to **17**: 504 mg (76%); NMR δ 1.98 (s, 3 H, $NHCOCH_3$), 2.27 (m, 2 H, $CH_2CH_2SO_2NH$), 2.33 (s, 3 H, CH_3), 2.45 (s, 3 H, CH_3), 3.08 (t, 1 H, C≡CH), 3.44 (m, 2 H, $CH_2CH_2SO_2NH$), 4.31 (br s, 2 H, CH_2C≡C), 4.50 (m, 1 H, CH), 4.72 (br s, 2 H, $ArCH_2N$<), 6.65 (m, 2 H, 3′-H & 5′-H), 7.47 (s, 1 H, quinazoline 8-H), 7.62 (dd, 1 H, 6′-H), 7.75 (s, 1 H, quinazoline 5-H), 7.90 (dd, 1 H, CONH); MS *m*/*z* 584 $[M - H]^-$. Anal. ($C_{27}H_{28}FN_5O_7S\cdot 1.2NaCl\cdot 0.5NH_4Cl$) C, H, N.

(*S*)-2-[*N*-(*tert*-Butoxycarbonyl)amino]-5-hydroxypentanoic Acid Benzyl Ester (48). A solution of *N*-(*tert*-butoxycarbonyl)-L-glutamic acid α-benzyl ester (13.5 g, 40 mmol) in anhydrous THF (200 mL) was stirred at −10 °C. Et_3N (5.61 mL, 40 mmol) was added followed by ethyl chloroformate (3.81 mL, 40 mmol). After 10 min, powdered $NaBH_4$ (4.52 g, 0.12 mol) was added in one portion. MeOH (400 mL) was then added dropwise over 10 min, keeping the temperature between −10 and 0 °C. The reaction mixture was stirred below 0 °C for a further 10 min, allowed to warm to room temperature, and acidified with 1 N HCl (80 mL). The organic solvents were removed from the mixture by rotary evaporation and the residue was partitioned between EtOAc (500 mL) and H_2O (200 mL) with acidification of the aqueous phase to pH 4.0. The EtOAc solution was washed (2×) with brine, dried, and evaporated to give crude **48** as a colorless viscous oil (14.28 g): NMR δ 1.35 (s, 9 H, Bu^t), 1.30−1.80 (m, 4 H, CH_2CH_2), 3.35 (t, 2 H, CH_2OH), 3.98 (m, 1 H, CH), 5.09 (d, 2 H, OCH_2Ph), 7.25 (d, 1 H, CONH), 7.36 (br, 5 H, ArH); MS *m*/*z* 324 $[MH]^+$. This oil was used without purification for the preparation of **49**.

(*S*)-5-(Acetylthio)-2-[*N*-(*tert*-butoxycarbonyl)amino]-pentanoic Acid Benzyl Ester (49). Et_3N (1.15 mL, 8.20 mmol) was added to a stirred solution of **48** (1.36 g, 4.10 mmol) in CH_2Cl_2 (15 mL) at 0 °C. Methanesulfonyl chloride (478 μL, 6.15 mmol) was added dropwise over 10 min. Stirring was continued for 2 h in the ice bath. The solution was washed with ice-cold H_2O, dried, and evaporated to a red oil (1.69 g). A solution of this oil in acetone (10 mL) was stirred 48 h with $KSCOCH_3$ (935 mg, 8.2 mmol). The dark brown reaction mixture was filtered through Celite and the filter cake was washed well with acetone. The filtrate was evaporated to dryness and the residue was purified by chromatography, eluting with 0−10% EtOAc in CH_2Cl_2 to give a pale brown gum: 1.348 g (86%); NMR δ 1.38 (s, 9 H, Bu^t), 1.45−1.80 (m, 4 H, CH_2CH_2), 2.30 (s, 3 H, $COCH_3$), 2.82 (t, 2 H, CH_2S), 4.00 (m, 1 H, CH), 5.11 (d, 2 H, OCH_2Ph), 7.30 (d, 1 H, CONH), 7.35 (br, 5 H, ArH); MS *m*/*z* 382 $[MH]^+$.

(*S*)-5-(Chlorosulfonyl)-2-[*N*-(*tert*-butoxycarbonyl)amino]pentanoic Acid Benzyl Ester (50). A solution of **49** (1.33 g, 3.54 mmol) and NaOAc (2.90 g, 35.4 mmol) in HOAc (20 mL) and H_2O (4 mL) was stirred below 10 °C. Liquid Cl_2 (2 mL) was condensed into a separate tube at −78 °C. The cooling bath was removed and the chlorine was slowly blown as a gas by a stream of argon into the reaction mixture. After 10 min argon was blown through the mixture for 10 min to remove excess Cl_2 and the solvent was evaporated. The residue was partitioned between EtOAc (2 × 25 mL) and H_2O (20 mL). The EtOAc solution was washed with brine, dried, and evaporated to the yellow oily sulfonyl chloride **50**, 1.57 g, contaminated with ca. 5% HOAc: NMR δ 1.37 (s, 9 H, Bu^t), 1.50−1.90 (m, 4 H, CH_2CH_2), 2.50 (t, 2 H, CH_2SO_2), 3.96 (m, 1 H, CH), 5.11 (d, 2 H, OCH_2Ph), 7.30 (d, 1 H, CONH), 7.36 (br, 5 H, ArH). This product was used without purification in the next stage.

(*S*)-5-(Aminosulfonyl)-2-[*N*-(*tert*-butoxycarbonyl)amino]pentanoic Acid Benzyl Ester (51). A solution of **50** (742 mg, 1.83 mmol) in $CHCl_3$ was added dropwise over 10 min to a stirred saturated solution of NH_3 in $CHCl_3$ (10 mL) below 10 °C. The mixture was stirred for 30 min, allowed to warm to room temperature, and evaporated to dryness. The residue was partitioned between EtOAc (2 × 25 mL) and H_2O. The EtOAc solution was washed with brine, dried, and evaporated to a pale yellow gum: 577 mg (82%); NMR δ 1.37 (s, 9 H, Bu^t), 1.60−1.90 (m, 4 H, CH_2CH_2), 3.03 (t, 2 H, CH_2SO_2), 4.00 (m, 1 H, CH), 5.12 (d, 2 H, OCH_2Ph), 6.76 (br, 2 H, NH_2), 7.32 (d, 1 H, CONH), 7.35 (br, 5 H, ArH).

(*S*)-5-(Aminosulfonyl)-2-[*N*-[4-[*N*-[[3,4-dihydro-2,7-dimethyl-4-oxo-3-[(pivaloyloxy)methyl]-6-quinazolinyl]-methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]amino]-pentanoic Acid Benzyl Ester (53). CF_3CO_2H (1 mL) was

added to a solution of **51** (250 mg, 0.64 mmol) in CH_2Cl_2 (3 mL). The mixture was swirled to give a homogeneous solution which was set aside for 1 h. The solvent was evaporated and the gummy residue was dried for 2 h at 0.1 mmHg. The crude amino ester CF_3CO_2H salt **52** was dissolved in DMA (5 mL) and stirred for 16 h with the pentafluorophenyl ester **38** (330 mg, 0.5 mmol), Et_3N (700 μL, 5.0 mmol), and HOBT (10 mg). The mixture was evaporated to dryness and the residue was partitioned between EtOAc (2 × 20 mL) and H_2O (10 mL), with acidification (2 N HCl) of the aqueous layer to pH 1.0. The EtOAc solution was washed with brine, dried, and evaporated. The residue was chromatographed, eluting with 0−6% EtOH in CH_2Cl_2 to yield a white foam: 240 mg (59%); NMR δ 1.11 (s, 9 H, Bu^t), 1.65−1.90 (m, 4 H, CH_2CH_2), 2.46 (s, 3 H, CH_3), 2.58 (s, 3 H, CH_3), 2.98 (t, 2 H, CH_2SO_2), 3.22 (t, 1 H, C≡CH), 4.30 (br s, 2 H, $CH_2C{\equiv}C$), 4.45 (m, 1 H, CH), 4.72 (br s, 2 H, $ArCH_2N<$), 5.14 (s, 2 H, OCH_2Ph), 6.00 (s, 2 H, OCH_2N), 6.64 (m, 2 H, 3′-H & 5′-H), 6.75 (br, 2 H, NH_2), 7.34 (br, 5 H, ArH), 7.48 (s, 1 H, quinazoline 8-H), 7.56 (dd, 1 H, 6′-H), 7.75 (s, 1 H, quinazoline 5-H), 8.20 (dd, 1 H, CONH); MS *m*/*z* 762 $[MH]^+$. Anal. ($C_{39}H_{44}FN_5O_8S{\cdot}3H_2O$) C, H, N.

(*S*)-5-(Acetylaminosulfonyl)-2-[*N*-[4-[*N*-[[3,4-dihydro-2,7-dimethyl-4-oxo-3-[(pivaloyloxy)methyl]-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]amino]-pentanoic Acid Benzyl Ester (54). Method I. Redistilled acetyl chloride (45 μL, 0.63 mmol) was added over 5 min to a stirred mixture of **53** (238 mg, 0.312 mmol), Pr^i_2EtN (109 μL, 0.63 mmol), and DMAP (77 mg, 0.63 mmol) in DMA (3 mL). Stirring was continued for 16 h and the resulting solution was evaporated to dryness. The residue was partitioned between EtOAc (2 × 10 mL) and H_2O (10 mL). The EtOAc solution was dried and evaporated. The crude product was purified by chromatography, eluting with 0−6% EtOH in CH_2Cl_2 to afford a sticky off-white solid: 221 mg (88%); NMR δ 1.12 (s, 9 H, Bu^t), 1.65−2.00 (m, 4 H, CH_2CH_2), 1.95 (s, 3 H, $COCH_3$), 2.46 (s, 3 H, CH_3), 2.58 (s, 3 H, CH_3), 3.24 (t, 1 H, C≡CH), 3.35 (t, 2 H, CH_2SO_2), 4.32 (br s, 2 H, $CH_2C{\equiv}C$), 4.45 (m, 1 H, CH), 4.72 (br s, 2 H, $ArCH_2N<$), 5.15 (s, 2 H, OCH_2Ph), 6.00 (s, 2 H, OCH_2N), 6.63 (m, 2 H, 3′-H & 5′-H), 7.34 (br, 5 H, ArH), 7.50 (s, 1 H, quinazoline 8-H), 7.54 (dd, 1 H, 6′-H), 7.75 (s, 1 H, quinazoline 5-H), 8.22 (dd, 1 H, CONH); MS *m*/*z* 804 $[MH]^+$.

(*S*)-5-(Acetylaminosulfonyl)-2-[*N*-[4-[*N*-[(3,4-dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]amino]pentanoic Acid (20). Method H. The benzyl ester **54** (215 mg, 0.27 mmol) was stirred for 2 h in a mixture of aqueous NaOH (2.4 mL, 2.4 mmol) and EtOH (6 mL). The reaction mixture was evaporated to dryness and the residue was dissolved in H_2O (4 mL). The solution was filtered into a centrifuge tube and acidified (2 N HCl) to pH 3.0. The gelatinous precipitate was isolated by centrifugation, washed (3×) with H_2O, and freeze-dried to a fluffy white solid: 130 mg (76%); NMR δ 1.60−2.00 (m, 4 H, CH_2CH_2), 1.95 (s, 3 H, $COCH_3$), 2.31 (s, 3 H, CH_3), 2.43 (s, 3 H, CH_3), 3.22 (t, 1 H, C≡CH), 3.35 (t, 2 H, CH_2SO_2), 4.30 (br s, 2 H, $CH_2C{\equiv}C$), 4.35 (m, 1 H, CH), 4.69 (br s, 2 H, $ArCH_2N<$), 6.65 (m, 2 H, 3′-H & 5′-H), 7.43 (s, 1 H, quinazoline 8-H), 7.55 (dd, 1 H, 6′-H), 7.70 (s, 1 H, quinazoline 5-H), 7.96 (dd, 1 H, CONH); MS *m*/*z* 598 $[M - H]^-$. Anal. ($C_{28}H_{30}FN_5O_7S{\cdot}1.75H_2O$) C, H, N.

(*RS*)-2-[*N*-(Benzyloxycarbonyl)amino]-3-(3*H*-1,2,3-triazol-4-ylthio)propanoic Acid (57). A solution of methyl 2-[*N*-(benzyloxycarbonyl)amino]acrylate (**56**)[20] (22.0 g, 93.6 mmol) in MeOH (50 mL) was stirred with 4-mercapto-1*H*-1,2,3-triazole disodium salt hexahydrate (38.0 g, 0.15 mol) at 45−50 °C for 3 h. The solvent was evaporated and the residue was partitioned between EtOAc (500 mL) and 2 N HCl (200 mL). The EtOAc solution was washed with brine, dried, and evaporated. Chromatography of the residue, eluting with 4:1 EtOAc/MeOH, yielded a complex mixture of high R_f products followed by **57** as a yellow gum: 4.40 g (15%); NMR δ 3.12, 3.43 (2 × dd, 2 × 1 H, CH_2S), 4.02 (m, 1 H, CH), 5.00 (s, 2 H, OCH_2Ph), 6.90 (d, 1 H, CONH), 7.32 (br, 5 H, ArH), 7.79 (s, 1 H, triazole-H); MS *m*/*z* 321 $[M - H]^-$.

(*RS*)-2-Amino-3-(3*H*-1,2,3-triazol-4-ylthio)propanoic Acid Triethylammonium Salt (58). A mixture of **57** (1.70 g, 5.28 mmol) and 48% aqueous HBr (50 mL) was stirred for 2 h at 25 °C. The reaction mixture was extracted with CH_2Cl_2 and the aqueous solution was evaporated to dryness at 0.1 mmHg: NMR δ (D_2O) 3.67 (m, 2 H, CH_2), 4.46 (m, 1 H, CH), 8.22 (br s, 1 H, CH). The residue was dissolved in H_2O (25 mL). The solution was filtered, treated with Et_3N (2.0 mL, 14.2 mmol), and evaporated at 0.1 mmHg to yield the crude Et_3N salt of **58** (1.5 g), an off-white solid, which was used immediately in the condensation with the pentafluorophenyl ester **38**.

(*RS,RS*)-2-[*N*-[4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]amino]-3-(3*H*-1,2,3-triazol-4-ylsulfinyl)propanoic Acid (23). Method J. Peracetic acid (32% w/w in HOAc, 250 μL) was added to a stirred solution of **22** (650 mg, 1.18 mmol) in $CHCl_3$ (100 mL) and MeOH (50 mL). Stirring was continued for 16 h. The solution was evaporated down to a thick white slurry which was sucked dry in a sinter. The white solid was vacuum-dried: 650 mg (70%); NMR (400 MHz) δ (Me_2SO-d_6/HOAc-d_6) 2.50 (s, 3 H, CH_3), 2.60 (s, 3 H, CH_3), 3.22 (t, 1 H, C≡CH), 3.50−3.85 (m, 2 H, CH_2SO), 4.34 (br s, 2 H, $CH_2C{\equiv}C$), 4.63, 4.84 (2 × m, 1 H, CH), 4.78 (br s, 2 H, $ArCH_2N<$), 6.64 (m, 2 H, 3′-H & 5′-H), 7.60 (dd, 1 H, 6′-H), 7.65 (s, 1 H, quinazoline 8-H), 7.75 (s, 1 H, quinazoline 5-H), 8.55, 8.62 (2 × br, 1 H, triazole-H); MS *m*/*z* 566 $[MH]^+$. Anal. ($C_{26}H_{24}FN_7O_5S{\cdot}CHCl_3{\cdot}CH_3OH$) H, N; C: calcd, 46.8; found, 47.3.

(*R*)-2-Amino-3-(1*H*-1,2,3,4-tetrazol-5-ylthio)propanoic Acid (62). A mixture of β-chloroalanine·HCl (1.60 g, 10 mmol), 5-mercapto-1*H*-1,2,3,4-tetrazole[21] (1.10 g, 10.8 mmol), and Bu_3P (100 μL) in 2 N aqueous NaOH (20 mL) was stirred 1 h at 80 °C. The reaction mixture was cooled and acidified (2 N HCl) to pH 3.0 and the solution was passed through a column of octadecyl-functionalized silica gel (Aldrich), eluting with 0.2% aqueous CF_3CO_2H. The fractions containing the product were pooled and concentrated by rotary evaporation to yield a white solid: 400 mg (21%); mp 150 °C dec; $[\alpha]_D^{25}$ −64.0° (MeOH); NMR δ 3.50, 3.57 (2 × dd, 2 × 1 H, CH_2), 4.19 (dd, 2 H, CH), 7.65 (br, 3 H, NH_2 + NH); MS *m*/*z* 188 $[M - H]^-$. Anal. ($C_4H_7N_5O_2S{\cdot}H_2O$) C, H, N, S.

(*S*)-2-Amino-4-(1-*tert*-butyl-1*H*-1,2,3,4-tetrazol-5-ylthio)-propanoic Acid (66). A mixture of l-homocystine (2.0 g, 7.4 mmol) and Bu_3P (4.0 mL, 16.0 mmol) in 2 N aqueous NaOH (16.0 mL) was stirred for 30 min. 1-*tert*-Butyl-5-chloro-1*H*-1,2,3,4-tetrazole[22] (2.60 g, 16.2 mmol) was added and stirring was continued for 48 h. The reaction mixture was partitioned between Et_2O (100 mL) and H_2O (100 mL). The aqueous layer was acidified (HOAc) to pH 5.0 and passed through a column of octadecyl-functionalized silica gel, eluting with H_2O then 70:30:0.2 MeOH/H_2O/CF_3CO_2H. The fractions containing the product were pooled and concentrated by rotary evaporation to afford the white solid CF_3CO_2H salt: 3.9 g (70%); NMR (D_2O) δ 1.88 (s, 9 H, Bu^t), 2.59 (m, 2 H, $CHCH_2$), 3.66 (m, 2 H, CH_2S), 4.22 (m, 1 H, CH); MS *m*/*z* 372 $[M - H + CF_3CO_2H]^-$.

(*S*)-2-Amino-4-(1*H*-1,2,3,4-tetrazol-5-ylthio)propanoic Acid (67) and (*S*)-2-Amino-4-(2-*tert*-butyl-2*H*-1,2,3,4-tetrazol-5-ylthio)propanoic Acid (68). A mixture of the amino acid CF_3CO_2H salt **66** (3.9 g, 10.45 mmol), anisole (2.5 mL, 23.0 mmol), and concd HCl (50 mL) was stirred at 80−90 °C for 2 h and evaporated to dryness. The residue was partitioned between Et_2O (100 mL) and H_2O (100 mL). The aqueous solution was adjusted (2 N NaOH) to pH 3.0 and passed through a column of octadecyl-functionalized silica gel, eluting with 0.2% aqueous CF_3CO_2H. Fractions containing the first product to be eluted from the column were pooled and evaporated to dryness and the residue was dissolved in MeOH. Evaporation of this solution gave **67** as the white amorphous CF_3CO_2H salt: 1.40 g (42%); NMR (D_2O) δ 2.55 (m, 2 H, $CHCH_2$), 3.60 (m, 2 H, CH_2S), 4.32 (dd, 1 H, CH); MS *m*/*z* 202 $[M - H]^-$. The second product to be eluted was **68**, obtained as the CF_3CO_2H salt: 1.80 g (46%); NMR (D_2O) δ 1.89 (s, 9 H, Bu^t), 2.51 (m, 2 H, $CHCH_2$), 3.51 (dd, 2 H, CH_2S), 4.25 (d, 1 H, CH); MS *m*/*z* 372 $[M - H + CF_3CO_2H]^-$ and 258 $[M - H]^-$.

Ethyl (*S*)-5-Hydroxy-2-phthalimidopentanoate (70). A solution of (*S*)-2-phthalimidoglutaric acid 1-ethyl ester (**69**)[23] (60.8 g, 0.20 mmol) in anhydrous THF (250 mL) was stirred mechanically using a PTFE paddle and cooled to −10 °C. Et_3N (41.1 mL, 0.30 mol) was added followed by ethyl chloroformate (23.7 mL, 0.25 mol). Stirring was continued for 10 min and powdered $NaBH_4$ (22.68 g, 0.60 mol) was added in one portion. MeOH (200 mL) was added dropwise over 10 min, keeping the temperature between −10 and 0 °C (**caution**: vigorous evolution of hydrogen gas occurred). Stirring was continued while the temperature of the reaction mixture rose to 20 °C. H_2O (80 mL) was carefully added and the pH was adjusted (2 N HCl) to 7.0. The organic solvents were removed by rotary evaporation and the aqueous residue was partitioned between EtOAc (2 × 200 mL) and H_2O (200 mL). The EtOAc solution was washed with brine, dried, and evaporated to a yellow oil. The above procedure was repeated on the same scale and the crude products (total yield 111 g) from the two experiments were combined and chromatographed using 4:1 isohexane/EtOAc as eluent to yield a colorless viscous oil, 50.2 g, which was shown to be 84% pure **70** by HPLC: NMR ($CDCl_3$) δ 1.23 (t, 3 H, OCH_2CH_3), 1.58 (m, 2 H, $CH_2CH_2CH_2$), 2.32 (m, 2 H, $CHCH_2$), 3.63 (t, 2 H, CH_2OH), 4.20 (q, 2 H, OCH_2CH_3), 4.87 (dd, 1 H, CH), 7.75 (m, 2H, ArH), 7.83 (m, 2 H, ArH); MS *m*/*z* 292 $[MH]^+$. The alcohol **70** was used in the next stage without further purification.

Ethyl (*S*)-2-Phthalimido-5-(1*H*-1,2,3,4-tetrazol-5-ylthio)-pentanoate (72). Methanesulfonyl chloride (5.00 mL, 64.4 mmol) was added dropwise to a stirred solution of the alcohol **70** (15.0 g of 84% pure, 43.3 mmol) in CH_2Cl_2 (250 mL) at 0−5 °C. The reaction mixture was stirred for 2 h at this temperature when TLC indicated complete reaction. The solution was washed with ice-cold H_2O (2 × 125 mL). The aqueous layers were back-extracted with CH_2Cl_2. The CH_2Cl_2 solution was dried and evaporated below 30 °C to give the crude mesylate **71** (20.2 g). This intermediate was immediately dissolved in a mixture of EtOH (250 mL) and DMF (20 mL) and stirred for 60 h with 5-mercapto-1*H*-1,2,3,4-tetrazole[21] (5.78 g, 56.7 mmol) and Et_3N (10.70 mL, 77.3 mmol). The EtOH was evaporated and the residue was partitioned between EtOAc (2 × 100 mL) and H_2O (100 mL), with acidification (2 N HCl) of the aqueous phase to pH 1.0. The EtOAc solution was washed with 0.5 N HCl and H_2O and dried and the solvent was evaporated. The crude product was chromatographed using 95:5 v/v CH_2Cl_2/MeOH as eluent. Fractions containing **72** were pooled and evaporated and the semisolid residue triturated with 1:1 Et_2O/isohexane to yield a white solid: 10.57 g (65%); mp 98−99 °C; NMR δ 1.25 (t, 3 H, OCH_2CH_3), 1.68 (m, 2 H, $CH_2CH_2CH_2$), 2.20 (m, 2 H, $CHCH_2$), 3.11 (t, 2 H, CH_2S), 4.25 (q, 2 H, OCH_2CH_3), 4.94 (dd, 1 H, CH), 7.91 (m, 4H, ArH); MS *m*/*z* 376 $[MH]^+$. Anal. ($C_{16}H_{17}N_5O_4S$) C, H, N.

Ethyl (*S*)-2-Amino-5-(1*H*-1,2,3,4-tetrazol-5-ylthio)pentanoate (73). A solution of the phthalimido compound **72** (10.0 g, 26.7 mmol) in EtOH (100 mL) was treated with $N_2H_4 \cdot H_2O$ (1.42 mL, 29.3 mmol) for 18 h. HPLC indicated that a small amount of starting material remained. Further $N_2H_4 \cdot H_2O$ (0.28 mL, 5.8 mmol) was added. After 1 h the solution was evaporated to dryness and the residue was stirred in CF_3CO_2H (100 mL) for 4 h. The CF_3CO_2H was evaporated and the residue was digested with H_2O (ca. 200 mL). The precipitated phthalazinedione was filtered off and the filtrate was evaporated to dryness to yield a colorless gum (10.0 g), the crude CF_3CO_2H salt of **73** which was used without purification.

(*S*)-2-[*N*-[4-[*N*-[[3,4-Dihydro-2,7-dimethyl-4-oxo-3-[(pivaloyloxy)methyl]-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]amino]-5-(1*H*-1,2,3,4-tetrazol-5-ylthio)pentanoic Acid Ethyl Ester (74). Method K. Et_3N (8.42 mL, 60 mmol), **73** CF_3CO_2H salt (7.20 g, 20 mmol), and 1-hydroxybenzotriazole (50 mg) were added to a stirred solution of the pentafluorophenyl ester **38** (8.11 g, 12.3 mmol) in DMF (80 mL). Stirring was continued for 4 days with the addition of further portions of **38** (1.0 g at 18 h and 5.5 g at 42 h). The reaction mixture was evaporated to dryness and the residue was partitioned between EtOAc (2 × 400 mL) and H_2O (400 mL) with acidification (2 N HCl) of the aqueous phase to pH 1.0. The organic solution was washed (2×) with brine and dried and the solvent was evaporated. Chromatographic purification, eluting with 5−15% MeOH in CH_2Cl_2, gave a pale brown foam: 4.72 g (33%); NMR δ 1.12 (s, 9 H, Bu^t), 1.16 (t, 3 H, OCH_2CH_3), 1.75 (m, 2 H, $CH_2CH_2CH_2$), 1.86 (m, 2 H, $CHCH_2$), 2.50 (s, 3 H, CH_3), 2.57 (s, 3 H, CH_3), 3.11 (t, 2 H, CH_2S), 3.22 (br s, 1 H, C≡CH), 4.08 (q, 2 H, OCH_2CH_3), 4.31 (br s, 2 H, $CH_2C{\equiv}C$), 4.35 (m, 1 H, CH), 4.71 (br s, 2 H, $ArCH_2N<$), 6.00 (s, 2 H, OCH_2N), 6.60 (dd, 1 H, 3′-H), 6.66 (dd, 1 H, 5′-H), 7.48 (s, 1 H, quinazoline 8-H), 7.55 (dd, 1 H, 6′-H), 7.76 (s, 1 H, quinazoline 5-H), 8.10 (dd, 1 H, CONH).

(*S*,*RS*)-2-[*N*-[4-[*N*-[[3,4-Dihydro-2,7-dimethyl-4-oxo-3-[(pivaloyloxy)methyl]-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]amino]-5-(1*H*-1,2,3,4-tetrazol-5-ylsulfinyl)pentanoic Acid Ethyl Ester (75). Method L. A solution of the sulfide **74** (4.71 g, 6.53 mmol) in CH_2Cl_2 (150 mL) was stirred with 3-chloroperbenzoic acid (1.35 g of 80%, 6.67 mmol) for 16 h. The reaction mixture was applied directly to a silica gel column which was eluted with 7.5% MeOH in CH_2Cl_2. The fractions containing pure product by TLC were evaporated to give **75** as a white foam: 1.74 g (36%); NMR δ 1.13 (s, 9 H, Bu^t), 1.17 (t, 3 H, OCH_2CH_3), 1.68 (m, 2 H, $CH_2CH_2CH_2$), 1.90 (m, 2 H, $CHCH_2$), 2.45 (s, 3 H, CH_3), 2.57 (s, 3 H, CH_3), 3.08 (dd, 2 H, CH_2SO), 3.20 (br, 1 H, C≡CH), 4.08 (q, 2 H, OCH_2CH_3), 4.30 (br s, 2 H, $CH_2C{\equiv}C$), 4.35 (m, 1 H, CH), 4.70 (br s, 2 H, $ArCH_2N<$), 5.99 (s, 2 H, OCH_2N), 6.59 (dd, 1 H, 3′-H), 6.63 (dd, 1 H, 5′-H), 7.47 (s, 1 H, quinazoline 8-H), 7.55 (dd, 1 H, 6′-H), 7.75 (s, 1 H, quinazoline 5-H), 8.13 (dd, 1 H, CONH); MS *m*/*z* 737 $[MH]^+$. Evaporation of the less pure fractions yielded a further 2.66 g of product, 80% pure by HPLC.

(*S*,*RS*)-2-[*N*-[4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzoyl]amino]-5-(1*H*-1,2,3,4-tetrazol-5-ylsulfinyl)pentanoic Acid (29). Method M. A mixture of **75** (1.74 g, 2.36 mmol), 2 N aqueous NaOH (30 mL, 60 mmol), and MeOH (20 mL) was stirred for 90 min, warmed to 50 °C for 10 min, and cooled back to 20 °C. The MeOH was evaporated and the resulting aqueous solution was filtered into a centrifuge tube. Acidification (2 N HCl) to pH 4.0 produced a gelatinous white precipitate which was isolated by centrifugation and washed (4×) with H_2O, during which process the precipitate became more granular. The resulting moist white solid was vacuum-dried overnight and then suspended in toluene which was then evaporated on a rotary evaporator. The solid was again vacuum-dried overnight: 1.02 g (67%); NMR δ 1.73 (m, 2 H, $CH_2CH_2CH_2$), 1.88 (m, 2 H, $CHCH_2$), 2.38 (s, 3 H, CH_3), 2.46 (s, 3 H, CH_3), 3.20 (t, 1 H, C≡CH), 3.29 (t, 2 H, CH_2SO), 4.30 (br s, 2 H, $CH_2C{\equiv}C$), 4.37 (dd, 1 H, CH), 4.71 (br s, 2 H, $ArCH_2N<$), 6.60 (dd, 1 H, 3′-H), 6.65 (dd, 1 H, 5′-H), 7.47 (s, 1 H, quinazoline 8-H), 7.54 (dd, 1 H, 6′-H), 7.72 (s, 1 H, quinazoline 5-H), 7.91 (dd, 1 H, CONH); MS *m*/*z* 595 $[MH]^+$. Anal. ($C_{27}H_{27}FN_8O_5S \cdot 0.5NaCl \cdot 1.2H_2O$) C, H, N.

Ethyl (*S*)-2-Phthalimido-5-[2*H*-1,2,4-triazol-3(4*H*)-ono-5-thio]pentanoate (77). A mixture of carbethoxythiosemicarbazide[43] (5.00 g, 30.7 mmol) and NaOH pellets (2.45 g, 61.4 mmol) in EtOH (150 mL) was stirred under reflux for 1 h to give a clear solution. The cooled solution was evaporated to dryness to give the crude disodium salt **76** of 5-mercapto-2*H*-1,2,4-triazol-3(4*H*)-one, 6.32 g, as a greenish solid which was used without purification.

A solution of the mesylate **71** (10.38 g, 28.14 mmol) in DMF (10 mL) was added over 10 min to a stirred solution of **76** (6.32 g) in EtOH (200 mL) at 50 °C. Stirring was continued under reflux for 1 h. The EtOH was evaporated and the residue was partitioned between EtOAc (2 × 100 mL) and H_2O (50 mL). Chromatographic purification of the crude product, eluting with 9:1 EtOAc/isohexane, yielded a white foam: 4.27 g (39%); NMR δ 1.17 (t, 3 H, OCH_2CH_3), 1.60 (m, 2 H, $CH_2CH_2CH_2$), 2.15 (m, 2 H, $CHCH_2$), 2.96 (t, 2 H, CH_2S), 4.10 (q, 2 H, OCH_2CH_3), 4.90 (m, 1 H, CH), 7.89 (m, 4 H, ArH), 11.40 (br, 1 H, NH), 11.56 (br, 1 H, NH); MS *m*/*z* 391 $[MH]^+$.

Ethyl (*S*)-2-Amino-5-[2*H*-1,2,4-triazol-3(4*H*)-ono-5-thio]-pentanoate·CF_3CO_2H Salt (78). Hydrazine hydrate (604 μL, 12.5 mmol) was added to a stirred solution of **77** (4.41 g, 11.3 mmol) in EtOH (100 mL). After 90 min the solution was evaporated to dryness to give a white foam (4.51 g) which was stirred in CF_3CO_2H (50 mL) for 2 h. The CF_3CO_2H was evaporated and the residue was dissolved in H_2O (100 mL). The solution was filtered to remove the insoluble phthalazinedione and the filtrate was evaporated to dryness to give an pale golden gum, 5.15 g (>100%), which was used without purification in the condensation with pentafluorophenyl ester **38** (method K).

Ethyl 2-Acetamido-2-carbethoxy-6-(1*H*-1,2,3,4-tetrazol-5-ylthio)hexanoate (80). Et_3N (3.36 mL, 24.3 mmol) was added in one portion to a stirred suspension of ethyl 2-acetamido-6-bromo-2-carbethoxyhexanoate (**79**)[24] (5.70 g, 16.2 mmol) and 5-mercaptotetrazole[21] (1.50 g, 14.7 mmol) in EtOH (60 mL). The resulting clear solution was kept at 20 °C for 48 h and the EtOH was evaporated to produce a yellow oil. The oil was partitioned between EtOAc (2 × 100 mL) and H_2O (50 mL) and acidified (2 N HCl) to pH 1.0. The EtOAc solution was washed with H_2O, dried, and evaporated to a sticky solid. Trituration with Et_2O produced a free flowing, amorphous white solid: 2.0 g (36%); NMR ($CDCl_3$) δ 1.26 (t, 2 H, 2 × OCH_2CH_3), 1.31 (m, 2 H, CH_2), 1.78 (m, 2 H, CH_2CH_2S), 2.13 (s, 3 H, $COCH_3$), 2.38 (m, 2 H, CCH_2), 3.23 (t, 2 H, CH_2S), 4.26 (q, 4 H, 2 × OCH_2CH_3), 7.04 (s, 1 H, NH); MS *m*/*z* 374 $[MH]^+$.

(*RS*)-2-Amino-6-(1*H*-1,2,3,4-tetrazol-5-ylthio)hexanoic Acid Hydrochloride (81). The ester **80** (2.00 g, 5.35 mmol) was stirred under reflux in 6 N HCl (50 mL) for 2 h to give a clear solution. The solution was cooled and evaporated to dryness on a rotary evaporator. The residue was dissolved in H_2O (50 mL) and this solution was evaporated to dryness. This was repeated with a further portion of H_2O followed by the evaporation of added toluene to give crude **81** as a white foam: 1.53 g; NMR δ 1.56 (m, 2 H, CH_2), 1.70 (m, 2 H, CH_2), 1.80 (m, 2 H, CH_2CH_2S), 3.25 (t, 2 H, CH_2S), 3.85 (m, 1 H, CH), 8.41 (br, 3 H, NH_3^+), 8.60 (br, 1 H, NH). This was used without purification in the preparation of the methyl ester **82**.

Methyl (*RS*)-2-Amino-6-(1*H*-1,2,3,4-tetrazol-5-ylthio)-hexanoate Hydrochloride (82). Thionyl chloride (740 μL, 8.58 mmol) was added to a stirred solution of the amino acid hydrochloride **81** (1.53 g) in MeOH (60 mL). After 18 h, further thionyl chloride (370 μL, 4.29 mmol) was added and the solution was boiled under reflux for 5 min. Evaporation of the solvent yielded a pale pink foam: 1.60 g (100%); NMR δ 1.47 (m, 2 H, CH_2), 1.69 (m, 2 H, CH_2), 1.77 (m, 2 H, CH_2CH_2S), 3.23 (t, 2 H, CH_2S), 3.74 (s, 3 H, OCH_3), 3.99 (m, 1 H, CH), 8.55 (br, 3 H, NH_3^+); MS *m*/*z* 246 $[MH]^+$. This was used without purification in the condensation with the pentafluorophenyl ester **38**.

(*S*)-2-[4-[*N*-[[3,4-Dihydro-2,7-dimethyl-4-oxo-3-[(pivaloyloxy)methyl]-6-quinazolinyl]methyl]-*N*-prop-2-ynylamino]-2-fluorobenzamido]-4-(1*H*-1,2,3,4-tetrazol-5-yl)butyric Acid Methyl Ester (89). A mixture of (*S*)-2-amino-4-(1*H*-1,2,3,4-tetrazol-5-yl)butyric acid methyl ester (**84**)[25,26] (19.4 g, 0.105 mol), pentafluorophenyl ester **38** (67.5 g, 0.102 mol), and HOBT (20 mg) in DMF (600 mL) was stirred for 60 h. The DMF was evaporated and the gummy residue was partitioned between EtOAc (1.5 L) and H_2O (1.0 L). The EtOAc solution was washed with brine, dried, and evaporated to an orange gum. Chromatographic purification, eluting with EtOAc followed by 9:1 CH_2Cl_2/MeOH, gave a white amorphous solid: 51.4 g (75%); NMR δ 1.22 (s, 9 H, Bu^t), 2.25 (m, 2 H, $CHCH_2$), 2.46 (s, 3 H, CH_3), 2.58 (s, 3 H, CH_3), 2.97 (t, 2 H, CH_2-tetrazole), 3.22 (t, 1 H, C≡CH), 3.65 (s, 3 H, OCH_3), 4.32 (br s, 2 H, $CH_2C{\equiv}C$), 4.48 (m, 1 H, CH), 4.72 (br s, 2 H, $ArCH_2N<$), 6.00 (s, 2 H, OCH_2N), 6.62 (dd, 1 H, 3′-H), 6.66 (dd, 1 H, 5′-H), 7.48 (s, 1 H, quinazoline 8-H), 7.55 (dd, 1 H, 6′-H), 7.75 (s, 1 H, quinazoline 5-H), 8.24 (dd, 1 H, CONH).

(*S*)-2-[4-[*N*-[(3,4-Dihydro-2,7-dimethyl-4-oxo-6-quinazolinyl)methyl]-*N*-prop-2-ynylamino]-2-fluorobenzamido]-4-(1*H*-1,2,3,4-tetrazol-5-yl)butyric Acid (35). The methyl ester **89** (65 g, 0.098 mol) was stirred with 2 N aqueous NaOH (400 mL, 0.8 mol) to give a clear pale yellow solution. After 3 h the solution was filtered and acidified (concd HCl) to pH 3.5. The resulting suspension was allowed to stand for 2 h and the precipitated solid was then filtered off and washed with H_2O (5 × 500 mL) in the sinter. The resulting paste was dried by rotary evaporation at 0.1 mmHg with the aid of added toluene to yield a white amorphous solid: 40.3 g (77%); NMR (400 MHz) δ 2.26 (m, 2 H, $CHCH_2$), 2.32 (s, 3 H, CH_3), 2.44 (s, 3 H, CH_3), 2.99 (t, 2 H, CH_2tetrazole), 3.23 (t, 1 H, C≡CH), 4.32 (br s, 2 H, $CH_2C{\equiv}C$), 4.46 (m, 1 H, CH), 4.71 (br s, 2 H, $ArCH_2N<$), 6.66 (dd, 1 H, 3′-H), 6.66 (dd, 1 H, 5′-H), 7.44 (s, 1 H, quinazoline 8-H), 7.59 (dd, 1 H, 6′-H), 7.72 (s, 1 H, quinazoline 5-H), 8.10 (dd, 1 H, CONH); MS *m*/*z* 531 $[M - H]^-$. Anal. ($C_{26}H_{25}FN_8O_4$) C, H, N.

Methyl (*S*)-2-Phthalimido-5-cyanopentanoate (87). Potassium cyanide (7.50 g, 0.115 mol) was added to a stirred solution of mesylate **86** (13.63 g, 38.4 mmol; prepared by the same method as for the ethyl ester **71**) in DMSO (200 mL). Stirring was continued overnight and the DMSO was removed by rotary evaporation at 0.1 mmHg. The orange gummy residue was partitioned between EtOAc (2 × 200 mL) and H_2O (100 mL). The EtOAc solutions were washed with brine, dried, and evaporated to an orange oil: 9.52 g (86%); NMR δ 1.57 (m, 2 H, $CH_2CH_2CH_2$), 2.26 (m, 2 H, $CHCH_2$), 2.50 (t, 2 H, CH_2CN), 3.65 (s, 3 H, OCH_3), 4.94 (dd, 1 H, CH), 7.91 (m, 4H, ArH); MS *m*/*z* 287 $[MH]^+$.

Methyl (*RS*)-2-Phthalimido-5-(1*H*-1,2,3,4-tetrazol-5-yl)-pentanoate (88). A mixture of the nitrile **87** (9.51 g, 33.3 mmol), NaN_3 (2.27 g, 34.9 mmol), and NH_4Cl (1.87 g, 34.9 mmol) in DMF (100 mL) was stirred at 90 °C for 16 h. HPLC showed that the mixture contained contained **88** and unreacted **87** in a ratio of 51:35. The DMF was removed by rotary evaporation at 0.1 mmHg and the orange oily residue was partitioned between EtOAc (3 × 100 mL) and H_2O (100 mL, acidified to pH 1.0 with concd HCl). The EtOAc solutions were washed with H_2O, dried, and evaporated to a light brown oil which was chromatographed using 2% MeOH in $CHCl_3$ as eluent to yield **88** as a colorless oil: 1.18 g (11%); NMR δ 1.70 (m, 2 H, $CH_2CH_2CH_2$), 2.12 (m, 2 H, $CHCH_2$), 2.90 (t, 2 H, CH_2tetrazole), 3.65 (s, 3 H, OCH_3), 4.94 (dd, 1 H, CH), 7.91 (m, 4H, ArH).

Methyl (*RS*)-2-Amino-5-(1*H*-1,2,3,4-tetrazol-5-yl)pentanoate (89). Hydrazine hydrate (180 μL, 3.68 mmol) was added to a stirred solution of **88** (1.10 g, 3.34 mmol) in MeOH (8 mL). The clear pale yellow solution was kept for 18 h and evaporated to dryness. The white solid residue was stirred with CF_3CO_2H (20 mL) for 2 h and the resulting solution was evaporated to a sticky white solid which was stirred with H_2O (20 mL) until no more material appeared to go into solution. The solution was filtered to remove the white insoluble phthalazinedione, the filtrate was evaporated to dryness, and the residue was azeotroped with toluene (3×) to give crude **89** (1.4 g) which was used without purification in the condensation with the pentafluorophenyl ester **38**.

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(1) Tomudex is a trade mark, the property of ZENECA Ltd.

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