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Effects of steric bulk and stereochemistry on the rates of diketopiperazine formation from *N*-aminoacyl-2,2-dimethylthiazolidine-4-carboxamides (Dmt dipeptide amides)—a model for a new prodrug linker system

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Abstract—A peptide-like self-immolative molecular clip is required for release of active drugs from prodrugs by endopeptidases. Upon cleavage from the carrier, this clip must collapse and release the drug rapidly. A series of aminoacyl-5,5-dimethylthiaproline (Aaa-Dmt) *N*-(2-(4-nitrophenyl)ethyl)amides were designed. Boc-L-aminoacyl fluorides were coupled with *R*-DmtOH to give Boc-L-Aaa-*R*-DmtOH, which were converted to the Boc-L-Aaa-*R*-Dmt *N*-(2-(4-nitrophenyl)ethyl)amides. The L,*S* diastereomeric series was prepared by the reaction of Boc-Aaa PFP esters with *S*-DmtOH. The L-Aaa-Dmt *N*-(2-(4-nitrophenyl)ethyl)amides were allowed to cyclise to diketopiperazines (DKPs) in aqueous buffers, expelling 2-(4-nitrophenyl)ethylamine as a model for amine-containing drugs. Reaction rates were dependant on pH. In the L,*R* diastereomeric series, increasing steric bulk of the Aaa side-chain (Gly, Ala, Phe, Val) led to decrease in the reaction rate. However, in the L,*S* series, the greatest rate of reaction was observed for the most bulky amino-acid (Val), with $t_{½}$=15 min at pH 8.0. The effects of steric bulk and stereochemistry are rationalised through conformational analysis (NMR and X-ray crystallography) of the starting dipeptide amides, the product diketopiperazines and key analogues. Since the dipeptides are (almost) exclusively in the *cis*-amide conformation, trans–cis interconversion is not relevant. The data suggest that steric interactions in the reacting conformations of the dipeptide amides, as they form the tetrahedral intermediates, are the controlling factors. Thus, L-Aaa-*S*-Dmt amides are shown to be excellent candidates for incorporation into the design of novel prodrugs.

1. Introduction

Prodrugs have an increasingly important role in the selective delivery of potent drugs to their intended site of action in the body. In a prodrug, a part or the whole of the pharmacophore is masked by a group, which is removed, usually by the catalytic activity of an enzyme, in the target site. The utility of the prodrug approach can be to increase the concentration of the active drug in the target tissue or to diminish the concentration in an organ to which the drug is toxic. The masking group can also carry functionality to increase aqueous solubility or to modify the biodistribution of the prodrug. For example, macromolecular prodrugs, in which many molecules of the drug are attached to a soluble polymer, are selectively retained in solid tumours owing to leaky vasculature and poor lymphatic drainage.[1] Prodrugs can be bipartite or tripartite in concept (Scheme 1). In bipartite prodrugs, the trigger (which is the masking group)[2] is joined directly to the effector (the drug moiety to be released); the activating enzyme modifies the trigger chemically, causing the effector to be released. Of course, the design of bipartite prodrugs relies on the possibility of appropriate modes of attachment of the trigger to the effector. Several reductively activated prodrug systems work in this way.[3,4] Where this is not possible, a linker is interposed between the trigger unit and the effector, making a tripartite prodrug (Scheme 1). In this system, the activating enzyme triggers release of the linker–effector unit; the linker then decomposes spontaneously to expel the effector (drug). Such linkers can be as simple as a carbamate ester,[5] providing a carbamate anion as a good leaving group from the trigger; carbon dioxide is then lost rapidly to give the amine drug. 4-Aminobenzyl carbamate esters are also useful linkers.[6]

Peptidases present particular problems as activating enzymes, in terms of prodrug design in general and linker design in particular. Exopeptidases, particularly carboxypeptidases,

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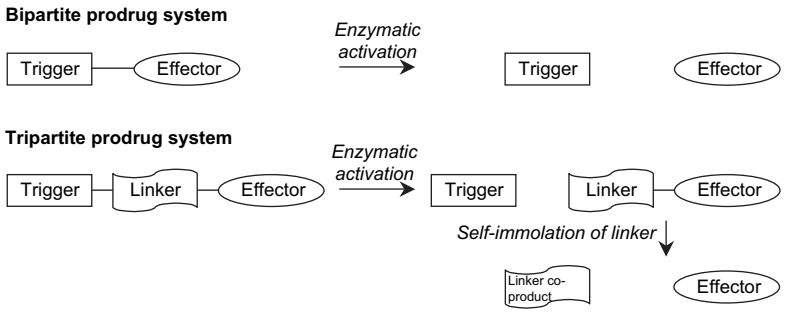
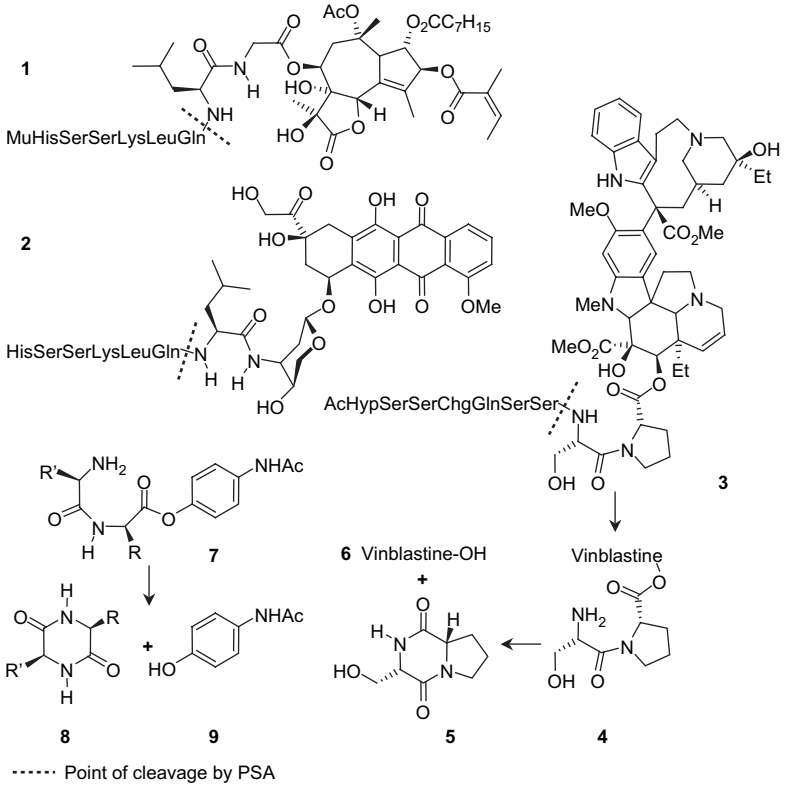


Scheme 1. Schematic representation of the release of drugs from bipartite and tripartite prodrugs systems. The trigger or the trigger–linker unit, respectively, comprises the group which masks the pharmacophore of the drug (effector).

can release amine-containing drugs directly from amides at the C-terminal of short peptides; for example, doxorubicin is released from the polymeric prodrug PK1 (in which it is linked to the polymeric backbone through the sequence GlyPheLeuGly) by cathepsin B.[7] However, some activating enzymes are endopeptidases, cleaving only amide bonds between amino acids. In several cases, this leads to the release of a drug molecule still carrying one or more aminoacyl unit; this may or may not be deleterious to the desired pharmacological activity. For example, prodrug **1** is cleaved by prostate-specific antigen (PSA) in malignant prostate tissue to release a thapsigargin analogue still carrying a Leu residue,[8] as shown in Scheme 2. Similarly, PSA-mediated cleavage of prodrug **2** releases leucyl-doxorubicin in prostate tumours.[9,10] Interestingly, PSA does not release doxorubicin from HisSerSerLysLeuGln-doxorubicin. In both cases, the released Leu-drug construct does have cytotoxic activity. To address this problem for a drug in which a pendant Leu would be deleterious to activity, prodrug **3** (Scheme 2) has been developed.[11] PSA causes cleavage of the Ser–Ser bond, releasing SerPro-vinblastine **4**. The N-terminal primary amine of this dipeptide ester then attacks the ester carbonyl, forming a diketopiperazine **5** and expelling the cytotoxic drug **6**. Similarly, dipeptide esters **7** of paracetamol have been proposed as prodrugs to release the analgesic **9** slowly by forming diketopiperazines **8**.[12]

In both of these systems where a dipeptide is the linker, the drug is a good leaving group, forming either an alkyl ester or a phenyl ester. Only a limited number of drugs carry an alcohol in the pharmacophore, whereas many carry a primary or secondary amine. Design of a linker where the drug is



Scheme 2. Examples of prodrugs triggered by an endopeptidase. The thapsigargin analogue prodrug **1** and the leucyl-doxorubicin prodrug **2** both release drug carrying one aminoacyl unit upon cleavage by prostate-specific antigen (PSA). PSA-mediated cleavage of the vinblastine prodrug **3** releases the Ser-Pro vinblastine ester **4**, which cyclises to form the diketopiperazine **5**, expelling the vinblastine alcohol **6**. The prodrug system **7** cyclises spontaneously, forming the diketopiperazines **8** and expelling paracetamol **9**.

attached to the C-terminus of a dipeptide through an amide is more challenging, owing to the much lower electrophilic reactivity of amides. In this paper, we report our development of a dipeptide linker suitable for amine-containing drugs.

2. Design of the dipeptide amide linker

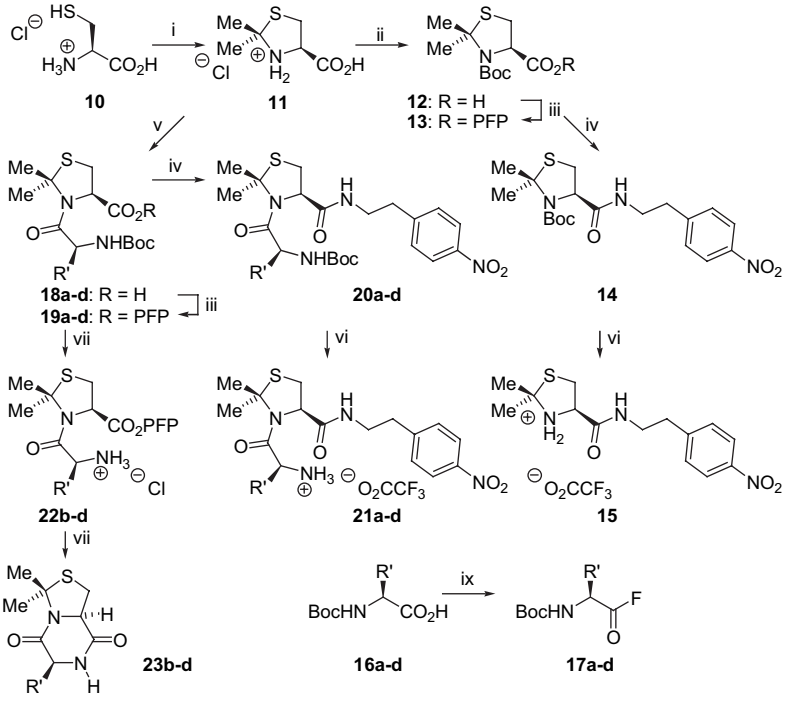
In peptides with free N-terminal amines, the N-terminal amino-acid pair can cyclise slowly to give a diketopiperazine (DKP); the C-terminal remainder of the peptide (an amine leaving group) is expelled. The rate of DKP formation depends on the proportion of the dipeptide in the reacting cis conformation.[13] However, most peptide sequences adopt only the trans conformation, because it is energetically favourable. The presence of proline at the penultimate position greatly enhances the rate of DKP formation and expulsion of the amine leaving group, owing to the greater propensity to adopt a *cis*-amide conformation (AlaPro is 13% cis[13,14]). This phenomenon was exploited in the SerPro linker in prodrug **3**; replacement of Pro with acyclic amino acids suppressed release of vinblastine.[11]

Interestingly, replacement of Pro with 2,2-dimethylthiazolidine-4-carboxylic acid (Dmt) has been reported to force peptides into 100% cis conformation,[14,15] putatively owing to the major steric clash between the side-chain of the N-terminal amino-acid and the geminal dimethyl unit. Since the conformation, and thus biological activity of peptides, is critically dependant on whether amino-acyl prolines are in the *cis* or *trans* amide rotamer, there exist several enzymes, which catalyse the interconversion, including cyclophilins and Pin1.[16–18] Control of conformation by 5,5-dimethylproline and by Dmt has been exploited in studying the biologically active conformations of peptides.[19–26]

Since peptides containing Dmt exist mainly in the *cis* (*Z*) tertiary amide rotamer, it is likely that aminoacyl-Dmt amides also adopt predominantly this conformation. We, therefore, postulated that, despite the lower electrophilicity of amides, these aminoacyl-Dmt amides should cyclise, expelling the amine (drug). To test this hypothesis and to study the effects of relative stereochemistry and steric bulk of the amino-acid side-chain on the rate of cyclisation and release, a series of N-terminal-protected aminoacyl-Dmt dipeptide amides were designed. In these dipeptide amides, 2-(4-nitrophenyl)-ethylamine was used as a simple model for drugs containing primary aliphatic amines. The protecting group was chosen to be Boc, which could be removed under acidic conditions, which prevent cyclisation. The dipeptide amide salts could then be placed in aqueous buffers of various pH values and the rates of cyclisation and release of the model drug from these candidate prodrug linkers could be measured.

3. Chemical synthesis

The synthetic approach to the first diastereomeric series of target dipeptide amide salts **21a–d** is shown in Scheme 3. In this series, L-amino acids carrying side chains with diverse steric bulk (Gly, L-Ala, L-Val, L-Phe) were coupled to *R*-2,2-dimethylthiazolidine-4-carboxylic acid derivatives. Firstly, 2,2-dimethylthiazolidine-4-carboxylic acid **11** (*R*-Dmt) was prepared in good yield from condensation of L-Cys hydrochloride **10** with 2,2-dimethoxypropane by the method of Kemp and Carey.[27] Initially, it was planned to carry out the peptide synthesis in the conventional manner for dipeptide



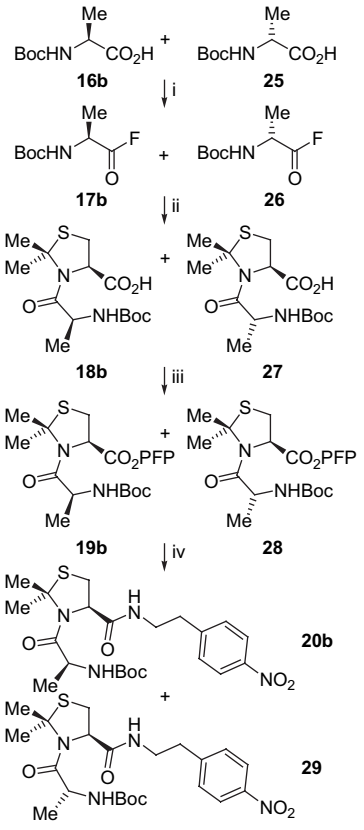
Scheme 3. Synthesis of dipeptide *N*-(2-(4-nitrophenyl)ethyl)amides **21a–d** and diketopiperazines **23b–d**. PFP=pentafluorophenyl. Reagents and conditions: (i) $(MeO)_2CMe_2$, acetone, N_2, Δ; (ii) Boc_2O, Pr^i_2NEt, MeCN; (iii) PFPOH, DCC, EtOAc, N_2; (iv) 2-(4-nitrophenyl)ethylamine hydrochloride (**24**), Et_3N, CH_2Cl_2; (v) **17a–d**, Pr^i_2NEt, DMF; (vi) CF_3CO_2H, CH_2Cl_2; (vii) HCl, CH_2Cl_2; (viii) Et_3N, CH_2Cl_2; (ix) 2,4,6-trifluoro-1,3,5-triazine, pyridine, CH_2Cl_2.

amides, by forming the C-terminal amide at the beginning of the synthesis. Protection of the secondary amine of **11** with Boc proceeded in poor yield, owing to the presence of three bulky substituents on the carbons adjacent to this nitrogen. Boc-*R*-DmtOH **12** was then activated as its pentafluorophenyl ester **13** for coupling with 2-(4-nitrophenyl)ethylamine **24** to form the required BocDmt *N*-(2-(4-nitrophenyl)ethyl)amide **14** in excellent yield. Removal of the Boc protection afforded the Dmt *N*-(2-(4-nitrophenyl)ethyl)amide salt **15** but all attempts to couple this with activated amino-acid derivatives led to opening of the thiazolidine ring, shown by observation of cysteinyl peptides in the crude multi-component reaction mixtures.

In view of the difficulty of achieving couplings to Dmt with the C-terminal amide already in place, an alternative strategy was investigated, coupling of *N*-protected amino acids with **11**, followed by installation of the *N*-(2-(4-nitrophenyl)ethyl)-amide. As noted above, the secondary amine of **11** is severely sterically hindered and is also deactivated as a nucleophile by the carboxylate and by the sulfur. Thus, a reactive and sterically small acylating agent is required for coupling. Wöhr et al.[28] have successfully coupled Fmoc-protected amino-acyl fluorides with **11** in moderate-to-good yields. Adapting this procedure to the required Boc-protected analogues, BocGlyOH **16a**, Boc-L-AlaOH **16b**, Boc-L-ValOH **16c** and Boc-L-PheOH **16d** were converted to the corresponding acyl fluorides **17a–d**, respectively (Scheme 3) by treatment with cyanuric fluoride in the presence of pyridine. These acyl fluorides proved to be unstable and were used immediately in crude form for reaction with **11** in dry DMF. The Boc-protected dipeptides **18a–d** were obtained in 27–35% yields after careful chromatography. Activation as the pentafluorophenyl esters **19a–d** and coupling with 2-(4-nitrophenyl)ethylamine then efficiently afforded the required Boc-protected amino-acyl Dmt *N*-(2-(4-nitrophenyl)ethyl)-amides **20a–d**. Brief treatment with trifluoroacetic acid in dichloromethane then afforded the amino-acyl Dmt *N*-(2-(4-nitrophenyl)ethyl)amide salts **21a–d**, which were sufficiently stable to be stored prior to the cyclisation rate studies.

The diketopiperazines **23b–d** were also required both as HPLC analytical standards for the kinetics studies of cyclisation and as compounds in their own right for study of their conformations, to aid in understanding the relative cyclisation reaction rates. Treatment of the Boc-dipeptide pentafluorophenyl esters **19b–d** with hydrogen chloride removed the Boc protection, giving the dipeptide PFP ester salts **22b–d**. Since all these carry the excellent pentafluorophenoxy leaving group, the corresponding free bases cyclised very rapidly to afford the required diketopiperazines **23b–d** in good yields.

To confirm that the two sets of signals seen in many of the NMR spectra did indeed correspond to rotamers about the tertiary amide bond and did not indicate racemisation of the N-terminal acyl fluoride prior to coupling, one synthetic sequence was conducted with a mixture of enantiomers of BocAla of known composition. This series of experiments would lead to mixtures of diastereoisomers; firstly, the NMR spectra of the individual diastereoisomers could be observed and compared with the spectra from the series starting with homochiral Boc-L-Ala and secondly, examination of the molar ratio of diastereoisomers at each step in the synthetic sequence would reveal and quantify any significant loss of stereochemical integrity. As shown in Scheme 4, the acid fluorides of a mixture comprising 40% Boc-L-AlaOH **16b** and 60% Boc-D-Ala **25** were prepared as above. This mixture of enantiomeric acyl fluorides **17b** and **26** was coupled with homochiral *R*-Dmt **11** to give a mixture of the L,*R* product **18b** and the D,*R* product **27** in 36% yield. As expected, the ^{1}H NMR spectrum of the mixture showed distinct sets of signals for the two diastereoisomers, in the molar ratio 2:3, respectively, *without prior chromatographic purification*. The ^{1}H–^{1}H COSY spectrum of this mixture highlighted these separate sets of signals, confirming that the diastereoisomers could be distinguished by this technique. Notably, only one set of signals corresponded to the product **18b** derived from homochiral Boc-L-AlaOH **16b** alone, confirming that the sets of signals in the spectra of the latter did correspond to rotamers and not to diastereoisomers. Furthermore, the similarity of the ratio of products to the ratio of starting Boc-Ala enantiomers confirms that there is little or no diastereoselectivity in the coupling reaction, despite the modest yield. Moving forward in the sequence, the mixture of **18b** and **27** was converted to a mixture of the corresponding pentafluorophenyl esters **19b** and **28** in high yield. The



Scheme 4. Experiments to demonstrate that no racemisation and no diastereomeric induction took place during the acyl fluoride coupling and subsequent steps towards the dipeptide *N*-(2-(4-nitrophenyl)ethyl)amides. PFP=pentafluorophenyl. The molar ratio of enantiomeric starting materials BocAlaOH **16b/25** was 2:3 in the mixture; molar ratios of diastereomeric pairs of intermediates and products **18b/27**, **19b/28** and **20b/29** were 2:3 in the mixtures, as determined by ^{1}H NMR. Reagents and conditions: (i) 2,4,6-trifluoro-1,3,5-triazine, pyridine, CH_2Cl_2; (ii) **11**, Pr^i_2NEt, DMF; (iii) PFPOH, DCC, EtOAc, N_2; (iv) 2-(4-nitrophenyl)ethylamine hydrochloride, Et_3N, CH_2Cl_2.

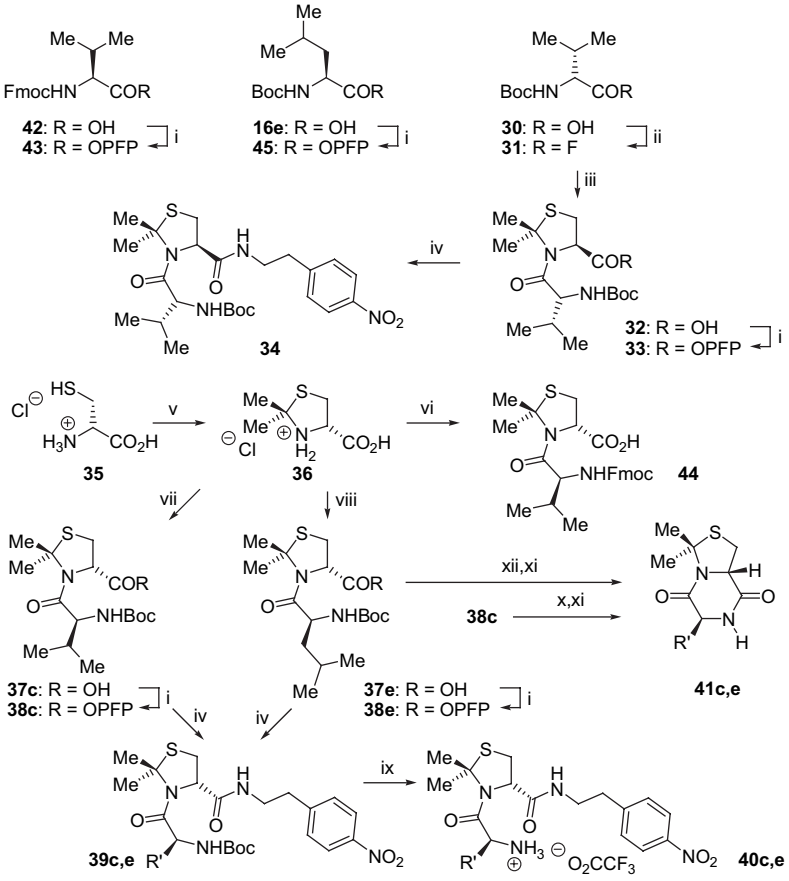
^{19}F NMR spectrum of this mixture was complex and indicated the presence of two conformers for each of the two diastereoisomers, with a diastereomeric ratio of 2:3 again. A similar ratio was seen in the ^{1}H NMR spectrum. These active esters then reacted with 2-(4-nitrophenyl)ethylamine **24** in the usual way to give a 2:3 mixture of **20b** and **29**. This series of experiments did show that the diastereoisomers were distinguishable by NMR at each stage and that little or no racemisation had taken place.

Scheme 5 shows the synthetic approaches to the two target dipeptide amides **40c** and **40e** in the L,*S* diastereomeric series. As a model experiment, D-ValOH **16c** was converted to its acyl fluoride **17c**, which was coupled with **11** in the usual way to give Boc-D-Val-*R*-DmtOH **32** in 50% yield. This dipeptide was then taken through to the pentafluorophenyl ester **33** and the Boc-protected dipeptide amide **34** in the usual way. In the target L,*S* series, condensation of D-Cys **35** with 2,2-dimethoxypropane gave *S*-DmtOH **36** and coupling with Boc-L-Val acyl fluoride **17c** gave the L,*S*-Boc-dipeptide **37c** in 35% yield. The corresponding pentafluorophenyl ester **38c** was then converted to the DKP **41c**, by deprotection and treatment with base, and to the protected dipeptide amide **39c** and through to the target L-Val-*S*-Dmt dipeptide amide salt **40c**, as for the L,*R* diastereoisomer **21c**, above. As expected, the ^{1}H NMR spectra of the enantiomers **34** and **39c** were identical.

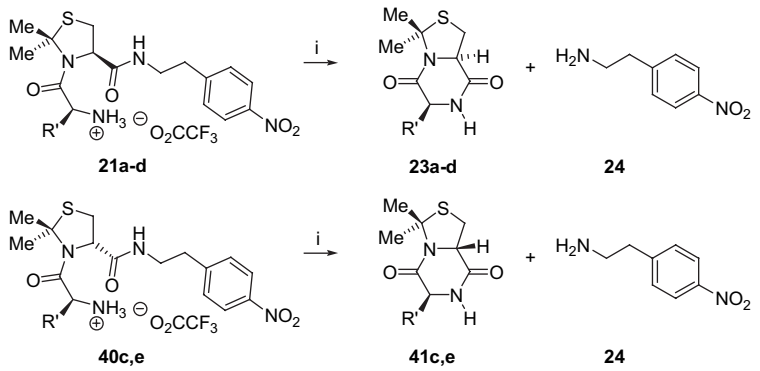
In the light of the relatively higher yields obtained for couplings of BocVal to the Dmt unit in this diastereomeric series, couplings with more sterically demanding pentafluorophenyl esters were explored. As a model, Fmoc-L-ValOH **42** was converted to its pentafluorophenyl ester **43**, which was coupled effectively with **36** to give Fmoc-L-Val-*S*-DmtOH **44**. Similarly, Boc-L-LeuOPFP **45** reacted smoothly with **36** to afford **37e**, from which the dipeptide pentafluorophenyl ester **38e** was readily obtained. As with the lower homologue **38c**, this material could be converted to the corresponding DKP **41e** and, through the Boc-dipeptide amide **39e**, to the target L-Leu-*S*-Dmt amide salt **40e**.

4. DKP formation studies

The rates of cyclisation of the dipeptide amides **21a–d**, **40c** and **40e** to form the DKPs **23a–d**, **41c** and **41e**, respectively, and to expel the model drug **24** (Scheme 6) were studied in aqueous buffer at pH 8.0 and 7.0, to reflect the physiological pH range. The reaction of the L-Val-*S*-Dmt dipeptide amide **40c** was also studied at pH 6.0. Experiments were conducted in duplicate or triplicate at 37 °C. HPLC analysis of the reaction mixtures was performed at appropriate time points to enable the determination of the rates of the reactions. UV detection at 275 nm reported on the loss of the starting dipeptide amides and production of **24** (through the



Scheme 5. Synthesis of the *S*-thiazolidine series of dipeptide amides **40c** and **40e** and diketopiperazines **41c** and **41e**. PFP=pentafluorophenyl. Reagents and conditions: (i) PFPOH, DCC, EtOAc, N_2; (ii) 2,4,6-trifluoro-1,3,5-triazine, pyridine, CH_2Cl_2; (iii) **11**, Pr^i_2NEt, DMF; (iv) 2-(4-nitrophenyl)ethylamine hydrochloride, Et_3N, CH_2Cl_2; (v) $(MeO)_2CMe_2$, acetone, N_2, Δ; (vi) **43**, Pr^i_2NEt, THF, DMF; (vii) **17c**, Pr^i_2NEt, DMF; (viii) **45**, Pr^i_2NEt, DMF; (ix) CF_3CO_2H, CH_2Cl_2; (x) HCl, CH_2Cl_2; (xi) Et_3N, CH_2Cl_2; (xii) CF_3CO_2H.



Scheme 6. Studies on rates of ring-closure of dipeptide amides **21a–d**, **40c** and **40e** to diketopiperazines **23a–d**, **41c** and **41e**, expelling the model drug 2-(4-nitrophenyl)ethylamine **24**. Reagent and conditions: (i) aqueous buffer pH 6.0, 7.0 or 8.0.

nitrophenyl chromophore), whereas simultaneous detection at 225 nm allowed quantification of the product DKPs. Examples of graphs showing consumption of the starting materials and formation of the products are shown in Figure 1. Each reaction followed first-order kinetics closely, as expected, and the calculated half lives are shown in Table 1. A wide range of half lives was seen at pH 8.0 (15 min to 28 h) and at pH 7.0 (1 h to >45 h).

Comparison of the cyclisation half lives at pH 8.0 with the corresponding values at pH 7.0 indicates that each dipeptide amide reacts some 2.5–4 times faster at higher pH. Since, it is only the unprotonated free-base dipeptide that carries the nucleophilic primary amine that attacks the secondary amide carbonyl, this consistently observed effect of pH on the overall reaction rate reflects the amount of this prototropic form that is present in solution. Since the pK_a values of the N-terminal amines are likely to be ca. 8–9, changing the pH by one pH unit will have a marked effect on the concentration of the free-base form in solution and, thus, on the reaction rates. In the case of **40c**, further lowering the pH to 6.0 slows the reaction a further 10-fold, reflecting the very low concentration of the reactive prototropic form in this acidic milieu.

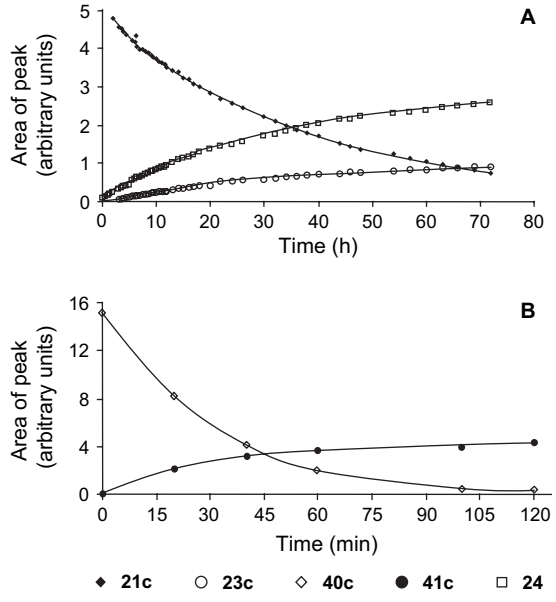


Figure 1. Examples of graphs obtained for typical experiments measuring consumption of dipeptide amides **21c** (A) and **40c** (B), formation of DKPs **23c** and **41a** and expulsion of 2-(4-nitrophenyl)ethylamine **24** in aqueous solution at pH 8.0.

Table 1. Half lives of cyclisation of dipeptide *N*-(2-(4-nitrophenyl)ethyl)-amides **21a–d**, **40c** and **40e** in aqueous buffer at pH 6.0, 7.0 and 8.0, forming the corresponding diketopiperazines **23a–d**, **41c** and **41e** and expelling 2-(4-nitrophenyl)ethylamine **24**

Compound	R	Stereoisomer[a]	$t_{½}$ (37 °C, h)		
			pH 8.0	pH 7.0	pH 6.0
21a	H	*R*	10–11[b]	42	ND[c]
21b	Me	L,*R*	3–4[b]	8–9[b]	ND[c]
21c	$CHMe_2$	L,*R*	26–27[b]	>45	ND[c]
21d	CH_2Ph	L,*R*	28–29[b]	ND[c]	ND[c]
40c	$CHMe_2$	L,*S*	0.25–0.3[b]	0.75–1.25[b]	10
40e	CH_2CHMe_2	L,*S*	1.0–1.5[b]	2.5	ND[c]

[a] L/D refers to the configuration of the N-terminal amino-acid; *R*/*S* refers to the configuration of the thiazolidine.
[b] Range of values from >1 experiment.
[c] Not determined.

In the L,*R* diastereomeric series, the effect of increasing the steric bulk of the amino-acid side-chain was not straightforward. It had been predicted that increasing the bulk would increase the rate of reaction by increasing the proportion of the reactive *Z* tertiary amide rotamer in solution. Comparison of the half-life of cyclisation of the Gly-*R*-Dmt amide **21a** with that of the L-Ala-*R*-Dmt analogue **21b** would suggest that this prediction was true. However, the L-Val-*R*-Dmt and L-Phe-*R*-Dmt dipeptide amides **21c** and **21d**, containing the much more bulky isopropyl and benzyl side chains, respectively, cyclise around nine times slower at pH 8.0 than does the L-Ala-*R*-Dmt analogue **21b**. Thus, with the exception of the Gly analogue, increase of steric bulk of the side-chain *decreases* the reaction rate in this series.

The converse is seen in other diastereomeric series. In general, the L,*S* dipeptide amides cyclise much faster than do the L,*R* analogues. Within the series, the L-Val-*S*-Dmt dipeptide amide **40c** reacts some 3–4 times faster than does the L-Leu-*S*-Dmt dipeptide amide **40e**. The isobutyl group of the latter, although formally larger than the isopropyl group of the former, is less sterically demanding at the critical β-carbon atom. The Gly-*R*-Dmt dipeptide amide **21a** is also formally a member of this diastereomeric series and its reaction rates are ca. 40 times slower than those of **40c** and 10–15 times slower than those of **40e**. Thus, in this

series, increasing the steric bulk of the side-chain *increases* the reaction rate.

Clearly, simple consideration of the *Z*/*E* tertiary amide rotamer equilibrium cannot provide the complete rationalisation of these effects. A detailed study of conformations of the dipeptide amides and related precursors was therefore undertaken, along with examination of conformations of likely intermediates in the courses of the cyclisations.

5. Conformational studies—dipeptides

5.1. NMR studies—L,*R* diastereomeric series

As expected, the ^{1}H NMR spectrum of **12** shows the presence of two rotamers about the Boc–N carbamate bond, in approximately equal amounts. Figure 2 shows the signals corresponding to the 4-H of the thiazolidine in $(CD_3)_2SO$ at 20 and 80 °C. At lower temperature, the signals for 4-H are distinct and, at higher temperature, the signals are fully coalesced.

The dipeptides were designed such that the geminal dimethyl unit should provide sufficient steric bulk to perturb the slow equilibrium between the rotamers about the tertiary amide peptide bond in favour of the cis (*Z*) conformer, which is the one required for the formation of the DKPs and expulsion of the 2-(4-nitrophenyl)ethylamine leaving group. As an indicator of the populations of the rotamers of the N-terminal free-base dipeptides (some of which were expected to cyclise too quickly for satisfactory measurement of rotamer populations), the ratios of the rotamers of the Boc-aminoacyl-DMT amides **20a–d**, **39c**,**e** were measured by ^{1}H NMR spectroscopy. Firstly, the spectrum (in $(CD_3)_2SO$ at 20 °C) of the simplest Boc-dipeptide amide (**20a**), which contains glycine and is thus a member of both diastereomeric series, showed two sharp singlets at δ 1.32 and 1.38 in the approximate ratio of 1:11, corresponding to the protons of the Boc group. The remainder of the spectrum also showed evidence for major and minor populations but the peaks were overlapping, precluding other measurements of the ratio of rotamers. To confirm that these populations did indeed correspond to amide rotamers, spectra were run at temperatures ranging from 20 to 100 °C. The energy barrier to rotation proved to be low for this type of restricted amide bond rotation, with significant broadening of the peaks at 30 °C and full coalescence to a sharp spectrum at 40 °C.

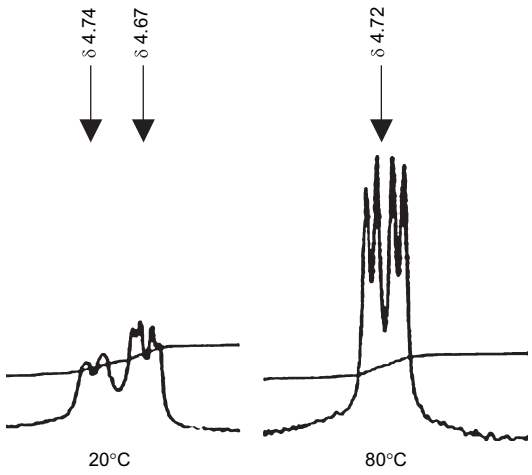


Figure 2. Parts of ^{1}H NMR spectra of BocDmtOH **12** in $(CD_3)_2SO$ at 20 and 80 °C.

Although this study did indicate that there was a strong preference for one of the rotamers, it did not show whether the more abundant rotamer was *Z* or *E* and identification of the conformation of the major rotamer required a NOESY spectrum at 20 °C. Firstly, the peaks of the major rotamer were fully assigned using a COSY spectrum and some conformational information was derived from the coupling constants. Interestingly, the signals for the Gly methylene protons were widely separated by some 0.47 ppm, which suggested a conformation in which they were located relatively close in space to the chiral 4-C of the thiazolidine. In the 2-(4-nitrophenyl)ethyl unit, the protons of the methylene adjacent to the amide nitrogen were magnetically inequivalent (δ 3.39 and 3.47), as expected, but the protons of the more remote methylene resonated as a simple triplet, reflecting its greater distance from the chiral centre. In the thiazolidine, a COSY cross-peak was seen between 4-H and 5_β-H (δ 3.31) but not between 4-H and 5_α-H (δ 3.10); this lack of a cross-peak is consistent with the multiplicity of the 5_α-H signal as a simple doublet, coupling only to its geminal partner. The lack of observed coupling between 4-H and 5_α-H points to a dihedral angle close to 90° between these protons. In the NOESY spectrum, a cross-peak was seen between 4-H and 5_α-H but no peak was seen between 4-H and 5_β-H, confirming the above assignment. A strong cross-peak diagnostic for the identity of the major rotamer was present between 4-H and the downfield Gly methylene proton, whereas a weak peak was seen between 4-H and the other Gly methylene proton. Cross peaks between the geminal dimethyl unit and the Gly methylene protons were absent, confirming that the glycine was close in space to the 4-C of the thiazolidine.

Similar conformational studies were carried out on the other three Boc-protected dipeptide amides in the *R*-Dmt series. The 1-D ^{1}H NMR spectra of **20b–d** in $(CD_3)_2SO$ at 20 °C each showed only one set of signals, indicating the presence of only one rotamer about the tertiary amide bond in each case. The identity of the single rotamer of the Ala analogue **20b** was established by a NOESY experiment, which showed a connectivity between Ala α-H and the thiazolidine 4-H. Correlations between the geminal methyls and all the protons of the BocAla moiety were correspondingly absent, confirming the *Z* tertiary amide conformation for the sole rotamer of **20b**. The spectra of **20c** and **20d** were similarly indicative of the *Z* rotamers. Thus, all four members of this L,*R* diastereomeric series exist either predominantly or exclusively in this conformation in DMSO solution, which is the one required for cyclisation.

5.2. Crystal structure of Boc-L-Ala-*R*-Dmt *N*-(2-(4-nitrophenyl)ethyl)amide 20b

Boc-L-Ala-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **20b** formed crystals, from ethyl acetate/hexane, of a suitable quality for X-ray crystallography. The crystal structure is shown in Figure 3. This molecule, which exists in DMSO solution as the *Z*

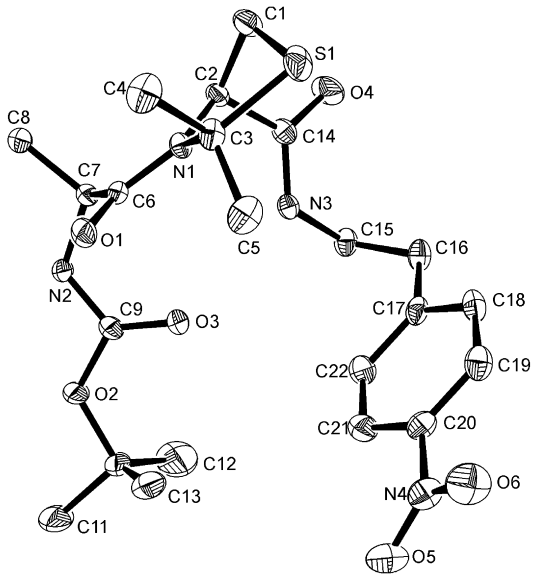


Figure 3. X-ray crystal structure plot of one molecule within the asymmetric unit of Boc-L-Ala-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **20b**, showing crystallographic numbering. Ellipsoids are represented at 30% probability and hydrogens are omitted for clarity.

rotamer, also adopts this conformation in the solid state. In addition to any conformational preference, which may be driven by steric factors, there is an intramolecular hydrogen bond tying the Boc carbonyl oxygen at the N-terminal of the dipeptide to the secondary amide N–H at the C-terminal. The intermolecular hydrogen bonds from the central tertiary amide carbonyl oxygen to the N-terminal Boc-N–H form a chain through the crystal.

5.3. NMR studies—L,*R* diastereomeric series

In the L,*S* diastereomeric series, the conformations of the tertiary amides of two examples were examined, in addition to that of BocGly-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **20a** which, as noted above, can be considered as a member of both series. ^{1}H NMR spectra of the enantiomers Boc-D-Val-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **34** and Boc-L-Val-*S*-DmtNH$(CH_2)_2C_6H_4NO_2$ **39c** showed the presence of two rotamers in the abundance ratio of 4:1; the major rotamer was again the *cis*-amide *Z* conformer, although a variable-temperature study showed that coalescence of the sets of signals was not yet complete at the highest temperature studied (80 °C). The signals for the rotamers of most tertiary amides coalesce below this temperature and that the current observation indicates a relatively high energy barrier to rotation in this molecule. The same ratio of tertiary amide rotamers was present in the solution of the homologue Boc-L-Leu-D-DmtNH$(CH_2)_2C_6H_4NO_2$ **39e**.

Thus, the *Z*/*E* ratio of rotamers is strongly in favour of the isomer in each case and the geminal dimethyl unit at the 4-position of the thiazolidine has been effective in achieving this desired outcome. However, direct comparison of the rotamer ratios for the diastereomers Boc-L-Val-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **20c** and Boc-L-Val-*S*-DmtNH$(CH_2)_2C_6H_4NO_2$ **39c** suggests that this sterically driven bias towards the *Z* rotamer is not as effective in the latter, owing to the greater distance between the isopropyl side-chain of the Val and the *pseudo*-axial 2-methyl group on the thiazolidine. Also noteworthy is the effect of steric bulk of the side-chain of the amino-acid on the chemical shifts of the diastereotopic thiazolidine 5-protons (Table 2). In the L,*R* series (**20b–d**), the simple doublet for 5-H cis to 4-H resonated downfield to the signal for 5-H trans to 4-H but in the L,*S* (and D,*R*) diastereomeric series (**34**, **39c**, **39e**), the chemical shifts are reversed; this may reflect a subtle conformational difference in the thiazolidine ring between the two series. The chemical shifts of the corresponding protons in the Gly analogue **20a** resembled those of the L,*S*/D,*R* series.

Table 2. ^{1}H NMR chemical shifts of the diastereotopic thiazolidine 5-protons in the Boc-Aaa-DmtNH$(CH_2)_2C_6H_4NO_2$ analogues **20a–d**, **34**, **39c** and **39e**

Compound	Stereoisomer[a]	δ (5-H_{trans})[b]	δ (5-H_{cis})[c]	$\Delta\delta$ (5_{trans}-H−5_{cis}-H)
20a	*R*	3.31	3.10	+0.21
20b	L,*R*	3.24	3.32	−0.08
20c	L,*R*	3.12	3.63	−0.41
20d	L,*R*	2.89	3.20	−0.31
34	D,*R*	3.36	3.04	+0.32
39c	L,*S*	3.36	3.04	+0.32
39e	L,*S*	3.45	3.05	+0.30

[a] L/D refers to the configuration of the N-terminal amino-acid; *R*/*S* refers to the configuration of the thiazolidine.
[b] 5-H trans to 4-H.
[c] 5-H cis to 4-H.

Examination of the ^{1}H NMR spectra of the dipeptide amide salts **21a–d** showed the presence of only one conformer in solution; this is presumably the *Z* tertiary amide rotamer, in the light of the similarity of the spectra to those of the *N*-Boc-protected precursors **20a–d**.

NOESY spectroscopy of the dipeptide pentafluorophenyl ester hydrochloride salts **22c** and **22d** gave some contrasting conformational information. The ^{1}H NMR spectrum of **22c** showed the presence of only one conformer in DMSO solution. Whereas the corresponding NOESY spectrum did allow assignment of the signals for the individual 5-H protons, no cross peaks were seen between signals for protons on the Val moiety and those on the thiazolidine; thus the sole rotamer could not be identified. However, in the case of the Phe analogue **22d**, the NOESY spectrum of a DMSO solution revealed through-space interaction between the upfield Me (δ 1.87) and the *ortho*-protons on the phenyl group. There is also connectivity between the downfield Me (δ 1.92) and the Phe α-H (δ 4.24). These data suggest that this dipeptide ester salt may be exclusively in the *E* amide conformation. This conformation may be favoured by the considerable steric bulk of the pentafluorophenyl ester driving the Phe residue towards the geminal dimethyl unit.

6. Conformational studies—diketopiperazines

As a contribution to understand the marked different rates of ring-closure to form the DKPs and to expel the 2-(4-nitrophenyl)ethylamine, NMR and crystallographic studies were carried out on the various DKPs. The *cyclo*-L-Ala-*R*-Dmt and *cyclo*-L-Val-*R*-Dmt DKPs **23b** and **23c** were crystalline

compounds and thus amenable for the determination of conformation in the solid state by X-ray crystallography. Although there have been several studies reported in the literature on the conformations of prolyl DKPs,[29–32] there are no reports to date for similar studies with their thiaproline analogues.

6.1. NMR studies—L,*R* diastereomeric series

DKP **23d**, derived from L-Phe and *R*-2,2-dimethylthiazolidine-4-carboxylic acid, formed a non-crystallisable gum and an attempt was made to determine its conformation in solution by NOESY spectroscopy and by examination of ^{1}H–^{1}H NMR coupling constants. Although the former was relatively uninformative about conformation, it did serve to assign the two singlet signals due to the methyl groups and to distinguish the signals for the two 1-H protons. This NOESY spectrum showed NOE connectivity between one 1-H double doublet (δ 3.26) and the 8a-H (δ 4.53); thus the signal at δ 3.26 is due to the 1-H on the lower (α) face of the molecule. NOE connectivity was also seen between the upfield methyl singlet at δ 1.87 and the β-face 1-H at δ 3.19; thus this upfield singlet signal (δ 1.87) is due to the methyl on the β-face. Another NOE connection was seen between the *ortho*-protons of the phenyl group and 6-H, suggesting that the phenyl group was pointing away from the heterocycle.

The boat conformation of the DKP ring of **23d** was confirmed by the presence of the five-bond coupling ^{5}J= 0.8 Hz between the axial 8a-H and the axial 6-H.[33] Therefore, the $PhCH_2$ side-chain is in an equatorial position and is far from the geminal dimethyl unit. The thiazolidine is probably in the half-chair conformation, with a *trans*-diaxial coupling ^{3}J=11.3 Hz between 8a-H and 1_{β}-H. The corresponding axial–equatorial coupling to 1_{α}-H is ^{3}J=5.9 Hz. A four-bond coupling ^{4}J=1.6 Hz is observed between 8a-H and NH, as seen for **23b**. The coupling constants from the Ph-CH_2 protons to 6-H are ^{3}J=10.2 and 3.9 Hz; thus the Ph cannot be antiperiplanar to the 6-H, as a coupling constant of 10.2 Hz is only consistent with antiperiplanar H–C–C–H; the phenyl is either orientated towards the nitrogen or towards the carbonyl oxygen in a staggered conformation. These data contrast with those reported[33] for the analogous ditryptophenaline, in which the two corresponding coupling constants between Ph-CH_2 and 6-H are ^{3}J=4.4 and 3.1 Hz; these values are used to support a conformation with Ph located over the DKP ring. We have also noted a similar conformation for *cyclo*-L-Phe-D-Pro.[34] However, the data for **23d** are consistent with the X-ray structure-derived conformation reported[35] for *cyclo*-L-Phe-L-Pro, in which the DKP is boat conformation, CH_2Ph is equatorial and the phenyl is antiperiplanar to the carbonyl. Further evidence for the boat conformation of the DKP ring is provided by the five-bond coupling ^{5}J=0.8 Hz between 8a-H and 6-H. Maes et al.[33] observed a similar coupling in ditryptophenaline, a natural product from *Aspergillus flavus* var. *columnaris*, which contains a *cyclo*-L-Phe-L-Pro unit fused to a heterocycle through the Pro side-chain loop, and used it to confirm that the DKP is in a boat conformation in solution with the corresponding protons in axial and cis. Thus 8a-H and 6-H in **23d** are also shown to be 1,4-diaxial and cis, an arrangement only available in a boat conformation.

6.2. Crystal structure of *cyclo*-L-Ala-*R*-Dmt 23b

A crystallographic quality crystal of *cyclo*-L-Ala-*R*-Dmt **23b** was grown from ethyl acetate/hexane and the X-ray crystal structure was determined. Figure 4 shows the intermolecular hydrogen-bonding pattern evident in the crystal and the structure of a single molecule, together with the crystallographic numbering scheme. The intermolecular hydrogen-bonding forms a ribbon through the crystal, with the N–H being hydrogen-bonded to the corresponding secondary amide carbonyl of the adjacent molecule. The tertiary amide carbonyl (from the dimethylthiaproline unit) does not participate in hydrogen-bonding. Within an individual molecule of **23b**, the DKP ring is in a flattened boat conformation, with the thiazolidine in a half-chair. This ring conformation places the geminal methyl groups in equatorial and axial positions; the 6-methyl occupies a *pseudo*-equatorial position. Notably, this 6-methyl group is remote in space from the geminal dimethyl unit and, indeed, from other sterically bulky groups.

NMR observations show that a similar conformation is also likely to be adopted in solution in chloroform. The coupling constants between the axial 8a-H and the 1_{β}-H and 1_{α}-H protons are ^{3}J=10.2 and 6.6 Hz, respectively. These values are consistent with the crystallographic dihedral angles (8a-H)-(8a-C)-(1-C)-(1_{β}-H) of 166° and (8a-H)-(8a-C)-(1-C)-(1_{α}-H) of 44°, according to the Karplus relationship. The axial 6-H couples with the adjacent N–H by ^{3}J=1.0 Hz, which corresponds to the crystallographic dihedral angle of 97°. Longer-range couplings are also observed. A four-bond coupling ^{4}J=1.6 Hz is seen between 8a-H and the NH; the coupling path between these atoms contains four atoms in one plane and one terminal atom out of that plane, thus the W-arrangement is almost adopted. A five-bond coupling

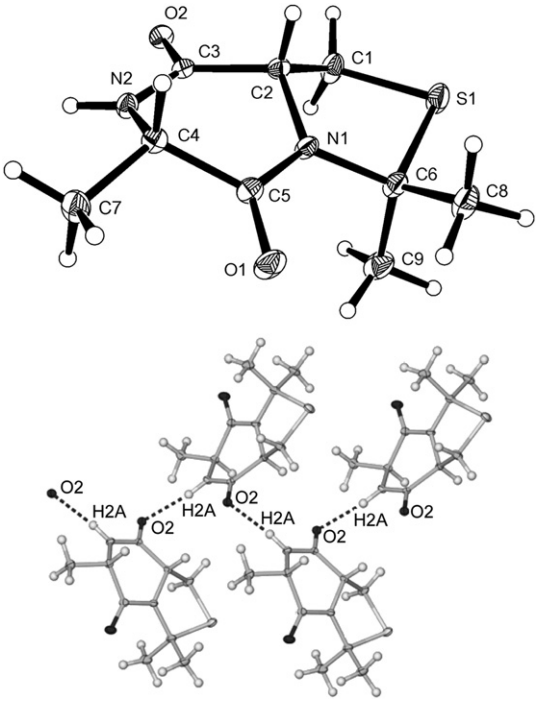


Figure 4. (Upper) Plot of the asymmetric unit in the X-ray crystal structure of *cyclo*-L-Ala-*S*-Dmt **23b**. Ellipsoids are represented at 30% probability. (Lower) Intermolecular hydrogen-bonding pattern for **23b** in the crystal.

^5J=0.8 Hz was observed between the axial 8a-H and the axial 6-H, confirming the boat conformation.[33]

6.3. Crystal structure of *cyclo*-L-Val-*R*-Dmt 23c

As for *cyclo*-L-Ala-*R*-Dmt **23b**, a crystal of *cyclo*-L-Val-*R*-Dmt **23c** was grown from ethyl acetate/hexane and the X-ray crystal structure was determined. Figure 5 shows the intermolecular hydrogen-bonding pattern evident in the crystal and the structure of a single molecule, together with the crystallographic numbering scheme. The intermolecular hydrogen-bonding motif in this crystal is different from that in the crystal of *cyclo*-L-Ala-*R*-Dmt **23b**, above, in that the hydrogen-bonded pairs are formed between the N–H hydrogens of each of a pair of molecules and the secondary amide carbonyls. Again, the tertiary amide carbonyls do not take part in hydrogen-bonding.

The X-ray crystallographic study of *cyclo*-L-Val-*R*-Dmt **23c** shows that this molecule adopts a conformation similar to that of *cyclo*-L-Ala-*R*-Dmt **23b**. Again, the DKP ring is in a boat conformation, with the thiazolidine in a half-chair. The geminal methyl groups at position-3 are in equatorial and axial positions; the 6-isopropyl occupies a *pseudo*-equatorial position. This 6-isopropyl group is again remote in space from the bulky geminal dimethyl unit. The solution conformation of *cyclo*-L-Val-*R*-Dmt **23c** in $(CD_3)_2SO$ is similar, as shown by the ^{1}H NMR spectrum. The ^3J coupling constants between the axial 8a-H and the 1_β-H and 1_α-H are 11.7 and 5.9 Hz, respectively, and are again consistent with the dihedral angles (8a-H)-(8a-C)-(1-C)-(1_β-H) of 169° and (8a-H)-(8a-C)-(1-C)-(1_α-H) of 41°. In this case, the coupling between the axial 6-H and the adjacent N–H is too small to be resolved; the dihedral angle in the crystal structure is 98°. The diagnostic five-bond coupling ^5J= 1.2 Hz was again observed between the axial 8a-H and the axial 6-H, confirming the boat conformation in solution.[33] Turning to the conformation of the side-chain, a ^3J=2.3 Hz coupling is seen between 6-H and the adjacent Me_2CH; in the solid state, the corresponding dihedral angle is 70°. Interestingly, Young et al.[32] reported an analogous coupling constant ^3J=2.7 Hz for coupling between the Val α-H and the Val β-H in *cyclo*-L-Val-L-Pro, which, together with lanthanide shift-reagent studies, is interpreted as showing that this DKP adopts the same conformation with respect to the isopropyl side-chain.

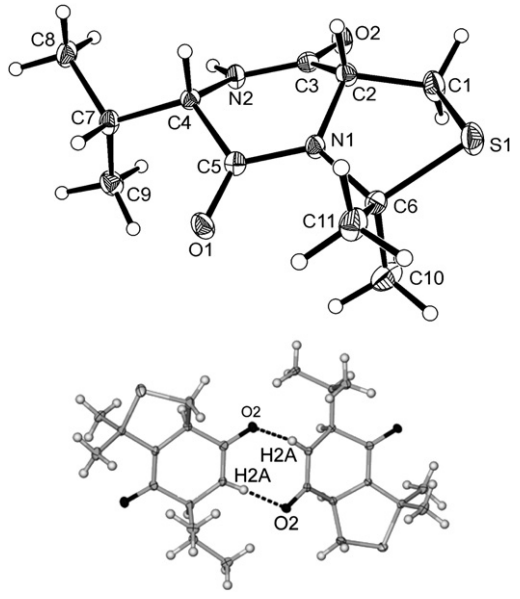


Figure 5. (Upper) Plot of one molecule in the asymmetric unit of *cyclo*-L-Val-*R*-Dmt **23c**. Ellipsoids are represented at 30% probability. (Lower) Intermolecular hydrogen-bonding pattern for **23b** in the crystal.

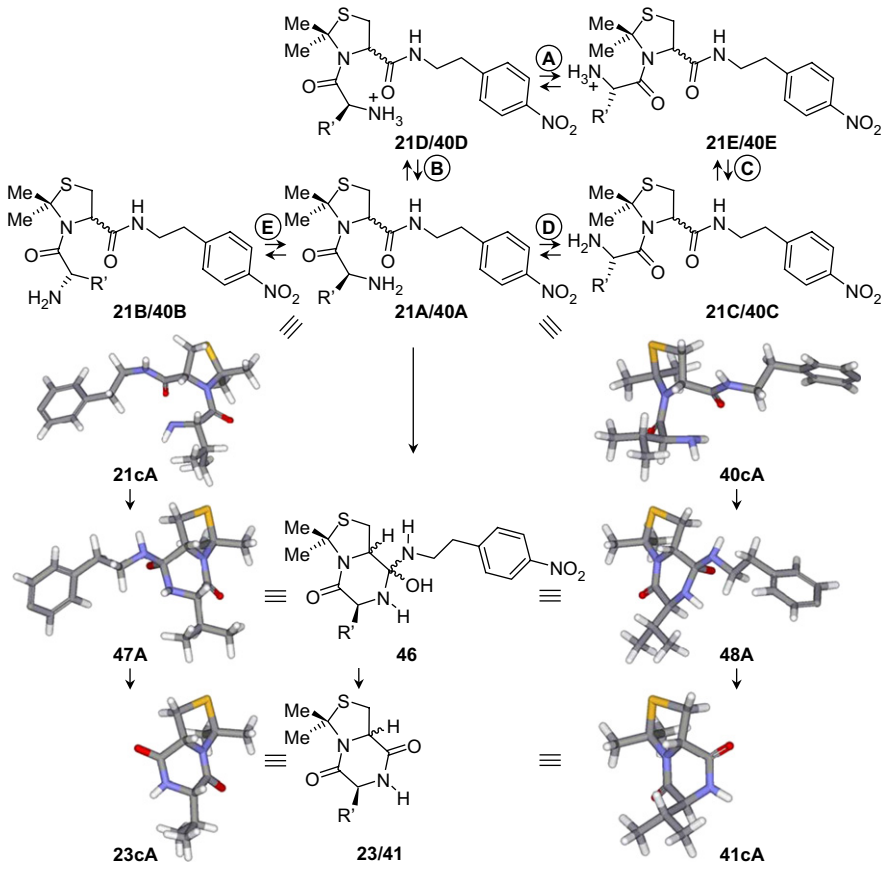
6.4. NMR studies—L,*S* diastereomeric series

In the L,*S* diastereomeric series, neither *cyclo*-L-Val-*S*-Dmt **41c** nor *cyclo*-L-Leu-*S*-Dmt **41e** formed crystals of a suitable quality for crystallography. However, ^{1}H NMR spectroscopy allowed some inferences to be made about their conformations in solution.

In contrast to the L,*R* series, where the coupling constant between 6-H and the adjacent N–H was very small, this coupling was much larger in the L,*S* series, with ^3J=3.5 Hz for **41c** and with ^3J=5.0 Hz for **41e**. These values are inconsistent with the boat conformations of the DKP rings but indicate that the DKPs are likely to be flattened from the boat form. This flattening would relieve steric compression between the 6-side-chain substituents (Me_2HC– or Me_2CHCH_2–) and 8a-H. Indeed the coupling constants show that the flattening is greater in **41c** than in **41e**, reflecting the greater steric demand of the isopropyl group compared to that of the 2-methylpropyl group. With the DKP tending towards planarity, the side chains are remote in space from the geminal dimethyl unit. As expected, 8a-H did not couple with 6-H in either compound in this series, since these protons are now trans and no longer 1,4-diaxial. The conformations of the five-membered rings are likely to be half chairs, as indicated by the coupling constants between 8a-H (now on the β-face) and 1_α-H (10.9 Hz for **41c** and 10.5 for **41e**) and between 8a-H and 1_β-H (^3J=5.5 Hz for **41c** and ^3J=5.9 Hz for **41e**).

7. Conclusions

At pH 8.0 and 7.0, there are trends relating the bulk of the side-chain of the N-terminal amino-acid and the rate of cyclisation. There are also marked differences in the rate of cyclisation between the diastereoisomers. As shown in Table 1, in the L,*R* series, the fastest rates are observed for L-Ala-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **21b** at both pH values. Increasing the steric bulk of the side-chain from Me to isopropyl or benzyl (in L-Val-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **21c** or L-Phe-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **21d**, respectively) slows the cyclisation by ca. seven-fold. Interestingly, the smallest analogue, Gly-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **21a**, also cyclised some three-fold slower than did **21b**. Scheme 7 shows some of the relevant conformational and prototropic equilibria involved in the cyclisation, which aid in the rationalisation of these observations. Firstly, cyclisation can only occur from the free-base forms **21A/40A**, **21B/40B** and **21C/40C**, in which the amine is nucleophilic, rather than from the cationic protonated forms **21D/40D** and **21E/40E**. However, the prototropic equilibria B and C would be rapid and the pK_a of **21D/40D** and **21E/40E** would be expected to be largely independent of the steric bulk of the



Scheme 7. Cyclisation of dipeptide *N*-(2-(4-nitrophenyl)ethyl)amides to form diketopiperazines, with expulsion of **24**, showing selected conformations and prototropic equilibria of starting dipeptide amides and reacting conformations of intermediates. The sequence **21A/40A**→**46**→**23/41** shows the general course of the reaction; sequences **21cA**→**47A**→**23cA** and **40cA**→**48A**→**41cA** show the specific structures for the diastereoisomers of the L-Val-derived analogues. Structures **21cA**, **47A**, **40cA**, **48A** and **41cA** are derived from energy-minimising MM2 calculations; structure **23cA** is an X-ray crystal structure. In structures **21cA**, **47A**, **40cA** and **48A**, the nitro group has been omitted for clarity.

side-chain R′. Thus, equilibria B and C can be discounted as sources of the differences in rates of ring-closure. As the core peptide bond in all the dipeptides is a tertiary amide, the populations of the *Z* and *E* amide rotamers in the reaction mixtures could be predicted to be an important determinant of the rate of cyclisation, since only the Z conformer places the primary amine sufficiently close to the secondary amide carbonyl carbon, the electrophile in the key step. Since the rate of interconversion of the tertiary amide rotamers is relatively slow at ambient temperature, the 2,2-dimethylthiazolidine unit was designed to bias the equilibrium (equilibrium D, Scheme 7) towards the required *Z* conformer **21A/40A**. As noted above, it was not possible to measure the populations of the tertiary amide bond rotamers directly in the free-base dipeptide amides **21A/40A**, owing to the possible perturbation of the equilibrium by the cyclisation reaction consuming one of the components. However, ^{1}H NMR spectra of the protonated (and thus unreactive) forms of salts **21a–d** showed that each existed solely in the *Z* tertiary amide conformation, irrespective of the bulk of the side-chain R′. Moreover, the state of protonation of L-Ala-L-ProNH_2 has been reported to have little effect on the cis/trans conformational equilibrium.[14] Thus, equilibrium A is not relevant to the effects of side-chain bulk on the rate of cyclisation. Similarly, as discussed above, the electrically neutral *N*-Boc precursors **20a–d** were shown to exist solely or >90% as the *Z* tertiary amide rotamers. Taken together, these conformational data suggest that the free-base dipeptide amides are likely to be all (almost) exclusively in the reactive *Z* tertiary amide conformation.

If the bulk of the side-chain R′ does not influence the rate of cyclisation by perturbing the *Z*/*E* tertiary amide rotamer population in the starting materials, then the question remains at which point in the cyclisation process does the nature of R′ have its effect? Since the primary amine has to be located close to the secondary amide carbonyl as the molecules move towards the first transition state, the conformation about the carbonyl–C_α bond in the N-terminal amino-acid is also important, i.e., the dipeptides must adopt conformer **21A** (Scheme 7), rather than conformers such as **21B**, in which the amine is pointing away from the target secondary amide. No data are available on the populations of these two types of conformers but one may speculate, on steric grounds, that the unreactive conformer **21** was more likely when R′=H in Gly-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **21a** and the reactive conformer **21** is more favoured when R′ is large, in L-Ala-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **21a**, L-Val-*R*-DmtNH$(CH_2)_2$-$C_6H_4NO_2$ **21c** and L-Phe-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **21d**. This would rationalise the slow cyclisation of the Gly analogue.

Now, comparing the rates of formation of DKPs from the remaining members of the L,*R* diastereomeric series, is the steric bulk of R′ exerting its effect on the first step of

the reaction, as the primary amine of the reactive conformers **21** attack the secondary amide carbonyl to form the tetrahedral intermediates **46** or is this effect evident in the second step of the reaction, the collapse of the intermediate to form the DKP **23/41** with expulsion of the leaving group 2-(4-nitrophenyl)ethylamine **24**? To answer this question, the structures of the key intermediates were compared. Structure **21cA** (Scheme 7) is a 3-D representation of the reacting conformation of **21c** (R′=isopropyl, derived from L-Val), generated using a MM2-minimised structure. Similarly, structure **47A** is a 3-D representation of the minimised structure of intermediate **46** (R′=isopropyl, derived from L-Val) and structure **23cA** is a 3-D representation of the crystal structure of DKP **23c** (R′=isopropyl, derived from L-Val). Examination of the DKP structure **23cA**, as discussed in detail above, shows that the isopropyl group is *pseudo*-equatorial in the boat conformation of the DKP ring and is thus remote from the geminal dimethyl unit and, indeed, from all other sterically demanding moieties. The conformations of the other DKPs in the L,*R* series are similar. Thus, increasing the steric bulk of R′ in this series would not cause increase in the steric crowding in the DKPs **23** and it is therefore highly unlikely that the effect of bulky R′ on slowing the overall reaction rate derives influences from the second step, the collapse of the tetrahedral intermediate. Similarly, the tetrahedral intermediates **46** are shown to have *pseudo*-chair conformations for the six-membered ring, as shown in the L-Val-derived analogue structure **47A**. In this conformation, again, the R′ group occupies a sterically open region of space and thus variations in steric bulk of R′ in this intermediate are similarly unlikely to influence the overall reaction rate significantly.

However, the detail of the conformation required for the starting dipeptide amide **21** as it enters the initial nucleophilic reaction reveals the origin of the rate-slowing effect of bulky R′. As can be seen in structure **21cA** (Scheme 7, derived from L-Val), the conformation in which the primary amine can approach the target electrophile requires a particular local conformation about the carbonyl–C_α bond in which R′ is fully eclipsing the carbonyl oxygen. Thus increasing the steric bulk of R′ will make this conformation increasingly unfavourable and thus slows the initial step of the cyclisation.

In the L,*S* diastereomeric series, which also includes Gly-*R*-DmtNH$(CH_2)_2C_6H_4NO_2$ **21a**, the opposite trend in overall cyclisation reaction rates is evident, with increasing *local* steric bulk of the side-chain R′ tending to speed the reaction (Table 1). The Gly analogue **21a** may be a special case, as noted above, involving unfavourable populations of the conformers in equilibrium E (Scheme 7). Additionally, the rates of cyclisation of the major members of this series, L-Val-*S*-DmtNH$(CH_2)_2C_6H_4NO_2$ **40c** and L-Leu-*S*-DmtNH$(CH_2)_2C_6H_4NO_2$ **40e**, are also much faster than those of the L,*R* series, with the L,*S* Val analogue **40c** reacting ca. 100 times faster than its diastereoisomer **21c**. As with the L,*R* series, examination of the structures of the reacting conformations enabled rationalisation of the relative rates. Structure **41cA** shows the conformation of the L-Val derived DKP, as discussed above. In this structure, the isopropyl group is not close in space to either of the geminal dimethyl groups, so variation of this group would not be expected to influence the energy of the DKP greatly. This group is also remote from the methyl groups in intermediate **48A**; thus steric crowding is also unimportant here. However, in the L,*S* series, as with the L,*R* series, it is the steric crowding in the reacting conformation of the initial dipeptide amide substrate that is critical. As shown in example structure **40cA** (Scheme 7), the reactive conformation of the carbonyl–C_α bond has the α-hydrogen almost eclipsing the tertiary amide carbonyl oxygen. This arrangement is clearly much more favourable on steric grounds than that in the L,*R* diastereoisomer **21cA**, with R′ eclipsing this oxygen. Thus the reacting conformation is more sterically accessible in the L,*S* series, facilitating the rapid formation of the tetrahedral cyclic intermediates. Comparing the rates of cyclisation of the L-Val analogue **40c** and the L-Leu analogue **40e**, one may then postulate that the greater steric demand of the tertiary isopropyl centre may drive the molecule more effectively into this reacting conformation than does the lesser demand of the secondary centre of the isobutyl group.

The cyclisation of peptides containing Dmt to give DKPs has not previously been studied. A related system, the cyclisation of L-alanyl-L-prolinamide in water to give *cyclo*-L-Ala-L-Pro and ammonia, was the subject of a detailed kinetic analysis by Capasso et al.[13] This is a relatively slower process than the cyclisations of aminoacyl-Dmt amides reported in this paper. In the $AlaProNH_2$ study, it was noted that the trans→cis conformational interchange of the tertiary amide was fast[36,37] and became rate-limiting only under conditions were the cyclisation was fast.[13] Interestingly, Sager et al.[38] note that the cis conformers of pentapeptides containing L-Pro or L-Dmt can be readily cyclised to give cyclic pentapeptides but the corresponding trans conformers tend to produce cyclic dimers (cyclic decapeptides) under the same conditions. Limitation of rate in this conformational interchange step may be important, in principle, in the cyclisations here, particularly those in the L-Aaa-*S*-DmtNH$(CH_2)_2C_6H_4NO_2$ diastereomeric series, but it is unlikely to affect the global rate of reaction since the vast majority of these reactant molecules are already in the cis conformation. As expected, pH had a marked effect on the rate of cyclisation of L-Ala-L-$ProNH_2$, where the trend was for the reaction to be faster at higher pH.[13] However, replotting the data to account for the fraction of the reactive free-base form in solution indicated that the cyclisation of this form was faster at high and low pH values and had a minimum between pH 7 and 10. These observations were used to support parallel mechanisms involving general-base catalysis and general-acid catalysis.[13] In the present case, there was a trend towards faster cyclisation at higher pH within the range pH 6.0–8.0, which correlated with increased fractions of the unprotonated reactive forms, but reactions at more extreme pH values (outside the physiological range) were not studied.

In this paper, we have reported the cyclisations of two diastereomeric series of aminoacyl-Dmt *N*-(2-(4-nitrophenyl)-ethyl)amides, giving the cyclic dipeptides (DKPs) and releasing 2-(4-nitrophenyl)ethylamine as a model for drugs carrying primary amines. The rate of ring-closure and release depends on the relative configurations of the two chiral centres, the L,*S* dipeptide amides react much more rapidly. Each of the Aaa-Dmt dipeptides adopts 80–100% the *cis* tertiary amide conformation; thus the rate of ring-closure is

effectively independent of cis–trans interconversion. Thus the L-Val-L-Dmt and L-Leu-L-Dmt units are shown to be highly effective self-immolative molecular clips for incorporation into prodrug linkers designed for cleavage/triggering by endopeptidases. These linkers release amine drugs rapidly, whereas the existing dipeptide cyclisation technology[11,12] is effective only in releasing OH-containing drugs where the ester is a better electrophile and the alcohol is a better leaving group. Aminoacyl-Dmt units, therefore, have great potential utility in prodrug design and development.

8. Experimental

8.1. 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, unless otherwise stated. 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 and 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 either with a Thermo Galen Kofler block (uncorrected) or with a differential scanning calorimeter.

8.2. *R*-2,2-Dimethyltetrahydrothiazole-4-carboxylic acid hydrochloride (11)

L-Cys·HCl·H_2O **10** (3.5 g, 20 mmol) was boiled under reflux in acetone (250 mL) and 2,2-dimethoxypropane (50 mL) for 6 h under N_2. The solid was collected by filtration to afford **11** (3.45 g, 87%) as a white solid: mp 125–130 °C (lit.[27] mp 165–168 °C); IR ν_{max} 3412, 2600, 1744 cm^{-1}; NMR (D_2O) δ_H 2.06 (6H, s, 2×Me), 2.92 (1H, dd, *J*=15.2, 3.9 Hz, 5-H), 3.00 (1H, dd, *J*=15.2, 5.9 Hz, 5-H), 4.06 (1H, dd, *J*=5.9, 3.9 Hz, 4-H).

8.3. *R*-3-(1,1-Dimethylethoxycarbonyl)-2,2-dimethyltetrahydrothiazole-4-carboxylic acid (12)

Compound **11** (1.0 g, 5.1 mmol) was stirred with Pr^i_2NEt (722 mg, 5.6 mmol) and di-*tert*-butyl dicarbonate (1.45 g, 6.6 mmol) in dry MeCN (10 mL) for 2 days. The evaporated residue, in Et_2O, was filtered (Celite®). The evaporated residue, in CH_2Cl_2, was washed with aq H_2SO_4 (100 mM, cold), H_2O and brine. Drying, evaporation and recrystallisation (hexane) afforded **12** (150 mg, 11%) as a white solid: mp 112–114 °C (lit.[27] mp 114–114.5 °C); NMR ($CDCl_3$, 20 °C) δ_H 1.43 (4.5H, br s) and 1.53 (4.5H, br s, Bu^t), 1.80 (4.5H, m) and 1.87 (1.5H, br s, 2×Me), 3.1–3.28 (2H, m, 5-H_2), 4.83 (1H, m) and 4.98 (1H, m, 4-H); NMR ($(CD_3)_2SO$, 20 °C) δ_H 1.35 (5.4H, br s) and 1.43 (3.6H, br s) (Bu^t), 1.70 (br s), 1.73 (br s) and 1.75 (br s, 2×Me), 3.03 (d, *J*=12.1 Hz) and 3.34 (m, 5-H_2), 4.67 (0.6H, dd, *J*=4, 2 Hz) and 4.74 (0.4H, br d, *J*=5 Hz, 4-H); NMR ($(CD_3)_2SO$, 80 °C) δ_H 1.41 (9H, s, Bu^t), 1.73 (3H, s, 2-Me), 1.77 (3H, s, 2-Me), 3.05 (1H, dd, *J*=12.1, 3.0 Hz, 5-H), 3.35 (1H, dd, *J*=12.1, 6.9 Hz, 5-H), 4.72 (1H, dd, *J*=6.9, 3.0 Hz, 4-H).

8.4. Pentafluorophenyl *R*-3-(1,1-dimethylethoxycarbonyl)-2,2-dimethyltetrahydrothiazole-4-carboxylate (13)

Compound **12** (1.10 g, 4.2 mmol) was stirred with pentafluorophenol (850 mg, 4.6 mmol) and DCC (850 mg, 4.6 mmol) in EtOAc (10 mL) at 0 °C under N_2 for 3 h. Filtration and evaporation gave **13** (1.62 g, 90%) as white needles: mp 107–109 °C (lit.[27] mp 104–105 °C); NMR δ_H 1.46 (6.3H, s, Bu^t), 1.52 (2.7H, s, Bu^t), 1.80 (0.9H, s, 2-Me), 1.83 (3H, s, 2-Me), 1.90 (2.1H, s, 2-Me), 3.28 (0.7H, br d, *J*=12.5 Hz, 5-H), 3.41 (0.3H, m, 5-H), 3.47 (1H, dd, *J*=12.5, 7.0 Hz, 5-H), 4.05 (0.3H, m, 5-H), 5.18 (0.7H, dd, *J*=6.6, 1.6 Hz, 4-H), 5.26 (0.3H, dd, *J*=6.6, 2.3 Hz, 4-H); NMR δ_F −162.2 (0.3F, m, 4′-F), −161.8 (0.7F, m, 4′-F), −157.8 (0.6F, t, *J*=21.1 Hz, 3′,5′-F_2), −157.3 (1.4F, t, *J*=21.1 Hz, 3′,5′-F_2), −152.6 (1.4F, d, *J*=17.2 Hz, 2′,6′-F_2), −151.90 (0.6F, d, *J*=17.2 Hz, 2′,6′-F_2).

8.5. *R*-2,2-Dimethyl-3-(1,1-dimethylethoxycarbonyl)-*N*-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (14)

2-(4-Nitrophenyl)ethylamine hydrochloride **24**·HCl (85 mg, 0.42 mmol) was stirred with Et_3N (85 mg, 0.84 mmol) and **13** (180 mg, 0.42 mmol) in CH_2Cl_2 (2.0 mL) for 2 h. Evaporation and chromatography ($CHCl_3$/MeOH, 9:1) afforded **14** (160 mg, 93%) as a viscous yellow oil: IR (film) ν_{max} 3325, 1681, 1519, 1346 cm^{-1}; NMR δ_H 1.43 (9H, s, Bu^t), 1.70 (3H, s, 2-Me), 1.75 (3H, s, 2-Me), 2.95 (2H, m, ArCH_2), 3.23 (2H, m, 5-H_2), 3.53 (1H, m) and 3.68 (1H, m, NHCH_2), 4.74 (1H, m, 4-H), 7.42 (2H, d, *J*=8.6 Hz, Ar 2,6-H_2), 8.18 (2H, d, *J*=8.6 Hz, Ar 3,5-H_2); NMR δ_C 19.6, 28.7, 36.0, 40.7, 67.5, 82.0, 93.7, 110.0, 124.0, 129.9, 146.9, 171.3; MS *m*/*z* 410.1764 (M+H) ($C_{19}H_{28}N_3O_5S$ requires 410.1750), 354 (M−Me_2C=CH_2), 336 (M−Bu^{t}O), 310 (M−Boc). Found: H, 6.59; N, 9.94. $C_{19}H_{27}N_3O_5S$ requires H, 6.65; N, 10.27%.

8.6. *R*-2,2-Dimethyl-*N*-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide trifluoroacetate salt (15)

Compound **14** (1.30 g, 3.2 mmol) was stirred with CF_3CO_2H (20 mL) and CH_2Cl_2 (10 mL) for 2 h. Evaporation afforded **15** (1.35 g, quant.) as a highly hygroscopic gum: NMR δ_H 1.82 (3H, s, 2-Me), 1.93 (3H, s, 2-Me), 2.97 (2H, m, ArCH_2), 3.20 (1H, dd, *J*=12.5, 6.2 Hz, 5-H), 3.59 (1H, dd, *J*=12.5, 8.2 Hz, 5-H), 3.64 (2H, m, NHCH_2), 5.22 (1H, dd, *J*=8.2, 6.2 Hz, 4-H), 7.35 (2H, d, *J*=8.6 Hz, Ar 2,6-H_2), 7.56 (1H, br t, *J*=5.9 Hz, NH), 8.15 (2H, d, *J*=8.6 Hz, Ar 3,5-H_2), 9.76 (2H, br, N^+H_2); MS *m*/*z* 310.1238 (M+H) ($C_{14}H_{20}N_3O_3S$ requires 310.1225).

8.7. 1,1-Dimethylethyl *N*-(fluorocarbonylmethyl)carbamate (17a)

BocGlyOH **16a** (1.0 g, 5.7 mmol) was stirred with pyridine (450 mg, 5.7 mmol) in dry CH_2Cl_2 (30 mL) and added

slowly to 2,4,6-trifluoro-1,3,5-triazine (1.54 g, 11.4 mmol) in dry CH_2Cl_2 (10 mL), then stirred for 3 h. The mixture was washed with ice-water (3×). Drying and evaporation afforded crude **17a** (800 mg) as a white gum: NMR δ_H 1.47 (9H, s, Bu^t), 4.11 (2H, t, J=5.5 Hz, CH_2), 5.05 (1H, br, NH); NMR δ_F 30.21 (1F, s, CO_2F).

8.8. 1,1-Dimethylethyl *S*-*N*-(1-fluorocarbonyl-2-methylpropyl)carbamate (17c)

BocValOH **16c** (2.5 g, 11 mmol) was stirred with pyridine (910 mg, 11.5 mmol) in dry CH_2Cl_2 (30 mL) and added slowly to 2,4,6-trifluoro-1,3,5-triazine (3.1 g, 23 mmol) in dry CH_2Cl_2 (30 mL). Stirring was continued under N_2 at −10 °C for 2 h. The mixture was washed with ice-water (3×100 mL). Drying and evaporation afforded crude **17c** (2.22 g) as a white solid: mp 38–42 °C (lit.[39] mp 36–38 °C); NMR δ_H 0.99 (3H, d, J=7.0 Hz, Val-Me), 1.04 (3H, d, J=6.6 Hz, Val-Me), 1.46 (9H, s, Bu^t), 2.25 (1H, m, Val β-H), 4.40 (1H, dd, J=8.6, 4.7 Hz, Val α-H), 4.96 (1H, br d, NH); NMR δ_F 32.29 (1F, s, CO_2F).

8.9. 1,1-Dimethylethyl *S*-*N*-(1-fluorocarbonyl-2-phenylethyl)carbamate (17d)

BocPheOH **16d** was treated with pyridine and 2,4,6-trifluoro-1,3,5-triazine, as for the synthesis of **17c**, to give crude **17d** (57%) as a colourless oil: NMR δ_H 1.42 (9H, s, Bu^t), 3.15 (1H, dd, J=14.4, 6.2 Hz, Phe β-H), 3.17 (1H, dd, J=14.4, 5.9 Hz, Phe β-H), 4.74 (1H, br d, J=5.9 Hz, Phe α-H), 4.86 (1H, d, J=6.6 Hz, NH), 7.10–7.30 (5H, m, Ph-H_5); NMR δ_F 29.21 (1F, s, CO_2F).

8.10. *R*-2,2-Dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)glycyl)-2,2-dimethyltetrahydrothiazole-4-carboxylic acid (18a)

Compound **17a** (810 mg, 4.6 mmol) was stirred with **11** (1.00 g, 5.1 mmol) and Pr^i_2NEt (1.24 g, 9.6 mmol) in dry DMF (100 mL) for 3 h. The evaporated residue, in EtOAc, was washed with cold citric acid and brine. Drying, evaporation and chromatography (EtOAc/Et_2O/AcOH, 13:6:1) afforded **18a** (560 mg, 35%) as a pale buff solid: mp 152–155 °C: IR ν_{max} 3385, 1748, 1672, 1625, 1539 cm^{-1}; NMR ($(CD_3)_2SO$) δ_H 1.38 (9H, s, Bu^t), 1.74 (3H, s, 2-Me), 1.76 (3H, s, 2-Me), 3.28 (2H, m, 5-H_2), 3.50 (1H, dd, J=17.2, 6.2 Hz) and 3.87 (1H, dd, J=16.8, 5.5 Hz, Gly-H_2), 5.10 (1H, m, 4-H), 6.78 (1H, t, J=6 Hz, NH); MS m/z 319.1339 (M+H) ($C_{13}H_{23}N_2O_5S$ requires 319.1328), 263 (M−Me_2C=CH_2). Found: C, 47.80; H, 6.72; N, 8.85. $C_{13}H_{22}N_2O_5S\cdot0.5H_2O$ requires C, 47.99; H, 6.51; N, 8.61%.

8.11. 1,1-Dimethylethyl *S*-*N*-(1-(fluorocarbonyl)ethyl)-carbamate (17b) and *R*-2,2-dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-alanyl)tetrahydrothiazole-4-carboxylic acid (18b)

BocAlaOH **16b** was treated with pyridine and 2,4,6-trifluoro-1,3,5-triazine, as for the synthesis of **17c**, to give crude **17b** as a white solid. This material was treated with **11**, as for the synthesis of **18a** except that the chromatographic eluant was EtOAc/Et_2O/AcOH (49:49:2), to give **18b** (30% from **11**) as a pale yellow solid: mp 93–94 °C: IR ν_{max} 3338, 1705, 1655, 1534 cm^{-1}; NMR δ_H 1.31 (3H, d, J=6.6 Hz, Ala-Me), 1.40 (9H, s, Bu^t), 1.81 (3H, s, 2-Me), 1.92 (3H, s, 2-Me), 3.24 (1H, dd, J=12.1, 5.5 Hz, 5-H), 3.45 (1H, d, J=12.1 Hz, 5-H), 4.53 (1H, qn, J=7 Hz, Ala α-H), 4.85 (1H, d, J=5.1 Hz, 4-H), 5.68 (1H, d, J=7.8 Hz, NH); MS m/z 333.1489 (M+H) ($C_{14}H_{25}N_2O_5S$ requires 333.1484), 277 (M−Me_2C=CH_2), 259 (M−Bu^{t}O).

8.12. *R*-2,2-Dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-valinyl)tetrahydrothiazole-4-carboxylic acid (18c)

Compound **17c** was treated with **11**, as for the synthesis of **18a** except that the chromatographic eluant was hexane/EtOAc/AcOH (70:29:1), to give **18c** (27%) as a colourless gummy solid: IR ν_{max} 3338, 1705, 1655, 1534 cm^{-1}; NMR δ_H 0.90 (3H, d, J=7.0 Hz, Val-Me), 1.0 (3H, d, J=6.6 Hz, Val-Me), 1.39 (9H, s, Bu^t), 1.82 (3H, s, 2-Me), 1.88 (1H, m, Val β-H), 1.92 (3H, s, 2-Me), 3.21 (1H, dd, J=12.1, 5.5 Hz, 5-H), 3.44 (1H, d, J=12.1 Hz, 5-H), 4.50 (1H, dd, J=9.4, 5.5 Hz, Val α-H), 5.02 (1H, d, J=5.1 Hz, 4-H), 5.60 (1H, d, J=9.4 Hz, NH), 8.52 (1H, br, OH); MS m/z 361.1822 (M+H) ($C_{16}H_{29}N_2O_5S$ requires 361.1797), 305 (M−Me_2C=CH_2), 261 (M−Boc).

8.13. *R*-2,2-Dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-phenylalanyl)tetrahydrothiazole-4-carboxylic acid (18d)

Compound **17d** was treated with **11**, as for the synthesis of **18a** except that the chromatographic eluant was EtOAc/AcOH (99:1), to give **18d** (30%) as a pale yellow gum: NMR δ_H 1.29 (9H, s, Bu^t), 1.68 (3H, s, 2-Me), 1.77 (3H, s, 2-Me), 2.80 (1H, dd, J=13.3, 7.1 Hz, Phe β-H), 2.88 (1H, dd, J=13.3, 6.1 Hz, Phe β-H), 2.97 (1H, dd, J=11.7, 5.9 Hz, 5-H), 3.12 (1H, d, J=11.7 Hz, 5-H), 4.34 (1H, ddd, J=9.0, 7.1, 6.1 Hz, Phe α-H), 4.68 (1H, d, J=5.9 Hz, 4-H), 6.50 (1H, d, J=9.0 Hz, NH), 7.26 (5H, m, Ph-H_5); MS m/z 409.1812 (M+H) ($C_{20}H_{29}N_2O_5S$ requires 409.1797), 353 (M−Me_2C=CH_2), 335 (M−Bu^{t}O), 309 (M−Boc).

8.14. Pentafluorophenyl *R*-2,2-dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)glycyl)tetrahydrothiazole-4-carboxylate (19a)

Compound **18a** (450 mg, 1.4 mmol) was stirred with pentafluorophenol (290 mg, 1.6 mmol) and DCC (330 mg, 1.6 mmol) in EtOAc (5.0 mL) at 0 °C under N_2 for 3 h. The mixture was filtered (Celite®) and the solid was washed with cold EtOAc. The evaporated residue, in hexane, was kept at 4 °C for 16 h and filtered. Evaporation gave **19a** (700 mg, 92%) as a colourless oil: NMR δ_H 1.45 (9H, s, Bu^t), 1.88 (3H, s, 2-Me), 1.92 (3H, s, 2-Me), 3.45 (1H, dd, J=12.5, 5.5 Hz, 5-H), 3.51 (1H, d, J=12.1 Hz, 5-H), 3.67 (1H, dd, J=17.2, 3 Hz) and 4.13 (1H, dd, J=17.2, 6.6 Hz) (Gly-H_2), 5.20 (1H, d, J=5.3 Hz, 4-H), 5.37 (1H, br, NH); NMR δ_F −161.3 (2F, m, 3′,5′-F_2), −156.4 (1F, t, J=21.4 Hz, 4′-F), −152.0 (2F, d, J=16.8 Hz, 2′,6′-F_2); MS m/z 485.1185 (M+H) ($C_{19}H_{22}F_5N_2O_5S$ requires 485.1170), 429 (M−Me_2C=CH_2), 411 (M−Bu^{t}O), 385 (M−Boc).

8.15. Pentafluorophenyl *R*-2,2-dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-alanyl)tetrahydrothiazole-4-carboxylate (19b)

Compound **18b** was treated with pentafluorophenol and DCC, as for the synthesis of **19a**, to give **19b** (89%) as a colourless oil: NMR δ_H 1.36 (2.1H, d, J=6.6 Hz, Ala-Me), 1.37 (0.9H, d, J=6.6 Hz, Ala β-H_3), 1.39 (6.3H, s, Bu^t), 1.43 (2.7H, s, Bu^t), 1.85 (2.1H, s, 2-Me), 1.94 (2.1H, s, 2-Me), 2.05 (0.9H, s, 2-Me), 2.09 (0.9H, s, 2-Me), 3.33 (0.3H, dd, J=12.7, 2.9 Hz, 5-H), 3.40 (0.7H, dd, J=12.5, 5.5 Hz, 5-H), 3.46 (0.3H, m, 4-H), 3.49 (0.7H, d, J=12.1 Hz, 4-H), 4.39 (0.7H, qn, J=7 Hz, Ala α-H), 4.79 (0.3H, qn, J=7 Hz, Ala α-H), 5.19 (0.7H, d, J=5.5 Hz, 4-H), 5.24 (0.3H, br, NH), 5.40 (0.7H, d, J=7.8 Hz, NH), 5.57 (0.3H, dd, J=6.6, 3.1 Hz, 4-H); NMR δ_F −161.7 (0.6F, m, 3′,5′-F_2), −161.6 (1.4F, dt, J=21.0, 18.4 Hz, 3′,5′-F_2), −157.1 (0.3F, t, J=21.0 Hz, 4′-F), −156.7 (0.7F, t, J=21.0 Hz, 4′-F), −151.9 (0.6F, d, J=17.1 Hz, 2′,6′-F_2), −150.8 (1.4F, d, J=17.1 Hz, 2′,6′-F_2); MS *m/z* 499.1335 (M+H) ($C_{20}H_{24}F_5N_2O_5S$ requires 499.1326), 443 (M−Me_2C=CH_2), 425 (M−Bu^{t}O).

8.16. Pentafluorophenyl 2,2-dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-valinyl)tetrahydrothiazole-4-carboxylate (19c)

Compound **18c** was treated with pentafluorophenol and DCC, as for the synthesis of **19a**, to give **19c** (93%) as a colourless oil: IR (film) ν_{max} 3440, 1718, 1669, 1521 cm^{-1}; NMR δ_H 0.94 (2.1H, d, J=6.6 Hz, Val-Me), 0.99 (2.1H, d, J=6.6 Hz, Val-Me), 1.03 (0.9H, d, J=7.0 Hz, Val-Me), 1.10 (0.9H, d, J=7.0 Hz, Val-Me), 1.33 (6.3H, s, Bu^t), 1.43 (2.7H, s, Bu^t), 1.86 (2.1H, s, 2-Me), 1.91 (0.7H, m, Val β-H), 1.95 (2.1H, s, 2-Me), 1.99 (1.8H, s, 2×2-Me), 3.32 (0.3H, dd, J=12.5, 3.5 Hz, 5-H), 3.40 (0.7H, dd, J=12.5, 5.5 Hz, 5-H), 3.44 (0.3H, d, J=6.2 Hz, 5-H), 3.50 (0.7H, d, J=12.1 Hz, 5-H), 4.28 (0.7H, dd, J=9.0, 6.2 Hz, Val α-H), 4.54 (0.3H, dd, J=9.8, 6.2 Hz, Val α-H), 5.12 (0.3H, d, J=10.2 Hz, NH), 5.24 (0.7H, d, J=9.0 Hz, NH), 5.38 (0.7H, d, J=4.7 Hz, 4-H), 5.62 (0.3H, dd, J=6.6, 3.5 Hz, 4-H); NMR δ_F −161.9 (1.4F, dd, J=22.4, 18.4 Hz, 3′,5′-F_2), −161.6 (0.6F, dd, J=21.0, 17.1 Hz, 3′,5′-F_2), −157.1 (0.7F, dt, J=22.4 Hz, 4′-F), −152.6 (0.3F, dt, J=17.1 Hz, 4′-F), −151.8 (0.6F, d, J=17.1 Hz, 2′,6′-F_2), −150.3 (1.4F, d, J=18.4 Hz, 2′,6′-F_2); MS *m/z* 527.1660 (M+H) ($C_{22}H_{28}F_5N_2O_5S$ requires 527.1639), 471 (M−Bu^{t}O), 427 (M−Boc), 328 (M−BocVal).

8.17. Pentafluorophenyl *R*-2,2-dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-phenylalanyl)tetrahydrothiazole-4-carboxylate (19d)

Compound **18d** was treated with pentafluorophenol and DCC, as for the synthesis of **19a**, to give **19d** (92%) as a white solid: mp 35-36 °C; IR ν_{max} 3442, 1792, 1716, 1659, 1523 cm^{-1}; NMR δ_H 1.39 (9H, s, Bu^t), 1.77 (3H, s, 2-Me), 1.86 (3H, s, 2-Me), 2.40 (1H, dd, J=12.5, 5.5 Hz, 5-H), 2.89 (1H, dd, J=12.5, 10.9 Hz, Phe β-H), 3.00 (1H, d, J=12.5 Hz, 5-H), 3.25 (1H, dd, 12.5, 3.9 Hz, Phe β-H), 4.37 (1H, d, J=5.5 Hz, 4-H), 4.51 (1H, m, Phe α-H), 5.42 (1H, d, J=7.8 Hz, NH), 7.33 (5H, m, Ph-H_5); NMR δ_F −161.8 (2F, dd, J=21.0, 17.1 Hz, 3′,5′-F_2), −156.9 (1F, t, J=21.0 Hz, 4′-F), −150.7 (1F, d, J=18.4 Hz, 2′,6′-F_2); MS *m/z* 575.1635 (M+H) ($C_{26}H_{28}F_5N_2O_5S$ requires 575.1639), 519 (M−Me_2C=CH_2), 501 (M−Bu^{t}O), 475 (M−Boc).

8.18. *R*-2,2-Dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)glycyl)-*N*-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (20a)

Compound **19a** was treated with **24**·HCl and Et_3N, as for the synthesis of **14** except that the chromatographic eluant was EtOAc, to give **20a** (76%) as a white solid: mp 65–66 °C; NMR ($(CD_3)_2SO$, 80 °C) δ_H 1.38 (9H, s, Bu^t), 1.72 (3H, s, 2-Me), 1.74 (3H, s, 2-Me), 2.91 (2H, t, J=7.4 Hz, $ArCH_2$), 3.10 (1H, d, J=12.9 Hz, 5$_α$-H), 3.20 (1H, dd, J=16.4, 5.5 Hz, Gly-H), 3.31 (1H, m, 5$_β$-H), 3.39 (1H, m) and 3.47 (1H, m) ($ArCH_2CH_2$), 3.67 (1H, dd, J=16.4, 5.9 Hz, Gly-H), 4.79 (1H, d, J=5.5 Hz, 4-H), 6.67 (1H, br t, NH), 7.50 (2H, d, J=8.6 Hz, Ar 2,6-H_2), 8.13 (2H, d, J=8.6 Hz, Ar 3,5-H_2); MS *m/z* 467.1943 (M+H) ($C_{21}H_{31}N_4O_6S$ requires 467.1964), 411 (M−Me_2C=CH_2), 393 (M−Bu^{t}O), 367 (M−Boc).

8.19. *R*-2,2-Dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-alanyl)-*N*-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (20b)

Compound **19b** was treated with **24**·HCl and Et_3N, as for the synthesis of **14** except that the chromatographic eluant was EtOAc/hexane (4:1), to give **20b** (75%) as a white solid: mp 156–158 °C: IR ν_{max} 3319, 1696, 1657, 1601, 1520 cm^{-1}; NMR ($(CD_3)_2SO$) δ_H 1.12 (3H, d, J=7.0 Hz, Ala-Me), 1.37 (9H, s, Bu^t), 1.61 (3H, s, 2-Me), 1.71 (3H, s, 2-Me), 2.95 (2H, m, $ArCH_2$), 3.24 (1H, dd, J=12.1, 5.5 Hz, 5$_β$-H), 3.32 (2H, m, 5$_α$-H+$ArCH_2CH$), 3.53 (1H, m, $ArCH_2CH$), 3.84 (1H, m, Ala α-H), 4.73 (1H, d, J=5.5 Hz, 4-H), 7.06 (1H, d, J=5.9 Hz, NH), 7.49 (2H, d, J=9.0 Hz, Ar 2,6-H_2), 8.13 (2H, d, J=8.6 Hz, Ar 3,5-H_2) 8.13 (1H, br, NH); MS *m/z* 481.2142 (M+H) ($C_{22}H_{33}N_4O_6S$ requires 481.2121), 425 (M−Me_2C=CH_2), 381 (M−Boc).

8.20. *R*-(2,2-Dimethyl-3-(1,1-dimethylethoxycarbonyl)-L-valinyl)-*N*-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (20c)

Compound **19c** was treated with **24**·HCl and Et_3N, as for the synthesis of **14** except that the chromatographic eluant was EtOAc/hexane (1:1), to give **20c** (73%) as a white solid: mp 233.8 °C (DSC); IR ν_{max} 3331, 1700, 1653, 1519 cm^{-1}; NMR δ_H 0.95 (3H, d, J=6.6 Hz, Val-Me), 1.01 (3H, d, J=6.6 Hz, Val-Me), 1.44 (9H, s, Bu^t), 1.65 (3H, s, 2-Me), 1.81 (3H, s, 2-Me), 1.87 (1H, m, Val β-H), 3.02 (2H, m, $ArCH_2$), 3.12 (1H, dd, J=12.1, 5.5 Hz, 5$_β$-H), 3.50 (1H, m, $ArCH_2CH$), 3.63 (1H, d, J=12.1 Hz, 5$_α$-H), 3.75 (1H, m, Val α-H), 3.79 (1H, m, $ArCH_2CH$), 4.67 (1H, d, J=5.5 Hz, 4-H), 4.90 (1H, d, J=7.8 Hz, NHBoc), 7.38 (2H, d, J=9.0 Hz, Ar 2,6-H_2), 7.98 (1H, br, N$H$$CH_2$), 8.14 (2H, d, J=8.6 Hz, Ar 3,5-H_2); MS *m/z* 509.2434 (M+H) ($C_{24}H_{37}N_4O_6S$ requires 509.2434), 453 (M−Bu^{t}O), 409 (M−Boc), 310 (M−BocVal).

8.21. *R*-2,2-Dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-phenylalanyl)-*N*-(2-(4-nitrophenyl)ethyl)-tetrahydrothiazole-4-carboxamide (20d)

Compound **19d** was treated with **24**·HCl and Et_3N, as for the synthesis of **20c**, to give **20d** (95%) as a white solid: mp 45–46 °C: IR ν_{max} 3442, 1699, 1652, 1518 cm^{-1}; NMR δ_H 1.43 (9H, s, Bu^t), 1.58 (3H, s, 2-Me), 1.60 (3H, s, 2-Me), 2.30 (1H, dd, J=11.7, 5.9 Hz, Phe β-H), 2.89 (2H, m, Phe β-H+5$_\beta$-H), 2.97 (2H, t, J=7.0 Hz, $ArCH_2$), 3.20 (1H, d, J=11.7 Hz, 5$_\alpha$-H), 3.41 (1H, m, $ArCH_2CH$), 3.69 (1H, m, $ArCH_2CH$), 3.74 (1H, d, J=5.5 Hz, 4-H), 4.13 (1H, m, Phe α-H), 4.98 (1H, d, J=5.9 Hz, Phe NH), 7.18 (2H, m, Phe 2′,6′-H_2), 7.33 (3H, m, Phe 3′,4′,5′-H_3), 7.35 (2H, d, J=8.6 Hz, Ar 2,6-H_2), 8.10 (2H, d, J=9 Hz, Ar 3,5-H_2), 8.10 (1H, m, NH); MS m/z 557.2433 (M+H) ($C_{28}H_{37}N_4O_6S$ requires 557.2434), 501 (M−Me_2C=CH_2), 457 (M−Boc).

8.22. *R*-2,2-Dimethyl-3-glycyl-*N*-(2-(4-nitrophenyl)-ethyl)tetrahydrothiazole-4-carboxamide trifluoroacetate salt (21a)

Compound **20a** (50 mg, 0.11 mmol) was stirred in CF_3CO_2H (0.4 mL) and CH_2Cl_2 (1.6 mL) for 45 min. Evaporation afforded **21a** (53 mg, quant.) as a highly hygroscopic colourless gum: NMR ($(CD_3)_2SO$) δ_H 1.76 (3H, s, 2-Me), 1.78 (3H, s, 2-Me), 2.90 (2H, t, J=6.6 Hz, $ArCH_2$), 3.18 (1H, m, Gly-H), 3.20 (d, J=12.1 Hz, 5-H), 3.34 (1H, dd, J=12.5, 5.9 Hz, 5-H), 3.42 (2H, br q, $ArCH_2CH_2$), 3.89 (1H, m, Gly-H), 4.85 (1H, d, J=6.2 Hz, 4-H), 7.50 (2H, d, J=9.0 Hz, Ar 2,6-H_2), 8.02 (3H, br, N^+H_3), 8.15 (2H, d, J=8.6 Hz, Ar 3,5-H_2), 8.26 (1H, t, J=6.2 Hz, NH); MS m/z 367.1448 (M+H) ($C_{16}H_{24}N_4O_4S$ requires 367.1400), 310 (M−Gly), 225 (M−$CH_2CH_2C_6H_4NO_2$).

8.23. 3-(L-Alanyl)-*R*-2,2-dimethyl-*N*-(2-(4-nitrophenyl)-ethyl)tetrahydrothiazole-4-carboxamide trifluoroacetate salt (21b)

Compound **20b** was treated with CF_3CO_2H, as for the synthesis of **21a** except that the reaction time was 20 min, to give **21b** (quant.) as a pale buff solid: mp 119–121 °C: IR ν_{max} 3410, 1655, 1600, 1517 cm^{-1}; NMR (CD_3OD) δ_H 1.34 (3H, d, J=6.6 Hz, Ala-Me), 1.69 (3H, s, 2-Me), 1.72 (3H, s, 2-Me), 2.85 (1H, dt, J=12.9, 6.6 Hz) and 2.89 (1H, dt, J=12.9, 6.6 Hz, $ArCH_2$), 3.10 (1H, dd, J=12.9, 1.6 Hz, 5-H), 3.33 (2H, m, 5-H, NC*H*H), 3.54–3.61 (1H, dt, J=13.7, 6.6 Hz, NC*H*H), 3.68 (1H, q, J=7.0 Hz, Ala α-H), 4.74 (1H, dd, J=5.9, 1.6 Hz, 4-H), 7.39 (2H, d, J=9.0 Hz, Ar 2,6-H_2), 8.07 (2H, d, J=9.0 Hz, Ar 3,5-H_2); MS m/z 381.1603 (M+H) ($C_{17}H_{25}N_4O_4S$ requires 381.1597).

8.24. *R*-2,2-Dimethyl-*N*-(2-(4-nitrophenyl)ethyl)-3-(L-valinyl)tetrahydrothiazole-4-carboxamide trifluoroacetate salt (21c)

Compound **20c** was treated with CF_3CO_2H, as for the synthesis of **21a** except that the reaction time was 15 min, to give **21c** (quant.) as a colourless highly hygroscopic gum: NMR ($(CD_3)_2SO$) δ_H 0.95 (3H, d, J=6.6 Hz, Val-Me), 1.00 (3H, d, J=6.6 Hz, Val-Me), 1.80 (3H, s, 2-Me), 1.82 (3H, s, 2-Me), 2.10 (1H, m, Val β-H), 2.98 (2H, t, J=7.0 Hz, $ArCH_2$), 3.32 (1H, dd, J=12.5, 2.3 Hz, 5-H), 3.45 (1H, dd, J=12.1, 5.9 Hz, 5-H), 3.53 (3H, m, NCH_2 and Val α-H), 4.95 (1H, dd, J=5.9, 2.3 Hz, 4-H), 7.57 (2H, d, J=8.6 Hz, Ar 2,6-H_2), 8.16 (3H, br, N^+H_3), 8.23 (2H, d, J=8.6 Hz, Ar 3,5-H_2), 8.33 (1H, t, J=5.9 Hz, NH); MS m/z 409.1911 (M+H) ($C_{19}H_{29}N_4O_4S$ requires 409.1910).

8.25. *R*-2,2-Dimethyl-*N*-(2-(4-nitrophenyl)ethyl)-3-(L-phenylalanyl)tetrahydrothiazole-4-carboxamide trifluoroacetate salt (21d)

Compound **20d** was treated with CF_3CO_2H, as for the synthesis of **21c**, to give **21d** (quant.) as a colourless highly hygroscopic gum: NMR (CD_3OD) δ_H 1.64 (3H, s, 2-Me), 1.70 (3H, s, 2-Me), 2.55 (1H, dd, J=12.5, 5.9 Hz, 5-H), 2.90 (2H, t, J=7.0 Hz, $ArCH_2$), 2.95 (1H, dd, J=13.3, 8.6 Hz, Phe β-H), 3.08 (1H, d, J=12.5 Hz, 5-H), 3.10 (1H, dd, J=13.3, 5.6 Hz, Phe β-H), 3.42 (2H, m, $NHCH_2$), 4.08 (1H, m, Phe α-H), 4.20 (1H, d, J=4.6 Hz, 4-H), 4.98 (1H, d, J=5.9 Hz, Phe NH), 7.27 (2H, m, Phe 2′,6′-H_2), 7.36 (3H, m, Phe 3′,4′,5′-H_3), 7.50 (2H, d, J=8.6 Hz, Ar 2,6-H_2), 8.18 (2H, d, J=8.6 Hz, Ar 3,5-H_2), 8.23 (1H, t, J=6.6 Hz, NH), 8.38 (3H, br, N^+H_3); MS m/z 457.1907 (M+H) ($C_{23}H_{29}N_4O_4S$ requires 457.1910).

8.26. Pentafluorophenyl 3-(L-alanyl)-*R*-2,2-dimethyl-tetrahydrothiazole-4-carboxylate hydrochloride (22b)

HCl was bubbled through **19b** (90 mg, 0.18 mmol) in CH_2Cl_2 (5.0 mL) for 30 min. Evaporation afforded **22b** (quant.) as a highly hygroscopic yellow solid: IR ν_{max} 3423, 1793, 1670, 1521 cm^{-1}; NMR ($(CD_3)_2SO$) δ_H 1.20 (3H, d, J=7.0 Hz, Ala-Me), 1.74 (3H, s, 2-Me), 1.77 (3H, s, 2-Me), 3.17 (1H, dd, J=12.1, 10.2 Hz, 5-H), 3.25 (1H, dd, J=12.1, 6.6 Hz, 5-H), 4.10 (1H, q, J=7.0 Hz, Ala α-H), 4.68 (1H, dd, J=10.4, 6.6 Hz, 4-H), 6.30 (3H, br, N^+H_3); MS m/z 215 (M−C_6F_5O).

8.27. Pentafluorophenyl *R*-2,2-dimethyl-3-(L-valinyl)-tetrahydrothiazole-2-carboxylate hydrochloride (22c)

Compound **19c** was treated with HCl, as for the synthesis of **22b** except that the reaction time was 1 h, to give **22c** (120 mg, 90%) as a white wax: IR ν_{max} 3433, 1790, 1667, 1520 cm^{-1}; NMR ($(CD_3)_2SO$) δ_H 0.82 (3H, d, J=6.6 Hz, Val-Me), 0.99 (3H, d, J=7.4 Hz, Val-Me), 1.75 (6H, s, 2×Me), 2.29–2.37 (1H, d septet, J=2.3, 7.0 Hz, Val β-H), 3.13 (1H, dd, J=11.3, 10.2 Hz, 5$_\beta$-H), 3.22 (1H, dd, J=11.7, 6.2 Hz, 5$_\alpha$-H), 3.89 (1H, m, Val α-H), 4.60–4.64 (1H, dd, J=6.2, 1.2 Hz, 4-H), 5.94 (3H, br, N^+H_3); NMR δ_F −171.5 (1F, tt, J=23.7, 6.8 Hz, 4′-F_2), −165.1 (2F, dd, J=23.7, 19.6 Hz, 3′,5′-F_2), −161.5 (2F, dd, J=19.6, 6.8 Hz, 2′,6′-F_2); MS m/z 427 (M+H), 243 (M−C_6F_5O).

8.28. Pentafluorophenyl *R*-2,2-dimethyl-3-(L-phenylalanyl)tetrahydrothiazole-2-carboxylate hydrochloride (22d)

Compound **19d** was treated with HCl, as for the synthesis of **22b** except that the reaction time was 1.5 h, to give **22d** (quant.) as a colourless highly hygroscopic viscous oil: IR ν_{max} 3434, 1791, 1669, 1520 cm^{-1}; NMR ($(CD_3)_2SO$) δ_H 1.66 (3H, s, 2-Me), 1.74 (3H, s, 2-Me), 2.75 (1H, t, J=10.9 Hz, 5$_\beta$-H), 3.04 (1H, dd, J=14.1, 4.7 Hz, Phe β-H),

3.06 (1H, dd, J=14.1, 4.3 Hz, Phe β-H), 3.13 (1H, dd, J=11.7, 5.9 Hz, 5_α-H), 4.34 (1H, t, J=4.3 Hz, Phe α-H), 4.46 (3H, br, N^+H_3), 4.60 (1H, dd, J=10.5, 5.5 Hz, 4-H), 7.25 (5H, m, Ph-H_5), 8.31 (1H, s, NH); NMR δ_F −171.5 (2F, tt, J=22.4, 6.6 Hz, 4′-F_2), −165.1 (1F, dd, J=22.4, 19.7 Hz, 3′,5′-F_2), −161.8 (2F, dd, J=19.7, 6.6 Hz, 2′,6′-F_2), MS m/z 475.1131 (M+H) ($C_{21}H_{20}F_5N_2O_3S$ requires 475.1115), 291 (M−C_6F_5O).

8.29. 6*S*,8a*R*-3,3,6-Trimethyltetrahydrothiazolo[3,4-*a*]-pyrazine-5,8-dione (23b)

Compound **22b** (190 mg, 480 μmol) was stirred with Et_3N (97 mg, 1.0 mmol) in CH_2Cl_2 (5.0 mL) for 5 min. Evaporation and chromatography (EtOAc) afforded **23b** (15 mg, 15%) as a white solid: mp 155.8 °C (DSC); IR ν_{max} 3436, 1693, 1652 cm^{-1}; NMR δ_H 1.46 (3H, d, J=7.0 Hz, 6-Me), 1.85 (3H, s, 3-Me), 1.89 (3H, s, 3-Me), 3.25 (1H, dd, J=12.5, 6.6 Hz, 1_α-H), 3.30 (1H, dd, J=12.5, 10.2 Hz, 1_β-H), 4.07 (1H, ddq, J=7.0, 1.0, 0.8 Hz, 6-H), 4.57 (1H, dddd, J=10.5, 6.6, 1.6, 0.8 Hz, 8a-H), 7.28 (1H, br, NH); MS m/z 368 (M+mNBA), 215.0862 (M+H) ($C_9H_{15}N_2O_2S$ requires 215.0854). Found C, 50.43; H, 6.55; N, 12.9. $C_9H_{14}N_2O_2S$ requires C, 50.45; H, 6.58; N, 13.07%.

8.30. 6*S*,8a*R*-3,3-Dimethyl-6-(1-methylethyl)tetrahydrothiazolo[3,4-*a*]pyrazine-5,8-dione (23c)

Compound **22c** was treated with Et_3N, as for the synthesis of **23b** except that the reaction time was 10 min, to give **23c** (87%) as a white solid: mp 186.4 °C (DSC); IR ν_{max} 3213, 1692 cm^{-1}; NMR ($(CD_3)_2SO$) δ_H 0.83 (3H, d, J=7.0 Hz, CHCH_3), 1.0 (3H, d, J=7.0 Hz, CHCH_3), 1.76 (3H, s, 3-Me), 1.77 (3H, s, 3-Me), 2.34 (1H, d septet, J=7.0, 2.3 Hz, CHMe$_2$), 3.14 (1H, dd, J=11.7, 10.2 Hz, 1_β-H), 3.23 (1H, dd, J=11.7, 5.9 Hz, 1_α-H), 3.34 (1H, br, NH), 3.89 (1H, dd, J=2.3, 1.2 Hz, 6-H), 4.64 (1H, ddd, J=11.7, 5.9, 1.2 Hz, 8a-H); MS m/z 396 (M+mNBA), 243.1168 (M+H) ($C_{11}H_{19}N_2O_2S$ requires 243.1167).

8.31. 6*S*,8a*R*-3,3-Dimethyl-6-phenylmethyltetrahydrothiazolo[3,4-*a*]pyrazine-5,8-dione (23d)

Compound **22d** was treated with Et_3N, as for the synthesis of **23b** except that the reaction time was 1.5 h, to give **23d** (81%) as a colourless gum: IR (film) ν_{max} 3377, 1794, 1688 cm^{-1}; NMR δ_H 1.87 (3H, s, 3-Me), 1.92 (3H, s, 3-Me), 2.82 (1H, dd, J=14.4, 10.2 Hz, PhCH), 3.19 (1H, dd, J=12.1, 10.5 Hz, 1_β-H), 3.26 (1H, dd, J=12.1, 5.9 Hz, 1_α-H), 3.56 (1H, dd, J=14.4, 3.9 Hz, PhCH), 4.24 (1H, ddd, J=10.2, 3.9, 0.8 Hz, 6-H), 4.53 (1H, dddd, J=11.3, 5.9, 1.6, 0.8 Hz, 8a-H), 5.82 (1H, s, NH), 7.21–7.37 (5H, m, Ph-H_5); MS m/z 291.1169 (M+H) ($C_{15}H_{18}N_2O_2S$ requires 291.1167).

8.32. *R*-2,2-Dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-D-alanyl)tetrahydrothiazole-4-carboxylic acid (27) and *R*-2,2-dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-alanyl)tetrahydrothiazole-4-carboxylic acid (18b)

A mixture of Boc(D-Ala)F **26** (308 mg, 1.6 mmol) and **17b** (154 mg, 800 μmol) was stirred with **11** (500 mg, 2.5 mmol) and Et_3N (480 mg, 4.8 mmol) in dry DMF (50 mL) for 16 h. The evaporated residue, in EtOAc, was washed with cold citric acid and brine. Drying and evaporation afforded a mixture of **27** and **18b** (300 mg, 36%) as a white solid: mp 153–155 and 160–162 °C; IR ν_{max} 3316, 1748, 1718, 1643, 1660, 1540, 1512 cm^{-1}; NMR δ_H 1.30 (1.2H, d, J=2.7 Hz, Ala-Me (**18b**)), 1.32 (1.8H, d, J=3.1 Hz, Ala-Me (**27**)), 1.40 (3.6H, s, Bu^t (**18b**)), 1.42 (5.4H, s, Bu^t (**27**)), 1.81 (1.2H, s, 2-Me (**18b**)), 1.84 (1.8H, s, 2-Me (**27**)), 1.87 (1.8H, s, 2-Me (**27**)), 1.91 (1.2H, s, 2-Me (**18b**)), 3.23 (0.4H, dd, J=11.7, 5.5 Hz, 5-H (**18b**)), 3.34 (0.6H, dd, J=12.1, 5.5 Hz, 5-H (**27**)), 3.40 (0.6H, d, J=12.1 Hz, 5-H (**27**)), 3.45 (0.4H, d, J=11.7 Hz, 5-H (**18b**)), 4.25 (0.6H, qn, J=6.6 Hz, Ala α-H (**27**)), 4.53 (0.4H, qn, J=7.0 Hz, Ala α-H (**18b**)), 4.87 (0.4H, d, J=5.1 Hz, 4-H (**18b**)), 5.36 (0.6H, d, J=8.6 Hz, NH (**27**)), 5.59 (0.6H, d, J=5.1 Hz, 4-H (**27**)), 5.79 (0.4H, d, J=8.2 Hz, NH (**18b**)); MS m/z 333.1494 (M+H) ($C_{14}H_{25}N_2O_5S$ requires 333.1484), 277 (M−Me_2C=CH_2).

8.33. Pentafluorophenyl *R*-2,2-dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-D-alanyl)tetrahydrothiazole-4-carboxylate (28) and pentafluorophenyl *R*-2,2-dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-alanyl)tetrahydrothiazole-4-carboxylate (19b)

The above mixture of **27** and **18b** was treated with pentafluorophenol and DCC, as for the synthesis of **19a**, to give a mixture of **28** and **19b** (80%) as a colourless gum: NMR δ_H 1.30 (2.1H, d, J=6.6 Hz, Ala-Me (**28**)), 1.37 (0.9H, d, J=6.6 Hz, Ala-Me (**19b**)), 1.39 (2.7H, s, Bu^t (**19b**)), 1.43 (6.3H, s, Bu^t (**28**)), 1.85 (0.9H, s, 2-Me (**19b**)), 1.87 (2.1H, s, 2-Me (**28**)), 1.89 (2.1H, s, 2-Me (**28**)), 1.94 (0.9H, s, 2-Me (**19b**)), 3.37 (0.3H, dd, J=12.5, 3.5 Hz, 5-H (**28**)), 3.40 (0.7H, dd, J=12.5, 5.9 Hz, 5-H (**28**)), 3.49 (1H, d, J=12.5 Hz, 5-H), 4.22 (0.7H, qn, J=7.0 Hz, Ala α-H (**28**)), 4.38 (0.3H, m, Ala α-H (**19b**)), 5.01 (0.7H, d, J=9.0 Hz, NH (**28**)), 5.19 (0.7H, d, J=5.5 Hz, 4-H (**28**)), 5.58 (0.3H, d, J=3.5 Hz, 4-H (**19b**)), 6.13 (0.3H, t, J=3.9 Hz, NH (**19b**)); NMR δ_F −163.8 (2F, t, J=21.0 Hz, 3′,5′-F_2), −162.1 (2F, t, J=21.0 Hz, 3′,5′-F_2), −161.5 (2F, m, 3′,5′-F_2), −161.1 (2F, t, J=22.4 Hz, 3′,5′-F_2), 156.4 (1F, t, J=21.0 Hz, 4′-F), −156.6 (1F, t, J=21.0 Hz, 4′-F), 157.1 (1F, t, J=21.0 Hz, 4′-F), −157.8 (1F, t, J=21.0 Hz, 4′-F), −150.9 (2F, d, J=17.1 Hz, 2′,6′-F_2), −151.9 (2F, d, J=17.1 Hz, 2′,6′-F_2), −152.1 (2F, d, J=18.4 Hz, 2′,6′-F_2), −152.6 (2F, d, J=17.1 Hz, 2′,6′-F_2); MS m/z 499.1340 (M+H) ($C_{20}H_{24}F_5N_2O_5S$ requires 499.1326), 443 (M−Me_2C=CH_2), 425 (M−Bu^{t}O).

8.34. *R*-(2,2-Dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-D-alanyl)-*N*-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (29) and *R*-2,2-dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-alanyl)-*N*-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (20b)

The above mixture of **28** and **19b** was treated with **24**·HCl and Et_3N, as for the synthesis of **20b**, to give a mixture of **29** and **20b** (220 mg, 76%) as a white solid: mp 69–80 °C; NMR ($(CD_3)_2SO$) δ_H 0.93 (1.8H, d, J=6.6 Hz, Ala-Me (**29**)), 1.11 (1.2H, d, J=6.6 Hz, Ala-Me (**20b**)), 1.36 (3.6H, s, Bu^t (**20b**)), 1.37 (5.4H, s, Bu^t (**20b**)), 1.59 (1.2H, s, 2-Me (**20b**)), 1.68 (1.8H, s, 2-Me (**29**)), 1.70 (1.8H, s, 2-Me

(**29**)), 1.71 (1.2H, s, 2-Me (**20b**)), 2.88 (0.8H, m, $ArCH_2$ (**20b**)), 2.95 (1.2H, m, $ArCH_2$ (**29**)), 3.00–4.00 (5H, m, 5-H_2, $NHCH_2$, Ala α-H), 4.72 (0.4H, d, *J*=4.1 Hz, 4-H (**20b**)), 5.25 (0.6H, d, *J*=4.3 Hz, 4-H (**29**)), 7.05 (0.4H, d, *J*=5.9 Hz, NH (**20b**)), 7.13 (0.6H, d, *J*=7.4 Hz, NH (**29**)), 7.49 (0.8H, d, *J*=8.2 Hz, Ar 2,6-H_2 (**20b**)), 7.51 (1.2H, d, *J*=8.6 Hz, Ar 2,6-H_2 (**29**)), 8.13 (2H, d, *J*=8.6 Hz, Ar 3,5-H_2), 8.17 (1H, d, *J*=5.9 Hz, Ala NH); MS *m*/*z* 481.2128 (M+H) ($C_{22}H_{33}N_4O_6S$ requires 481.2121), 425 (M−Me_2C=CH_2), 381 (M−Boc).

8.35. *S*-2,2-Dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-D-valinyl)tetrahydrothiazole-4-carboxylic acid (32)

Compound **31** (prepared as **17c**) (270 mg, 1.1 mmol) was stirred with **11** (250 mg, 1.1 mmol) and Pr^i_2NEt (310 mg, 2.4 mmol) in dry DMF (10 mL) for 16 h. The evaporated residue, in EtOAc, was washed with cold citric acid, cold H_2O and brine. Drying, evaporation and chromatography (hexane/EtOAc/AcOH, 49:49:2) afforded **32** (200 mg, 50%) as a colourless gum: IR (film) ν_{max} 3329, 1714, 1657, 1462 cm^{-1}; NMR δ_H 0.92 (3H, d, *J*=6.2 Hz, Val-Me), 0.94 (3H, d, *J*=6.6 Hz, Val-Me), 1.43 (9H, s, Bu^t), 1.85 (3H, s, 2-Me), 1.89 (3H, s, 2-Me), 2.05 (1H, m, Val β-H), 3.27 (1H, dd, *J*=12.1, 5.7 Hz, 5-H), 3.40 (1H, d, *J*=12.1 Hz, 5-H), 3.86 (1H, t, *J*=9.6 Hz, Val α-H), 5.42 (1H, d, *J*=10.1 Hz, NH), 5.66 (1H, d, *J*=5.7 Hz, 4-H); MS *m*/*z* 361.1810 (M+H) ($C_{16}H_{29}N_2O_5S$ requires 361.1797), 305 (M−Me_2C=CH_2).

8.36. Pentafluorophenyl *R*-2,2-dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-D-valinyl)tetrahydrothiazole-4-carboxylate (33)

Compound **32** was treated with pentafluorophenol and DCC, as for the synthesis of **19a**, to give **33** (41%) as a colourless oil: NMR δ_H 0.89 (2.1H, d, *J*=7.0 Hz, Val-Me), 0.98 (2.1H, d, *J*=6.6 Hz, Val-Me), 1.04 (0.9H, d, *J*=7.0 Hz, Val-Me), 1.10 (0.9H, d, *J*=7.0 Hz, Val-Me), 1.45 (6.3H, s, Bu^t), 1.48 (2.7H, s, Bu^t), 1.89 (2.1H, s, 2-Me), 1.93 (2.1H, s, 2-Me), 2.00 (0.9H, s, 2-Me), 2.02 (1H, m, Val β-H), 2.06 (0.9H, s, 2-Me), 3.30 (0.3H, dd, *J*=12.9, 3.1 Hz, 5-H), 3.43 (0.3H, d, *J*=4.7 Hz, 5-H), 3.54 (0.7H, dd, *J*=12.5, 6.2 Hz, 5-H), 3.83 (0.7H, t, *J*=9.4, 5-H), 5.03 (0.7H, d, *J*=9.8 Hz, 4-H), 5.08 (0.3H, d, *J*=9.0 Hz, 4-H), 5.62 (0.3H, dd, *J*=6.6, 2.3 Hz, Val α-H), 5.67 (0.7H, dd, *J*=3.9, 2.0 Hz, Val α-H), 6.2 (1H, m, NH).

8.37. *R*-(2,2-Dimethyl-3-(1,1-dimethylethoxycarbonyl)-D-valinyl)-*N*-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (34)

Compound **33** was treated with **24**·HCl and Et_3N, as for the synthesis of **20c**, to give **34** (17%) as a colourless gum: IR (film) ν_{max} 3320, 1702, 1655, 1514 cm^{-1}; NMR δ_H 0.83 (3H, d, *J*=6.6 Hz, Val-Me), 0.92 (3H, d, *J*=6.6 Hz, Val-Me), 1.42 (7.2H, s, Bu^t), 1.55 (1.8H, s, Bu^t), 1.79 (2.4H, s, 2-Me), 1.81 (2.4H, s, 2-Me), 1.88 (0.6H, s, 2-Me), 1.91 (0.6H, s, 2-Me), 1.92 (1H, m, Val β-H), 2.97 (2H, t, *J*=7.0 Hz, $ArCH_2$), 3.04 (1H, d, *J*=12.5 Hz, 5_α-H), 3.36 (1H, dd, *J*=12.5, 7.0 Hz, 5_β-H), 3.44 (1H, m, $ArCH_2CH$), 3.80 (1H, m, $ArCH_2CH$), 3.86 (1H, t, *J*=9.0 Hz, Val α-H), 4.85 (1H, d, *J*=9.4 H, ValNH), 5.07 (0.2, d, *J*=6.2 Hz, 4-H), 5.36 (0.8H, d, *J*=6.6 Hz, 4-H), 6.03 (0.2H, br, $NHCH_2$), 6.41 (0.8H, br, $NHCH_2$), 7.35 (0.4H, d, *J*=8.6 Hz, Ar 2,6-H_2), 7.40 (1.6H, d, *J*=8.6 Hz, Ar 2,6-H_2), 8.15 (0.4H, d, *J*=9.0 Hz, Ar 3,5-H_2), 8.17 (1.6H, d, *J*=8.6 Hz, Ar 3,5-H_2); MS *m*/*z* 509.2437 (M+H) ($C_{24}H_{37}N_4O_6S$ requires 509.2434).

8.38. *S*-2,2-Dimethyltetrahydrothiazole-4-carboxylic acid hydrochloride (36)

D-Cys·HCl·H_2O **35** was treated with acetone and 2,2-dimethoxypropane, as for the synthesis of **11**, to give **36** (78%) as a white solid: mp 166–170 °C (lit.[39] mp 165–168 °C for *R* enantiomer); IR ν_{max} 3412, 2600, 1743 cm^{-1}; NMR (D_2O) δ_H 2.06 (6H, s, 2×Me), 2.92 (1H, dd, *J*=15.2, 3.9 Hz, 5-H), 3.00 (1H, dd, *J*=15.2, 5.9 Hz, 5-H), 4.06 (1H, dd, *J*=5.9, 3.9 Hz, 4-H).

8.39. *S*-2,2-Dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-valinyl)tetrahydrothiazole-4-carboxylic acid (37c)

Compound **17c** (1.08 g, 4.6 mmol) was stirred with **36** (1.00 g, 5.1 mmol) and Pr^i_2NEt (1.24 g, 9.6 mmol) in dry DMF (100 mL) for 16 h. The evaporated residue, in EtOAc, was washed with cold citric acid, cold H_2O and brine. Drying, evaporation and chromatography (hexane/EtOAc/AcOH, 70:28:2) afforded **37c** (630 mg, 35%) as a colourless gummy solid: IR ν_{max} 3434, 2970, 1723, 1605, 1513 cm^{-1}; NMR δ_H 0.87–0.95 (6H, m, 2×Val-Me), 1.43 (9H, s, Bu^t), 1.85 (3H, s, 2-Me), 1.88 (3H, s, 2-Me), 2.00 (1H, m, Val β-H), 3.20–3.50 (2H, m, 5-H+Val α-H), 3.85 (1H, t, *J*=9.8 Hz, 5-H), 5.36 (1H, d, *J*=10.2 Hz, 4-H), 5.68 (0.7H, d, *J*=5.1 Hz, NH), 8.63 (1H, br, OH); MS *m*/*z* 361.1810 (M+H) ($C_{16}H_{29}N_2O_5S$ requires 361.1797), 305 (M−Me_2C=CH_2).

8.40. *S*-2,2-Dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-leucyl)tetrahydrothiazole-4-carboxylic acid (37e)

Compound **45** (1.5 g, 3.8 mmol) was stirred with **36** (833 mg, 4.2 mmol) and Pr^i_2NEt (1.63 g, 12.6 mmol) in dry DMF (40 mL) for 16 h. The evaporated residue, in EtOAc, was washed with cold 5% aq citric acid, cold H_2O and brine. Drying and evaporation afforded crude **37e** (2.4 g) as a gummy oil: NMR δ_H 0.81–0.90 (6H, m, 2×Leu-Me), 1.40 (9H, s, Bu^t), 1.50–1.60 (2H, m, Leu β-H_2+Leu γ-H), 1.83 (3H, s, 2-Me), 1.86 (3H, s, 2-Me), 3.28 (2H, m, 5-H_2), 4.17 (1H, m, Leu α-H), 5.30 (1H, d, *J*=5.92 Hz, NH), 5.70 (1H, m, 4-H); MS *m*/*z* 375.1948 (M+H) ($C_{17}H_{31}N_2O_5S$ requires 375.1954), 319 (M−Me_2C=CH_2).

8.41. Pentafluorophenyl *S*-2,2-dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-valinyl)tetrahydrothiazole-4-carboxylate (38c)

Compound **37c** was treated with pentafluorophenol and DCC, as for the synthesis of **19a**, to give gave **38c** (68%) as a colourless oil: NMR δ_H 0.88 (3H, d, *J*=6.6 Hz, Val-Me), 0.97 (3H, d, *J*=6.6 Hz, Val-Me), 1.45 (9H, s, Bu^t), 1.88 (3H, s, 2-Me), 1.92 (3H, s, 2-Me), 2.04 (1H, m, Val

β-H), 3.55 (1H, dd, *J*=12.5, 6.6 Hz, 5-H), 3.82 (1H, t, *J*=9.4 Hz, 5-H), 5.08 (1H, br d, *J*=9.8 Hz, 4-H), 5.61 and 5.66 (1H, 2×d, *J*=4.7 Hz, Val α-H), 6.22 (1H, br d, *J*=3.9 Hz, NH); MS *m/z* 527.1656 (M+H) ($C_{22}H_{28}F_5N_2O_5S$ requires 527.1639), 471 (M−Bu^{t}O).

8.42. Pentafluorophenyl *S*-2,2-dimethyl-3-(*N*-(1,1-dimethylethoxycarbonyl)-L-leucyl)tetrahydrothiazole-4-carboxylate (38e)

Compound **37e** was treated with pentafluorophenol and DCC, as for the synthesis of **19a**, to give crude **38e** as a pale yellow oil: NMR δ_H 0.88 (3H, d, *J*=6.4 Hz, Leu-Me), 0.90 (3H, d, *J*=6.4 Hz, Leu-Me), 1.44 (9H, s, Bu^t), 1.55–1.65 (2H, m, Leu β,γ-H_3), 1.86 (3H, s, 2-Me), 1.91 (3H, s, 2-Me), 3.47 (1H, dd, *J*=12.7, 9.8 Hz, $5_β$-H), 3.54 (1H, dd, *J*=12.7, 4.8 Hz, $5_α$-H), 4.17 (1H, dt, *J*=2.7, 8.7 Hz, Leu α-H), 4.96 (1H, d, *J*=9.0 Hz, 4-H), 6.17 (1H, d, *J*=9.0 Hz, NH); NMR δ_F −161.5 (2F, t, *J*=17.1 Hz, 3′,5′-F_2), −156.6 (1F, t, *J*=22.4 Hz, 4′-F), −152.1 (2F, d, *J*=18.4 Hz, 2′,6′-F_2). MS *m/z* 541.1797 (M+H) ($C_{23}H_{30}N_2O_5F_5S$ requires 541.1796), 485 (M−Me_2C=CH_2), 441 (M−Boc).

8.43. *S*-(2,2-Dimethyl-3-(1,1-dimethylethoxycarbonyl)-L-valinyl)-*N*-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (39c)

Compound **38c** was treated with **24**·HCl and Et_3N, as for the synthesis of **20c**, to give **39c** (49%) as a colourless oil: IR ν_{max} 3323, 1698, 1605, 1520 cm^{-1}; NMR δ_H 0.83 (3H, d, *J*=6.6 Hz, Val-Me), 0.92 (3H, d, *J*=6.6 Hz, Val-Me), 1.42 (7.2H, s, Bu^t), 1.55 (1.8H, s, Bu^t), 1.80 (2.4H, s), 1.82 (2.4H, s), 1.89 (0.6H, s) and 1.91 (0.6H, s, 2-Me_2), 1.95 (1H, m, Val β-H), 2.81 (1.6H, dt, *J*=14.1, 7.0 Hz, ArCH_2), 3.04 (0.8H, d, *J*=12.5 Hz, $5_β$-H), 3.36 (1H, dd, *J*=12.5, 6.6 Hz, $5_α$-H), 3.44 (1H, m, ArCH_2*CH*), 3.77 (1H, m, ArCH_2*CH*), 3.86 (1H, t, *J*=9.0 Hz, Val α-H), 4.92 (1H, d, *J*=9.8 Hz, Val NH), 5.07 (0.2H, d, *J*=7.0 Hz, 4-H), 5.36 (0.8H, d, *J*=6.2 Hz, 4-H), 6.09 (0.2H, br, N*H*CH_2), 6.47 (0.8H, t, *J*=5.5 Hz, N*H*CH_2), 7.35 (0.4H, d, *J*=8.6 Hz, Ar 2,6-H_2), 7.40 (1.6H, d, *J*=8.6 Hz, Ar 2,6-H_2), 8.13 (0.4H, d, *J*=8.2 Hz, Ar 3,5-H_2), 8.16 (1.6H, d, *J*=8.6 Hz, Ar 3,5-H_2); MS *m/z* 509.2432 (M+H) ($C_{24}H_{37}N_4O_6S$ requires 509.2434), 453 (M−Bu^{t}O). Some ^{1}H NMR peaks from the minor isomer overlapped with other peaks from the major isomer and thus were not identifiable.

8.44. *S*-(2,2-Dimethyl-3-(1,1-dimethylethoxycarbonyl)-L-leucyl)-*N*-(2-(4-nitrophenyl)ethyl)tetrahydrothiazole-4-carboxamide (39e)

Compound **38e** was treated with **24**·HCl and Et_3N, as for the synthesis of **20c**, to give **39e** (58%) as a white solid: mp 108–109 °C; NMR δ_H 0.84 (2.4H, d, *J*=6.4 Hz, Leu-Me), 0.90 (2.4H, d, *J*=6.4 Hz, Leu-Me), 0.98 (1.2H, d, *J*=6.4 Hz, 2×Leu-Me), 1.41 (7.2H, s, Bu^t), 1.43 (1.8H, s, Bu^t), 1.60–1.70 (3H, m, Leu β,γ-H_3), 1.78 (2.4H, s, 2-Me), 1.80 (2.4H, s, 2-Me), 1.84 (0.6H, s, 2-Me), 1.85 (0.6H, s, 2-Me), 2.97 (2H, t, *J*=7.0 Hz, ArCH_2), 3.05 (1H, d, *J*=12.3 Hz, $5_β$-H), 3.45 (2H, m, ArCH_2*CH*, $5_α$-H), 3.85 (1H, m, ArCH_2*CH*), 4.11 (0.8H, m, Leu α-H), 4.30 (0.2H, m, Leu α-H), 4.81 (0.8H, d, *J*=8.6 Hz, LeuNH), 4.92 (0.2H, d, *J*=8.6 Hz, LeuNH), 5.30 (0.2H, d, *J*=7.0 Hz, 4-H), 5.42 (0.8H, d, *J*=7.0 Hz, 4-H), 6.15 (1H, t, *J*=5.1 Hz, N*H*CH_2), 7.41 (2H, d, *J*=8.6 Hz, Ar 2,6-H_2), 8.15 (0.4H, d, *J*=8.3 Hz, Ar 3,5-H_2), 8.19 (1.6H, d, *J*=8.6 Hz, Ar 3,5-H_2); MS *m/z* 523.2596 (M+H) ($C_{25}H_{39}N_4O_6S$ requires 523.2590), 467 (M−Me_2C=CH_2), 423 (M−Boc).

8.45. *S*-2,2-Dimethyl-*N*-(2-(4-nitrophenyl)ethyl)-3-(L-valinyl)tetrahydrothiazole-4-carboxamide trifluoroacetate salt (40c)

Compound **39c** was treated with CF_3CO_2H, as for the synthesis of **21c**, to give **40c** (quant.) as a highly hygroscopic viscous oil: NMR ($(CD_3)_2SO$) δ_H 0.82 (3H, d, *J*=7.4 Hz, Val-Me), 0.84 (3H, d, *J*=7.0 Hz, Val-Me), 1.80 (6H, s, 2×2-Me), 2.02 (1H, m, Val β-H), 2.88 (2H, t, *J*=7.0 Hz, ArCH_2), 3.08 (1H, d, *J*=12.1 Hz, 5-H), 3.38 (2H, m, NHCH_2), 3.52 (1H, dd, *J*=12.1, 4.7 Hz, 5-H), 4.60 (1H, m, Val α-H), 5.05 (1H, d, *J*=5.9 Hz, 4-H), 7.49 (2H, d, *J*=8.6 Hz, Ar 2,6-H_2), 8.15 (2H, d, *J*=8.6 Hz, Ar 3,5-H_2), 8.23 (1H, t, *J*=5.9 Hz, NH); MS *m/z* 409.1911 (M+H) ($C_{19}H_{29}N_4O_4S$ requires 409.1910).

8.46. *S*-2,2-Dimethyl-*N*-(2-(4-nitrophenyl)ethyl)-3-(L-leucyl)tetrahydrothiazole-4-carboxamide trifluoroacetate salt (40e)

Compound **39e** was treated with CF_3CO_2H, as for the synthesis of **21c**, to give **40e** (quant.) as colourless highly hygroscopic gum: NMR (CD_3OD) δ_H 0.90 (3H, d, *J*=4.7 Hz, Leu-Me), 0.98 (3H, d, *J*=4.3 Hz, Leu-Me), 1.60–1.70 (3H, m, Leu β,γ-H_3), 1.89 (3H, s, 2-Me), 1.92 (3H, s, 2-Me), 2.95–3.07 (3H, m, 5-H, ArCH_2), 3.38–3.52 (2H, m, ArCH_2*CH*, 5-H), 3.70 (1H, m, ArCH_2*CH*), 3.95 (1H, m, Leu α-H), 5.08 (1H, d, *J*=6.2 Hz, 4-H), 7.52 (2H, d, *J*=7.0 Hz, Ar 2,6-H_2), 8.20 (2H, d, *J*=7.4 Hz, Ar 3,5-H_2); MS *m/z* 423.2077 (M+H) ($C_{20}H_{31}N_4O_4S$ requires 423.2066).

8.47. 6*S*,8a*S*-3,3-Dimethyl-6-(1-methylethyl)tetrahydrothiazolo[3,4-*a*]pyrazine-5,8-dione (41c)

Compound **38c** was treated with HCl, as for the synthesis of **22b**, to give crude pentafluorophenyl *S*-2,2-dimethyl-3-(*N*-(L-valinyl)tetrahydrothiazole-2-carboxylate hydrochloride (quant.) as a pale yellow gummy solid: IR ν_{max} 3412 cm^{-1}; NMR ($(CD_3)_2SO$) δ_H 0.88 (3H, d, *J*=6.6 Hz, Val-Me), 0.92 (3H, d, *J*=7.0 Hz, Val-Me), 1.78 (3H, s, 2-Me), 1.82 (3H, s, 2-Me), 2.06 (1H, m, Val β-H), 3.11 (1H, dd, *J*=11.7, 10.9 Hz, 5-H), 3.22 (1H, dd, *J*=11.7, 5.5 Hz, 5-H), 3.32 (3H, br, N^+H_3), 4.64 (1H, dd, *J*=10.9, 5.5 Hz, 4-H), 5.19 (1H, m, Val α-H); NMR δ_F −171.46 (2F, m, 3′,5′-F_2), −165.12 (1F, dt, *J*=23.7 Hz, 4′-F), −161.53 (2F, dd, *J*=19.7, 6.6 Hz, 2′,6′-F_2); MS *m/z* 427 (M+H), 243 (M−C_6F_5O). This material was treated with Et_3N, as for the synthesis of **23b**, to give **41c** (61%) as a white solid: mp 180–182 °C; NMR ($(CD_3)_2SO$) δ_H 0.88 (3H, d, *J*=6.6 Hz, 6-CMe), 0.91 (3H, d, *J*=6.6 Hz, 6-CMe), 1.78 (3H, s, 3-Me), 1.82 (3H, s, 3-Me), 2.07 (1H, m, 6-CH), 3.11 (1H, dd, *J*=11.7, 10.5 Hz, $1_α$-H), 3.23 (1H, dd, *J*=11.7, 5.5 Hz, $1_β$-H), 3.40 (1H, dd, *J*=5.9, 3.5 Hz, 6-H), 4.64 (1H, dd, *J*=10.9, 5.5 Hz, 8a-H), 8.90 (1H, d, *J*=3.5 Hz, NH); MS *m/z* 396 (M+*m*NBA), 243.1176 (M+H) ($C_{11}H_{19}N_2O_2S$

requires 243.1167). Found C, 54.60; H, 7.39; N, 11.4. $C_{11}H_{18}N_2O_2S$ requires C, 54.52; H, 7.49; N, 11.56%.

8.48. 6*S*,8a*S*-3,3-dimethyl-6-(2-methylpropyl)tetrahydrothiazolo[3,4-*a*]pyrazine-5,8-dione (41e)

Compound **38e** (700 mg, 1.30 mmol) was stirred with CF_3CO_2H (1 mL) and CH_2Cl_2 (4 mL) for 20 min. Evaporation afforded crude pentafluorophenyl *S*-2,2-dimethyl-3-(*N*-(L-leucyl)tetrahydrothiazole-2-carboxylate trifluoroacetate salt (quant.) as a gummy solid. This material (554 mg, 1 mmol) was stirred with Et_3N (202 mg, 2 mmol) in CH_2Cl_2 (3.0 mL) for 30 min. Evaporation and chromatography (EtOAc) afforded **41e** (150 mg, 59%) as a white solid: mp 153–155 °C; NMR δ_H 0.95 (3H, d, *J*=6.6 Hz, CHC*H*$_3$), 1.00 (3H, d, *J*=6.6 Hz, CHC*H*$_3$), 1.60–1.78 (3H, m, 6-CH_2CH), 1.88 (3H, s, 3-Me), 1.89 (3H, s, 3-Me), 3.23 (1H, dd, *J*=12.1, 10.9 Hz, 1$_\alpha$-H), 3.32 (1H, dd, *J*=12.1, 5.9 Hz, 1$_\beta$-H), 3.87 (1H, dt, *J*=10.0, 5.0 Hz, 6-H), 4.55 (1H, dd, *J*=10.5, 5.5 Hz, 8a-H), 6.29 (1H, br, NH); MS *m*/*z* 257.1316 ($C_{12}H_{21}N_2O_2S$ requires 257.1324).

8.49. *N*-(Fluoren-9-ylmethoxycarbonyl)-L-valine pentafluorophenyl ester (43)

FmocValOH **42** was treated with pentafluorophenol and DCC, as for the synthesis of **19a**, to give **43** (70%) as a white solid: mp 114–117 °C (lit.[40] mp 122–123 °C); NMR δ_H 1.03 (3H, d, *J*=6.6 Hz, Val-Me), 1.10 (3H, d, *J*=6.6 Hz, Val-Me), 2.40 (1H, m, Val β-H), 4.24 (1H, t, *J*=6.6 Hz, C*H*CH$_2$O), 4.46 (2H, d, *J*=6.6 Hz, CH$_2$O), 4.67 (1H, dd, *J*=9.4, 5.1 Hz, Val α-H), 5.27 (1H, d, *J*=9.4 Hz, NH), 7.30 (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$_2$), 7.75 (2H, d, *J*=7.8 Hz, Ar-H$_2$); NMR δ_F −161.2 (2F, t, *J*=20.9 Hz, 3′,5′-F$_2$), −156.6 (1F, t, *J*=20.9 Hz, 4′-F), −151.5 (2F, d, *J*=18.0 Hz, 2′,6′-F$_2$).

8.50. *S*-2,2-Dimethyl-3-(*N*-(fluoren-9-ylmethoxycarbonyl)-L-valinyl)tetrahydrothiazole-4-carboxylic acid (44)

Compound **43** (1.16 g, 2.3 mmol), in dry THF (10 mL), was added slowly to **36** (495 mg, 2.5 mmol) and Pr^i_2NEt (970 mg, 7.5 mmol) in dry DMF (30 mL) at 0 °C under N_2 for 3 h. The mixture was then slowly warmed to 20 °C and stirred for 16 h. The evaporated residue, in EtOAc, was washed with cold 5% aq citric acid and brine. Drying, evaporation and chromatography (hexane/EtOAc/AcOH, 25:25:1) afforded **44** (600 mg, 50%) as a white solid: mp 95–97 °C; NMR δ_H 0.92 (3H, d, *J*=7.0 Hz, Val-Me), 0.94 (3H, d, *J*=6.6 Hz, Val-Me), 1.84 (3H, s, 2-Me), 1.89 (3H, s, 2-Me), 2.10 (1H, m, Val β-H), 3.25 (1H, dd, *J*=12.1, 5.5 Hz, 5-H), 3.35 (1H, d, *J*=12.5 Hz, 5-H), 3.90 (1H, t, *J*=9.8 Hz, Val α-H), 4.20 (1H, t, *J*=7.0 Hz, C*H*CH$_2$O), 4.37 (1H, dd, *J*=10.9, 7.0 Hz, CHO), 4.45 (1H, dd, *J*=10.5, 7.0 Hz, CHO), 5.43 (1H, d, *J*=10.1 Hz, NH), 5.52 (1H, d, *J*=5.1 Hz, 4-H), 7.28 (2H, t, *J*=7.4 Hz, Ar-H$_2$), 7.38 (2H, t, *J*=7.4 Hz, Ar-H$_2$), 7.54 (2H, d, *J*=6.6 Hz, Ar-H$_2$), 7.75 (2H, d, *J*=7.4 Hz, Ar-H$_2$).

8.51. *N*-(1,1-Dimethylethoxycarbonyl)-L-leucine pentafluorophenyl ester (45)

Boc-L-LeuOH **16e** was treated with pentafluorophenol and DCC, as for the synthesis of **19a**, to give **45** (79%) as a colourless oil (lit.[41] oil): NMR δ_H 1.02 (6H, d, *J*=6.3 Hz, 2×Leu-Me), 1.47 (9H, s, Bu^t), 1.67 (2H, dd, *J*=9.7, 8.2 Hz, Leu β-H), 1.8 (2H, m, Leu β-H+Leu γ-H), 4.41 (0.1H, m, Leu α-H), 4.62 (0.9H, m, Leu α-H), 5.75 (0.1H, br, NH), 4.92 (0.9H, d, *J*=8.2 Hz, NH); NMR δ_F −162.1 (1.8F, t, *J*=21 Hz, 3′,5′-F$_2$), −161.9 (0.2F, t, *J*=21.0 Hz, 3′,5′-F$_2$), −157.6 (0.9F, t, *J*=22.4 Hz, 4′-F), −157.4 (0.1F, t, *J*=22.4 Hz, 4′-F), −152.7 (0.2F, d, *J*=19.7 Hz, 2′,6′-F$_2$),

Table 3. Crystal data and structure refinement of **20b**, **23b** and **23c**

Compound	**20b**	**23b**	**23c**
Empirical formula	$C_{22}H_{32}N_4O_6S$	$C_9H_{14}N_2O_2S$	$C_{11}H_{18}N_2O_2S$
Formula weight	480.58	214.28	242.33
Crystal system	Monoclinic	Orthorhombic	Triclinic
Space group	$P2_1$	$P2_12_12_1$	$P1$
a/Å	7.2780(1)	6.1600(1)	5.7440(2)
b/Å	21.0700(2)	6.5280(1)	9.7410(3)
c/Å	16.5780(2)	25.9050(5)	11.0190(4)
α/°	—	—	91.399(1)
β/°	94.700(1)	—	94.980(1)
γ/°	—	—	98.341(2)
U/Å^3	2533.65(5)	1041.70(3)	607.29(4)
Z	4	4	2
D_c/g cm^{-3}	1.260	1.366	1.325
M/mm^{-1}	0.170	0.287	0.255
F(000)	1024	456	260
Crystal size/mm	0.22×0.08×0.08	0.40×0.20×0.10	0.15×0.10×0.10
Theta min, max/°	3.81, 27.47	4.06, 27.86	3.60, 27.45
Index ranges	$-9\le h\le 9$; $-27\le k\le 27$; $-21\le l\le 21$	$-8\le h\le 8$; $-8\le k\le 8$; $-34\le l\le 34$	$-7\le h\le 7$; $-12\le k\le 12$; $-14\le l\le 14$
Reflections collected	48362	15756	9382
Independent reflections, *R*(int)	11591, 0.0738	2475, 0.0470	5031, 0.0391
Reflections observed (>2)	7928	2180	4185
Data/restraints/parameters	11591/5/625	2475/1/136	5031/5/307
Goodness-of-fit on F^2	1.005	1.037	1.040
Final *R*1, *wR*2 indices [*I*>2(*I*)]	0.0451, 0.0789	0.0298, 0.0697	0.0364, 0.0760
Final *R*1, *wR*2 indices (all data)	0.0886, 0.0904	0.0386, 0.0730	0.0513, 0.0818
Flack parameter	0.01(5)	0.02(8)	0.04(5)
Largest diff. peak and hole/eÅ^{-3}	0.226, −0.284	0.192, −0.219	0.253, −0.242

−152.2 (1.8F, d, J=18.4 Hz, 2′,6′-F_2); MS m/z 795 (2 M), 398.1394 (M+H) ($C_{17}H_{21}N_1O_4S$ requires 398.1391).

8.52. X-ray crystallography

Single crystals of compounds **20b**, **23b** and **23c** were analysed at 150(2) K using graphite-monochromated Mo Kα radiation and a Nonius Kappa CCD diffractometer. Details of the data collections, solutions and refinements are given in Table 3. The structures were uniformly solved using SHELXS-97[42] and refined using full-matrix least squares in SHELXL-97.[43]

Convergence was uneventful, other than for the following noteworthy points:

The asymmetric unit in **20b** and **23c** consists of two molecules. In **23b** and **23c**, the NH hydrogens were located and refined at 0.89 Å from the relevant parent nitrogens.

In all three structures, the NH and carbonyl groups are implicated in defining the supramolecular topology. In particular, the lattices in **20b** and **23b** are dominated by the hydrogen-bonded chains of molecules, whereas in **23c**, discrete intermolecular interactions occur between pairs of adjacent molecules in the gross array. Intermolecular hydrogen-bonding is also evident in **20b.**

Crystallographic data for all three compounds have been deposited with the Cambridge Crystallographic Data Centre as supplementary publications CCDC 603701–603703. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 1223 336033, e-mail: deposit@ccdc.cam.ac.uk].

8.53. Cyclisation studies

The HPLC equipment comprised a Dionex autosampler (Model ASl—100/ASl—100T), Dionex pump (Model P 580), Dionex column thermostat for HPLC and GPC (Model 585) and a Dionex UV–vis detector (Model UVD 170S/340S). A C_{18} Hypersil (Thermoquest BDS) column (250×4.6 mm) was used. The mobile phase was composed of a mixture (65/35, v/v) of acetonitrile and aqueous phosphate buffer (0.066 M), adjusted to pH 6.0, 7.0 and 8.0 with aqueous potassium hydroxide (19 M) using a Hanaa pH meter (Model 210). The phosphate buffer was filtered in a Vacuubrand GmbH vacuum system (Model ME 4) with a Schleicher and Schuell filter membrane (pore size and diameter of 0.45 and 47 mm). The flow rate during the assays was 0.8 mL min^{-1} and detection was accomplished at λ=275 and 225 nm. For each kinetic run, the dipeptide amide salt **21a**, **21b**, **21c**, **21d**, **40c** or **40e** (1.0 mg) was added to the buffer (1.0 mL) at the appropriate pH and the mixture was stirred at 37 °C. Samples were withdrawn at appropriate time points for direct HPLC analysis. The HPLC runs were also conducted at 37 °C.

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