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Bioorganic & Medicinal Chemistry 15 (2007) 3474–3488
Bioorganic & Medicinal Chemistry

Synthetic approaches to peptides containing the L-Gln-L-Val-D(*S*)-Dmt motif

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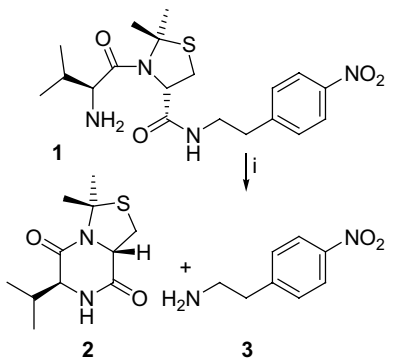
Received 6 November 2006; revised 16 February 2007; accepted 2 March 2007
Available online 6 March 2007

Abstract—The *pseudo*prolines *S*-Dmo (5,5-dimethyl-4-oxaproline) and *R*-Dmt (5,5-dimethyl-4-thiaproline) have been used to study the effects of forcing a fully *cis* conformation in peptides. Synthesis of peptides containing these (which have the same configuration as L-Pro) is straightforward. However, synthesis of peptides containing *S*-Dmt is difficult, owing to the rapid cyclisation of L-Aaa-*S*-Dmt amides and esters to form the corresponding diketopiperazines (DKP); thus the intermediacy of L-Aaa-*S*-Dmt amides and esters must be avoided in the synthetic sequence. Peptides containing the L-Gln-L-Val-D(*S*)-Dmt motif are particularly difficult, owing to the insolubility of coupling partners containing Gln. Introduction of Gln as *N*-Boc-pyroglutamate overcame the latter difficulty and the dipeptide active ester BocPygValOC$_6$F$_5$ coupled in good yield with *S*-DmtOH. BocPygVal-*S*-DmtNH(CH$_2$)$_2$C$_6$H$_4$NO$_2$ was converted quantitatively to BocGlnVal-*S*-DmtNH(CH$_2$)$_2$C$_6$H$_4$NO$_2$ with ammonia, demonstrating the utility of this approach. Two peptide derivatives (CbzSerLysLeuGlnVal-*S*-DmtNH(CH$_2$)$_2$C$_6$H$_4$NO$_2$ and CbzSerSerLysLeuGln-Val-*S*- DmtNH(CH$_2$)$_2$C$_6$H$_4$NO$_2$) were assembled, using these new methods of coupling a dipeptide acid active ester with *S*-DmtOH and introduction of Gln as Pyg, followed by conventional peptide couplings. The presence of the Val caused these peptides to be cleaved very slowly by prostate-specific antigen (PSA) at Leu ↓ Gln, rather than the expected Gln ↓ Val.

1. Introduction

In our previous paper,[1] we reported the rapid cyclisation of L-Val-*S*-Dmt *N*-(2-(4-nitrophenyl)ethyl)amide **1** to give the diketopiperazine **2**, expelling the amine **3** with first-order kinetics in aqueous buffers ($t_{1/2}$ (pH 8.0) = 15 min, $t_{1/2}$ (pH 7.0) = 45 min, $t_{1/2}$ (pH 6.0) = 600 min) (Scheme 1). This dipeptide unit was proposed as a potential molecular clip for use in prodrug systems. Other dipeptides in this *L*,*S* diastereomeric series also cyclised rapidly to give diketopiperazines (DKPs) but the dipeptide derivatives in the *L*,*R* series cyclised much more slowly (for L-Val-*R*-Dmt *N*-(2-(4-nitrophenyl)ethyl)amide: $t_{1/2}$ (pH 8.0) = 1600 min, $t_{1/2}$ (pH 7.0) > 2700 min). Thus, in order to build longer peptides containing this L-Val-*S*-Dmt feature, the synthetic sequence must not involve generation of L-Val-*S*-Dmt amides or esters as intermediates.

Sterically demanding *pseudo*prolines, such as 5,5-dimethyl-4-oxaproline (Dmo) and 5,5-dimethyl-4-thiaproline (Dmt), have been used to mimic proline in bio-active peptides to force the conformation of the Aaa-ψPro wholly or largely into the *cis* tertiary amide conformation. These peptides have mostly included *S*-Dmo or *R*-Dmt, where the configuration matches that of L-Pro. For



Scheme 1. Rapid spontaneous cyclisation of the L-Val-*S*-Dmt amide **1** to form the DKP **2**, expelling the primary amine **3**. Reagent and conditions: (i) aq buffer pH 6.0, pH 7.0 or pH 8.0.

Keywords: Dimethylthiaproline; Pseudoproline; Prostate-specific antigen; Peptide synthesis; Pyroglutamate.
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doi:10.1016/j.bmc.2007.03.005

example, *R*-Dmt has been incorporated into peptides mimicking amyloid protein,[2] into a neuroprotective tripeptide,[3] into an oxytocin analogue,[4] into a δ-conotoxin analogue,[5] into analogues of morphiceptin and endomorphin-2[6] and into an analogue of aspergillamide.[7] Interestingly, although the L-Aaa-*R*-Dmt unit exists in virtually exclusively the *cis*-amide conformation,[1,4–6,8,9] up to 10% of the *trans*-amide rotamer is present in the diastereomeric L-Aaa-*S*-Dmt series.[1] *S*-Dmo has been used frequently in peptide synthesis as a masked form of serine to avoid problems of insolubility and aggregation during assembly of medium-to-large peptides[10–12] and has itself been used to control conformation in peptides.[8,13] Owing to the relatively slow cyclisation of L-amino-acyl-*R*-Dmt derivatives, it has been possible to use *R*-Dmt in conventional peptide synthesis in solution and on the solid phase.[2–6] Indeed, some L-amino-acyl-*R*-Dmt amides have been sufficiently stable to allow (presumably rapid) biochemical and biological evaluation.[3,6] Notably, there have been no previous reports of assembly of peptides containing *S*-Dmt, presumably since conventional C → N stepwise synthesis is precluded by the rapid formation of DKPs from L-amino-acyl-*S*-Dmt amides.[1]

In this paper, we report our development of an effective synthetic route to peptides containing L-Aaa-*S*-Dmt units, overcoming issues of poor reactivity and poor solubility. The sequences Cbz-L-Ser-L-Lys-L-Leu-L-Gln-L-Val-*S*-DmtNH$(CH_2)_2C_6H_4NO_2$ **47** and Cbz-L-Ser-L-Ser-L-Lys-L-Leu-L-Gln-L-Val-*S*-DmtNH$(CH_2)_2$ $C_6H_4NO_2$ **51** (Scheme 5) were selected as targets for synthesis, as these were required as part of a study on development of prodrugs from which active drugs are released by the peptidase activity of prostate-specific antigen (PSA). The sequences HisSerSerLysLeuGln ↓ Aaa and SerLysLeuGln ↓ Aaa are cleaved by PSA at the points shown ↓ and are not substrates for cleavage by other peptidases.[14] Peptidases present special problems as activating enzymes, in terms of prodrug design in general and linker design in particular. Exopeptidases, particularly carboxypeptidases, can release amine-containing drugs directly from amides at the C-terminal of short peptides.[15] However, some activating enzymes, including PSA, are endopeptidases, cleaving only amide bonds between amino-acids. In several cases, this leads to release of a drug molecule still carrying one or more amino-acyl unit; this may or may not be deleterious to the desired pharmacological activity.[14,16,17] Our design of a molecular clip[1] to overcome this problem involves placing a L-Val-*S*-Dmt unit to the C-terminal side of the point at which the endopeptidase cuts the peptide; when this is released by the peptide cleavage, it rapidly forms a DKP and expels the active drug.

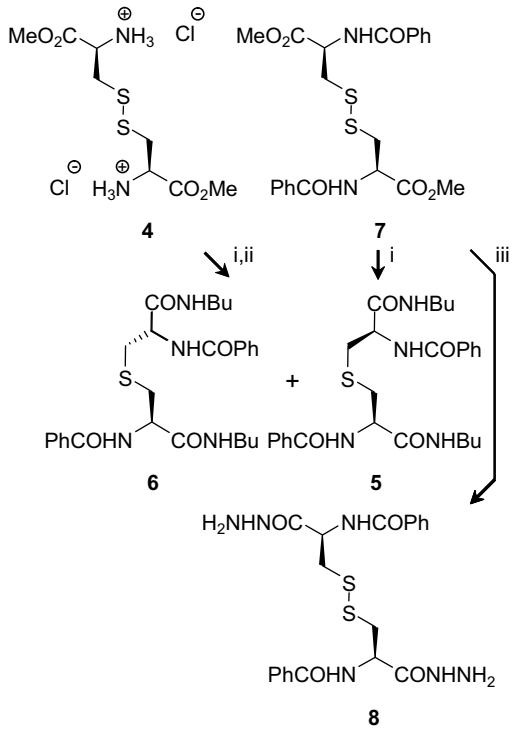
2. Synthesis of peptides

Three alternatives were explored to avoid the synthetic pitfall of the rapid cyclisation of L-Val-*S*-Dmt amides and esters. *First*, the rapid formation of the DKP is promoted by the very high proportion of L-Val-*S*-Dmt dipeptides in the *cis* amide conformation; in turn, this is caused by the tertiary nature of the amide and by the presence of the buttressing geminal dimethyl unit. Introduction of the amino-acid as D-Cys and ring-closure to the thiazolidine late in the synthetic sequence may obviate this problem. *Second*, no DKP formation occurs from L-Val-*S*-Dmt dipeptides under acidic conditions, as the primary amine is protonated and, hence, not nucleophilic. Alternatively, it might be expected that DKP formation could be diminished by diminishing the electrophilicity of the carbonyl electrophile. This could potentially be achieved by attempting the couplings of the required amino-acids with L-Val-*S*-DmtOH, which, under the basic conditions of the couplings, should form a C-terminal carboxylate salt with much reduced electrophilicity. *Third*, appropriately protected dipeptides could be coupled with *S*-DmtOH, although this approach may lead to problems of loss of stereochemical integrity in the central amino-acid of the tripeptides formed.

2.1. Cyclocondensation of pre-formed Aaa-D-CysNHR peptides

Dmo residues have been used as temporary protection for Ser and Thr in peptide synthesis and can be constructed by treatment of the appropriate Aaa-Ser or Aaa-Thr carboxylic acid or ester with 2,2-dimethoxypropane under acidic conditions.[18] Similarly, Aaa-Cys carboxylic acid and esters can be converted into Aaa-Dmt.[13,19] However, there are no reports of cyclocondensation of in-chain Cys residues of the type -Aaa-Cys-Aaa- to give the corresponding -Aaa-Dmt-Aaa- peptides. A short study was therefore undertaken to investigate the feasibility of this process. The first target as a model substrate for reaction with 2,2-dimethoxypropane was N-benzoyl-L-Cys N-butylamide; this substrate would test both the feasibility and the regiochemistry of the cyclisation (thiazolidine or tetrahydrothiazine). However, as shown in Scheme 2, treatment of L-cystine dimethyl ester **4** with butylamine, followed by benzoylation, gave the sulfides **5** and **6** in low yields as the sole identifiable products. The ^{1}H NMR spectra of these two compounds were very similar; they were distinguished principally through the observation of an optical rotation $[\alpha]_D^{20} + 41°$ for **5**, whereas the more-polar **6** was optically inactive, as is consistent with its *meso* relative stereochemistry. Carrying out the two reactions in the opposite order, that is, treatment of N,N′-dibenzoyl-L,L-cystine dimethyl ester **7**[20] with butylamine at elevated temperature, gave only the optically active sulfide **5** in poor yield. The mechanism of the loss of one sulfur atom in these processes is unclear but the formation of the meso **6** suggests that a planar dehydroalanine-like intermediate may be involved. Interestingly, **7** did react conventionally with the alternative nitrogen nucleophile hydrazine to give the expected N,N-dibenzoyl-L,L-cystine dihydrazide **8** in good yield.

Replacing the N-butylamide in the target with a naphth-2-ylamide, L,L-cystine N,N-bis(naphth-2-yl)amide **9** was acetylated to give the bis-acetamide **10** (Scheme 3). Reductive cleavage of the disulfide with propane-1,3-dithiol under basic conditions afforded a good yield of



Scheme 2. Model experiments to investigate the possibility of forming the tetrahydrothiazole ring late in the synthetic sequence. Part A. Reagents: (i) $BuNH_2$; (ii) PhCOCl, NaOAc, H_2O, Et_2O; (iii) $N_2H_4{\cdot}H_2O$, MeOH.

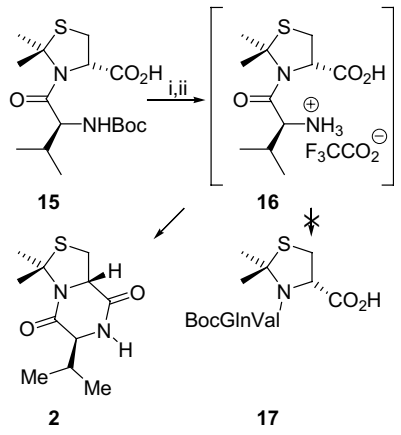
N-acetylcysteine *N*-(naphth-2-yl)amide **11**. However, treatment with 2,2-dimethoxypropane did not lead to the desired thiazolidine **12**; the isolated monothioacetal **13** and the enol thioether **14** arose from reaction at the sulfur nucleophile only. This result suggested that formation of the 2,2-thiazolidine unit late in the synthetic sequence was not feasible.

2.2. Coupling of Aaa-D-DmtOH

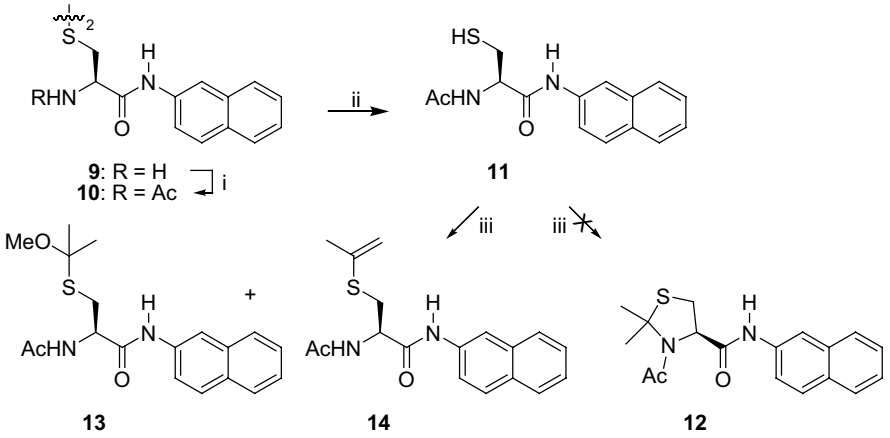
The second approach towards synthesis of the Dmt peptides was to keep the dipeptide Aaa-Dmt with a carboxylic acid at the C-terminal. Under acidic conditions, the N-terminal amine should be protonated and not react as a nucleophile to trigger formation of DKPs. Under basic conditions, the C-terminal carboxylate would be expected to be ionised and not be electrophilic. Under intermediate conditions, an unreactive zwitterion should be present, rather than a free amine and a carboxylic acid. To test this concept, an attempt was made to couple Boc-L-Gln to L-Val-*S*-Dmt (Scheme 4); this provides the most severe test of the sequence, as L-Val-*S*-Dmt *N*-(2-(4-nitrophenyl)ethyl)amide cyclises to form the DKP **17** and 2-(4-nitrophenyl)ethylamine with $t_{1/2}$ = 15 min at pH 8.0.[1] Deprotection of Boc-L-Val-*S*-DmtOH **15**[1] with trifluoroacetic acid, followed by reaction of the intermediate **16** with Boc-L-GlnOSu, however, gave only the DKP **2**. Thus couplings involving Aaa-Dmt dipeptides are unlikely to be successful in the L,*S* relative configuration.

2.3. Assembly of target SKLQ-S-Dmt and SSKLQ-S-Dmt amides through coupling of dipeptides to S-DmtOH

In the light of the failure to convert Cys to Dmt in a preassembled model peptide and of the formation of DKPs upon attempted coupling to Aaa-DmtOH dipeptides, it

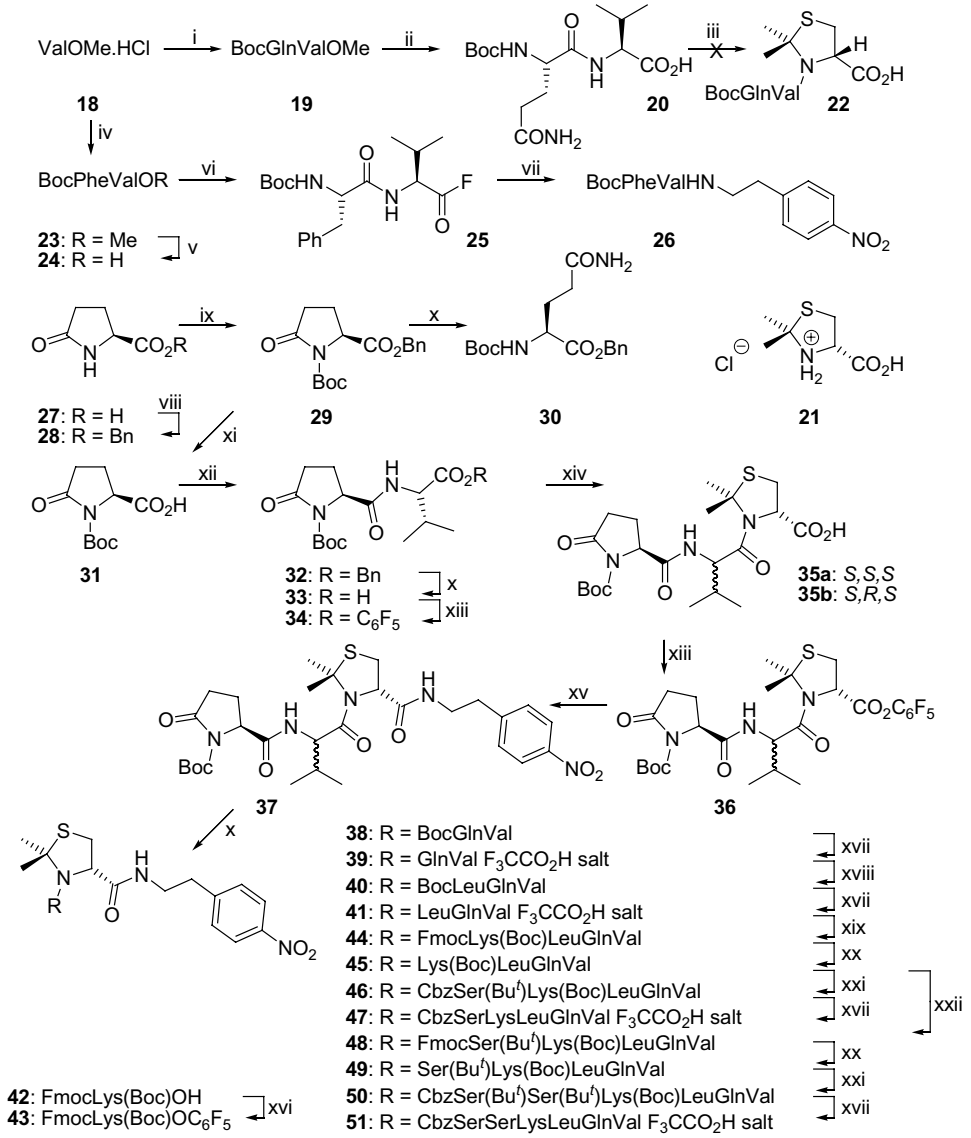


Scheme 4. Formation of diketopiperazine **2** during attempted peptide coupling of Val-*S*-DmtOH **16**. Reagents: (i) CF_3CO_2H; (ii) BocGlnOSu, Et_3N, THF, DMF.



Scheme 3. Model experiments to investigate the possibility of forming the tetrahydrothiazole ring late in the synthetic sequence. Part B. Reagents: (i) Ac_2O, Et_3N; (ii) $CH_2(CH_2SH)_2$, Pr^i_2NEt, THF; (iii) $Me_2C(OMe)_2$, TsOH, CH_2Cl_2.

was necessary to devise a synthetic sequence which avoided Aaa-Dmt dipeptides with free amines at the N-terminus. The strategy of attaching dipeptides to Dmt was developed, as shown in Scheme 5, despite the usual propensity for loss of stereochemical integrity in fragment-wise assembly of peptides. As noted previously,[1] attempted couplings to Dmt amides lead to ring-opening of the thiazolidine, so the C-terminal N-(2-(4-nitrophenyl)ethyl)amide was introduced later in the sequence and couplings of dipeptides to *S*-DmtOH **21** were studied. L-ValOMe **18** was coupled with Boc-L-Gln by the DCC method to give Boc-L-Gln-L-ValOMe **19**. Base-hydrolysis of the methyl ester gave the acid **20**, which failed to couple with **21** using a variety of coupling systems, including the acyl fluoride method which had been successful[1] in coupling single amino-acids to Dmt. To check that acyl fluorides derived from dipeptides were, indeed, stable and capable of coupling effectively with amines, L-ValOMe **18** was coupled with Boc-L-PheOH by the DCC method to give Boc-L-Phe-L-ValOMe **23**. Hydrolysis of the ester and formation of the acyl fluoride **25** from the dipeptide acid **24**, using cyanuric fluoride, were uneventful. This dipeptide acyl fluoride reacted efficiently with 2-(4-nitrophenyl)ethylamine **3** to give the dipeptide amide **26**, without any evidence of loss of stereochemical integrity. Thus couplings with dipeptide acyl fluorides are feasible[21] but either the side-chain primary amide of the Gln or the



Scheme 5. Synthesis of target peptide derivatives containing *S*-Dmt-NH$(CH_2)_2C_6N_4NO_2$. Reagents: (i) BocGlnOH, DCC, HOBt, CH_2Cl_2; (ii) NaOH, MeOH; (iii) various coupling conditions (see text); (iv) BocPheOH, DCC, HOBt, CH_2Cl_2; (v) NaOH, aq MeOH; (vi) cyanuric fluoride, pyridine; (vii) $O_2NC_6H_4(CH_2)_2NH_2{\cdot}HCl$, Et_3N, DMAP, CH_2Cl_2; (viii) BnCl, Et_3N, THF; (ix) Boc_2O, Et_3N, DMAP, CH_2Cl_2; (x) NH_3, aq THF; (xi) H_2, Pd/C, MeOH; (xii) DCC, HOBt, CH_2Cl_2, then ValOBn, Pr^i_2NEt; (xiii) $C_6F_5O_2CCF_3$, pyridine, DMF; (xiv) **21**, Pr^i_2NEt, DMF; (xv) $O_2NC_6H_4(CH_2)_2NH_2{\cdot}HCl$, Et_3N, CH_2Cl_2; (xvi) C_6F_5OH, DCC, EtOAc; (xvii) F_3CCO_2H, CH_2Cl_2; (xviii) BocLeuOSu, Et_3N, DMAP, THF, DMF; (xix) **43**, Et_3N, DMAP, THF, DMF; (xx) Et_2NH, CH_2Cl_2; (xxi) CbzSer(But)OSu, Et_3N, DMAP, THF, DMF; (xxii) FmocSer(But)OSu, Et_3N, DMAP, THF, DMF.

steric crowding of the secondary amine in Dmt prevented the desired coupling.

We have reported[1] that *S*-DmtOH also couples with Boc-L-Leu pentafluorophenyl (PFP) ester. The coupling of a suitable dipeptide PFP ester was therefore examined. Since incorporation of the required Gln had lowered yields and impeded coupling reactions owing to poor solubility of the primary amide in organic solvents, we sought to introduce the carbon framework of the Gln in a more lipophilic form. N-Boc pyroglutamates (Boc-Pygs) are known[22,23] to react with carbon nucleophiles (enolates, Grignard reagents) at the lactam carbonyl; this system also reacts with primary and heterocyclic amines to give N^{δ}-substituted Gln derivatives.[24] We rationalised that Boc-Pyg could also act as a lipophilic synthon for Gln, forming Boc-Gln on treatment with ammonia. As a model sequence, L-PygOH **27** was converted to its benzyl ester **28** by alkylation of the carboxylate anion with benzyl chloride. Boc was then introduced to the lactam nitrogen, giving the Boc-L-Pyg benzyl ester **31**. Ring opening of this lactam with aqueous ammonia in THF gave Boc-L-Gln benzyl ester **30** in very high yield, indicating that this strategy may be applied to introduction of Gln at the N-terminal of large and more complex peptides as the more soluble Boc-Pyg, followed by treatment with ammonia.

Taking this tactic forward, hydrogenolysis of the benzyl ester of **29** gave the carboxylic acid **31**, which was coupled with L-Val-OBn via the hydroxybenzotriazole ester, affording Boc-L-Pyg-L-ValOBn **32**. Removal of the benzyl ester protection (giving Boc-L-Pyg-L-ValOH **33**) was followed by activation of the carboxylic acid as the PFP ester **34**, using pentafluorophenyl trifluoroacetate[25] which avoided the isolation difficulties sometimes associated with the formation of PFP esters using DCC. This active ester acylated the hindered secondary amine of *S*-DmtOH **21**, giving two isomeric products in 52% total isolated yield, showing very good efficiency for this type of coupling.[1] These two isomers were shown by ^{1}H NMR to be the diastereoisomers **35a** and **35b** (diastereomeric ratio 4.2:1); the major isomer was ascribed to be the LL*S* isomer **35a** with the minor LD*S* isomer **35b** arising from partial racemisation of the Val α-chiral centre in the active ester **34** under the basic reaction conditions, a known problem in assembly of peptides from preformed segments. Conversion of the BocPygValDmt **35a** to the PFP active ester **36** and reaction with **3** introduced the model for the amine-containing drug in **37**, without any evidence of racemisation as is usual for such couplings with Dmt active esters.[1] At this point in the synthetic sequence, the Pyg ring was opened quantitatively with ammonia to give the corresponding BocGln peptide **38**. Thus Pg is shown to be a very effective synthon for Gln, providing suitable solubility in organic solvents to allow the more challenging couplings to take place. In the present sequence, the lipophilicity of the ValDmtNH$(CH_2)_2C_6H_4NO_2$ unit provided sufficient solubility to facilitate the remaining couplings. The following steps used conventional solution-phase stepwise N-deprotection and active-ester coupling cycles through acid-catalysed removal of Boc (giving **39**), coupling with Boc-L-LeuOSu (giving **40**), deprotection (giving **41**) and coupling with Fmoc-L-Lys(Boc)OPFP **43** (prepared from Fmoc-L-Lys(Boc)OH **42**) to afford the Fmoc-L-Lys-L-Leu-L-Gln-L-Val-*S*-Dmt amide **44**. The change from Boc to Fmoc in the main-chain protection strategy was necessitated by the need for simultaneous acid-catalysed cleavage of all the side-chain protecting groups in the target peptides. Cbz is unavailable for protection, as hydrogenolysis would also reduce the nitro group and hydrogen bromide is known to be deleterious to the thiazolidine ring. The Fmoc was removed very rapidly and efficiently with diethylamine, rather than the more usual piperidine; this approach using a very volatile secondary amine greatly facilitated the isolation of the N-unprotected pentapeptide amide **45**.

Now, simple coupling of **45** with Cbz-L-Ser(Bu^t)OSu and removal of the side-chain protecting groups with trifluoroacetic acid gave an excellent yield of the hexapeptide target CbzSerLysLeuGlnVal-*S*-DmtNH$(CH_2)_2C_6H_4NO_2$ **47**. This carries the SerLysLeuGln↓ minimal sequence claimed[14] to be required for recognition and cleavage by PSA, with suitable chromophoric markers for the fates of the N-terminal (Cbz) and C-terminal (nitrophenyl) products of the enzymic cleavage studies. The corresponding heptapeptide target **51**, carrying two serines, was accessed by coupling of **45** with FmocSerOSu, removal of Fmoc with diethylamine (giving **49**), coupling with CbzSerOSu and exposure of the side-chain functional groups with trifluoroacetic acid.

2.4. Synthesis of control peptide Cbz-SKLQV-OMe

The peptide amides **47** and **51** were designed as substrates for cleavage by PSA. However, these peptide constructs are modified in two ways from the construct SerLysLeuGlnLeu-Drug reported by Denmeade to be cleaved by this enzyme.[14] First and more importantly, the highly bulky Dmt unit is present at the C-terminus of the sequence recognised by PSA; second, Leu has been replaced by the (apparently) very similar Val (lipophilic, aliphatic) to the C-terminal side of the expected cleavage-point in **47** and **51**. It was therefore necessary to prepare and evaluate a control peptide ester **58** in which the Dmt is absent but the Val is present to elucidate which feature may be responsible for any modification of the sequence-selectivity of the enzymic cleavage. This control peptide CbzSerLysLeuGlnValOMe **58** was assembled largely by conventional stepwise C → N solution-phase synthesis, as outlined in Scheme 6. In this sequence, it was unnecessary to employ the tactic of incorporating Gln as Pyg, as there were no difficult couplings. As before, this target peptide carried Cbz at the N-terminal.

3. Cleavage with PSA

Samples of the peptides **47**, **51** and **58** were incubated with PSA in aqueous buffer at pH 7.4 at 37 °C and the compositions of the reaction mixtures were monitored by HPLC at various time-points for up to 7 d for consumption of the starting peptide constructs. The rates

19: BocGlnValOMe
52: GlnValOMe F_3CCO_2H salt
53: BocLeuGlnValOMe
54: LeuGlnValOMe F_3CCO_2H salt
55: FmocLys(Boc)LeuGlnValOMe
56: Lys(Boc)LeuGlnValOMe
57: R = CbzSer(Bu^t)Lys(Boc)LeuGlnValOMe
58: R = CbzSerLysLeuGlnValOMe F_3CCO_2H salt

i, ii, i, iii, iv, v, i

Scheme 6. Synthesis of control peptide **58**. Reagents: (i) F_3CCO_2H, CH_2Cl_2; (ii) BocLeuOSu, Et_3N, DMAP, THF, DMF; (iii) **43**, Et_3N, DMAP, THF, DMF; (iv) Et_2NH, CH_2Cl_2; (v) CbzSer(^{t}Bu)OSu, Et_3N, DMAP, THF, DMF.

of cleavage by different batches of the PSA enzyme varied markedly; the results of typical incubation experiments are shown in Figure 1. It is immediately evident that the peptides **47**, **51** and **58** (containing -SerLysLeuGlnVal-) were cleaved very slowly, with half-lives of several days; this contrasts with the rapid cleavage of peptides containing -SerLysLeuGlnLeu- under similar conditions reported by Denmeade et al.[14] However, even though peptides **47**, **51** and **58** were poor substrates, was the cleavage point Gln ↓ Val, as predicted by the results of Denmeade showing cleavage at Gln ↓ Leu?[14] If **47** and **51** were cleaved at Gln ↓ Val, then L-Val-*S*-Dmt N-(2-(4-nitrophenyl)ethyl)amide **1** would be released; this dipeptide amide should cyclise very rapidly (<1 h at pH 7.4) to give the DKP **2** and 2-(4-nitrophenyl)ethylamine **3**, as shown in Scheme 7. Similarly, cleavage of **58** at Gln ↓ Val should release L-valine methyl ester **18**. However, HPLC analysis of the reaction mixtures from **47** and **51** showed that **2** and **3** were absent but that the sole product containing the nitrophenyl chromophore had retention time equal to that of GlnValDmt N-(2-(4-nitrophenyl)ethyl)amide **39**. Similarly, GlnValOMe **52** was identified as the product of cleavage of **58** by PSA. These data indicate that not only does replacement of Leu by Val make the peptides poor substrates for PSA-mediated cleavage but also that the cleavage point is changed from SerLysLeuGln ↓ Leu to SerLysLeu ↓ GlnVal. The cleavage of the control peptide confirms that the effects of slowing and change in site of the cleavage are not due to the presence of Dmt.

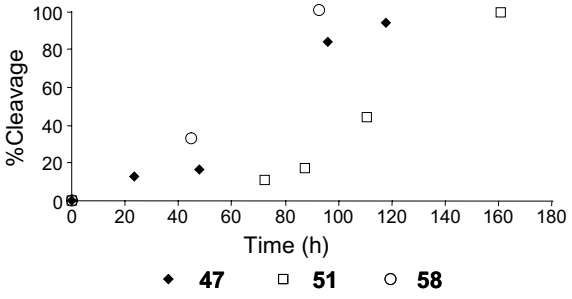
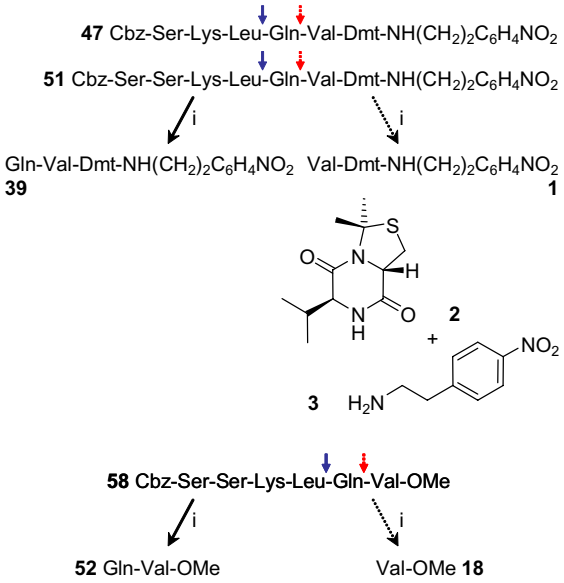


Figure 1. Time courses of degradation of peptide derivatives CbzSKLQVDmtNPEA **47**, CbzSSKLQVDmtNPEA **51** and CbzSKLQVOMe **58** by PSA. Data are taken from typical experimental runs.



Scheme 7. Predicted outcomes and actual outcomes of cleavage of peptide constructs **47**, **51** and **58** with PSA. The red arrows indicate the predicted cleavage points whereas the blue arrows show the actual cleavage points. Reagent and condition: (i) PSA, aq buffer pH 7.4.

4. Conclusions

In this paper, we have reported the development of an efficient synthetic route to peptide derivatives containing L-Val-*S*-Dmt. Although this dipeptide is of considerable potential for use as a molecular clip in the design of endopeptidase-activated prodrugs, its synthesis and incorporation into longer peptides presents some challenges owing to the very rapid cyclisation of L-Val-*S*-Dmt amides and esters to the corresponding DKP **2**.[1] First, it proved impossible to carry out a cyclocondensation with 2,2-dimethoxypropane on a model peptide containing Cys with the aim of generating the target peptide containing D-Cys, then converting the latter into *S*-Dmt at the end of the sequence. The isolated products arose from reaction at sulfur only. An interesting desulfurisation was noted during the preparation of a model Cys-containing peptide. Second, it was observed that the drive to cyclisation of L-Val-*S*-Dmt is so great that even the unmasked dipeptide **16** (which should not exist in the cyclisable H_2N–CO_2H prototropic form) cyclised to the corresponding DKP **2** before coupling with an amino-acid active ester could be achieved.

Assembly of the required peptides containing L-Val-*S*-Dmt was achieved by coupling of dipeptide acid pentafluorophenyl esters to *S*-DmtOH **21**. Yields of this critical coupling were satisfactory but were achieved at the expense of some loss of stereochemical integrity at the Val of the Pyg-Val acylating unit. The diastereoisomers were readily separable. Dipeptide acid fluorides were shown for the first time to be stable and useful in simple peptide couplings but failed with DmtOH.

Gln could not be employed directly in this sequence owing to the poor solubility of intermediates containing this residue in solvents compatible with the formation

of the dipeptide acid pentafluorophenyl ester and with the coupling reaction. N-Boc-pyroglutamic acid (Boc-Pyg) was used as a masked form of Gln in the difficult coupling steps where solubility and ease of chromatographic isolation were essential. Simple treatment of BocPyg peptides with ammonia gave the corresponding BocGln peptides in quantitative yields, pointing to the more general utility of this tactic in incorporating the poorly soluble and potentially aggregating Gln residue in 'difficult' peptides. A hexapeptide amide CbzSerLysLeuGlnVal-*S*-Dmt-NH$(CH_2)_2C_6H_4NO_2$ **47** and a heptapeptide amide CbzSerSerLysLeuGlnVal-*S*-Dmt-NH$(CH_2)_2C_6H_4NO_2$ **51** were successfully assembled using these methods, together with an appropriate protecting-groups strategy; thus the L-Val-*S*-Dmt unit can be efficiently incorporated into peptides by our new approach.

Incubation of these two peptides and the control peptide **58** showed, surprisingly, that the apparently conservative replacement of Leu in previously published PSA-sensitive peptides[14] with Val led to the present SerLysLeuGlnVal peptides being cleaved only very slowly by PSA and at the N-terminal side of Gln, rather than at the previously observed C-terminal side[14] of this residue. Further design of PSA-cleavable peptide analogues will need to reflect the apparently tight requirements in this region.

5. Experimental

5.1. General

^{1}H NMR spectra were recorded on Varian GX270 or EX400 spectrometers of samples in $CDCl_3$, unless otherwise stated. IR spectra were recorded on a Perkin-Elmer 782 spectrometer as KBr discs, unless otherwise stated. Mass spectra were obtained using fast atom bombardment (FAB) ionisation in the positive ion mode. The chromatographic stationary phase was silica gel. DCC refers to *N*,*N*′-dicyclohexylcarbodiimide, THF refers to tetrahydrofuran, DMF refers to dimethylformamide, HOBt refers to 1-hydroxybenzotriazole, DMAP refers to 4-dimethylaminopyridine, citric acid refers to a 5% aqueous solution of 3-carboxy-3-hydroxypentanedioic acid. THF was dried with Na. Solutions in organic solvents were dried with $MgSO_4$. Solvents were evaporated under reduced pressure. The aqueous $NaHCO_3$ and brine were saturated. Experiments were conducted at ambient temperature, unless otherwise stated. Melting points were measured with a Thermo Galen Kofler block and are uncorrected. All amino-acids and peptides are of the L-configuration, except where noted. PSA was purchased from Aldrich.

5.2. N,N-Dibenzoylcystine dihydrazide (8)

N,N′-Dibenzoylcystine dimethyl ester **7**[26] (500 mg, 1.1 mmol) was stirred with $N_2H_4{\cdot}H_2O$ (820 mg, 25 mmol) in MeOH (10 mL) for 16 h. The filtered solid was washed with MeOH. Drying afforded **8** (300 mg, 60%) as a white solid: mp 205–207 °C (lit.[27] mp 206–207 °C); NMR δ_H 3.02 (2H, dd, J = 13.3, 9.8 Hz, 2× β-H), 3.20 (2H, dd, J = 13.3, 5.1 Hz, 2× β-H), 4.73 (2H, ddd, J = 9.8, 8.2, 5.1 Hz, 2× α-H), 7.44 (2H, t, J = 7.4 Hz, 2× Ph 4-H), 7.52 (4H, t, J = 7.4 Hz, 2× Ph 3,5-H_2), 7.85 (4H, d, J = 7.4 Hz, 2× 2,6-H_2), 8.62 (2H, d, J = 8.2 Hz, 2× PhCONH), 9.37 (2H, br, 2× N*H*NH_2); MS *m*/*z* 477.1370 (M+H) ($C_{20}H_{25}N_6O_2S_2$ requires 477.1379).

5.3. *R*,*R*-Bis(2-benzoylamino-3-butylamino-3-oxopropyl)sulfide (5) and *R*,*S*-bis(2-benzoylamino-3-butylamino-3-oxopropyl)sulfide (6)

Cystine dimethyl ester dihydrochloride **4** (5.0 g, 15 mmol) was dissolved in $BuNH_2$ (100 mL) at −78 °C. The solution was heated under reflux for 5 h. The evaporation residue, in MeOH, was acidified (HCl). Evaporation afforded crude cystine N,N′-dibutyl amide (14.8 g) as a yellow viscous oil: MS *m*/*z* 351.1874 (M+H) ($C_{14}H_{31}N_4O_2S_2$ requires 351.1888). This material was stirred with NaOAc (10 g) in H_2O (100 mL) and Et_2O (100 mL). Benzoyl chloride (9.1 g, 65 mmol) was added at 0 °C and the mixture was stirred vigorously for 4 h. The Et_2O layer was separated. Drying, evaporation and chromatography (EtOAc/hexane 1:1) afforded **5** (500 mg, 6.5%) as a white solid: mp 154–156 °C; $[\alpha]_D^{20}$ +41° (c 3.2×10^{-3}, $CHCl_3$); IR ν_{max} 3286, 1634, 1602, 1557, 1540 cm^{-1}; NMR δ_H 0.90 (6H, t, J = 7.0 Hz, 2× Me), 1.41 (4H, sextet, J = 7.0 Hz, 2× Bu 3-CH_2), 1.58 (4H, quintet, J = 7.0 Hz, 2× Bu 2-CH_2), 3.01 (2H, dd, J = 14.8, 6.2 Hz, 2× 1-H), 3.07 (2H, dd, J = 14.8, 6.2 Hz, 2× 1-H), 3.33 (4H, m, 2× Bu 1-CH_2), 5.43 (2H, q, J = 6.2 Hz, 2× 2-H), 7.46–7.58 (8H, m, 2× Ar 3,4,5-H_3+2× NH), 7.85 (4H, m, 2× Ar 2,6-H_2), 7.96 (2H, t, J = 5.5 Hz, 2× NHBu); MS *m*/*z* 527.2696 (M+H) ($C_{28}H_{39}N_4O_4S$ requires 527.2692); found: C, 62.30; H, 7.32; N, 10.37; $C_{28}H_{38}N_4O_4S$ requires C, 63.85; H, 7.27; N, 10.63%. Further elution gave **6** (310 mg, 4.0%) as a white solid: mp 178–180 °C; $[\alpha]_D^{20}$ +0.0° (c 0.003, $CHCl_3$); IR ν_{max} 3287, 1633, 1602, 1560, 1532 cm^{-1}; NMR δ_H 0.93 (6H, t, J = 7.4 Hz, 2× Me), 1.40 (4H, sextet, J = 7.4 Hz, 2× Bu 3-CH_2), 1.56 (4H, quintet, J = 7.4 Hz, 2× Bu 2-CH_2), 3.14 (4H, d, J = 6.2 Hz, 2× 2-H_2), 3.33 (4H, m, 2× Bu 1-CH_2), 4.90 (2H, q, J = 6.2 Hz, 2× 1-H), 7.33 (2H, t, J = 5.5 Hz, 2× NHBu), 7.44 (6H, m, 2× Ph 3,5-H_2+2× NH), 7.53 (2H, m, 2× Ph 4-H), 7.81 (4H, m, 2× Ph 2,6-H_2); MS *m*/*z* 527.2689 (M+H) ($C_{14}H_{31}N_4O_2S_2$ requires 527.2692).

5.4. N,N-Diacetylcystine bis(naphth-2-yl)amide (10)

Cystine bis(naphth-2-yl)amide **9** (500 mg, 1.0 mmol) was stirred with Ac_2O (7 mL) and Et_3N (206 mg, 2.0 mmol) for 16 h. The evaporation residue was washed with H_2O. Drying afforded **10** (0.54 g, 92%) as a white solid: mp 250–252 °C; IR ν_{max} 3289, 1647, 1604, 1536, 1505 cm^{-1}; NMR ($(CD_3)_2SO$) δ_H 1.91 (6H, s, 2× Me), 3.03 (2H, dd, J = 13.3, 8.4 Hz, 2× β-H), 3.25 (2H, dd, J = 13.3, 5.9 Hz, 2× β-H), 4.78 (2H, dt, J = 8.4, 5.9 Hz, 2× α-H), 7.40 (2H, dt, J = 1.2 Hz, 7.0 Hz) and 7.46 (2H, dt, J = 1.2, 7.0 Hz) (2× Ar 6,7-H_2), 7.63 (2H, dd, J = 8.6, 2.0 Hz, 2× Ar 3-H), 7.85 (6H, m, 2× Ar 4,5,8-H_3), 8.29 (2H, d, J = 2.0 Hz, 2× Ar 1-H), 8.45 (2H, d,

J = 7.8 Hz, 2× NHAc), 10.41 (2H, s, 2× NH); MS *m/z* 575.1782 (M+H) ($C_{30}H_{31}N_4O_4S_2$ requires 575.1787); found: C, 60.70; H, 5.31; N, 9.18; $C_{30}H_{30}N_4O_4S_2$ 1.0 H_2O requires C, 60.79; H, 5.44; N, 9.45%.

5.5. N-Acetylcysteine naphth-2-ylamide (11)

Compound **10** (250 mg, 0.44 mmol) was boiled under reflux with propane-1,3-dithiol (480 mg, 4.4 mmol) and Pr^i_2NEt (142 mg, 1.1 mmol) in THF (10 mL) for 48 h. The evaporation residue, in CH_2Cl_2, was washed with cold aq H_2SO_4 (1 M, twice) and brine and was dried. The evaporation residue was washed with hexane and dried to afford **11** (190 mg, 75%) as a white solid: mp 204–207 °C; IR ν_{max} 3271, 1646, 1542, 1508 cm^{-1}; NMR δ_H 1.94 (1H, dd, J = 10.5, 7.0 Hz, SH), 2.15 (3H, s, Me), 2.81 (1H, ddd, J = 10.5, 7.4, 3.7 Hz, β-H), 3.24 (1H, ddd, J = 7.4, 7.0, 3.7 Hz, β-H), 4.80 (1H, dt, J = 3.7, 7.0 Hz, α-H), 6.61 (1H, d, J = 6.6 Hz, NHAc), 7.47 (3H, m, Ar-H_3), 7.79 (3H, m, Ar-H_3), 8.19 (1H, d, J = 2.0 Hz, Ar 1-H), 8.74 (1H, br s, CONH); MS *m/z* 288.0932 (M) ($C_{15}H_{16}N_2O_2S$ requires 288.0932).

5.6. N-Acetyl-S-(1-methoxy-1-methylethyl)cysteine naphth-2-ylamide (13) and N-acetyl-S-(1-methylethenyl)cysteine naphth-2-ylamide (14)

Compound **11** (50 mg, 0.17 mmol) was boiled under reflux in 2,2-dimethoxypropane (0.7 mL), 4-methylbenzenesulfonic acid hydrate (8.0 mg, 40 μmol) and CH_2Cl_2 (3 mL) for 3 h, after which CH_2Cl_2 (20 mL) and H_2O (10 mL) were added. The organic layer was extracted and washed with H_2O. Drying, evaporation and chromatography (EtOAc/hexane 9:1) afforded **14** (25 mg, 50%) as a colourless oil: NMR δ_H 2.03 (3H, s, Me), 2.10 (3H, s, COMe), 3.17 (1H, dd, J = 14.1, 7.4 Hz, β-H), 3.30 (1H, dd, J = 14.1, 6.2 Hz, β-H), 4.87 (1H, dd, J = 7.4, 6.2 Hz, α-H), 5.04 (1H, br s, =CH), 5.13 (1H, s, =CH), 6.59 (1H, d, J = 7.0 Hz, AcNH), 7.45 (3H, m, Ar 3,6,7-H_3), 7.75 (3H, m, Ar 4,5,8-H_3), 8.19 (1H, d, J = 2.0 Hz, Ar 1-H), 8.85 (1H, br s, NH); MS *m/z* 329.1329 (M+H) ($C_{19}H_{25}N_2O_3S$ requires 329.1324). Further elution gave **13** (30 mg, 49%) as a white solid: mp 137–138 °C; NMR δ_H 1.56 (3H, s, Me), 1.58 (3H, s, Me), 2.09 (3H, s, COMe), 3.03 (1H, dd, J = 13.7, 6.2 Hz, β-H), 3.10 (1H, dd, J = 13.7, 6.2 Hz, β-H), 3.67 (3H, s, OMe), 4.93 (1H, dt, J = 7.8, 6.2 Hz, α-H), 7.01 (1H, d, J = 7.8 Hz, AcNH), 7.39 (2H, m, Ar 6,7-H_2), 7.46 (1H, dd, J = 9.0, 2.0 Hz, Ar 3-H), 8.20 (1H, d, J = 1.6 Hz, Ar 1-H), 9.20 (1H, br s, NH); MS *m/z* 361.1589 (M+H) ($C_{19}H_{25}N_2O_3S$ requires 361.1586).

5.7. N-(N-(1,1-Dimethylethoxycarbonyl)glutaminyl)valine methyl ester (19)

BocGlnOH (2.78 g, 11.3 mmol) was stirred with HOBt (1.68 g, 12.4 mmol) and DCC (3.49 g, 16.9 mmol) in CH_2Cl_2 (150 mL) at 0 °C for 1 h. Pr^i_2NEt (2.92 g, 22.6 mmol) was added, followed by **18** (1.89 g, 11.3 mmol) in CH_2Cl_2 (50 mL) and the mixture was stirred for 24 h. The mixture was kept at 4 °C for 16 h and filtered (Celite®). The filtrate was washed with aq citric acid (10%), aq $NaHCO_3$ and H_2O. Drying, evaporation and chromatography (EtOAc → EtOAc/MeOH 1:1) afforded **19** (2.90 g, 71%) as a white solid: mp 145–148 °C; NMR δ_H 0.93 (3H, d, J = 6.6 Hz, Val-Me), 0.96 (3H, d, J = 6.6 Hz, Val-Me), 1.43 (9H, s, ^{t}Bu), 2.04 (2H, m, Gln β-H_2), 2.21 (1H, m, Val β-H), 2.42 (2H, t, J = 6.6 Hz, Gln γ-H_2), 3.73 (3H, s, OMe), 4.26 (1H, br q, J = 7.4 Hz, Gln α-H), 4.48 (1H, dd, J = 8.4, 5.1 Hz, Val α-H), 5.59 (1H, d, J = 7.4 Hz, Gln-NH), 5.84 (1H, s, CONH), 6.40 (1H, s, CONH), 7.59 (1H, d, J = 8.4 Hz, Val NH); MS *m/z* 741 (2M+Na), 719 (2M+H), 382 (M+Na), 360.2142 (M+H) ($C_{16}H_{30}N_3O_6$ requires 360.2135); found C, 52.00; H, 7.82; N, 11.60; $C_{16}H_{29}N_3O_6$ requires C, 52.14; H, 7.82; N, 11.60%.

5.8. N-(N-(1,1-Dimethylethoxycarbonyl)glutaminyl)-valine (20)

Compound **19** (890 mg, 2.5 mmol) was stirred with NaOH (2.0 g, 50 mmol) in MeOH (30 mL) for 2 h. H_2O (10 mL) was added and MeOH was evaporated. The solution was brought to neutrality with cold aq citric acid (10%) and extracted four times with EtOAc. The aqueous layer was saturated with NaCl and extracted further with EtOAc. Drying and evaporation of the combined extracts afforded **20** (550 mg, 64%) as a white solid: mp 86–89 °C (lit.[28] solid); NMR ($(CD_3)_2SO$) δ_H 0.87 (6H, d, J = 7.0 Hz, 2× Val-Me), 1.37 (9H, s, Boc), 1.66 (1H, m, Gln β-H), 1.83 (1H, m, Gln β-H), 2.10 (3H, m, Gln γ-H_2,Val β-H), 3.94 (1H, dt, J = 8.6, 5.5 Hz, Gln α-H), 4.15 (1H, dd, J = 8.6, 5.5 Hz, Val α-H), 6.76 (1H, s, CONH), 6.94 (1H, d, J = 8.2 Hz, Gln NH), 7.25 (1H, s, CONH), 7.75 (1H, d, J = 8.6 Hz, Val NH); MS *m/z* 346.1975 (M+H) ($C_{15}H_{28}N_3O_6$ requires 346.1978).

5.9. N-(N-(1,1-Dimethylethoxycarbonyl)phenylalanyl)-valine methyl ester (23)

BocPheOH (10.0 g, 37.7 mmol) was stirred with HOBt (5.10 g, 37.7 mmol) and DCC (7.80 g, 37.7 mmol) in CH_2Cl_2 (250 mL) at 0 °C for 1 h. Compound **18** (6.30 g, 37.7 mmol) in CH_2Cl_2 (100 mL) was added in the presence of Et_3N (7.60 g, 75.4 mmol) and the mixture was stirred for 48 h. The mixture was kept at 4 °C for 16 h and filtered through Celite®. The solution was washed with 10% aq citric acid, aq $NaHCO_3$ and H_2O. Drying, evaporation and recrystallisation (EtOAc/hexane) afforded **23** (4.50 g, 33%) as a white solid: mp 115–118 °C (lit.[29] mp 115–117 °C); NMR δ_H 0.84 (3H, d, J = 7.0 Hz, Val-Me), 0.87 (3H, d, J = 7.0 Hz, Val-Me), 1.42 (9H, s, Boc), 2.11 (1H, d septet, J = 5.0, 7.0 Hz, Val β-H), 3.07 (2H, d, J = 7.0 Hz, Phe β-H_2), 3.68 (3H, s, OMe), 4.34 (1H, br q, J = 6.6 Hz, Phe α-H), 4.46 (1H, dd, J = 8.8, 5.1 Hz, Val α-H), 4.99 (1H, br, Phe NH), 6.34 (1H, d, J = 8.8 Hz, Val NH), 7.25 (5H, m, Ph-H_5).

5.10. N-(N-(1,1-Dimethylethoxycarbonyl)phenylalanyl)-valine (24)

Compound **23** (1.0 g, 2.6 mmol) was stirred with NaOH (1.0 g, 26 mmol) in MeOH (15 mL) and H_2O (15 mL) for 1 h. The mixture was concentrated and EtOAc

(25 mL) was added. The mixture was brought to neutrality with cold 10% aq citric acid and extracted with EtOAc. Drying and evaporation afforded **24** (850 mg, 88%) as a white solid: mp 77–78 °C (lit.[30] mp 77–78 °C); NMR δ_H 0.84 (3H, d, J = 7.0 Hz, Val-Me), 0.91 (3H, d, J = 7.0 Hz, Val-Me), 1.4 (9H, s, Boc), 2.17 (1H, d septet, J = 4.7, 6.7 Hz, Val β-H), 3.07 (2H, m, Phe β-H_2), 4.34 (1 H, m, Phe α-H), 4.46 (1H, dd, J = 8.4, 4.7 Hz, Val α-H), 5.10 (1H, br, Phe NH), 6.55 (1H, d, J = 8.4 Hz, Val NH), 7.25 (5H, m, Ph-H_5).

5.11. N-(N-(N-1,1-Dimethylethoxycarbonyl)phenylalanyl)valine N-(2-(4-nitrophenyl)ethyl)amide (26)

BocPheValOH **24** (200 mg, 0.55 mmol) was stirred with pyridine (44 mg, 0.55 mmol) in dry CH_2Cl_2 (5 mL) and added slowly to cyanuric fluoride (222 mg, 1.6 mmol) in dry CH_2Cl_2 (5 mL) at −10 °C. The mixture was stirred under N_2 at −10 °C for 2 h. The mixture was washed thrice with ice-water. Drying and evaporation afforded crude **25**. This material, in CH_2Cl_2 (3 mL), was added to 2-(4-nitrophenyl)ethylamine hydrochloride **3**·HCl (168 mg, 830 μmol), Et_3N (172 mg, 1.7 mmol) and DMAP (1 mg) in CH_2Cl_2 (3 mL) under N_2. The mixture was stirred for 16 h and the evaporation residue was washed with EtOAc to afford **26** (100 mg, 36%) as a buff solid: mp 202–203 °C; NMR ($(CD_3)_2SO$) δ_H 0.76 (6H, d, J = 6.7 Hz, 2× Val-Me), 1.28 (9H, s, Boc), 1.85 (1H, m, Val β-H), 2.71 (1H, dd, J = 13.5, 10.6 Hz, Phe β-H), 2.85 (2H, t, J = 7.0 Hz, $ArCH_2$), 2.91 (1H, dd, J = 13.5, 4.1 Hz, Phe β-H), 3.34 (2H, m, NCH_2), 4.07 (1H, dd, J = 9.1, 7.0 Hz, Val α-H), 4.17 (1H, ddd, J = 10.3, 8.5, 4.1 Hz, Phe β-H), 7.03 (1H, d, J = 8.5 Hz, Phe NH), 7.12–7.26 (5H, m, Ph-H_5), 7.47 (2H, d, J = 8.8 Hz, Ar 2,6-H_2), 7.64 (1H, d, J = 8.8 Hz, Val NH), 8.06 (1H, t, J = 5.9 Hz, NH), 8.11 (2H, d, J = 8.8 Hz, Ar 3,5-H_2); MS *m/z* 513.2714 (M+H) ($C_{27}H_{37}N_4O_6$ requires 513.2713), 457 (M−H_2C=CMe_2), 413 (M−Boc).

5.12. Phenylmethyl *S*-1-(1,1-dimethylethoxycarbonyl)-5-oxopyrrolidine-2-carboxylate (29)

S-5-oxopyrrolidine-2-carboxylic acid **27** (5.0 g, 39 mmol) was boiled under reflux with Et_3N (3.92 g, 39 mmol) and benzyl chloride (5.4 g, 43 mmol) in THF (50 mL) for 5 d. After cooling, H_2O (50 mL) was added and the THF was evaporated. The evaporation residue was extracted thrice with CH_2Cl_2. The evaporation residue was stirred with Et_3N (3.92 g, 39 mmol), Boc_2O (16.9 g, 77.6 mmol) and DMAP (2.8 g, 39 mmol) in CH_2Cl_2 (150 mL) at 0 °C for 1 h and at 20 °C for 16 h. The evaporation residue, in CH_2Cl_2, was washed with cold 5% aq citric acid and brine. Drying and evaporation afforded **29** (9.0 g, 72%) as pale buff solid: mp 62–65 °C (lit.[31] mp 57–59 °C); NMR δ_H 1.41 (9H, s, Bu^t), 2.05 (1H, ddt, J = 12.8, 10.3, 3.3 Hz, Pyg β-H), 2.32 (1H, ddt, J = 12.8, 9.8, 9.4 Hz, Pyg β-H), 2.47 (1H, ddd, J = 17.6, 9.4, 3.5 Hz, Pyg γ-H), 2.60 (1H, ddd, J = 17.5, 10.3, 9.8 Hz, Pyg γ-H), 4.62 (1H, dd, J = 9.4, 3.3 Hz, Pyg α-H), 5.18 (1H, d, J = 12.1 Hz) and 5.20 (1H, d, J = 12.1 Hz) (CH_2Ph), 7.34 (5H, m, Ph-H_5).

5.13. *S*-1-(1,1-Dimethylethoxycarbonyl)-5-oxopyrrolidine-2-carboxylic acid (31)

Compound **29** (15.0 g, 47 mmol) was stirred with 10% Pd/C (450 mg) in MeOH (300 mL) under H_2 for 6 h. Filtration (Celite®) and evaporation afforded **31** (10.0 g, 93%) as a white solid: mp 65–67 °C (lit.[32] mp 77–80 °C); NMR ($(CD_3)_2SO$) δ_H 1.42 (9H, s, ^{t}Bu), 1.90 (1H, m, Pyg β-H), 2.30–2.55 (3H, m, Pyg β,γ-H_3), 4.50 (1H, dd, J = 9.4, 3.5 Hz, Pyg α-H).

5.14. N-(*S*-1-(1,1-Dimethylethoxycarbonyl)-5-oxopyrrolidine-2-carbonyl)valine phenylmethyl ester (32)

Compound **31** (10.0 g, 43.7 mmol) was stirred with HOBt (6.50 g, 48.1 mmol) and DCC (9.92 g, 48.1 mmol) in CH_2Cl_2 (200 mL) at 0 °C for 1 h. ValOBn (16.60 g, 43.7 mmol) and Pr^i_2NEt (11.30 g, 87.4 mmol) in CH_2Cl_2 (50 mL) were added and the mixture was stirred for 24 h. The mixture was kept at 4 °C for 16 h and filtered (Celite®). The solution was washed with cold 5% aq citric acid, aq $NaHCO_3$ and H_2O. Drying, evaporation and chromatography (EtOAc) afforded **32** (13.0 g, 71%) as a white solid: mp 96–98 °C; NMR δ_H 0.87 (3H, d, J = 7.0 Hz, Val-Me), 0.93 (3H, d, J = 7.0 Hz, Val-Me), 1.51 (9H, s, Boc), 2.20 (3H, m, Val β-H, Pyg β-H_2), 2.46 (1H, ddd, J = 17.6, 8.5, 3.5 Hz, Pyg γ-H), 2.73 (1H, dt, J = 17.6, 10.5 Hz, Pyg γ-H), 4.57 (1H, dd, J = 7.8, 3.1 Hz, Pyg α-H), 4.60 (1H, dd, J = 9.0, 4.7 Hz, Val α-H), 5.14 (1H, d, J = 12.1 Hz) and 5.20 (1H, d, J = 12.1 Hz) (CH_2Ph), 6.46 (1H, d, J = 9.0 Hz, Val NH), 7.35 (5H, m, Ph-H_5); MS *m/z* 859 (2M+Na), 837 (2M+H), 737 (2M+H–Boc), 441 (M+Na), 419.2192 (M+H) ($C_{22}H_{31}N_2O_6$ requires 410.2182), 319 (M−Boc).

5.15. N-(*S*-1-(1,1-Dimethylethoxycarbonyl)-5-oxopyrrolidine-2-carbonyl)valine (33)

Compound **32** (7.50 g, 18 mmol) was stirred with 10% Pd/C (400 mg) in MeOH (300 mL) under H_2 for 16 h. Filtration (Celite®) and evaporation afforded **33** (5.60 g, quant.) as a white solid: mp 152–155 °C; NMR ($(CD_3)_2SO$) δ_H 0.91 (3H, d, J = 7.0 Hz, Val-Me), 0.92 (3H, d, J = 7.0 Hz, Val-Me), 1.39 (9H, s, Boc), 1.80 (1H, m, Pyg β-H), 2.10 (1H, octet, J = 7.0 Hz, Val β-H), 2.2–2.4 (3 H, m, Pyg β,γ-H_3), 4.16 (1H, dd, J = 8.2, 7.0 Hz, Val α-H), 4.68 (1H, dd, J = 9.0, 2.3 Hz, Pyg α-H), 8.32 (1H, d, J = 8.6 Hz, NH); MS *m/z* 679 (2M+Na), 657 (2M+H), 557 (2M+H–Boc), 351 (M+Na), 329.1721 (M+H) ($C_{15}H_{25}N_2O_6$ requires 329.1713), 229 (M−Boc).

5.16. N-(*S*-1-(1,1-Dimethylethoxycarbonyl)-5-oxopyrrolidine-2-carbonyl)valine pentafluorophenyl ester (34)

Compound **33** (200 mg, 610 μmol) was stirred with pentafluorophenyl trifluoroacetate (188 mg, 671 μmol) and pyridine (53 mg, 671 μmol) in dry DMF for 1 h. The mixture was diluted with EtOAc (20 mL) and washed with cold 5% aq citric acid and 5% aq $NaHCO_3$. Drying and evaporation afforded **34** (250 mg, 83%) as a highly viscous colourless oil; NMR δ_H 1.05 (3H, d, J = 7.0 Hz, Val-Me), 1.07 (3H, d, J = 6.6 Hz, Val-Me),

1.52 (9H, s, Boc), 2.26 (2H, m, Val β-H, Pyg β-H), 2.42 (1H, m, Pyg β-H), 2.50 (1H, m, Pyg γ-H), 2.75 (1H, m, Pyg γ-H), 4.62 (1H, dd, J = 7.8, 3.1 Hz, Pyg α-H), 4.89 (1H, dd, J = 8.6, 5.1 Hz, Val α-H), 6.64 (1H, d, J = 8.6 Hz, NH); NMR δ_F −161.2 (2F, dd, J = 20.9, 18.0 Hz, 3′,5′-F_2), −156.4 (1F, t, J = 20.9 Hz, 4′-F), −151.6 (2 F, d, J = 18.0 Hz, 2′,6′-F_2); MS m/z 495.1571 (M+H) ($C_{21}H_{24}N_2O_6F_5$ requires 495.1555), 395 (M−Boc).

5.17. *S*-N-(N-(*S*-1-(1,1-Dimethylethoxycarbonyl)-5-oxopyrrolidine-2-carbonyl)valyl)-2,2-dimethyltetrahydrothiazole-4-carboxylic acid (35a) and *S*-N-(N-(*S*-1-(1,1-dimethylethoxycarbonyl)-5-oxopyrrolidine-2-carbonyl)-D-valyl)-2,2-dimethyltetrahydrothiazole-4-carboxylic acid (35b)

Compound **34** (750 mg, 1.52 mmol) was stirred with *S*-2,2-dimethyltetrahydrothiazole-4-carboxylic acid **21** (326 mg, 1.65 mmol) and Pr^i_2NEt (640 mg, 4.95 mmol) in dry DMF (100 mL) for 16 h. The evaporation residue, in EtOAc, was washed with cold 5% aq citric acid and brine. Drying, evaporation and chromatography (EtOAc/AcOH 49:1) afforded **35a** (300 mg, 42%) as a white solid: mp 166–168 °C; NMR δ_H 0.92 (3H, d, J = 6.6 Hz, Val-Me), 0.95 (3H, d, J = 6.6 Hz, Val-Me), 1.50 (9H, s, Boc), 1.83 (3H, s, 2-Me), 1.88 (3H, s, 2-Me), 2.00–2.16 (2H, m, Val β-H, Pyg β-H), 2.25 (1H, m, Pyg β-H), 2.45 (1H, ddd, J = 17.7, 9.4, 2.7 Hz, Pyg γ-H), 2.69 (1H, dt, J = 17.7, 9.8 Hz, Pyg γ-H), 3.26 (1H, dd, J = 12.1, 5.9 Hz, 5-H), 3.39 (1H, d, J = 12.1 Hz, 5-H), 4.25 (1H, t, J = 9.4 Hz, Val α-H), 4.55 (1H, dd, J = 9.0, 2.0 Hz, Pyg α-H), 5.54 (1H, d, J = 5.1 Hz, 4-H), 6.83 (1H, d, J = 9.4 Hz, Val-NH) (Minor peaks were observed corresponding to a rotamer); MS m/z 472.2129 (M+H) ($C_{21}H_{34}N_3O_7S$ requires 472.2117), 372 (M−Boc), 625 (M+mNBA+H), 742 (2× (M−Boc)). Further elution gave **35b** (70 mg, 10%) as a white solid: mp 149–151 °C; NMR δ_H 0.93 (3H, d, J = 6.6 Hz, Val-Me), 1.01 (3H, d, J = 6.6 Hz, Val-Me), 1.50 (9H, s, Boc), 1.83 (3H, s, 2-Me), 1.87 (3H, s, 2-Me), 1.95 (2H, m, Val β-H, Pyg β-H), 2.45 (1H, m, Pyg β-H), 2.48 (1H, ddd, J = 17.4, 9.4, 2.7 Hz, Pyg γ-H), 2.70 (1H, dt, J = 17.4, 9.8 Hz, Pyg γ-H), 3.26 (1H, dd, J = 11.7, 5.5 Hz, 5-H), 3.43 (1H, d, J = 11.7 Hz, 5-H), 4.52 (1H, dd, J = 9.4, 2.7 Hz, Pyg α-H), 4.78 (1H, dd, J = 9.4, 6.6 Hz, Val α-H), 5.02 (1H, d, J = 5.5 Hz, 4-H), 7.07 (1H, d, J = 9.4 Hz, Val-NH); MS m/z 471.2002 (M−H) ($C_{21}H_{32}N_3O_7S$ requires 471.2039).

5.18. Pentafluorophenyl *S*-N-(N-(*S*-1-(1,1-Dimethylethoxycarbonyl)-5-oxopyrrolidine-2-carbonyl)valyl)-2,2-dimethyltetrahydrothiazole-4-carboxylate (36)

Compound **35a** (100 mg, 212 μmol) was stirred with pentafluorophenyl trifluoroacetate (65 mg, 233 μmol) and pyridine (18 mg, 233 μmol) in dry DMF (1 mL) for 1 h. The mixture was diluted with EtOAc (20 mL) and washed with cold 5% aq citric acid and 5% aq $NaHCO_3$. Drying, evaporation and recrystallisation (EtOAc/hexane) afforded **36** (125 mg, 93%) as a white solid: mp 150–152 °C; NMR δ_H 0.92 (3H, d, J = 6.6 Hz, Val-Me), 0.96 (3H, d, J = 6.6 Hz, Val-Me), 1.54 (9H, s, Boc), 1.87 (3H, s, 2-Me), 1.92 (3H, s, 2-Me), 2.15 (2H, m, Val β-H, Pyg β-H), 2.26 (1H, m, Pyg β-H), 2.50 (1H, ddd, J = 17.2, 9.0, 2.7 Hz, Pyg γ-H), 2.71 (1H, ddd, J = 17.2, 10.5, 9.4 Hz, Pyg γ-H), 3.46 (2H, m, 5-H_2), 4.12 (1H, t, J = 8.6 Hz, Val α-H), 4.58 (1H, dd, J = 9.0, 2.3 Hz, Pyg α-H), 6.14 (1H, dd, J = 4.3, 2.3 Hz, 4-H), 6.83 (1 H, d, J = 8.6 Hz, Val NH); NMR δ_F −161.1 (2F, dd, J = 21.0, 18.4 Hz, 3′,5′-F_2), −156.4 (1F, t, J = 21.0 Hz, 4′-F), −151.4 (2F, d, J = 17.1 Hz, 2′,6′-F_2); MS m/z 1298 (2M+Na), 1276 (2M+H), 660.1779 (M+Na) ($C_{27}H_{32}F_5N_3NaO_7$ requires 660.1795), 560 (M+Na–Boc), 538 (M−^{t}BuO).

5.19. *S*-3-(N-(*S*-1-(1,1-Dimethylethoxycarbonyl)-5-oxopyrrolidine-2-carbonyl)valyl)-2,2-dimethyl-N-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (37)

Compound **3**: HCl (426 mg, 2.1 mmol) was stirred with Et_3N (425 mg, 4.2 mmol) and **36** (1.34 g, 2.1 mmol) in CH_2Cl_2 (10 mL) for 5 h. Evaporation and chromatography (EtOAc) afforded **37** (900 mg, 69%) as a pale buff solid: mp 115–117 °C; NMR δ_H 0.77 (3 H, d, J = 6.6 Hz, Val-Me), 0.83 (3H, d, J = 6.6 Hz, Val-Me), 1.43 (9H, s, Boc), 1.70 (3H, s, 2-Me), 1.74 (3H, s, 2-Me), 1.9–2.2 (3H, m, Val β-H, Pyg β-H_2), 2.36 (1H, ddd, J = 17.6, 9.4, 2.7 Hz, Pyg γ-H), 2.57 (1H, m, Pyg γ-H), 2.90 (2H, t, J = 7.4 Hz, $ArCH_2$), 2.93 (1H, d, J = 12.5 Hz, 5-H), 3.28 (1H, dd, J = 12.5, 6.6 Hz, 5-H), 3.40 (1H, m, NH*CH*), 3.65 (1H, m, NH*CH*), 4.13 (1H, t, J = 8.2 Hz, Val α-H), 4.46 (1H, dd, J = 9.0, 2.0 Hz, Pyg α-H), 5.24 (1H, t, J = 6.6 Hz, 4-H), 6.39 (1H, d, J = 8.2 Hz, Val NH), 6.50 (1H, t, J = 5.9 Hz, N*H*CH_2), 7.31 (2H, d, J = 8.4 Hz, Ar 2,6-H_2), 8.08 (2H, d, J = 8.4 Hz, Ar 3,5-H_2); MS m/z 520.2225 (M−Boc) ($C_{24}H_{34}N_5O_6S$ requires 520.2230).

5.20. *S*-3-(N-(N-(Dimethylethoxycarbonyl)glutaminyl)valyl)-2,2-dimethyl-N-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (38)

Compound **37** (100 mg, 162 μmol) was stirred in aq NH_3 (35%, 1.0 mL) and THF (10 mL) for 18 h. Evaporation afforded **38** (quant.) as a white solid: mp 95–97 °C; NMR δ_H 0.84 (3H, d, J = 6.6 Hz, Val-Me), 0.91 (3H, d, J = 6.6 Hz, Val-Me), 1.42 (9H, s, Boc), 1.77 (3H, s, 2-Me), 1.79 (3H, s, 2-Me), 2.0 (3 H, m, Val β-H, Gln β-H_2), 2.25 (1H, m, Gln γ-H), 2.35 (1H, m, Gln γ-H), 2.95–3.00 (3H, m, 5-H, $ArCH_2$), 3.37 (1H, dd, J = 12.5, 6.2 Hz, 5-H), 3.45 (1H, m, NHC*H*), 3.75 (1H, m, NHC*H*), 4.11 (2H, m, Val α-H, Gln α-H), 5.35 (1H, d, J = 6.2 Hz, 4-H), 5.50 (1H, d, J = 7.4 Hz, NH), 5.69 (1H, s, CONH), 6.21 (1H, s, CONH), 6.50 (1H, br t, N*H*CH_2), 7.27 (1H, d, J = 8.2 Hz, NH), 7.38 (2H, d, J = 8.6 Hz, Ar 2,6-H_2), 8.16 (2H, d, J = 8.6 Hz, Ar 3,5-H_2); MS m/z 637.3020 (M+H) ($C_{29}H_{45}N_6O_8S$ requires 637.3021), 537 (M−Boc).

5.21. *S*-2,2-Dimethyl-3-(N-glutaminylvalyl)-N-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide trifluoroacetate salt (39)

Compound **38** (300 mg, 472 μmol) was stirred in CF_3CO_2H (900 μL) and CH_2Cl_2 (3.6 mL) for 1 h.

Evaporation and trituration (Et_2O) afforded **39** (300 mg, 98%) as a white solid: mp 108–110 °C; NMR (CD_3OD) δ_H 0.89 (3H, d, J = 6.6 Hz, Val-Me), 0.91 (3H, d, J = 6.6 Hz, Val-Me), 1.77 (3H, s, 2-Me), 1.88 (3H, s, 2-Me), 2.10 (3H, m, Val β-H, Gln β-H_2), 2.40 (2H, m, Gln γ-H_2), 2.99 (3H, m, 5-H, ArCH_2), 3.37 (1H, dd, J = 12.5, 6.2 Hz, 5-H), 3.51 (2H, m, NHCH_2), 3.96 (1H, t, J = 6.6 Hz, Gln α-H), 3.98 (1H, d, J = 8.6 Hz, Val α-H), 5.38 (1H, d, J = 5.5 Hz, 4-H), 7.48 (2H, d, J = 8.8 Hz, Ar 2,6-H_2), 8.16 (2H, d, J = 8.8 Hz, Ar 3,5-H_2); MS *m/z* 537.2495 (M+H) ($C_{24}H_{37}N_6O_6S$ requires 537.2493).

5.22. *S*-2,2-Dimethyl-3-(N-(N-(N-(dimethylethoxycarbonyl)leucyl)glutaminyl)valyl)-N-(2-(4- nitrophenyl)ethyl)-tetrahydrothiazole-4-carboxamide (40)

BocLeuOSu (157 mg, 477 μmol) in dry THF (2.0 mL) was added to **39** (310 mg, 477 μmol), Et_3N (97 mg, 954 μmol) and DMAP (1 mg) in dry DMF (1.5 mL) at 0 °C and the mixture was stirred for 30 min. The mixture was warmed to 20 °C and stirred for 24 h. The evaporation residue, in EtOAc, was washed with cold 5% aq citric acid, H_2O and brine. Drying, evaporation and recrystallisation (EtOAc/hexane) afforded **40** (330 mg, 92%) as white solid: mp 109–111 °C; NMR (CD_3OD) δ_H 0.85 (3H, d, J = 6.6 Hz, Val-Me), 0.86 (3H, d, J = 6.6 Hz, Val-Me), 0.93 (3H, d, J = 6.6 Hz, Leu-Me), 0.95 (3H, d, J = 6.6 Hz, Leu-Me), 1.45 (9H, s, Boc), 1.52 (2H, t, J = 7.0 Hz, Leu β-H_2), 1.79 (3H, s, 2-Me), 1.88 (3H, s, 2-Me), 1.70–2.10 (4H, m, Val β-H, Leu γ-H, Glnβ-H_2), 2.30 (2H, m, Gln γ-H_2), 2.94–3.04 (3H, m, 5-H, ArCH_2), 3.42 (1H, dd, J = 12.1, 5.9 Hz, 5-H), 3.55 (2H, br q, J = 5.5 Hz, NHCH_2), 3.95 (1H, t, J = 9.0 Hz, Val α-H), 4.04 (1H, t, J = 7.4 Hz, Leu α-H), 4.30 (1H, br q, J = 6.6 Hz, Gln α-H), 5.21 (1H, d, J = 5.9 Hz, 4-H), 7.50 (2H, d, J = 8.6 Hz, Ar 2,6-H_2), 8.07 (1H, t, J = 5.5 Hz, N*H*CH_2), 8.16 (2H, d, J = 8.6 Hz, Ar 3,5-H_2), 8.18 (2H, m, 2× NH); MS *m/z* 772 (M+Na), 750.3860 (M+H) ($C_{35}H_{56}N_7O_9S$ requires 750.3901), 650 (M−Boc).

5.23. *S*-2,2-Dimethyl-3-(N-(N-leucylglutaminyl)valyl)-N-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (41)

Compound **40** (300 mg, 401 μmol) was stirred in CF_3CO_2H (0.9 mL) and CH_2Cl_2 (3.6 mL) for 30 min. Evaporation and trituration (Et_2O) afforded **41** (300 mg, 98%) as a white solid: mp 121–123 °C; NMR (CD_3OD) δ_H 0.76 (3H, d, J = 7.0 Hz, Val-Me), 0.77 (3H, d, J = 6.6 Hz, Val-Me), 1.00 (3H, d, J = 5.5 Hz, Leu-Me), 1.01 (3H, d, J = 5.5 Hz, Leu-Me), 1.54–1.74 (3H, m, Leu β-H_2, Leu γ-H), 1.70 (3H, s, 2-Me), 1.79 (3H, s, 2-Me), 1.82–2.04 (3H, m, Val β-H, Gln β-H_2), 2.20 (2H, m, Gln γ-H_2), 2.89 (2H, t, J = 6.6 Hz, ArCH_2), 2.91 (1H, d, J = 12.1 Hz, 5-H), 3.30 (1H, dd, J = 12.1, 5.9 Hz, 5-H), 3.50 (2H, m, NHCH_2), 3.80 (1H, br t, J = 8.2 Hz, Leu α-H), 3.87 (1H, d, J = 9.4 Hz, Val α-H), 4.31 (1H, t, J = 7.8 Hz, Gln α-H), 5.20 (1H, d, J = 5.9 Hz, 4-H), 7.40 (2H, d, J = 9.0 Hz, Ar 2,6-H_2), 7.97 (1H, t, J = 5.5 Hz, N*H*CH_2), 8.06 (2H, d, J = 9.0 Hz, Ar 3,5-H_2), 8.26 (1H, d, J = 9.0 Hz, NH); MS *m/z* 650.3343 (M+H) ($C_{30}H_{48}N_7O_7S$ requires 650.3336), 723 (M+Na).

5.24. N^{ε}-(1,1-Dimethylethoxycarbonyl)-N^{α}-(fluoren-9-ylmethoxycarbonyl)lysine pentafluorophenyl ester (43)

N^{α}-Fmoc-N^{ε}-BocLysOH **42** (1.0 g, 2.1 mmol) was stirred with pentafluorophenol (432 mg, 2.3 mmol) and DCC (483 g, 2.3 mmol) in EtOAc (5 mL) and THF (5 mL) at 0 °C under N_2 for 4 h for 16 h. The mixture was filtered (Celite®) and the precipitate was washed with cold EtOAc. Evaporation afforded **43** (2.10 g, 70%) as a white solid: mp 102–104 °C (lit.[33] mp 99–101 °C); NMR δ_H 1.43 (9H, s, Boc), 1.50–2.85 (8H, m, β,γ,δ,ε-H_8), 4.23 (1H, t, J = 7.0 Hz, C*H*CH_2O), 4.41 (1H, dd, J = 10.5, 7.0 Hz, CHO), 4.49 (1H, dd, J = 10.5, 7.0 Hz, CHO), 4.61 (1H, br, NH), 4.69 (1H, q, J = 7.8 Hz, α-H), 5.57 (1H, d, J = 7.0 Hz, NH), 7.29 (2H, t, J = 7.4 Hz, Ar-H_2), 7.38 (2H, t, J = 7.4 Hz, Ar-H_2), 7.58 (2H, d, J = 7.4 Hz, Ar-H_2), 7.74 (2H, d, J = 7.4 Hz, Ar-H_2); NMR δ_F −161.7 (2F, t, J = 20.7 Hz, 3′,5′-F_2), −157.1 (1F, t, J = 20.7 Hz, 4′-F), −152.2 (2F, d, J = 18.4 Hz, 2′,6F_2).

5.25. *S*-2,2-Dimethyl-3-(N-(N-(N-(N$^{\varepsilon}$-(1,1-dimethylethoxycarbonyl)-N$^{\alpha}$-(fluoren-9-ylmethoxycarbonyl)lysyl)-leucyl)glutaminyl)valyl)-N-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (44)

Compound **43** (290 mg, 380 μmol) in dry THF (1.5 mL) was stirred with **41** (241 mg, 380 μmol), Et_3N (77 mg, 760 μmol) and DMAP (1 mg) in dry DMF (1.5 mL) at 0 °C for 30 min. The mixture was warmed to 20 °C and stirred for 16 h. The evaporation residue, in EtOAc, was washed with cold 5% aq citric acid, H_2O and brine. Drying and recrystallisation (EtOAc/hexane) afforded **44** (395 mg, 95%) as a white solid: mp 72–75 °C; NMR δ_H 0.75–0.85 (12H, m, 2× Val-Me, 2× Leu-Me), 1.20–1.40 (6H, m, Lys β,γ,δ-H_6), 1.43 (9H, s, Boc), 1.50–1.65 (3H, m, Leu β,γ-H_3), 1.73 (3H, s, 2-Me), 1.83 (3H, s, 2-Me), 1.90–2.10 (3H, m, Val β-H, Gln β-H_2), 2.30 (2H, m, Gln γ-H_2), 2.78 (2H, t, J = 7.4 Hz, Lys ε-H_2), 2.89–3.03 (4H, m, 5-H_2, ArCH_2), 3.35 (1H, m, NH*CH*CH_2), 3.65 (1H, m, NH*CH*CH_2), 4.04 (1H, t, J = 9.0 Hz, C*H*CH_2O), 4.15–4.50 (6H, m, CH_2O, Val α-H, Gln α-H, Leu α-H, Lys α-H), 5.11 (1H, m, 4-H), 7.24–7.55 (8H, m, Ar-H_8), 7.72 (2H, d, J = 7.4 Hz, Ar 2,6-H_2), 8.10 (2H, d, J = 7.4 Hz, Ar 3,5-H_2); MS *m/z* 1100.5544 (M+H) ($C_{56}H_{78}N_9O_{12}S$ requires 1100.5491), 1000 (M−Boc).

5.26. *S*-2,2-Dimethyl-3-(N-(N-(N-(N$^{\varepsilon}$-(1,1-dimethylethoxycarbonyl)lysyl)leucyl)glutaminyl)valyl)-*N*-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (45)

Compound **44** (250 mg, 227 μmol) was stirred in CH_2Cl_2 (4.5 mL) and diethylamine (500 μL) for 30 min. The evaporation residue was washed with Et_2O and dried to afford **45** (180 mg, 90%) as a white solid: mp 100–103 °C; NMR (CD_3OD) δ_H 0.85 (3H, d, J = 6.6 Hz, Val-Me), 0.86 (3H, d, J = 6.6 Hz, Val-Me), 0.92 (3H, d, J = 6.6 Hz, Leu-Me), 0.96 (3H, d, J = 6.6 Hz, Leu-Me), 1.40–1.65 (18H, m, Boc, Lys β,γ,δ-H_6, Leu β,γ-H_3), 1.79 (3H, s, 2-Me), 1.88 (3H, s, 2-Me), 1.90–2.12 (3H, m, Val β-H, Gln β-H_2), 2.30 (2H, m, Gln γ-H_2), 2.96–3.04 (6H, m, Lys ε-H_2, 5-H_2, ArCH_2), 3.40 (1H, m, NH*CH*CH_2), 3.55 (1H, m, NH*CH*CH_2), 3.92–4.42

(4H, m, Val α-H, Gln α-H, Leu α-H, Lys α-H), 5.24 (1H, d, J = 5.4 Hz, 4-H), 7.50 (2H, d, J = 8.6 Hz, Ar 2,6-H_2), 8.15 (2H, d, J = 8.6 Hz, Ar 3,5-H_2); MS m/z 878.4831 (M+H) ($C_{41}H_{68}N_9O_{10}S$ requires 878.4810), 778 (M−Boc).

5.27. *S*-2,2-Dimethyl-3-(N-(N-(N-(N$^{\varepsilon}$-(1,1-dimethylethoxycarbonyl)-N$^{\alpha}$-(O-(1,1-dimethylethyl)-N-(phenylmethoxycarbonyl)serinyl)lysyl)leucyl)glutaminyl)valyl)-N-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (46)

CbzSer(Bu^t)OSu (13.3 mg, 34 μmol) in dry THF (700 μL) was stirred with **45** (30 mg, 34 μmol), Et_3N (6.9 mg, 68 μmol) and DMAP (1 mg) in dry DMF (300 μL) at 0 °C for 30 min and at 20 °C for 16 h. The evaporation residue, in EtOAc, was washed with cold 5% aq citric acid, H_2O and brine. Drying and recrystallisation (EtOAc/hexane) afforded **46** (20 mg, 51%) as a white solid: mp 128–130 °C; NMR (CD_3OD) δ_H 0.84 (3H, d, J = 6.6 Hz, Val-Me), 0.86 (3H, d, J = 6.6 Hz, Val-Me), 0.89 (3H, d, J = 6.2 Hz, Leu-Me), 0.90 (3H, d, J = 6.2 Hz, Leu-Me), 1.20 (9H, s, SerOBu^t), 1.42 (9H, s, Boc), 1.45–1.75 (9H, m, Lys β,γ,δ-H_6, Leu β,γ-H_3), 1.79 (3H, s, 2-Me), 1.88 (3H, s, 2-Me), 1.90–2.15 (3H, m, Val β-H, Gln β-H_2), 2.30 (2H, m, Gln γ-H_2), 2.96–3.04 (4H, m, Lys ε-H_2, $ArCH_2$), 3.42 (1H, dd, J = 12.5, 5.9 Hz, 5-H), 3.55 (3H, m, 5-H, NHCH_2), 3.76 (1H, m, Ser β-H), 3.85 (1H, m, Ser β-H), 3.95 (1H, d, J = 9.4 Hz, Val α-H), 4.15–4.44 (4H, m, Gln α-H, Leu α-H, Lys α-H, Ser α-H), 5.09 (1H, d, J = 12.5 Hz) and 5.14 (1H, d, J = 12.5 Hz) (CH_2Ph), 5.21 (1H, d, J = 5.9 Hz, 4-H), 7.36 (5H, m, Ph-H_5), 7.51 (2H, d, J = 8.8 Hz, Ar 2,6-H_2), 8.06 (1H, br t, J = 7 Hz, NH), 8.16 (2H, d, J = 8.8 Hz, Ar 3,5-H_2); MS m/z 1155.6162 (M+H) ($C_{56}H_{87}N_{10}O_{14}S$ requires 1155.6124), 1055 (M−Boc), 846 (M− DmtNHCH$_2$CH$_2$PhNO$_2$).

5.28. *S*-2,2-Dimethyl-3-(N-(N-(N-(N-(phenylmethoxycarbonyl)serinyl)lysyl)leucyl)glutaminyl)valyl)-N-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide trifluoroacetate salt (47)

Compound **46** (5.0 mg, 4.3 μmol) was stirred in CF_3CO_2H (100 μL) and CH_2Cl_2 (400 μL) for 2 h. Evaporation and trituration (Et_2O) afforded **47** (4.7 mg, 98%) as a white solid: mp 107–109 °C; NMR (CD_3OD) δ_H 0.84 (3H, d, J = 6.6 Hz, Val-Me), 0.85 (3H, d, J = 6.6 Hz, Val-Me), 0.90 (3H, d, J = 6.2 Hz, Leu-Me), 0.95 (3H, d, J = 6.2 Hz, Leu-Me), 1.45–1.70 (9H, m, Lys β,γ,δ-H_6, Leu β,γ-H_3), 1.78 (3H, s, 2-Me), 1.88 (3H, s, 2-Me), 1.90–2.15 (3H, m, Val β-H, Gln β-H_2), 2.30 (2H, t, J = 7.4 Hz, Gln γ-H_2), 2.39 (2H, t, J = 7.0 Hz, $ArCH_2$), 2.98 (2H, t, J = 6.5 Hz, Lys ε-H_2), 3.03 (1H, J = 12.1 Hz, 5-H), 3.41 (1H, dd, J = 12.1, 5.5 Hz, 5-H), 3.55 (2H, m, NHCH_2), 3.75 (1H, m, Ser β-H), 3.85 (1H, m, Ser β-H), 3.95 (1H, d, J = 9.4 Hz, Val α-H), 4.20–4.40 (4H, m, Gln α-H, Leu α-H, Lys α-H, Ser α-H), 5.07 (1H, d, J = 12.5 Hz) and 5.12 (1H, d, J = 12.5 Hz) (CH_2Ph), 5.21 (1H, d, J = 5.5 Hz, 4-H), 7.36 (5H, m, Ph-H_5), 7.51 (2H, d, J = 8.8 Hz, Ar 2,6-H_2), 8.06 (1H, br t, J = 7 Hz, NH), 8.16 (2H, d, J = 8.8 Hz, Ar 3,5-H_2); MS m/z 999.4964 (M+H) ($C_{57}H_{71}N_{10}O_{12}S$ requires 999.4974).

5.29. *S*-2,2-Dimethyl-3-(N-(N-(N-(N$^{\varepsilon}$-(1,1-dimethylethoxycarbonyl)-N$^{\alpha}$-(N-(1,1-dimethylethyl)-N-(fluoren-9-ylmethoxycarbonyl)serinyl)lysyl)leucyl)glutaminyl)valyl)-N-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (48)

FmocSer(Bu^t)OSu (44 mg, 91 μmol) in dry THF (500 μL) was stirred with **45** (80 mg, 91 μmol), Et_3N (18.4 mg, 182 μmol) and DMAP (1 mg) in dry DMF (500 μL) at 0 °C for 30 min and at 20 °C for 16 h. The evaporation residue, in EtOAc, was washed with cold 5% aq citric acid, H_2O and brine. Drying and recrystallisation (EtOAc/hexane) afforded **48** (70 mg, 62%) as a white solid: mp 184–186 °C; NMR (CD_3OD) δ_H 0.85 (3H, d, J = 6.6 Hz, Val-Me), 0.86 (3H, d, J = 6.6 Hz, Val-Me), 0.90 (3H, d, J = 6.2 Hz, Leu-Me), 0.95 (3H, d, J = 6.2 Hz, Leu-Me), 1.18 (9H, s, SerOBu^t), 1.43 (9H, s, Boc), 1.45–1.75 (9H, m, Lys β,γ,δ-H_6, Leu β,γ-H_3), 1.79 (3H, s, 2-Me), 1.88 (3H, s, 2-Me), 1.90–2.15 (3H, m, Val β-H, Gln β-H_2), 2.30 (2H, m, Gln γ-H_2), 2.96–3.02 (4H, m, $ArCH_2$, Ser β-H_2), 3.15–3.25 (2H, m, 5-H_2), 3.55 (2H, m, Lys ε-H_2), 3.62 (2H, m, NHCH_2), 3.94 (1H, d, J = 9.4 Hz, CHCH_2O), 4.21–4.35 (5H, m, Gln α-H, Leu α-H, Lys α-H, Ser α-H, fluorene 9-H), 5.20 (1H, d, J = 5.1 Hz, 4-H), 7.30 (2H, t, J = 7.4 Hz, Ar-H_2), 7.39 (2H, t, J = 7.4 Hz, Ar-H_2), 7.50 (2H, d, J = 8.6 Hz, Ar 2,6-H_2), 7.66 (2H, J = 7.4 Hz, Ar-H_2), 7.80 (2H, J = 7.4 Hz, Ar-H_2),8.16 (2H, d, J = 8.6 Hz, Ar 3,5-H_2); MS m/z 1243.6437 (M+H) ($C_{63}H_{91}N_{10}O_{14}S$ requires 1243.6477).

5.30. *S*-2,2-Dimethyl-3-(N-(N-(N-(N$^{\varepsilon}$-(1,1-dimethylethoxycarbonyl)-N$^{\alpha}$-(*N*-(1,1-dimethylethyl)serinyl)lysyl)leucyl)glutaminyl)valyl)-*N*-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (49)

Compound **48** (70 mg, 56 μmol) was stirred in CH_2Cl_2 (1.35 mL) and Et_2NH (150 μL) for 30 min. The evaporation residue was washed with Et_2O and dried to afford **49** (50 mg, 88%) as a white solid: mp 94–96 °C; NMR (CD_3OD) δ_H 0.85 (3H, d, J = 6.6 Hz, Val-Me), 0.86 (3H, d, J = 6.6 Hz, Val-Me), 0.92 (3H, d, J = 6.2 Hz, Leu-Me), 0.96 (3H, d, J = 6.2 Hz, Leu-Me), 1.31 (9H, s, SerOBu^t), 1.44 (9H, s, Boc), 1.45–1.75 (9H, m, Lys β,γ,δ-H_6, Leu β,γ-H_3), 1.78 (3H, s, 2-Me), 1.88 (3H, s, 2-Me), 1.90–2.15 (3H, m, Val β-H, Gln β-H_2), 2.30 (2H, t, J = 7.0 Hz, Gln γ-H_2), 2.96–3.05 (5H, m, 5-H, $ArCH_2$, Ser β-H_2), 3.42 (1H, dd, J = 12.1, 5.3 Hz, 5-H), 3.55 (2H, m, Lys ε-H_2), 3.58–3.55 (2H, m, NHCH_2), 3.95 (1H, d, J = 9.0 Hz, Val α-H), 4.20–4.40 (4H, m, Gln α-H, Leu α-H, Lys α-H, Ser α-H), 5.21 (1H, d, J = 5.3 Hz, 4-H), 7.51 (2H, d, J = 8.6 Hz, Ar 2,6-H_2), 8.16 (2H, d, J = 8.6 Hz, Ar 3,5-H_2); MS m/z 1021.5756 (M+H) ($C_{48}H_{81}N_{10}O_{12}S$ requires 1021.5762).

5.31. *S*-2,2-Dimethyl-3-(N-(N-(N-(N$^{\varepsilon}$-(1,1-dimethylethoxycarbonyl)-N$^{\alpha}$-(N-(1,1-dimethylethyl)-N-(N-(1,1-dimethylethyl)-N-(phenylmethoxycarbonyl)serinyl)serinyl)lysyl)leucyl)glutaminyl)valyl)-N-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (50)

CbzSer(Bu^t)OSu (9.8 mg, 25 μmol) in dry THF (700 μL) was added to compound **49** (25 mg, 25 μmol), Et_3N (5.1 mg, 50 μmol) and DMAP (1 mg) in dry DMF

(300 μL) at 0 °C. The mixture was stirred for 30 min at 0 °C and for 16 h at 20 °C. The evaporation residue, in EtOAc, was washed with cold 5% aq citric acid, H_2O and brine. Drying and evaporation afforded **50** (20 mg, 62%) as a white solid: mp 202–205 °C; NMR (CD_3OD) δ_H 0.86 (3H, d, J = 6.6 Hz, Val-Me), 0.89 (3H, d, J = 6.6 Hz, Val-Me), 0.91 (3H, d, J = 5.9 Hz, Leu-Me), 0.95 (3H, d, J = 5.9 Hz, Leu-Me), 1.20 (18H, s, 2× SerO-Bu^t), 1.42 (9H, s, Boc), 1.45–1.75 (9H, m, Lys β,γ,δ-H_6, Leu β,γ-H_3), 1.80 (3H, s, 2-Me), 1.88 (3H, s, 2-Me), 1.90–2.15 (3H, m, Val β-H, Gln β-H_2), 2.30 (2H, m, Gln γ-H_2), 2.96–3.04 (4H, m, Lys ε-H_2, ArCH_2), 3.45–3.8 (8H, m, 5-H_2, NHCH_2, 2× Ser β-H_2), 3.95 (1H, d, J = 9.4 Hz, Val α-H), 4.15–4.80 (6H, m, Gln α-H, Leu α-H, Lys α-H, 2× Ser α-H), 5.09 (2H, m, CH_2Ph), 5.21 (1H, d, J = 5.5 Hz, 4-H), 7.35 (5H, m, Ph-H_5), 7.51 (2H, d, J = 8.8 Hz, Ar 2,6-H_2), 8.16 (2H, d, J = 8.8 Hz, Ar 3,5-H_2); MS m/z 1199.6588 (M−Boc) ($^{13}C_1^{12}C_{57}H_{92}N_{11}O_{14}S$ requires 1199.6579), 1297 (M−H).

5.32. *S*-2,2-Dimethyl-3-(N-(N-(N-(N-(N-(N-(phenylmethoxycarbonyl)serinyl)serinyl)lysyl)leucyl)glutaminyl)valyl)-N-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide trifluoroacetate (51)

Compound **50** (5.0 mg, 3.9 μmol) was stirred in CF_3CO_2H (100 μL) and CH_2Cl_2 (400 μL) for 2 h. Evaporation afforded **51** (quant.) as a highly hygroscopic gummy solid: NMR (CD_3OD) δ_H 0.86 (3H, d, J = 6.6 Hz, Val-Me), 0.89 (3H, d, J = 6.6 Hz, Val-Me), 0.91 (3H, d, J = 5.9 Hz, Leu-Me), 0.95 (3H, d, J = 5.9 Hz, Leu-Me), 1.45–1.75 (9H, m, Lys β,γ,δ-H_6, Leu β,γ-H_3), 1.80 (3H, s, 2-Me), 1.88 (3H, s, 2-Me), 1.90–2.15 (3H, m, Val β-H, Gln β-H_2), 2.30 (2H, m, Gln γ-H_2), 2.96–3.04 (4H, m, Lys ε-H_2, ArCH_2), 3.45–3.8 (8H, m, 5-H_2, NHCH_2, 2× Ser β-H_2), 3.95 (1H, d, J = 9.4 Hz, Val α-H), 4.00–4.80 (6H, m, Gln α-H, Leu α-H, Lys α-H, 2× Ser α-H), 5.10 (2H, m, CH_2Ph), 5.21 (1H, d, J = 5.5 Hz, 4-H), 7.35 (5H, m, Ph-H_5), 7.51 (2H, d, J = 8.8 Hz, Ar 2,6-H_2), 8.16 (2H, d, J = 8.8 Hz, Ar 3,5-H_2).

5.33. N-Glutaminylvaline methyl ester trifluoroacetate salt (52)

Compound **19** (1.00 g, 2.8 mmol) was stirred in CF_3CO_2H (3 mL) and CH_2Cl_2 (3 mL) for 45 min. Evaporation afforded crude **52** (quant.) as a highly hygroscopic yellow viscous oil: NMR ($(CD_3)_2SO$) δ_H 0.85 (6H, d, J = 7.0 Hz, 2× Val-Me), 1.88 (2H, br q, J = 5.7 Hz, Gln β-H_2), 2.00 (1H, m, Val β-H), 2.20 (2H, t, J = 7.4 Hz, Gln γ-H_2), 3.59 (3H, s, OMe), 3.87 (1H, br q, J = 5.7 Hz, Gln α-H), 4.16 (1H, dd, J = 7.8, 5.9 Hz, Val α-H), 6.93 (1H, s, CONH), 7.42 (1H, s, CONH), 8.14 (3H, s, N^+H_3), 8.68 (1H, d, J = 7.8 Hz, Val NH).

5.34. N-(N-(N-(1,1-Dimethylethoxycarbonyl)leucyl)glutaminyl)valine methyl ester (53)

BocLeuOSu (1.14 g, 3.5 mmol) in dry THF (5 mL) was stirred with **52** (1.29 g, 3.5 mmol), Et_3N (708 mg, 7 mmol) and DMAP (1 mg) in dry DMF (5 mL) at 0 °C for 30 min and at 20 °C for 24 h. The evaporation residue, in EtOAc, was washed with cold aq citric acid (5%) and brine. Drying, evaporation and chromatography (EtOAc → EtOAc/MeOH 1:1) afforded **53** (900 mg, 54%) as a white solid: mp 135–139 °C; NMR δ_H 0.83–0.87 (12H, m, 2× Val-Me, 2× Leu-Me), 1.34 (9H, s, Boc), 1.41 (1H, m, Leu β-H), 1.57 (1H, m, Leu γ-H), 1.70 (2H, m, Gln β-H, Leu β-H), 1.85 (1H, m, Gln β-H), 2.03 (1H, m, Val β-H), 2.08 (2H, m, Gln γ-H_2), 3.62 (3H, s, OMe), 3.94 (1H, dt, J = 8.2, 6.6 Hz, Leu α-H), 4.14 (1H, dd, J = 7.8, 5.9 Hz, Val α-H), 4.34 (1H, dt, J = 7.8, 5.5 Hz, Gln α-H), 6.77 (1H, s, CONH), 6.95 (1H, d, J = 8.2 Hz, Leu-NH), 7.25 (1H, s, CONH), 7.82 (1H, d, J = 7.8 Hz, Gln NH), 8.12 (1H, d, J = 7.8 Hz, Val NH); MS m/z 495.2793 (M+Na) ($C_{22}H_{40}N_4O_7$ requires 495.2795), 395 (M+Na–Boc).

5.35. N-(N-Leucylglutaminyl)valine methyl ester trifluoroacetate salt (54)

Compound **53** (650 mg, 1.4 mmol) was stirred in CF_3CO_2H (2 mL) and CH_2Cl_2 (2 mL) for 2 h. Evaporation afforded **54** (quant.) as a highly hygroscopic colourless viscous oil: NMR ($(CD_3)_2SO$) δ_H 0.85–0.87 (12H, m, 2× Val-Me, 2× Leu-Me), 1.26 (1H, m, Leu β-H), 1.62 (1H, m, Leu γ-H), 1.73 (2H, m, Gln β-H, Leu β-H), 1.85 (1H, m, Gln β-H), 2.04 (1H, m, Val β-H), 2.15 (2H, m, Gln γ-H_2), 3.62 (3H, s, OMe), 3.80 (1H, m, Leu α-H), 4.16 (1H, dd, J = 8.2, 6.2 Hz, Val α-H), 4.42 (1H, dt, J = 7.8, 5.5 Hz Gln α-H), 6.82 (1H, s, CONH), 7.31 (1H, s, CONH), 8.14 (3H, br, N^+H_3), 8.28 (1H, d, J = 8.2 Hz, Val NH), 8.66 (1H, d, J = 7.8 Hz, Gln NH); MS m/z 525 (M+mNBA), 395 (M+Na), 373.2443 (M+H) ($C_{17}H_{33}N_4O_5$ requires 373.2451).

5.36. N-(N-(N-(N$^{\varepsilon}$-(1,1-Dimethylethoxycarbonyl)-N$^{\alpha}$-(fluoren-9-ylmethoxycarbonyl)lysyl)leucyl)glutaminyl)valine methyl ester (55)

Compound **43** (700 mg, 1.1 mmol) in dry THF (5 mL) was stirred with **54** (826 mg, 1.7 mmol), Et_3N (344 mg, 3.4 mmol) and DMAP (1 mg) in dry DMF (3 mL) at 0 °C for 30 min and at 20 °C for 16 h. The evaporation residue was washed with EtOAc, cold aq citric acid (5%) and H_2O. Drying and evaporation afforded **55** (600 mg, 68%) as a white solid: mp 200–202 °C; NMR ($(CD_3)_2SO$) δ_H 0.80–0.86 (12H, m, 2× Val-Me, 2× Leu-Me), 1.35 (9 H, s, Boc), 1.44–1.52 (6H, m, Lys β,γ,δ-H_6), 1.54 (1H, m, Gln β-H), 1.57 (1H, m, Gln β-H), 2.05 (1H, m, Val β-H), 2.22 (2H, m, Gln γ-H_2), 2.88 (2H, m, Lys ε-H_2), 3.61 (3H, s, OMe), 3.94–4.32 (7H, m, Leu α-H, Gln α-H, Val α-H, Lys α-H, fluorene 9-H, CH_2O), 6.77 (2H, m, CONH, NH), 7.23 (1H, s, CONH), 7.30 (2H, t, J = 7.4 Hz, Ar-H_2), 7.39 (2H, t, J = 7.4 Hz, Ar-H_2), 7.46 (1H, d, J = 8.2 Hz, NH), 7.69 (2H, t, J = 7.4 Hz Ar-H_2), 7.86 (2H, d, J = 7.4 Hz, Ar-H_2), 7.89 (1H, d, J = 8.2 Hz, NH), 7.94 (1H, d, J = 7.4 Hz, NH), 8.04 (1H, d, J = 8.2 Hz, NH); MS m/z 823.4597 (M+H) ($C_{43}H_{63}N_6O_{10}$ requires 823.4606).

5.37. N-(N-(N-(N$^{\varepsilon}$-(1,1-Dimethylethoxycarbonyl)lysyl)-leucyl)glutaminyl)valine methyl ester (56)

Compound **55** (52 mg, 64 μmol) was stirred in DMF (900 μL) and Et_2NH (100 μL) for 30 min. The evaporation

residue was washed with Et_2O and dried to afford **56** (35 mg, 91%) as a white solid: mp 162-165 °C; NMR (CD_3OD) δ_H 0.94–0.98 (12H, m, 2× Val-Me, 2× Leu-Me), 1.38–1.62 (17H, m, Boc, Lys β,γ,δ-H_6, Gln β-H_2), 1.76 (1H, m, Leu γ-H), 1.95 (1H, m, Leu β-H), 2.08–2.20 (2H, m, Val β-H, Leu β-H), 2.35 (2H, t, J = 7.4 Hz, Gln γ-H_2), 3.04 (2H, t, J = 6.6 Hz, Lys ε-H_2), 3.37 (1H, t, J = 6.6 Hz, Lys α-H), 3.72 (3H, s, OMe), 4.31 (1H, d, J = 5.9 Hz, Val α-H), 4.38 (1H, t, J = 7.4, Gln α-H), 4.45 (1H, dd, J = 8.9, 5.1 Hz, Leu α-H); MS *m/z* 601.3948 (M+H) ($C_{28}H_{53}N_6O_8$ requires 601.3925).

5.38. N-(N-(N-(N$^{\varepsilon}$-(1,1-Dimethylethoxycarbonyl)-N$^{\alpha}$-(O-(1,1-dimethylethyl)-N-(phenylmethoxycarbonyl)seri-nyl)lysyl)leucyl)glutaminyl)valine methyl ester (57)

CbzSer(Bu^t)OSu (19 mg, 48 μmol) in dry THF (700 μL) was stirred with **56** (29 mg, 48 μmol), Et_3N (9.8 mg, 96 μmol) and DMAP (1 mg) in dry DMF (300 μL) at 0 °C for 30 min and at 20 °C for 72 h. The evaporation residue, in EtOAc, was washed with cold aq citric acid (5%) and H_2O and was dried to afford **57** (30 mg, 68%) as a white solid: mp 234–236 °C; NMR ($(CD_3)_2SO$) δ_H 0.83–0.87 (12H, m, 2× Val-Me, 2× Leu-Me), 1.10 (9H, s, CH_2OBu^t), 1.25–2.11 (21H, Boc, Val β-H, Gln β-H_2, Leu β,γ-H_3, Lys β,γ,δ-H_6), 2.85 (2H, t, J = 6.2 Hz, Gln γ-H_2), 3.32 (2H, m, CH_2OBu^t), 3.44 (2H, t, J = 5.5 Hz, Lys ε-H_2), 3.63 (3H, s, OMe), 4.10–4.24 (5H, Val α-H, Gln α-H, Leu α-H, Lys α-H, Ser α-H), 5.02 (1H, d, J = 12.5 Hz) and 5.06 (1H, d, J = 12.5 Hz) (CH_2Ph), 6.72–8.06 (13H, m, 8× NH, Ph-H_5); MS *m/z* 878.5264 (M+H) ($C_{43}H_{72}N_7O_{12}$ requires 878.5239).

5.39. N-(N-(N-(N$^{\alpha}$-(N-(Phenylmethoxycarbonyl)seri-nyl)lysyl)leucyl)glutaminyl)valine methyl ester trifluoro-acetate salt (58)

Compound **57** (3.0 mg, 3.4 μmol) was stirred in CF_3CO_2H (300 μL) and CH_2Cl_2 (700 μL) for 4 h. Evaporation afforded **58** (quant.) as a highly hygroscopic colourless gum: NMR ($(CD_3)_2SO$) δ_H 0.83 (3H, d, J = 6.6 Hz, Val-Me), 0.87 (3H, d, J = 6.2 Hz, Leu-Me), 0.89 (6H, d, J = 5.9 Hz) (Val-Me, Leu-Me), 1.25–1.80 (10H, m, Lys β,γ,δ-H_6, Leu β-H_2, Gln β-H, Val β-H), 1.85 (1H, m, Gln β-H), 2.04 (1H, nonet, J = 6.2 Hz, Leu γ-H), 2.11 (2H, br t, J = 7 Hz, Gln γ-H_2), 2.85 (2H, m, Lys ε-H_2), 3.57 (2H, br d, J = 5.5 Hz, Ser β-H_2), 3.63 (3H, s, CO_2Me), 4.11 (1H, br q, J = 7.4 Hz, Ser α-H), 4.15 (1H, dd, J = 8.1, 6.2 Hz), 4.20–4.35 (3H, m, Gln α-H, Leu α-H, Lys α-H), 5.00 (1H, d, J = 12.5 Hz) and 5.04 (1H, d, J = 12.5 Hz) (CH_2Ph), 6.81 (1H, s), 7.28 (1H, s) ($CONH_2$), 7.32 (6H, m, Ser NH, Ph-H_5), 7.65 (3H, br, NH_3), 7.91 (1H, d, J = 5.9 Hz, NH), 7.93 (1H, d, J = 7.4 Hz, NH), 8.04 (1H, d, J = 7.8 Hz, Val NH), 8.10 (1H, d, J = 8.2 Hz, NH); MS *m/z* 722.4099 (M+H) ($C_{34}H_{56}N_7O_{10}$ requires 722.4089).

5.40. Incubations of peptide constructs with PSA

The peptide constructs **47**, **51** and **58** were incubated with PSA at a molar ratio of 100:1 in a buffer containing Tris–HCl (50 mM) and NaCl (140 mM) at pH 7.4 at 37 °C. The mixtures were incubated for the appropriate time periods before the enzymic reaction was stopped by precipitation of the protein by addition of $ZnCl_2$ to a final concentration of 10 mM. The composition of the mixture was then analysed by HPLC, using the same mobile phase and conditions as in our previous paper.[1] The retention times of the product peaks were compared with those of synthetic samples.

Acknowledgments

We thank Dr. Steven J. Black and Dr. Timothy Woodman (University of Bath) for the NMR spectra and the EPSRC Mass Spectrometry Centre (Swansea) for some of the mass spectra. We are very grateful to the Association for International Cancer Research for generous funding in support of the project.

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