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Functionalised 2,3-dimethyl-3-aminotetrahydrofuran-4-one and *N*-(3-oxo-hexahydrocyclopenta[*b*]furan-3*a*-yl)acylamide based scaffolds: synthesis and cysteinyl proteinase inhibition

John Watts, Alex Benn, Nick Flinn, Tracy Monk, Manoj Ramjee, Peter Ray,[†] Yikang Wang and Martin Quibell[*]

Amura Therapeutics Limited, Incenta House, Horizon Park, Barton Road, Comberton, Cambridge CB3 7AJ, UK

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Abstract—A stereoselective synthesis of functionalised (2*R*,3*R*)-2,3-dimethyl-3-amidotetrahydrofuran-4-one, its (2*S*,3*R*)-epimer and (3*aR*,6*aR*)-*N*-(3-oxo-hexahydrocyclopenta[*b*]furan-3*a*-yl)acylamide cysteinyl proteinase inhibitors has been developed using Fmoc-protected scaffolds **6**–**8** in a solid-phase combinatorial strategy. Within these scaffolds, the introduction of an alkyl substituent α to the ketone affords chiral stability to an otherwise configurationally labile molecule. Preparation of scaffolds **6**–**8** required stereoselective syntheses of suitably protected α-diazomethylketone intermediates **9**–**11**, derived from appropriately protected α-methylthreonines (2*R*,3*R*)-**12**, (2*R*,3*S*)-**13** and a protected analogue of (1*R*,2*R*)-1-amino-2-hydroxycyclopentanecarboxylic acid **14**. Application of standard methods for the preparation of amino acid α-diazomethylketones, through treatment of the mixed anhydride or pre-formed acyl fluorides of intermediates **12**–**14** with diazomethane, proved troublesome giving complex mixtures. However, the desired α-diazomethylketones were isolated and following a lithium chloride/acetic acid promoted insertion reaction provided scaffolds **6**–**8**. Elaboration of **6**–**8** on the solid phase gave α,β-dimethyl monocyclic ketone based inhibitors **38a**–**f**, **39a**,**b**,**d**,**e**,**f** and bicyclic inhibitors **40a**–**e** that exhibited low micromolar activity against a variety of cysteinyl proteinases.

1. Introduction

Over the last two decades, cysteinyl proteinases have been shown to exhibit a wide range of disease-related biological functions and as such the inhibition of cysteinyl proteinase activity has evolved into an area of intense current interest.[1] In particular, proteinases of the clan CA/family C1 (CAC1) have been implicated in a multitude of disease processes.[2,3] Examples include human proteinases such as cathepsin K (osteoporosis), cathepsins S and F (autoimmune disorders), cathepsin B (tumour invasion/metastases) and cathepsin L (metastases/autoimmune disorders), as well as parasitic proteinases such as falcipain (malaria parasite *Plasmodium falciparum*), cruzipain (*Trypanosoma cruzi* infection) and the CPB proteinases associated with *Leishmaniasis*.[2] Selective inhibition of any of these CAC1 proteinases offers enormous therapeutic potential and consequently there has been a concerted drive within the pharmaceutical industry towards the development of compounds suitable for human administration.[4] These efforts have largely focused on low molecular weight substrate based peptidomimetics and to date the most advanced inhibitors are in early clinical assessment.[5] The GSK candidate SB-462795 **1**, a potent inhibitor of cathepsin K indicated for the treatment of osteoporosis, emerged from the progression of early linear peptidomimetic ketones through to cyclic constrained ketone inhibitors.[6] The initial cyclic inhibitors of GSK were based upon potent, selective and reversible 3-amidotetrahydrofuran-4-ones **2a**, 3-amidopyrrolidin-4-ones **2b**, 4-amidotetrahydropyran-3-ones **2c** and 4-amido piperidin-3-ones **2d** (Fig. 1).

Upon further examination, it became apparent that cyclic ketones **2**, in particular the five-membered ring analogues **2a** and **2b**, suffered from configurational

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* Corresponding author. Tel.: +44-01223-264211; fax: +44-01223-265662; e-mail: martin.quibell@incenta.co.uk
[†] Present address: Organon Research Scotland, Newhouse, Lanarkshire, Scotland ML1 5SH, UK.

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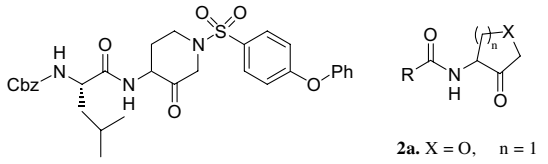
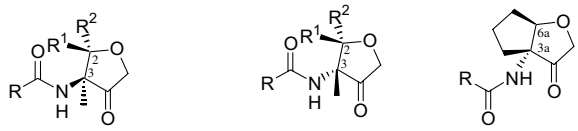


1. SB-462795

2a. X = O, n = 1
2b. X = NR', n = 1
2c. X = O, n = 2
2d. X = NR', n = 2
2e. X = NR', n = 3

Figure 1. GSK cyclic ketone inhibitors of cathepsin K.



3a. R^1, R^2 = H (3*S*)
3b. R^1 = Me, R^2 = H (2*R*, 3*S*)
3c. R^1 = H, R^2 = Me (2*S*, 3*S*)

4a. R^1, R^2 = H (3*R*)
4b. R^1 = Me, R^2 = H (2*R*, 3*R*)
4c. R^1 = H, R^2 = Me (2*S*, 3*R*)

5 (3*aR*, 6*aR*)

Figure 2. Stereochemical assignment of α,β-dimethyl monocyclic and *N*-(3-oxo-hexahydrocyclopenta[*b*]furan-3*a*-yl)acylamide bicyclic ketone inhibitors of CAC1 cysteinyl proteinases.

instability due to facile epimerisation at the centre situated α to the ketone.[6b,7,8] This physiochemical characteristic generally precluded the pre-clinical optimisation of inhibitors of formulae **2a**–**d** and led to the development of the configurationally stable azepanone series **2e**.[6b] As an alternative to the ring expansion approach, alkylation of the α-carbon would remove the ability of cyclic ketones **2** to undergo α-enolisation and hence lead to configurational stability. A preliminary examination of α-methylation in the 3-amidopyrrolidin-4-one **2b** system has been reported, resulting in a substantial loss in potency versus cathepsin K from $K_{i,app} \approx 0.18$ to 50 nM.[6b] However, it was unclear from this single example whether the α-stereochemistry examined was of the *S* configuration as depicted in **3** or the *R* configuration as depicted in **4** (Fig. 2). Our in-house molecular modelling of 3-methyl-3-amidotetrahydrofuran-4-ones **3a** and **4a** has highlighted the importance of the α-stereochemistry within these cyclic systems.[9] Herein we report our design strategy, building block preparation, solid-phase synthesis and the inhibition kinetics for a series of configurationally stable α,β-dimethyl monocyclic **4** and *N*-(3-oxo-hexahydrocyclopenta[*b*]furan-3*a*-yl)acylamide bicyclic ketones **5** as inhibitors of CAC1 proteinases.[10]

2. Results and discussion

2.1. Design strategy

Our design strategy commenced with in silico construction of each of the four possible hemithioketal tetrahedral intermediates (*si* or *re* stereofacial thiolate addition to both prime and nonprime binding orientations) for 3-methyl-3-amidotetrahydrofuran-4-one **3a**, then repeating the process for stereoisomer **4a**.[9] When considering the possible nonprime binding models, a

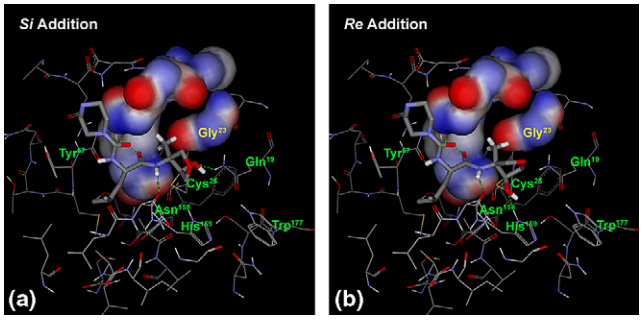


Figure 3. Predicted binding conformations for *si* (a) and *re* (b) stereofacial thiolate addition to a model α-methyl monocyclic **3a**, piperazine-1-carboxylic acid [3-methyl-1*S*-(3*S*-methyl-4-oxo-tetrahydrofuran-3-yl-carbamoyl)butyl]amide.[9,11] The residues Gly^{23}, Trp^{26}, Cys^{63}, Gly^{64} and Gly^{65} that make the P1 wall of cathepsin K structure 1mem are shown as an electrostatic potential coloured surface. The hydrogen atoms of the intermediate hydroxyl, α-methyl and α-NH are added and residue Gly^{23} labelled.

number of conclusions were apparent.[11] Stereoisomer **3a** modelled through either the *si* (addition of thiolate to face opposite the α-Me) or *re* (addition of thiolate to same face as the α-Me) tetrahedral intermediate produced an unfavourable spatial clash between the S1 wall of the proteinase comprised of Gly^{23}, Trp^{26}, Cys^{63}, Gly^{64} and Gly^{65} and the α-methyl group. In particular, there was a clear spatial overlap with the carbonyl of Gly^{23} (Fig. 3).

Molecular modelling of *si* thiolate addition to stereoisomer **3a** (Fig. 3a) predicted that the oxygen of the hemithioketal would be stabilised by a hydrogen bond to the NH_2 of the primary carboxamide side chain of glutamine[19] and a second hydrogen bond to the backbone NH of cysteine[25]. Additional hydrogen bonds were predicted between the P1 NH of **3a** and the backbone C=O of asparagine[158]; the P2 carbonyl oxygen of **3a** and the backbone NH of glycine[66] and the P2 NH of **3a** and the backbone carbonyl of glycine[66]. In contrast, *re* thiolate addition to stereoisomer **3a** (Fig. 3b) was predicted to exhibit the same P2/P1 interactions as for *si* addition, however the hydroxyl of the hemithioketal was now stabilised by two hydrogen bonds from the π-NH of the imidazole side chain of histidine[159].

When considering stereoisomer **4a**, binding conformations for both *si* (addition of thiolate to same face as the α-Me) and *re* (addition of thiolate to face opposite the α-Me) thiolate addition were predicted to retain the P2/P1 hydrogen-bonding network between the proteinase backbone and the inhibitor (compare Figs. 3 and 4). However, in contrast to stereoisomer **3a**, both binding modes predicted that the α-methyl of *R*-stereochemistry projects away from the proteinase and into the solvent exposed cavity (Fig. 4). A further contrast between stereoisomers **3a** and **4a** was evident in the *si* thiolate adduct (compare Figs. 3a and 4a), which predicted that the hydroxyl of the hemithioketal would again be stabilised by one hydrogen bond to the backbone NH of cysteine[25] but for **4a** the second hydrogen bond would now be to the backbone carbonyl of glycine[23].

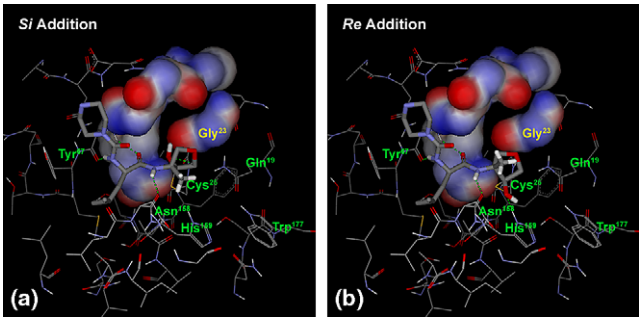


Figure 4. Predicted binding conformations for *si* (a) and *re* (b) stereofacial thiolate addition to a model α-methyl monocyclic **4a**, piperazine-1-carboxylic acid [3-methyl-1*S*-(3*R*-methyl-4-oxo-tetrahydrofuran-3-yl-carbamoyl)butyl]amide.[9,11] The residues Gly^{23}, Trp^{26}, Cys^{63}, Gly^{64} and Gly^{65} that make the P1 wall of cathepsin K structure 1mem are shown as an electrostatic potential coloured surface. The hydrogen atoms of the intermediate hydroxyl, α-methyl and α-NH are added and residue Gly^{23} labelled.

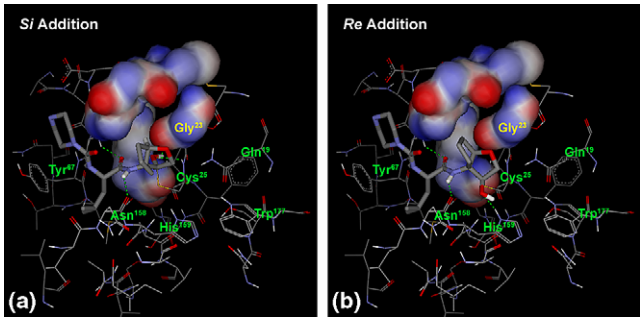


Figure 5. Predicted binding conformations for *si* (a) and *re* (b) stereofacial thiolate addition to a model bicycle **5**, (3*aR*,6*aR*) piperazine-1 carboxylic acid [3-methyl-1*S*-(3-oxo-hexahydrocyclopenta[*b*]furan-3*a*-ylcarbamoyl)butyl]amide.[9,10a] The residues Gly^{23}, Trp^{26}, Cys^{63}, Gly^{64} and Gly^{65} that make the P1 wall of cathepsin K structure 1mem are shown as an electrostatic potential coloured surface. The hydrogen atoms of the intermediate hydroxyl, ring bridgehead 6a and -NH are added.

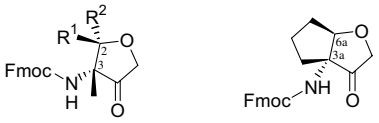
When considering the possible prime-side binding models, a number of conclusions were apparent.[11] The general spatial fit of models generated from **3a** with *si* or *re* thiolate addition and **4a** with *re* thiolate addition were predicted to be reasonable with no obvious clash with the proteinase. However, apart from the tetrahedral hydroxyl, few hydrogen-bonding interactions were predicted between the compounds and proteinase.[12] Potential binding modes of this nature, with the loss of major backbone hydrogen-bonding networks would probably lead to a dramatic loss of potency. Prime-side binding models for stereoisomer **4a** with *si* thiolate addition were predicted to give an unfavourable clash with Gly^{23} of the proteinase. Thus, from the eight binding conformations considered for 3-methyl-3-amidotetrahydrofuran-4-ones **3a** and **4a**, only the nonprime-side models of **4a** are predicted to exhibit a reasonable energy conformer that retained a good hydrogen-bonding network to the proteinase backbone along with the absence of spatial clashes (Fig. 4).[13]

The design process was further extended through the introduction of an alkyl substituent to *C*-2 within general formulae **3** and **4**. Building upon the models depicted in Figures 3 and 4, a methyl substituent was added to give **3b** (*C*-2 *R*), **3c** (*C*-2 *S*), **4b** (*C*-2 *R*) and **4c** (*C*-2 *S*) and modelling assessment of all 16 possible binding conformations conducted. As detailed previously, results indicated that nonprime-side binding of all compounds of general formulae **3** gave an unfavourable overlap between the α-methyl substituent and the proteinase. In contrast, nonprime-side binding of compounds of general formulae **4** gave a mixture of results. Compound **4b** was predicted to bind similarly to **4a** (Fig. 4a and b), with the addition of a synclinal relationship (−45° looking along the bond from *C*-2 → *C*-3) between the *C*-2 and *C*-3 methyl substituents. However, if compound **4c** bound in a similar orientation to **4a** (Fig. 4), the addition of an antiperiplanar relationship (180°) between the *C*-2 and *C*-3 methyl, would result in the *C*-2 methyl exhibiting an unfavourable clash with the proteinase. Prime-side binding models for compounds **3b**,**c** and **4b**,**c** were complicated by predicted clashes between either the *C*-2 or *C*-3 methyl with either Gly^{23} or Gly^{64} of the proteinase for six of the eight possible conformations. Only **3b** *si* and **3c** *re* gave solvent exposed methyl substituents, however, as before, all prime-side models were predicted to lose the major backbone hydrogen-bonding network. Thus, from the 16 binding conformations considered for 2,3-dimethyl-3-amidotetra-hydrofuran-4-ones **3b**,**c** and **4b**,**c** only the nonprime-side model of **4b** was predicted to exhibit a reasonable energy conformer that retained a good hydrogen-bonding network to the proteinase backbone along with the absence of spatial clashes.[14]

In the final part of our design process, the nonprime-side models of **4b** were used to formulate the bicyclic ketone **5**, containing the *cis*-fused *N*-(3-oxo-hexahydrocyclopenta[*b*]furan-3*a*-yl)acylamide. Models of *si* thiolate addition to diastereoisomer **5** (Fig. 5a) predict stabilisation of the oxygen of the hemithioketal by a hydrogen bond to the backbone NH of cysteine^{25} and a second hydrogen bond between the hydroxyl and the backbone carbonyl of glycine^{23}, along with the standard P2/P1 interactions (e.g., see earlier description for **3a**). Again, similar to the observations made for *re* addition to stereoisomer **3a**, *re* thiolate addition to diastereoisomer **5** (Fig. 5a) was predicted to exhibit the same P2/P1 interactions as for *si* addition, with the hydroxyl of the hemithioketal being stabilised by two hydrogen bonds from the π-NH of the imidazole side chain of histidine^{159}.

2.2. Chemistry

2.2.1. Synthesis of Fmoc-protected scaffolds 6–8. Synthesis of compounds of general formulae **4** and **5** was envisaged via solid-phase chemistries utilising the key scaffold intermediates (2*R*,3*R*)-**6**, (2*S*,3*R*)-**7** and (3*aR*,6*aR*)-**8** (Fig. 6).



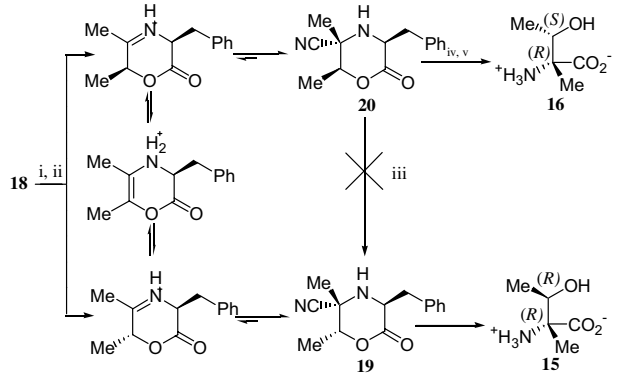
6. R^1 = Me, R^2 = H
7. R^1 = H, R^2 = Me

8

Figure 6. Protected mono- and bicyclic building blocks towards solid-phase assembly of compounds of general formulae **4** and **5**.

Retrosynthetic analysis indicated that the adaptation of a literature method for the preparation of compounds of general formula **2a**, through a lithium chloride/acetic acid promoted insertion reaction of protected amino acid α-diazomethylketones,[8] may provide scaffolds **6**–**8** via α-diazomethylketones **9**–**11**. It was thought that α-diazomethylketones **9**–**11** would be available from protected amino acids **12**–**14** (Fig. 7), which in turn are available from the free amino acids **15**–**17**.

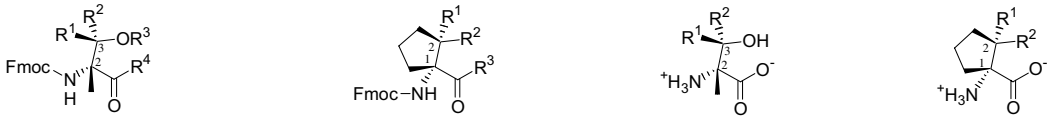
Routes towards amino acids **15**–**17** were sought and a survey of the literature identified the work of Ohfune et al. who have described the synthesis of α-alkylated serine and threonine analogues[15] and the cyclic amino acid (1*R*,2*S*)-**17b**.[16] The reported synthesis of (2*R*,3*R*)-**15** and (2*R*,3*S*)-**16** was appealing for our synthetic strategy since it offered a stereodivergent route to the desired diastereoisomers (Scheme 1). However, following the literature procedure, we were unable to achieve the quoted yields, particularly for the intramolecular Strecker reaction and equilibrium isomerisation (it may be that the methodology is highly sensitive to scale-up). Therefore, we adapted the literature procedures as follows. Treatment of diastereomeric *N*-Boc-L-phenylalanine acetoin ester **18** (78% yield by the coupling of *dl*-acetoin and *N*-Boc-L-phenylalanine using 1-[3-(dimethylamino)propyl]-3-carbodiimide·HCl (EDC) activation in the presence of 4-(dimethylamino)pyridine (DMAP)) with trifluoroacetic acid (TFA) in dichloromethane gave the corresponding TFA salt. Treatment of the TFA salt



Scheme 1. Synthesis of (2*R*,3*S*)-**16**. Reagents and conditions: (i) TFA, CH_2Cl_2; (ii) 2 equiv powdered NaCN, 2-propanol, 2 h; (iii) 1 equiv TFA, 2-propanol then recrystallisation from Et_2O/hexane; (iv) 2 equiv *tert*-BuOCl, Et_2O, then triethylamine; (v) cHCl; Dowex® 50W×4 (elution with 1 M NH_3), then recrystallisation from H_2O/EtOH/Et_2O.

in anhydrous 2-propanol with powdered sodium cyanide for 2 h, in our hands resulted in an improved formation of the mixture of nitriles **19** and **20** (46%). Flash chromatography followed by recrystallisation from diethyl ether/heptane gave enantiopure **20** (18%). Despite the low overall yield of **20**, the above modification was reproducible and provided sufficient quantities of material (~3 g). Attempts to reproduce the reported isomerisation of enantiopure **20** (as well as the mother liquor obtained after recrystallisation, which contained **19** and **20**) failed to provide any useful quantities of **19**.[17] Finally, removal of the phenylalanyl moiety from **20** was accomplished by oxidation with freshly prepared *tert*-butylhypochlorite and triethylamine, followed by hydrolysis with concentrated hydrochloric acid to give (2*R*,3*S*)-**16** (33% from **20**) after ion-exchange chromatography and recrystallisation from water (trace)/ethanol/diethyl ether.

Due to the difficulties encountered with the above preparation of **19**, the methodology reported by Seebach



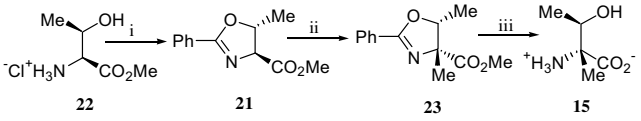
9a. R^1 = Me, R^2 = H, R^3 = H, R^4 = -CH=N_2
9b. R^1 = Me, R^2 = H, R^3 = Bu^t, R^4 = -CH=N_2
10a. R^1 = H, R^2 = Me, R^3 = H, R^4 = -CH=N_2
10b. R^1 = H, R^2 = Me, R^3 = Bu^t, R^4 = -CH=N_2
12a. R^1 = Me, R^2 = H, R^3 = H, R^4 = OH
12b. R^1 = Me, R^2 = H, R^3 = Bu^t, R^4 = OH
12c. R^1 = Me, R^2 = H, R^3 = H, R^4 = OAllyl
12d. R^1 = Me, R^2 = H, R^3 = Bu^t, R^4 = OAllyl
13a. R^1 = H, R^2 = Me, R^3 = H, R^4 = OH
13b. R^1 = H, R^2 = Me, R^3 = Bu^t, R^4 = OH
13c. R^1 = H, R^2 = Me, R^3 = H, R^4 = OAllyl
13d. R^1 = H, R^2 = Me, R^3 = Bu^t, R^4 = OAllyl
13e. R^1 = H, R^2 = Me, R^3 = Bu^t, R^4 = F

11a. R^1 = H, R^2 = OH, R^3 = -CH=N_2
11b. R^1 = H, R^2 = OBu^t, R^3 = -CH=N_2
14a. R^1 = H, R^2 = OH, R^3 = OH
14b. R^1 = H, R^2 = OBu^t, R^3 = OH
14c. R^1 = OH, R^2 = H, R^3 = OAllyl
14d. R^1 = H, R^2 = OH, R^3 = OAllyl
14e. R^1 = H, R^2 = OBu^t, R^3 = OAllyl
14f. R^1 = H, R^2 = OBu^t, R^3 = F

15 (2*R*, 3*R*) R^1 = Me, R^2 = H
16 (2*R*, 3*S*) R^1 = H, R^2 = Me

17a (1*R*, 2*R*) R^1 = H, R^2 = OH
17b (1*R*, 2*S*) R^1 = OH, R^2 = H

Figure 7. Amino acid building blocks towards mono- and bicyclic scaffolds **6**–**8**.

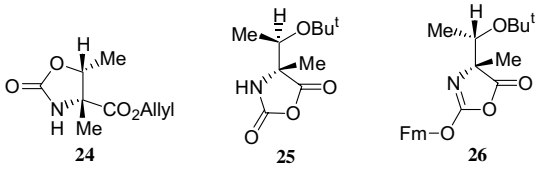


Scheme 2. Synthesis of (2*R*,3*R*)-**15**. Reagents and conditions: (i) H_2O, EtOCNHPh, Et_2O; (ii) Li(*i*-Pr)$_2$N, THF, hexane, then CH_3I; (iii) 6 M HCl, then Dowex® 50W×4 (elution with 1 M NH_3) and recrystallisation from H_2O/EtOH/Et_2O.

et al. (Scheme 2) was used in the synthesis of (2*R*,3*R*)-**15**.[18] 5*R*-Methyl-2-phenyl-4,5-dihydrooxazole-4*S*-carboxylic acid methyl ester **21** was prepared (67%) by a slight modification of the literature protocol, from the commercially available ethyl benzimidate hydrochloride salt (which was rendered salt free by washing a diethyl ether solution with saturated aqueous sodium hydrogen carbonate) and L-threonine methyl ester hydrochloride **22**. Stereo-controlled methylation gave **23** (88%), which underwent facile hydrolysis with 6 M hydrochloric acid to give (2*R*,3*R*)-**15** (99%) after ion-exchange chromatography. Recrystallisation from water (trace)/ethanol/diethyl ether gave **15** in multigram quantities.

Fmoc Nα-protection of amino acids **15** and **16** proceeded smoothly under standard Schotten–Baumann conditions to obtain amino acids **12a** (92%) and **13a** (80%). In situ activation of **12a** as the mixed anhydride using *iso*-butylchloroformate and *N*-methylmorpholine also proceeded smoothly (HPLC-MS and analytical HPLC monitoring showed >95% activation). However, the addition of freshly prepared diazomethane to the mixed anhydride of **12a** gave a complex mixture of products from which the desired α-diazomethylketone **9a** (11%), methyl ester (**12a** wherein $R^4 = OMe$, 18%) and an uncharacterised mixture of products, which appeared to polymerise on standing/attempted recrystallisation were isolated.[19] Literature precedent appears to suggest that the formation of methyl esters under these conditions is common for hindered carboxylic acids, even though the activation of **12a** appeared near quantitative.[20] Treatment of **9a** with a solution of lithium chloride in water and acetic acid gave the desired scaffold (2*R*,3*R*)-(2,3-dimethyl-4-oxo-tetrahydrofuran-3-yl)carbamic acid 9*H*-fluoren-9-ylmethyl ester **6** (50%, overall yield 5.5% from **12a**) following chromatography.

Since formation of α-diazomethylketone **9a** was low yielding, we investigated the preparation of α-diazomethylketone **9b** containing *tert*-butyl ether protection of the β-hydroxyl. It was envisaged that the precursor acid **12b** would be available from the intermediate allyl ester **12d**, in turn prepared by *tert*-butylation of allyl ester **12c**. Preparation of **12c** was attempted from **12a** using *para*-toluenesulfonic acid, allyl alcohol and toluene under Dean–Stark reflux conditions. However, the carbamate 4,5-dimethyl-2-oxo-oxazolidine-4-carboxylic acid allyl ester **24** was obtained as the major product (84%), together with a minor amount of the desired allyl ester **12c** (2%). The formation of carbamate was avoided by the use of allyl bromide under mild phase transfer catalysed conditions described by Kunz and co-workers,[21] providing **12c** in excellent yield (94%). Protection of the hydroxyl group of **12c** as the *tert*-butyl ether to give **12d** was accomplished using isobutene in the presence of catalytic concd sulfuric acid (87% yield based on recovered starting material). The allyl ester was then smoothly cleaved using Pd-catalysed allyl transfer to give acid **12b** (98%).[22] Activation of **12b** as the mixed anhydride using *iso*-butylchloroformate and *N*-methylmorpholine proceeded smoothly (similar to that observed for **12a** with HPLC-MS and analytical HPLC monitoring showing >95% activation). However, in contrast to activated **12a**, the addition of freshly prepared diazomethane to the mixed anhydride of **12b** gave no indication of the desired α-diazomethylketone **9b**, with a new major product identified as the *N*-carboxyanhydride **25** isolated (61%).[23] This route was not examined further since the desired scaffold **6** was already in hand.

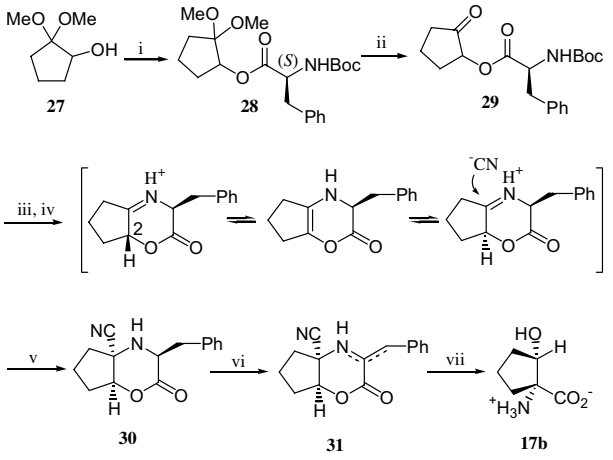


For the synthesis of scaffold (2*S*,3*R*)-**7** we took into consideration the observations and conclusions detailed above, in particular the low yield of α-diazomethylketone **9a** from **12a** and the high yield of *N*-carboxyanhydride **25** from the activation of **12b**. We chose to investigate the preparation of α-diazomethylketone **10b** from protected acid **13b**, through acyl fluoride **13e**. Literature reported that activation of *N*-protected hindered α,α-disubstituted amino acids as acyl fluorides was highly favourable due to their inherent stability after purification (even in the presence of *tert*-butyl-based protecting groups), their slow conversion to 4*H*-oxazol-5-one in the presence of tertiary bases and their ability to react in high yield using relatively short reaction times.[24] Thus, Nα-protected amino acid **13a** was converted to **13b** following the optimised protocols described for the conversion of **12a** to **12b**. Treatment of acid **13b** with pyridine and cyanuric fluoride gave the corresponding acyl fluoride **13e** in near quantitative yield. Frustratingly, in contrast to the general literature precedence,[24] treatment of the **13e** with diazomethane gave 4-(1*S*-*tert*-butoxyethyl)-2-(9*H*-fluoren-9-ylmethoxy)-4*R*-methyl-4*H*-oxazol-5-one **26** as the major product (45% yield from **13e**), which was partially separated from a mixture of the desired α-diazomethylketone **10b** and methyl ester (**13b** wherein $R^4 = OMe$). Treatment of **10b** with a solution of lithium chloride in acetic acid and water gave the desired scaffold (2*S*,3*R*)-**7** (5.4% from acid **13b**) following chromatography. Furthermore, 4*H*-oxazol-5-one **26** could be converted back into acyl fluoride **13e** using a combination of HF/pyridine and cyanuric fluoride,[24] which opens the possibility of recycling of **26** into **13e**.

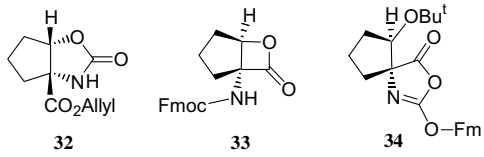
Having synthesised scaffolds **6** and **7**, we turned our attention to synthesis of the ring constrained analogue **8**.

It was envisaged that **8** would be obtained from the cyclisation of α-diazomethylketones **11a** or **11b**, that in turn would be obtained from the corresponding β-hydroxy amino acid **17a** (see Fig. 7). Synthesis of the epimeric β-hydroxy amino acid **17b** has been reported by Ohfune et al. and the method is outlined in Scheme 3.[16] The methodology makes use of the previously discussed intramolecular Strecker reaction. Since there were no literature methods for the preparation of **17a**, we chose to synthesise **17b** according to Scheme 3 with the aim of inverting the hydroxyl group at a later stage. Thus, alcohol **27**, prepared by oxidation of cyclopentanone, was coupled to BocPheOH (using EDC and DMAP) to give **28**. Hydrolysis of the dimethyl ketal was accomplished with *para*-toluenesulfonic acid monohydrate in wet acetone to give the corresponding ketone **29** (40% yield from cyclopentanone). Removal of the Boc protecting group and cyclisation under a modification of the reported literature protocol gave the desired (4*aS*,7*aS*)-carbonitrile **30** (36%), together with ketimine intermediates (see parentheses in Scheme 3). Carbonitrile **30** was then oxidised with freshly prepared *tert*-butylhypochlorite and triethylamine to give crude **31** and the resultant product mixture was hydrolysed with concentrated hydrochloric acid and purified by ion-exchange chromatography to obtain the desired β-hydroxy amino acid **17b** (67%).

Fmoc protection of **17b**, followed by allylation under the previously described phase transfer conditions gave allyl ester **14c** (68%). Attempts to invert the hydroxyl group of **14c** under Mitsunobu conditions using formic acid as the nucleophile were unsuccessful.[25] However, inversion of the hydroxyl was achieved by Swern oxidation of **14c**, followed by sodium borohydride reduction to give a mixture of 1*R*-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2*R*-hydroxycyclopentanecarboxylic acid allyl ester **14d** and 1*R*-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2*S*-hydroxycyclopentanecarboxylic acid allyl ester **14c** (approximately 9:1, 55% yield in total). To facilitate purification, the mixture of alcohols was heated at 100 °C in toluene in the presence of *para*-toluenesulfonic acid. Whilst the *trans*-(*R*,*R*)-epimer **14d** remained intact, the *cis*-(*R*,*S*)-epimer **14c** cyclised to form the bicycle oxazolidine **32** that was readily separated from the desired product by chromatography. Additionally, following the Fmoc protection of **17b**, further evidence for the stereochemical assignment of alcohols **14c** and **14d** was obtained upon treatment with allyl alcohol and *para*-toluenesulfonic acid in refluxing toluene. Here the isolated products from the reaction included **32** (49%) together with a *trans* epimer, which based upon the precedence of Ohfune et al.[16] was assigned as the desired allyl-protected (*R*,*R*)-epimer **14d** (9%), presumably formed from a minor component present in the starting material, namely the (*R*,*R*)-acid **14a.** It is likely that this (*R*,*R*)-acid **14a** would have arisen from a small amount of the (*R*,*R*)-epimer **17a** being present following HCl hydrolysis of **31** (Scheme 3). The minor (*R*,*R*)-component was probably formed from one of the alternative ketimine intermediates shown in Scheme 3, with the stereochemistry of the *C*-2 carbon atom being incompletely controlled by the stereochemistry of the phenylalanine.



Scheme 3. Synthesis of **17b**. Reagents and conditions: (i) BocPheOH, EDC, DMAP, dichloromethane; (ii) *para*-toluenesulfonic acid monohydrate, $(CH_3)_2CO$; (iii) TFA, CH_2Cl_2, then concentration in vacuo and azeotropic removal of excess TFA with toluene; (iv) $MgSO_4$, NaOAc CH_3CN, then filtration and concentration in vacuo; (v) TMSCN, $ZnCl_2$; (vi) 2 equiv *tert*-BuOCl, Et_2O, followed by triethylamine; (vii) cHCl, Dowex® 50W×4 (elution with 1 M NH_3).

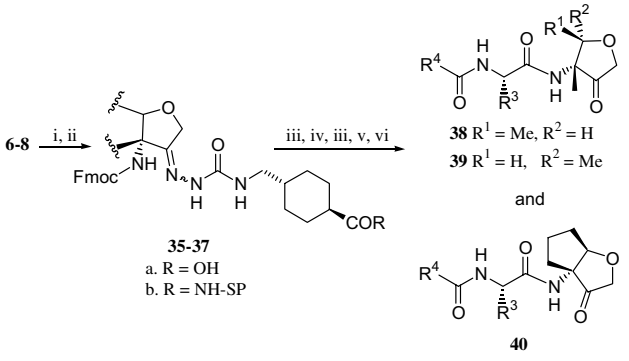


The alcohol functionality of the (*R*,*R*)-epimer **14d** was protected as the *tert*-butyl ether using isobutene in the presence of concentrated sulfuric acid to obtain **14e** (75%). Then the allyl ester of both **14d** and **14e** were smoothly cleaved using Pd-catalysed allyl transfer to give acids **14a** (80%) and **14b** (78%).[22] In situ activation of acid **14a** as the mixed anhydride using *iso*-butylchloroformate and *N*-methylmorpholine proceeded smoothly (HPLC-MS and analytical HPLC monitoring showed >95% activation). However, following treatment with diazomethane, no evidence for the desired diazomethylketone **11a** was observed,[19] with the only isolable product being tentatively identified as the 4,5-bicyclic lactone **33** (41%). Similarly, in situ activation of acid **14b** as the mixed anhydride proceeded smoothly, but following treatment with diazomethane a complex inseparable mixture was now observed.[19] The desired scaffold **8** was however prepared via the pre-formed acyl fluoride **14f**, which was prepared from acid **14b** by treatment with pyridine and cyanuric fluoride. In a similar manner to that described earlier for acyl fluoride **13e**, treatment of the crude acyl fluoride **14f** with diazomethane led to the recovery of mixtures of **14f**, oxazolone **34** and (1*R*,2*R*)-2-*tert*-butoxy-1-(9*H*-fluoren-9-ylmethoxycarbonylamino)cyclopentanecarboxylic acid methyl ester (**14b** wherein $R^3 = OMe$) as the major products (approximately 50%), together with the desired α-diazomethylketone **11b** (13% yield from **14f**). The oxazolone **34** containing mixture above could again be

re-cycled to the acyl fluoride **14f** by using a combination of HF/pyridine and cyanuric fluoride. Finally, **11b** was treated with a solution of lithium chloride in acetic acid and water to give the desired ring constrained analogue **8** (83% from **11b**).

2.2.2. Solid-phase synthesis. Two main strategies towards the solid-phase assembly of ketones have recently been developed.[26] Firstly, the ketone functionality has been transiently protected as the dimethylketal, then the scaffold linked to the solid phase through an amine via an acid labile backbone linker.[26b] Alternatively, the ketone containing scaffold has been derivatised as an acid labile semicarbazone, through reaction with a hydrazide.[26c,d] Our solid-phase strategy was based upon the utilisation of the hydrazide linker described by Murphy et al.,[27] (Scheme 4).

Semicarbazones **35a**–**37a** were prepared by refluxing the respective ketones **6**–**8** with the hydrazide linker of Murphy et al.,[27] in aqueous ethanol/sodium acetate. For scaffolds **6** and **7** optimal formation of the linker constructs **35a** and **36a** was obtained after 24 h. For bicyclic scaffold **8** optimal formation of the linker construct **37a** was obtained after 8 h.[28a] Semicarbazones **35a**–**37a** provided a carboxylic acid for attachment to an aminomethyl functionalised polymer support (SP) and also protection of the ketone from nucleophilic attack during the synthetic steps prior to cleavage. The efficiency of formation of loaded linker constructs **35b**–**37b** was monitored based by spectrophotometric determination of the Fmoc-derived chromophore liberated upon treatment with 20% piperidine in dimethylformamide and shown to be essentially quantitative.[29] The efficiency of acidolytic cleavage was monitored by treatment of the loaded construct with TFA/H_2O, followed by an assessment of the residual (i.e., noncleaved) Fmoc-derived chromophore. Constructs **35b**–**37b** were found to require significantly extended cleavage times when



Scheme 4. Synthesis and use of supported linker constructs **35b**–**37b**. Reagents and conditions: (i) *trans*-4[(hydrazinocarbonyl)amino]methylcyclohexanecarboxylic acid. trifluoro-acetate, EtOH, H_2O, NaOAc, reflux; (ii) 3 equiv **35a**–**37a**, 3 equiv HBTU, 3 equiv HOBt, 6 equiv NMM, H_2N-solid phase, DMF; (iii) 20% piperidine/DMF (v/v), 30 min; (iv) 10 equiv Fmoc-NHCHR^3-COOH, 10 equiv HBTU, 10 equiv HOBt, 20 equiv NMM, DMF, rt, o/n; (v) 10 equiv R^4-COOH, 10 equiv HBTU, 10 equiv HOBt, 20 equiv NMM, DMF, rt, o/n; (vi) TFA/H_2O, (95:5, v/v), rt, 2×24 h (**35b**, **36b**), 1×24 h (**37b**).

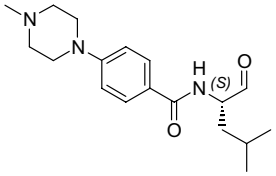
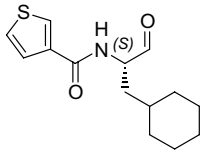
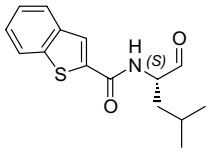
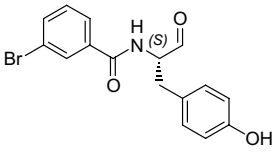
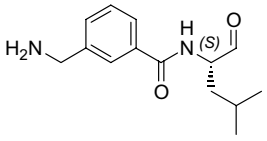
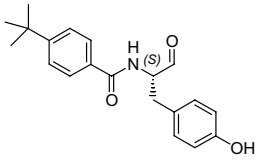
compared to nonhindered ketones.[28b] Final compounds **38**–**40** were prepared by a series of sequential washing and coupling reaction steps involving removal of Fmoc, coupling of an activated Fmoc-NHCHR^3-COOH, removal of Fmoc, coupling of an activated R^4-COOH and acidolytic cleavage. The coupling of activated Fmoc-amino acids to the amine functionality of the α,α-dialkylated loaded scaffolds was expected to be a difficult reaction due to steric hinderance.[30] However, the coupling was found to be surprisingly facile, exhibiting complete acylation under standard uronium activation conditions. This facile acylation is probably due to the restricted conformational freedom of the loaded scaffold providing relatively easy access to the α-amine functionality.

2.2.3. Enzyme inhibition studies. Following the methods detailed in Scheme 4, monocyclic inhibitors **38a**–**f**, **39a**,**b**,**d**,**e**,**f** and bicyclic inhibitors **40a**–**e** were prepared and then screened against cathepsins B, K, L and S as well as the parasitic proteinases cruzain and CPB.[10b] The preliminary steady-state inhibition constants (K_i^{ss}) are shown in Table 1 (mean of $n = 3$ determinations). The substituents detailed in Table 1 were chosen to exemplify binding groups that provide potent inhibitors when combined with the unsubstituted monocyclic scaffold **2a** of GSK.[31]

In general, the results show a marked loss of potency for both the α-methyl monocyclic analogues (**38** and **39**) and the *cis*-fused bicyclic analogues (**40**) when compared with the non-α-substituted equivalents. However, clear structure–activity trends are observed within an active series against a particular proteinase. For example, when comparing series *a* for cathepsin K, series *b* for cathepsin S, series *d* for cathepsin L and series *e* for cathepsins K and L, generally bicyclic analogues **40** were more potent than the monocyclic analogues (2*R*,3*R*)-**38**, which in turn were more potent than monocyclic analogues (2*S*,3*R*)-**39**. These experimental finding are consistent with the modelling conclusions formulated during our design process. As detailed previously, it was clear that stereoisomer (2*R*,3*R*)-**4b** was predicted to have superior binding when compared to stereoisomer (2*S*,3*R*)-**4c** and this has translated into generally improved potency for series (2*R*,3*R*)-**38** when compared to series (2*S*,3*R*)-**39**. Also, during the design process the possibility of cyclising the *cis*-methyl substituents of (2*R*,3*R*)-**4b** providing bicyclic **5** was proposed (see Fig. 5). Experimentally, this has translated into improved potency for series (3*aR*,6*aR*)-**40** when compared to series (2*R*,3*R*)-**38**. Given the reasonable binding conformations[9] and comparable scoring functions[11] for inhibitors such as **38a** and **40a** against cathepsin K or **38b** and **40b** against cathepsin S when compared with the equivalent non-α-methylated analogues, we were intrigued to understand why such a marked loss of potency has occurred. Thus, individual association (k_{on}) and dissociation (k_{off}) rate constants were determined for series *b* against cathepsin S and the results detailed in Table 2.

Clearly, once associated, inhibitors **38b**, **40b** and **41** exhibit comparable hydrolysis rates as reflected by their

Table 1. Preliminary inhibitory activities (K_i^{ss}, μM) for mono- and bicyclic inhibitors **38**–**40**[31]

Compound no	P3–P2 Structure	Cat. K	Cat. L	Cat. S	Cat. B	Cruz.	CPB
38a		8.7	>65	NI[a]	NI	NI	>50
39a		NI	26	NI	NI	NI	NI
40a		1.8	21	>40	NI	12.3	21
38b		NI	>80	9.9	NI	NI	NI
39b		NI	>60	>85	NI	NI	NI
40b		NI	>50	5.6	NI	NI	NI
38c		NI	>65	NI	NI	NI	NI
40c		2.8	>50	>40	>50	>75	>50
38d		NI	>35	NI	NI	NI	NI
39d		NI	>30	NI	NI	NI	NI
40d		NI	2.8	>60	NI	>55	>70
38e		22	11.3	>40	NI	>70	>60
39e		NI	>80	NI	NI	NI	NI
40e		15.7	9.2	>70	NI	>60	>60
38f		>50	>30	>30	>30	>30	5.5
39f		NI	>60	NI	NI	NI	NI

[a] Where NI stands for no observed inhibition.

broadly similar k_{off} rates. However, the association rate of inhibitors **38b** and **40b** was found to be significantly slower than that of the parent non-α-methylated analogue **41** and this explains the loss in potency since the steady-state inhibition constant $K_i^{ss} \approx k_{off}/k_{on}$. The effect on association rate is most clearly seen in **39b** where a dramatically reduced k_{on} is observed, which is consistent with the modelling conclusions formulated during our design process.

3. Conclusions

The introduction of an α-alkyl group into the 3-amido-tetrahydrofuran-4-one scaffold removed the possibility of enolisation, thus stabilising the α-stereochemistry. However, introduction of the simple α-methyl group or cyclisation of simple α,β-dimethyl substituents had a profound effect upon both the synthesis and cysteinyl proteinase inhibition properties of these new scaffolds. Synthetically, manipulation of α-methylthreonines towards the (2*R* or *S*,3*R*)-2,3-dimethyl-3-amido-tetrahydrofuran-4-one scaffolds proved challenging due to their tendency to undergo multiple strain-relieving side reactions that were not observed when reacting the corresponding threonine analogues. Similarly, multiple side reactions were observed when manipulating the cyclic amino acid (1*R*,2*R*)-1-amino-2-hydroxycyclopentanecarboxylic acid towards the bicyclic (3*aR*,6*aR*)-*N*-(3-oxo-hexahydrocyclopenta[*b*]furan-3*a*-yl)-acylamide scaffold. Additionally, utilisation of the new α-methyl monocyclic and *cis*-5,5-bicyclic scaffolds on the solid

Table 2. Association (k_{on}) and dissociation rate constants (k_{off}) for series *b* inhibitors against cathepsin S

Compound	k_{on} (M^{-1} s^{-1}) ($\times 10^3$)	k_{off} (s^{-1}) ($\times 10^{-3}$)	K_i (M) ($\times 10^{-6}$)[a]	K_i^{ss} (M) ($\times 10^{-6}$)
38b	2.6 ± 0.2	8.8 ± 1.8	3.3	9.9 ± 2.5
39b	0.009 ± 0.001	4.9 ± 0.9	544	86.2 ± 24.2
40b	1.1 ± 0.6	16 ± 2	14.5	5.6 ± 0.4
41[b]	100 ± 30	37 ± 8	0.37	0.23 ± 0.08

[a] Inhibition constants calculated from the individual k_{on} and k_{off} rates.
[b] The parent non-α-methylated analogue (2*R*,3*R*)-furan-3-carboxylic acid [2-cyclohexyl-1*S*-(2-methyl-4-oxo-tetrahydrofuran-3-ylcarbamoyl)-ethyl]amide.

phase highlighted that the introduction of a simple α-methyl group had led to a dramatic effect upon the steric accessibility of the ketone moiety through significantly extended linker formation and cleavage times. The increased chemical steric hinderance of the ketone moiety was observed biologically through significantly reduced association rates for the α-alkylated inhibitor analogues with the active site thiolate nucleophile. However, low micromolar potency analogues were obtained, with good selectivity, for a range of therapeutically attractive CAC1 proteinases. Further exemplification of the α-alkylation principle that ensures α-chiral integrity, through spanning of the active site into both prime and nonprime sides, could possibly access additional binding parameters leading to an improvement in inhibitor potency into the nanomolar range.

4. Experimental

4.1. General procedures

Standard vacuum techniques were used in handling of air sensitive materials. Solvents were purchased from ROMIL Ltd, UK at SpS or Hi-Dry grade unless otherwise stated. ^{1}H NMR and ^{13}C NMR: Bruker DPX400 (400 MHz ^{1}H frequency and 100 MHz ^{13}C frequency; QXI probe) in the solvents indicated. Chemical shifts are expressed in parts per million (δ) and are referenced to residual signals of the solvent. Coupling constants (*J*) are expressed in Hz. All analytical HPLC were obtained on Phenomenex Jupiter C_4, 5 μm, 300 Å, 250 × 4.6 mm, using mixtures of solvent A = 0.1% aq trifluoroacetic acid (TFA) and solvent B = 90% acetonitrile/10% solvent A on automated Agilent systems with 215 and/or 254 nm UV detection. Unless otherwise stated a gradient of 10–90% B in A over 25 min at 1.5 mL/min was performed for full analytical HPLC. HPLC-MS analysis was performed on an Agilent 1100 series LC/MSD, using automated Agilent HPLC systems, with a gradient of 10–90% B in A over 10 min on Phenomenex Columbus C_8, 5 μm, 300 Å, 50 × 2.0 mm at 0.4 mL/min. Semipreparative HPLC purification was performed on Phenomenex Jupiter C_4, 5 μm, 300 Å, 250 × 10 mm, using a gradient of 10–90% B in A over 25 min at 4 mL/min on automated Agilent systems with 215 and/or 254 nm UV detection. Flash column purification was performed on silica gel 60 (Merck 9385). Multipins (polyamide 1.2 or 10 μmol loadings, see www.mimotopes.com) were used for the solid-phase synthesis. Biochemical protocols together with enzyme assays were carried out as previously described.[10b] Substrates utilising fluorescence resonance energy transfer methodology (i.e., FRET-based substrates) were synthesised using standard solid-phase Fmoc chemistry methods,[32] and employed Abz (2-aminobenzoyl) as the fluorescence donor and 3-nitrotyrosine [Tyr(NO_2)] as the fluorescence quencher.[33]

4.1.1. (2*S*)-2-*tert*-Butoxycarbonylamino-3-phenylpropionic acid 1-methyl-2-oxopropyl ester (18). 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (10.9 g, 57 mmol) and 4-(dimethylamino)pyridine (700 mg, 5.7 mmol) were added to a solution of (2*R*)-2-*tert*-butoxycarbonylamino-3-phenylpropionic acid (11 g, 41.8 mmol) in anhydrous dichloromethane (20 mL) at 0 °C and under a nitrogen atmosphere. The mixture was stirred for 5 min and then 3-hydroxybutan-2-one (6.6 g, 75 mmol, assuming monomeric form) was added at 0 °C. The mixture was allowed to warm to ambient temperature then stirred for 3 days. The mixture was then evaporated in vacuo to give a residue. Flash chromatography of the residue over silica gel (500 g) using ethyl acetate/heptane (3:7) as the eluent gave **18** (10.9 g, 78%), TLC (single UV spot, $R_f = 0.40$, 50% ethyl acetate in heptane) and HPLC-MS (single main UV peak with $R_t = 9.16$ min, 358.2 $[M+Na]^+$, 693.3 $[2M+Na]^+$) (lit.[15]).

4.1.2. (2*S*,3*S*,5*S*)-5-Benzyl-2,3-dimethyl-6-oxomorpholine-3-carbonitrile (20). Trifluoroacetic acid (77 mL) was added to a solution of (2*R*)-2-*tert*-butoxycarbonylamino-3-phenylpropionic acid 1-methyl-2-oxopropyl ester **18** (7.99 g, 23.8 mmol) in anhydrous dichloromethane (140 mL) at 0 °C under a nitrogen atmosphere. The mixture was stirred for 1 h at 0 °C then evaporated in vacuo to give a residue. Toluene (40 mL) was then added to the residue and evaporated in vacuo. This procedure was repeated twice, to remove excess trifluoroacetic acid. The residue was then dissolved in anhydrous 2-propanol (390 mL) under a nitrogen atmosphere. Powdered sodium cyanide (2.6 g, 53 mmol) was then added in one portion and the mixture stirred for 2 h at ambient temperature. The mixture was then rapidly filtered through anhydrous sodium sulfate and evaporated in vacuo. Flash chromatography over silica gel (200 g) using ethyl acetate/heptane (3:7) as eluent gave crude 5-benzyl-2,3-dimethyl-6-oxo-morpholine-3-carbonitrile (2.7 g). Recrystallisation (diethyl ether–heptane) gave crystalline (2*S*,3*S*,5*S*)-**20** (1.05 g), TLC

(single UV spot, $R_f = 0.38$, 50% ethyl acetate in heptane), analytical HPLC $R_t = 15.94$ min, HPLC-MS (single main UV peak with $R_t = 7.53$ min, 245.1 $[M+H]^+$, 267.1 $[M+Na]^+$); ^1H NMR (400 MHz, $CDCl_3$): δ 1.35 (d, $J = 6.50$ Hz, *2*-$CH_3$3H), 1.48 (s, *3*-CH_3, 3H,), 3.01 (dd, $J = 9.10$ and 13.80 Hz, CH_2Ph, 1H), 3.48 (dd, $J = 3.55$ and 13.85 Hz, CH_2Ph, 1H), 4.17 (dd, $J = 3.60$ and 9.10 Hz, *5*-H, 1H), 4.57 (q, $J = 6.50$ Hz, *2*-H, 1H), 7.29–7.42 (m, C_6H_5, 5H) (lit.[15]).

4.1.3. (2*R*,3*S*)-2-Amino-3-hydroxy-2-methylbutyric acid (16). To a solution of **20** (1.38 g, 5.63 mmol) in anhydrous diethyl ether (120 mL) was added *tert*-butylhypochlorite (1.26 mL, 11.25 mmol) at 0 °C under a nitrogen atmosphere. The solution was stirred at ambient temperature for 2 h, then triethylamine (1.26 mL) was added at 0 °C. The mixture was then allowed to warm to ambient temperature and stirred for 20 h. The resulting suspension was filtered and the solid residue washed with diethyl ether (20 mL). Water (100 mL) was then added to the filtrate and the product extracted into ether (50 mL × 3). The combined ethereal layers were dried ($MgSO_4$) and evaporated in vacuo to give a residue (1.9 g). Concentrated hydrochloric acid (70 mL) was then added to the residue at 0 °C. The mixture was stirred for 1 h at 0 °C then at ambient temperature for 24 h. The mixture was then transferred to a sealed pressure tube and heated at 80 °C for 2 days. The mixture was then cooled and extracted with diethyl ether (50 mL × 3). The aqueous layer was then concentrated under reduced pressure then purified over Dowex® 50W × 4 (activated with 0.01 M hydrochloric acid). Elution with water (until the eluent approached ≈ pH 7), followed by ammonium hydroxide (1 M) gave impure **16** (555 mg). Recrystallisation from H_2O(trace)/EtOH/Et_2O gave **16** (250 mg, 33%), TLC (single UV spot, $R_f = 0.27$, 4:1:1 *n*-BuOH/H_2O/AcOH); ^1H NMR (400 MHz, D_2O): δ 0.92 (d, $J = 6.65$ Hz, $CHCH_3$, 3H), 1.06 (s, *2*-CH_3, 3H), 3.85 (q, $J = 6.60$ Hz, *3*-H, 1H); HPLC-MS (single main UV peak with $R_t = 0.45$ min, 134 $[M+H]^+$) (lit.[15]).

4.1.4. (4*S*,5*R*)-5-Methyl-2-phenyl-4,5-dihydrooxazole-4-carboxylic acid methyl ester (21). Saturated aqueous sodium hydrogen carbonate (100 mL) was added to a solution of ethyl benzimidate hydrochloride (4 g, 21.25 mmol) in diethyl ether (100 mL). The phases were vigorously mixed and then separated. The ethereal layer was dried (Na_2SO_4) and evaporated in vacuo to give a crude residue. The residue was then dissolved in diethyl ether (8.5 mL) and then added to a solution of L-threonine methyl ester hydrochloride **22** (2.1 g, 12.4 mmol) in water (1.5 mL). The mixture was stirred for 20 h then water (20 mL) added. The mixture was then extracted with diethyl ether (40 mL × 3) and the combined ethereal layers washed with brine (20 mL), dried (Na_2SO_4) and evaporated in vacuo to give a residue. The excess ethyl benzimidate was removed by distillation at 100 °C under reduced pressure (~0.5 mbar). The remaining material was purified by flash chromatographed on silica gel (300 g) using ethyl acetate/heptane (1:3) as eluent to give **21** (1.83 g, 67%), TLC (single UV spot, $R_f = 0.47$, 50% ethyl acetate in heptane); ^1H NMR (400 MHz, $CDCl_3$): δ 1.59 (d, $J = 6.30$ Hz, *5*-CH_3, 3H), 3.86 (s, $COCH_3$ 3H), 4.53 (d, $J = 7.50$ Hz, *4*-H, 1H), 5.02–5.09 (m, *5*-H, 1H), 7.45–7.57 (m, phenyl C*H*, 3H) and 8.03–8.05 (m, phenyl C*H*, 2H); analytical HPLC $R_t = 10.33$ min, HPLC-MS (single main UV peak with $R_t = 5.76$ min, 220 $[M+H]^+$) (lit.[18]).

4.1.5. (4*R*,5*R*)-4,5-Dimethyl-2-phenyl-4,5-dihydrooxazole-4-carboxylic acid methyl ester (23). A solution of **21** (8.0 g, 36.5 mmol) in anhydrous tetrahydrofuran (43 mL) was added to a solution of lithium diisopropylamine (20.2 mL, 2 M solution in heptanes and tetrahydrofuran) in anhydrous tetrahydrofuran/hexane (243 mL, 10:1) at −78 °C under an atmosphere of nitrogen. After 1 h of stirring at −78 °C iodomethane (5.70 mL, 91.74 mmol) was added. The mixture was stirred for a further 4 h at −78 °C and then allowed to warm to ambient temperature over 18 h. Saturated aqueous ammonium chloride solution (200 mL) was then added, followed by heptane (300 mL). The phases were separated and the organic phase washed with brine, dried (Na_2SO_4) and evaporated in vacuo to give a residue. Flash chromatography over silica (300 g) using ethyl acetate/heptane (3:7) as the eluent gave **23** (7.5 g, 88%), TLC (single UV spot, $R_f = 0.49$, 50% EtOAc in heptane); ^1H NMR (400 MHz, $CDCl_3$): δ 1.07 (d, $J = 6.55$ Hz, *5*-CH_3, 3H), 1.38 (s, *4*-CH_3, 3H), 3.46 (s, $COCH_3$, 3H), 4.27 (q, $J = 6.55$ Hz, *5*-H, 1H), 7.12–7.24 (m, phenyl C*H*, 3H) and 7.69–7.72 (m, phenyl C*H*, 2H); analytical HPLC $R_t = 10.99$ min, HPLC-MS (single main peak, 234 $[M+H]^+$) (lit.[18]).

4.1.6. (2*R*,3*R*)-2-Amino-3-hydroxy-2-methylbutyric acid (15). Hydrochloric acid (6 M, 10 mL) was added to **23** (1.12 g, 4.81 mmol), and the mixture was then heated under reflux for 18 h. The mixture was cooled to ambient temperature then water (7 mL) added, followed by diethyl ether (14 mL). The phases were thoroughly mixed and then separated. The aqueous phase was concentrated under reduced pressure then purified over Dowex® 50W × 4 (activated with 0.01 M HCl). Elution with water (100 mL), followed by 1 M NH_4OH gave **15** (640 mg, 99%). Recrystallisation from H_2O (trace)/EtOH/Et_2O gave (2*R*,3*R*)-**15**, TLC (single UV spot, $R_f = 0.27$, 4:1:1 *N*-BuOH/H_2O/AcOH); ^1H NMR (400 MHz, D_2O): δ 0.84 (d, $J = 6.60$ Hz, $CHCH_3$, 3H), 1.13 (s, *2*-CH_3, 3H), 3.69 (q, $J = 6.60$ Hz, *3*-H, 1H); HPLC-MS (single main UV peak with $R_t = 0.45$ min, 134 $[M+H]^+$) (lit.[18]).

4.1.7. (2*R*,3*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-2-methylbutyric acid (12a). Amino acid **15** (1.54 g, 11.6 mmol) was added to a vigorously stirred solution of sodium carbonate (3.05 g, 28.9 mmol) in water (66 mL) at 0 °C. 1,4-Dioxan (32 mL) was then added, providing an opaque mobile mixture. 9-Fluorenylmethyl chloroformate (3.13 g, 12.16 mmol) in 1,4-dioxan (35 mL) was then added over 40 min. The mixture was then allowed to warm to ambient temperature over

30 min. Water (300 mL) was then added, and the reaction mixture washed with chloroform (2×250 mL) and the combined organic layers discarded. The aqueous phase was acidified with 1 M HCl (≈pH 2), providing a thick opaque mixture. The acidified aqueous mixture was extracted with chloroform (3×250 mL) and the combined organic phase dried (Na_2SO_4) and evaporated in vacuo to give a residue (4.2 g). Recrystallisation (from ethyl acetate/heptane) gave **12a** (3.8 g, 92%), TLC (single UV spot, R_f =, 20% MeOH in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.17 (d, $J = 5.60$ Hz, CHCH_3, 3H), 1.63 (s, *2*-CH_3, 3H), 4.20–4.30 (m, *3*-H and fluorenyl CHCH$_2$O, 2H), 4.44 (br, CHCH_2O, 2H), 6.09 (s, NH, 1H), 7.31–7.35 (m, 2×fluorenyl CH, 2H), 7.40–7.43 (m, 2×fluorenyl CH, 2H), 7.60 (d, $J = 7.45$ Hz, 2×fluorenyl CH, 2H) and 7.78 (d, $J = 7.50$ Hz, 2×fluorenyl CH, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.6 (CHCH$_3$), 20.4 (*2*-CH_3), 47.5 (CHCH$_2$O), 64.0 (C-*2*), 67.6 (CH$_2$O), 71.6 (C-*3*), 120.5, 125.4, 127.5, 128.2 (8×fluorenyl CH), 141.7, 144.0 (4×fluorenyl quaternary C), 157.0 (OCON) and 176.4 (C-*1*); analytical HPLC $R_t = 16.09$ min, HPLC-MS (single main peak, 356 $[M+H]^+$, 378 $[M+Na]^+$). Anal. Calcd for $C_{20}H_{21}NO_5$: C, 67.59; H, 5.96; N, 3.94. Found: C, 67.25; H, 6.00; N, 3.95. Exact mass calcd for $C_{20}H_{21}NO_5Na$: 378.1312, found 378.1327, δ +4.11 ppm.

4.1.8. (1*R*,1′*R*)-[3-Diazo-1-(1-hydroxyethyl)-1-methyl-2-oxopropyl]carbamic acid 9*H*-fluoren-9-ylmethyl ester (9a). The acid **12a** (1.77 g, 5.0 mmol) was dissolved with stirring in anhydrous dichloromethane (30 mL) and tetrahydrofuran (10 mL). The reaction was flushed with nitrogen and cooled to −15 °C. *iso*-Butylchloroformate (0.74 mL, 5.44 mmol) in anhydrous dichloromethane (5 mL) and *N*-methylmorpholine (1.03 mL, 10.0 mmol) in anhydrous dichloromethane (5 mL) were added simultaneously in 1 mL aliquots over 15 min. Etheral diazomethane [generated from addition of diazald® (4.7 g, ∼15 mmol) in diethyl ether (75 mL) onto sodium hydroxide (5.25 g) in water (7.5 mL)/ethanol (15 mL) at 60 °C] was added to the activated amino acid solution at −20 °C. The mixture was then allowed to warm to ambient temperature and stirred for 20 h. A few drops of acetic acid were added to the mixture. *tert*-Butyl methyl ether (100 mL) was then added to the mixture. The ethereal layers were then washed with water (3×75 mL), dried (Na_2SO_4) and the solvents removed under reduced pressure to give a yellow residue (2 g). Flash chromatography of the residue over silica (100 g) using gradient elution with ethyl acetate/heptane in the ratios of (1:3) to (1:0) gave an unidentified fraction (236 mg), followed by an unstable unidentified product (790 mg), followed by (2*R*,3*R*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-2-methylbutyric acid methyl ester (344 mg, 18%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.10 (d, $J = 5.90$ Hz, CHC$H_3$3H), 1.61 (s, *2*-CH_3, 3H), 3.69 (s, OCH_3, 3H), 3.90–4.08 (br, *3*-H, 1H), 4.23 (t, $J = 6.80$ Hz, fluorenyl CHCH$_2$O, 1H), 4.69 (br s, CHCH_2O, 2H), 5.95 (s, NH, 1H), 7.31–7.35 (m, 2×fluorenyl CH, 2H), 7.42 (t, $J = 7.40$Hz, 2×fluorenyl CH, 2H), 7.60 (d, $J = 7.40$ Hz, 2×fluorenyl CH, 2H) and 7.68 (d, $J = 7.40$ Hz, 2×fluorenyl CH, 2H); analytical HPLC $R_t = 17.47$ min, HPLC-MS (single main peak, 370 $[M+H]^+$, 392 $[M+Na]^+$), followed by **9a** (220 mg, 11%), HPLC-MS (main UV peak with $R_t = 8.84$ min, 352.2 $[M-N_2+H]^+$, 374.2 $[M-N_2+Na]^+$).

4.1.9. (2*R*,3*R*)-(2,3-Dimethyl-4-oxo-tetrahydro-furan-3-yl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester (6). A solution of lithium chloride (247 mg, 5.7 mmol) in water (1.5 mL) and acetic acid (6.0 mL) was added to **9a** (220 mg, 0.57 mmol). A gas evolved and after 1 h, chloroform (75 mL) was added and the organic phase washed with saturated aqueous sodium hydrogen carbonate (2×70 mL) and brine (70 mL). The chloroform layer was dried (Na_2SO_4) and evaporated in vacuo to give a residue (220 mg). Flash chromatography of the residue over silica (35 g) using ethyl acetate/heptane (1:4) as the eluent gave **6** (100 mg, 50%), TLC (single UV spot, $R_f = 0.72$, ethyl acetate/heptane, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.19 (s, *3*-CH_3, 3H), 1.29 (d, $J = 6.25$ Hz, *2*-CH_3, 3H), 4.19–4.23 (m, *5*-H_2 and fluorenyl CHCH$_2$O, 3H), 4.40 (d, $J = 6.60$ Hz, CHCH_2O, 2H), 4.48 (q, $J = 6.20$ Hz, *2*-H, 1H), 4.97 (s, NH, 1H), 7.31–7.70 (m, fluorenyl CH, 8H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.5 (*2*-CH_3), 16.3 (*3*-CH_3), 47.5 (CHCH$_2$O), 61.4 (C-*2* and C-*3*), 67.3 (CHCH$_2$O), 70.7 (C-*5*), 120.4, 125.4, 127.5, 128.2 (8×fluorenyl CH), 141.7, 143.8 and 144.2 (4×fluorenyl quaternary C), 155.1 (OCON) and 214.1 (C-*4*); analytical HPLC $R_t = 18.21$ min, HPLC-MS (single main peak, 352 $[M+H]^+$, 374 $[M+Na]^+$). Anal. Calcd for $C_{21}H_{21}NO_4$ 0.1EtOAc: C, 70.05; H, 6.33; N, 3.59. Found: C, 70.35; H, 5.95; N, 3.91. Exact mass calcd for $C_{21}H_{22}NO_4$: 352.1543, found 352.1554, δ +3.03 ppm.

4.1.10. (2*R*,3*R*)-2-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-2-methylbutyric acid allyl ester (12c). (a) A solution of **12a** (1.92 g, 5.41 mmol) and tricaprylmethylammonium chloride (2.19 g, 5.41 mmol) in dichloromethane (8 mL) was added to a stirred solution of sodium hydrogen carbonate (454 mg, 5.41 mmol) in water (8 mL), then allyl bromide (0.47 mL, 5.41 mmol) was added in one portion. The biphasic mixture was vigorously stirred for 5 days then diluted with water (80 mL) and the product extracted into dichloromethane (3×100 mL). The combined organic layers were dried (Na_2SO_4) and evaporated in vacuo to give a residue (4.9 g). Flash chromatography of the residue over silica gel (200 g) using ethyl acetate/heptane (3:7) as the eluent gave **12c** (2.02 g, 94%), TLC (single UV spot, $R_f = 0.48$, 50% ethyl acetate in heptane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.13 (d, $J = 5.00$ Hz, CHCH_3, 3H), 1.61 (s, *2*-CH_3, 3H), 4.10–4.30 (m, OH, *3*-H and fluorenyl CHCH$_2$O, 3H), 4.35–4.55 (m, fluorenyl CHCH_2O, 2H), 4.68 (br s, OCH_2CHCH$_2$, 2H), 5.25–5.43 (m, OCH$_2$CHC$H_2$2H), 5.83–6.09 (m, NH and OCH$_2$CHCH$_2$, 2H), 7.30–7.39 (m, 2×fluorenyl CH, 2H), 7.40–7.50 (m, 2×fluorenyl CH, 2H), 7.62 (d, $J = 7.45$ Hz, 2×fluorenyl CH, 2H) and 7.79 (d, $J = 7.55$ Hz, 2×fluorenyl CH, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.9 (CHCH$_3$), 20.6 (*2*-CH_3), 47.8 (CHCH$_2$O), 64.6 (C-*2*), 67.3 (OCH$_2$CHCH$_2$), 67.7

($CHCH_2O$), 71.8 (C-*3*), 120.0 (OCH_2CHCH_2), 120.7, 125.7, 127.8, 128.3 and 128.5 (8×fluorenyl *C*H), 131.8 (OCH_2CHCH_2), 142.0 and 144.3 (4×fluorenyl quaternary *C*), 156.8 (O*C*ON) and 173.9 (C-*1*); analytical HPLC $R_t = 18.33$ min, HPLC-MS (single main UV peak, 396.2 $[M+H]^+$ and 418.2 $[M+Na]^+$).

(b) Toluene (50 mL) was added to **12a** (1.01 g, 2.84 mmol), followed by allyl alcohol (3.74 mL) and *para*-toluenesulfonic acid (710 mg). The mixture was refluxed using Dean–Stark conditions for 1.5 h. Dilute hydrochloric acid (1 M, 100 mL) was added and the mixture extracted with ethyl acetate (3×100 mL). The combined organics were dried (Na_2SO_4) and evaporated in vacuo to give a residue. Flash chromatography of the residue over silica gel (250 g) using ethyl acetate/heptane (1:9) as the eluent gave a mixture of uncharacterised products (400 mg), followed by **12c** (22 mg, 2%), followed by 4,5-dimethyl-2-oxo-oxazolidine-4-carboxylic acid allyl ester **24** (476 mg, 84%), TLC (single spot, $R_f = 0.20$, 50% ethyl acetate in heptane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.27 (d, $J = 6.55$ Hz, *5*-CH_3, 3H), 1.50 (s, *4*-CH_3, 3H), 4.38 (q, C*H*CH_3, 1H), 4.55–4.65 (m, O*C*H_2CHCH_2, 2H), 5.20–5.33 (m, OCH_2CHCH_2, 2H), 5.78–5.92 (m, OCH_2CHCH_2, 1H) and 5.99 (s, N*H*, 1H); HPLC-MS (single main peak, 200.1 $[M+H]^+$, 399.2 $[2M+H]^+$ and 421.2 $[2M+Na]^+$); Exact mass calcd for $C_9H_{13}NO_4$: 200.0917, found 200.0926, δ +4.56 ppm.

4.1.11. (2*R*,3*R*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2-methyl butyric acid allyl ester (12d). A stirred solution of **12c** (1.55 g, 3.92 mmol) in dichloromethane (18 mL) was cooled in a pressure vessel to −78 °C then isobutylene gas (~10 mL) condensed into the solution. Concentrated sulfuric acid (89 μL) was added then the pressure vessel sealed. The mixture was stirred at ambient temperature for 3 days then cooled to −78 °C. *N*-Methylmorpholine (187 μL) was added then the unsealed pressure vessel allowed to warm to ambient temperature. The mixture was diluted with saturated aqueous sodium hydrogen carbonate solution (100 mL) then the product extracted into dichloromethane (2×100 mL). The combined dichloromethane layers were washed with brine (40 mL), dried (Na_2SO_4) and evaporated in vacuo to give a residue. Flash chromatography of the residue over silica gel (150 g) using ethyl acetate/heptane (1:3) as the eluent gave **12d** (1.28 g, 72%), TLC (single UV spot, $R_f = 0.70$, 50% ethyl acetate in heptane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95–1.28 (m, $CHCH_3$ and $C(CH_3)_3$, 12H), 1.55 (br, *2*-CH_3, 3H), 3.85–4.90 (m, *3*-H, fluorenyl C*H*CH_2O and fluorenyl $CHCH_2O$, 4H), 4.56 (br, OCH_2CHCH_2, 2H), 5.10–5.13 (m, OCH_2CHCH_2, 1H), 5.27 (br d, $J = 17.15$ Hz, OCH_2CHCH_2, 1H), 5.70–5.89 (m, N*H* and OCH_2CHCH_2, 1H), 7.21–7.27 (m, 2×fluorenyl C*H*, 2H), 7.29–7.36 (m, 2×fluorenyl C*H*, 2H), 7.50–7.59 (m, 2×fluorenyl C*H*, 2H) and 7.68 (d, $J = 7.50$ Hz, 2×fluorenyl C*H*, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.1 ($CHCH_3$), 20.7 (*2*-CH_3), 29.11 ($C(CH_3)_3$), 47.6 ($CHCH_2O$), 64.5 (C-*2*), 66.4 (OCH_2CHCH_2), 66.9 ($CHCH_2O$), 71.6 (C-*3*), 74.8 ($C(CH_3)_3$), 118.6 (OCH_2CHCH_2), 120.4, 125.5, 125.6, 127.4, 127.5 and 128.1 (8×fluorenyl *C*H), 132.2 (OCH_2CHCH_2), 141.7, 144.4 and 144.5 (4×fluorenyl quaternary *C*), 155.6 (O*C*ON) and 172.5 (C-*1*); analytical HPLC $R_t = 23.30$ min, HPLC-MS (single main UV peak, 452.3 $[M+H]^+$ and 474.2 $[M+Na]^+$); Exact mass calcd for $C_{27}H_{34}NO_5$: 452.2431, found 452.2447, δ +3.40 ppm, followed by recovered **12c** (260 mg, 17%).

4.1.12. (2*R*,3*R*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2-methyl butyric acid (12b). Tetrakistriphenylphosphine palladium(0) (69 mg, 0.06 mmol), dichloromethane (35 mL) then phenyltrihydrosilane (0.72 mL, 5.84 mmol) were added consecutively to **12d** (1.2 g, 2.66 mmol) under nitrogen. The mixture was stirred for 1 h then saturated aqueous sodium hydrogen carbonate (100 mL) added and the mixture extracted with chloroform (100 mL×3). The combined organics were washed with dilute aqueous hydrochloric acid (0.01 M, 100 mL), dried (Na_2SO_4) and evaporated in vacuo to give a residue. Flash chromatography of the absorbed residue over silica gel (100 g) using ethyl acetate/heptane (2:3), followed by (1:1), followed by (7:3) as the eluent gave **12b** (1.07 g, 98%), TLC (single UV spot, $R_f = 0.32$, 50% ethyl acetate in heptane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.00–1.25 (m, $CHCH_3$ and $C(CH_3)_3$, 12H), 1.49 (s, *2*-CH_3, 3H), 4.01–4.19 (m, fluorenyl C*H*CH_2O and $CHCH_2O$, 3H), 4.22–4.45 (m, *3*-H, 1H), 5.59 (s, N*H*, 1H), 7.31–7.33 (m, 2×fluorenyl C*H*, 2H), 7.37–7.41 (m, 2×fluorenyl C*H*, 2H), 7.55–7.60 (m, 2×fluorenyl C*H*, 2H) and 7.75 (d, $J = 7.55$ Hz, 2×fluorenyl C*H*, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.7 ($CHCH_3$), 21.5 (*2*-CH_3), 29.1 ($C(CH_3)_3$), 47.6 ($CHCH_2O$), 60.9 ($C(CH_3)_3$), 64.2 (C-*2*), 67.2 (CH_2O), 71.1 (C-*3*), 120.4, 125.4, 127.5, 128.1 (8×fluorenyl *C*H), 134.58, 141.7, 144.2 (4×fluorenyl quaternary *C*), 156.3 (O*C*ON) and 175.5 (C-*1*); analytical HPLC $R_t = 19.90$ min, HPLC-MS (single main peak, 434.2 $[M+Na]^+$). Anal. Calcd for $C_{24}H_{29}NO_5$ 0.07$CDCl_3$: C, 65.85; H, 6.60; N, 3.16. Found: C, 66.04; H, 6.47; N, 2.66; Exact mass calcd for $C_{24}H_{30}NO_5$: 412.2118, found 412.2136, δ +4.16 ppm.

4.1.13. (4*R*,4′*R*)-4-(1-*tert*-Butoxyethyl)-4-methyl-oxazolidine-2,5-dione (25). The acid **12b** (1.01 g, 2.46 mmol) was dissolved with stirring in anhydrous dichloromethane (16 mL) and tetrahydrofuran (6 mL). The reaction was flushed with nitrogen and cooled to −15 °C. *iso*-Butylchloroformate (0.35 mL, 2.71 mmol) in anhydrous dichloromethane (5 mL) and *N*-methylmorpholine (0.54 mL, 4.92 mmol) in anhydrous dichloromethane (5 mL) were added simultaneously in portions over 15 min. The mixture was stirred at −15 °C for 30 min (>95% activation was observed by HPLC-MS and analytical HPLC). Etheral diazomethane [generated from addition of diazald® (2.11 g, ~9.8 mmol) in diethyl ether (43 mL) onto sodium hydroxide (3.0 g) in water (4.3 mL)/ethanol (8.7 mL) at 60 °C] was added to the activated amino acid solution at −15 °C. The mixture was then allowed to warm to ambient temperature and stirred for 20 h. A few drops of acetic acid were added to the mixture. Ethyl acetate (150 mL) was then added to

the mixture. The organics were then washed with saturated aqueous sodium hydrogen carbonate (80 mL), saturated aqueous ammonium chloride (80 mL), dried (Na_2SO_4) and the solvents removed under reduced pressure to give a yellow residue (1.2 g). Flash chromatography of the residue over silica (100 g) using gradient elution with ethyl acetate/heptane in the ratios of (1:9) to (1:4) gave 9-methylene-9-fluorene (160 mg, 36%), TLC (single UV spot, $R_f = 0.82$, 20% ethyl acetate in heptane), HPLC-MS (single main UV peak, 179.1 $[M+H]^+$), followed by an unidentified fraction (345 mg), followed by **25** (325 mg, 61%), TLC (single spot, $R_f = 0.10$, 20% ethyl acetate in heptane); ^1H NMR (400 MHz, $CDCl_3$): δ 1.21 (s, $C(CH_3)_3$, 9H), 1.23 (d, $J = 6.15$ Hz, *4′*-CH_3, 3H), 1.52 (s, *4*-CH_3, 3H), 3.78 (q, $J = 6.20$ Hz, $CHCH_3$, 1H) and 6.39 (br s, NH, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.8 (*4′*-CH_3), 22.3 (*4*-CH_3), 29.6 ($C(CH_3)_3$), 68.5 ($HNCCH_3$), 71.8 (OCH), 76.1 ($OCCH_3$), 153.2 (OCON) and 172.4 (C_2); HPLC-MS (single main peak, 216.1 $[M+H]^+$ and 453.1 $[2M+Na]^+$); Exact mass calcd for $C_{10}H_{17}NO_4Na$: 238.1050, found 238.1058, δ +3.35 ppm.

4.1.14. (2*R*,3*S*)-3-*tert*-Butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2-methyl butyric acid (13b).

(a) Amino acid **16** (288 mg, 2.17 mmol) was added to a vigorously stirred solution of sodium carbonate (570 mg, 5.41 mmol) in water (12 mL) at 0 °C. 1,4-Dioxan (6 mL) was then added, providing an opaque mobile mixture. 9-Fluorenylmethyl chloroformate (585 mg, 2.27 mmol) in 1,4-dioxan (6 mL) was then added over 40 min. The mixture was then allowed to warm to ambient temperature over 40 min. Water (100 mL) was then added, the reaction mixture washed with chloroform (100 mL) and the organic layer discarded. The aqueous phase was acidified with 1 M HCl (≈pH 2), providing a thick opaque mixture. The acidified aqueous mixture was extracted with chloroform (5 × 80 mL) and the combined organic phase dried (Na_2SO_4) and evaporated in vacuo to give **13a** (616 mg, 80%), TLC (single UV spot, $R_f = 0.24$, 20% methanol in chloroform), analytical HPLC $R_t = 15.54$ min, HPLC-MS (single main UV peak with $R_t = 7.70$ min, 356.2 $[M+H]^+$, 378.1 $[M+Na]^+$).

(b) A solution of acid **13a** (616 mg, 1.74 mmol) and tricaprylmethylammonium chloride (701 mg, 1.74 mmol) in dichloromethane (2.6 mL) was added to a stirred solution of sodium hydrogen carbonate (146 mg, 1.74 mmol) in water (2.6 mL), then allyl bromide (0.15 mL, 1.74 mmol) was added in one portion. The biphasic mixture was vigorously stirred for 5 days then diluted with water (40 mL) and the product extracted into dichloromethane (3 × 80 mL). The combined organic layers were dried (Na_2SO_4) and evaporated in vacuo to give a residue (1.1 g). Flash chromatography of the residue over silica gel (200 g) using ethyl acetate/heptane (3:7) as the eluent gave (2*R*,3*S*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-2-methyl butyric acid allyl ester **13c** (360 mg, 52%), TLC (single UV spot, $R_f = 0.48$, 50% ethyl acetate in heptane); ^1H NMR (400 MHz, $CDCl_3$): δ 1.21 (d, $J = 6.20$ Hz, $CHCH_3$, 3H), 1.52 (s, *2*-CH_3, 3H), 3.38 (br s, OH, 1H), 3.47–4.16 (m, *3*-H, 1H), 4.25 (t, $J = 6.85$ Hz, fluorenyl $CHCH_2O$, 1H), 4.38–4.42 (m, fluorenyl $CHCH_2O$, 2H), 4.67 (br, OCH_2CHCH_2, 2H), 5.24 (dd, $J = 1.15$ and 10.45 Hz, OCH_2CHCH_2, 1H), 5.35 (m, OCH_2CHCH_2, 1H), 5.52 (s, NH, 1H), 5.87–5.95 (m, OCH_2CHCH_2, 1H), 7.31–7.35 (m, 2 × fluorenyl CH, 2H), 7.40–7.43 (m, 2 × fluorenyl CH, 2H), 7.60 (d, $J = 7.40$ Hz, 2 × fluorenyl CH, 2H) and 7.78 (d, $J = 7.50$ Hz, 2 × fluorenyl CH, 2H); analytical HPLC $R_t = 18.42$ min, HPLC-MS (single main peak, 396.2 $[M+H]^+$, 418.2 $[M+Na]^+$). Anal. Calcd for $C_{23}H_{25}NO_5$ 0.06EtOAc: C, 68.94; H, 6.54; N, 3.33. Found: C, 68.56; H, 6.24; N, 3.30; Exact mass calcd for $C_{23}H_{26}NO_5$: 396.1805, found 396.1802, δ −0.10 ppm.

(c) A stirred solution of (2*R*,3*S*)-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-2-methylbutyric acid allyl ester **13c** (312 mg, 0.79 mmol) in dichloromethane (10 mL) was cooled in a pressure vessel to −78 °C then isobutylene gas (~8 mL) condensed into the solution. Concentrated sulfuric acid (27 μL) was added then the pressure vessel sealed. The mixture was stirred at ambient temperature for 3 days then cooled to −78 °C. *N*-Methylmorpholine (56 μL) was added then the unsealed pressure vessel allowed to warm to ambient temperature. The mixture was diluted with saturated aqueous sodium hydrogen carbonate solution (100 mL) then the product extracted into dichloromethane (3 × 100 mL). The combined dichloromethane layers were washed with brine (50 mL), dried (Na_2SO_4) and evaporated in vacuo to give a residue. Flash chromatography of the residue over silica gel (35 g) using ethyl acetate/heptane (3:7) as the eluent gave (2*R*,3*S*)-3-*tert*-butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2-methyl butyric acid allyl ester **13d** (310 mg, 87%), TLC (single UV spot, $R_f = 0.70$, 50% ethyl acetate in heptane); ^1H NMR (400 MHz, $CDCl_3$): δ 1.16–1.25 (m, $CHCH_3$ and $C(CH_3)_3$, 12H), 1.62 (s, *2*-CH_3, 3H), 3.95–4.05 (m, *3*-H, 1H), 4.23–4.43 (m, fluorenyl $CHCH_2O$ and fluorenyl $CHCH_2O$, 3H), 4.55–4.71 (m, OCH_2CHCH_2, 2H), 5.21 (dd, $J = 1.20$, 2.50 Hz, OCH_2CHCH_2, 1H), 5.34 (d, $J = 17.15$ Hz, OCH_2CHCH_2, 1H), 5.72 (s, NH, 1H), 5.86–5.95 (m, OCH_2CHCH_2, 1H), 7.31–7.35 (m, 2 × fluorenyl CH, 2H), 7.40–7.43 (m, 2 × fluorenyl CH, 2H), 7.62–7.70 (m, 2 × fluorenyl CH, 2H) and 7.78 (d, $J = 7.50$ Hz, 2 × fluorenyl CH, 2H); analytical HPLC $R_t = 23.59$ min, HPLC-MS (single main peak, 474.1 $[M+Na]^+$); Exact mass calcd for $C_{27}H_{34}NO_5$: 452.2431, found 452.2425, δ −1.39 ppm.

(d) Tetrakistriphenylphosphine palladium(0) (17.25 mg, 0.015 mmol), dichloromethane (10 mL) then phenyltrihydrosilane (0.18 mL, 1.33 mmol) were added consecutively to (2*R*,3*S*)-3-*tert*-butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonyl amino)-2-methyl butyric acid allyl ester **13d** (300 mg, 0.665 mmol) under nitrogen. The mixture was stirred for 90 min then absorbed onto silica gel (2 g). Flash chromatography of the absorbed residue over silica gel (35 g) using ethyl acetate/heptane (2:3), followed by (1:1) as the eluent gave **13b** (140 mg, 51%), TLC (single UV spot, $R_f = 0.33$, 50% ethyl acetate in heptane); ^1H NMR (400 MHz, $CDCl_3$): δ 1.19 (d,

$J = 6.10$ Hz, $CHCH_3$, 3H), 1.32 (s, $C(CH_3)_3$, 9H), 1.69 (s, *2*-CH_3, 3H), 4.23 (t, $J = 7.05$ Hz, fluorenyl $CHCH_2O$, 1H), 4.37 (d, $J = 6.95$ Hz, fluorenyl $CHCH_2O$, 2H), 4.61–4.65 (m, *3*-H, 1H), 6.12 (s, N*H*, 1H), 7.31–7.34 (m, 2×fluorenyl C*H*, 2H), 7.39–7.43 (m, 2×fluorenyl C*H*, 2H), 7.59–7.62 (m, 2×fluorenyl C*H*, 2H) and 7.77 (d, $J = 7.50$ Hz, 2×fluorenyl C*H*, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.3 ($CHCH_3$), 21.3 (*2*-CH_3), 28.7 ($C(CH_3)_3$), 47.2 ($CHCH_2O$), 60.5 ($C(CH_3)_3$), 62.7 (C-*2*), 66.7 (CH_2O), 69.5 (C-*3*), 120.1, 125.2, 127.2, 127.8 (8×fluorenyl *C*H), 141.4, 143.8 (4×fluorenyl quaternary *C*), 154.6 (O*C*ON) and 174.8 (C-*1*); analytical HPLC $R_t = 20.20$ min, HPLC-MS (single main peak, 434.1 $[M+Na]^+$).

4.1.15. (1*R*,2*S*)-(2-*tert*-Butoxy-1-fluorocarbonyl-1-methylpropyl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester (13e). Pyridine (47 μL, 0.55 mmol) then cyanuric fluoride (63 μL, 0.71 mmol) were added consecutively at 0 °C to a stirred solution of **13b** (130 mg, 0.316 mmol) in dichloromethane (8 mL) under nitrogen. The suspension was slowly warmed to ambient temperature and stirred for 20 h. Crushed ice (~10 mL) and ice-chilled water (10 mL) were added, then the product was extracted into dichloromethane (40 mL). The dichloromethane layer was dried ($MgSO_4$) and evaporated in vacuo to give **13e** (138 mg), TLC (single UV spot, $R_f = 0.75$, 50% ethyl acetate in heptane), HPLC-MS (single main UV peak with $R_t = 11.66$ min, 436.1 $[M+Na]^+$).

4.1.16. (1*R*,1′*S*)-[1-(1-*tert*-Butoxyethyl)-3-diazo-1-methyl-2-oxopropyl]carbamic acid 9*H*-fluoren-9-ylmethyl ester (10b). Ethereal diazomethane [generated from diazald® (~15 mmol) addition in diethyl ether (75 mL) to sodium hydroxide (5.25 g) in water (7.5 mL)/ethanol (15 mL) at 60 °C and dried over potassium hydroxide pellets] was added to a stirred solution of **13e** (138 mg, ~0.316 mmol) in dichloromethane (3 mL) at 0 °C. The mixture was then allowed to warm to ambient temperature and stirred for 20 h. A few drops of acetic acid were added to quench any excess diazomethane then the solution was stirred for 5 min before adding *tert*-butyl methyl ether (100 mL). The ethereal layer was washed with saturated aqueous sodium hydrogen carbonate (2×50 mL), dried (Na_2SO_4) and evaporated in vacuo to give a residue. Flash chromatography of the residue over silica gel (35 g) using ethyl acetate/heptane (1:19) as the eluent gave 4-(1*S*-*tert*-butoxyethyl)-2-(9*H*-fluoren-9-ylmethoxy)-4*R*-methyl-4*H*-oxazol-5-one **26** (55 mg, 45%), TLC (single UV spot, $R_f = 0.77$, 50% ethyl acetate in heptane); ^1H NMR (400 MHz, $CDCl_3$): δ 1.13 (s, $C(CH_3)_3$, 9H), 1.25 (d, $J = 6.10$ Hz, $CHCH_3$, 3H), 1.35 (s, *4*-CH_3, 3H), 3.81 (q, $J = 6.10$ Hz, $CHCH_3$, 1H), 4.41 (dd, fluorenyl $CHCH_2O$, 1H), 4.50 (m, fluorenyl $CHCH_2O$, 1H), 4.80 (dd, fluorenyl $CHCH_2O$, 1H), 7.31–7.35 (m, 2×fluorenyl C*H*, 2H), 7.41–7.45 (m, 2×fluorenyl C*H*, 2H), 7.67 (d, $J = 7.50$ Hz, 2×fluorenyl C*H*, 2H) and 7.79 (d, $J = 7.55$ Hz, 2×fluorenyl C*H*, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.3 ($CHCH_3$), 20.5 (*4*-CH_3), 28.8 ($C(CH_3)_3$), 46.3 ($CHCH_2O$), 71.1 (C-*4*), 72.1 (CH_2O), 74.4 ($CHCH_3$), 75.6 ($C(CH_3)_3$), 120.2, 125.4, 125.7, 127.3, 128.0, 128.1 (8×fluorenyl *C*H), 141.4, 141.5, 143.0, 143.6 (4×fluorenyl quaternary *C*), 157.6 (O*C*ON) and 178.8 (C-*5*); analytical HPLC $R_t = 21.15$ min and HPLC-MS (single main peak with $R_t = 11.65$ min, 434.2 $[M+H_2O+Na]^+$). Anal. Calcd for $C_{24}H_{27}NO_4$ 0.13DCM: C, 65.57; H, 6.33; N, 3.10. Found: C, 65.78; H, 6.69; N, 3.16; Exact mass calcd for $C_{27}H_{27}NO_4$ $Na{\cdot}H_2O$: 434.1938, found 434.1948, δ +2.22 ppm, followed by a mixed fraction containing **26**, **10b** [TLC UV spot, $R_f = 0.75$, 50% ethyl acetate in heptane] and (2*S*,3*R*)-3-*tert*-butoxy-2-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2-methyl butyric acid methyl ester (35 mg).

4.1.17. (2*S*,3*R*)-(2,3-Dimethyl-4-oxo-tetrahydrofuran-3-yl)carbamic acid 9*H*-fluoren-9-ylmethyl ester (7). A solution of lithium chloride (21.7 mg, 0.51 mmol) in water (0.25 mL) and acetic acid (1 mL) was added to the above mixed fractions containing **10b** (35 mg). A gas evolved and after 1 h, chloroform (40 mL) was added and the organic phase washed with saturated aqueous sodium hydrogen carbonate (20 mL). The chloroform layer was dried (Na_2SO_4) and evaporated in vacuo to give a residue (40 mg). Flash chromatography of the residue over silica (20 g) using ethyl acetate/heptane (1:4), followed by (1:1) as the eluent gave the methyl ester of **7** (10 mg, 7.4% from **13b**), TLC (single UV spot, $R_f = 0.73$, 50% ethyl acetate in heptane); ^1H NMR (400 MHz, $CDCl_3$): δ 1.20–1.21 (br, $CHCH_3$ and $C(CH_3)_3$, 12H), 1.57 (s, *2*-CH_3, 3H), 3.72 (br, $OCH_3$3H), 3.96 (br d, $J = 5.35$ Hz, *3*-H, 1H), 4.21–4.31 (m, fluorenyl $CHCH_2O$ and fluorenyl $CHCH_2O$, 2H), 4.39–4.44 (m, fluorenyl $CHCH_2O$, 1H), 5.68 (s, N*H*, 1H), 7.30–7.34 (m, 2×fluorenyl C*H*, 2H), 7.39–7.42 (m, 2×fluorenyl C*H*, 2H), 7.60–7.65 (m, 2×fluorenyl C*H*, 2H) and 7.77 (d, $J = 7.55$ Hz, 2×fluorenyl C*H*, 2H); analytical HPLC $R_t = 23.01$ min, HPLC-MS (single main peak, 448.2 $[M+Na]^+$), followed by **7** (6 mg, 5.4% from **13b**), TLC (single UV spot, $R_f = 0.57$, ethyl acetate/heptane 1:1); ^1H NMR (400 MHz, $CDCl_3$): δ 1.12 (d, $J = 6.25$ Hz, *2*-CH_3, 3H), 1.50 (s, *3*-CH_3, 3H), 4.05 (d, $J = 17.85$ Hz, *5*-H, 1H), 4.16–4.24 (m, *5*-H and fluorenyl $CHCH_2O$, 2H), 4.38–4.48 (m, $CHCH_2O$, 2H), 4.50–4.65 (br, *2*-H, 1H), 4.84 (s, N*H*, 1H), 7.31–7.33 (m, 2×fluorenyl C*H*, 2H), 7.39–7.43 (m, 2×fluorenyl C*H*, 2H), 7.58 (d, $J = 7.40$ Hz, 2×fluorenyl C*H*, 2H) and 7.78 (d, $J = 7.40$ Hz, 2×fluorenyl C*H*, 2H); analytical HPLC $R_t = 18.22$ min, HPLC-MS (single main UV peak with $R_t = 8.92$ min, 352 $[M+H]^+$, 374 $[M+Na]^+$), followed by recovered **13b** (8 mg, 6.2%).

4.1.18. (2*S*)-2-*tert*-Butoxycarbonylamino-3-phenylpropionic acid 2-oxo-cyclopentyl ester (29). (a) A solution of cyclopentanone (11.6 mL, 130 mmol) in methanol (250 mL) was added dropwise at 0 °C over 20 min to a stirred solution of potassium hydroxide (85% tech., 22.1 g, 335 mmol) in methanol (75 mL). The mixture was stirred at 0 °C for 30 min then 2-iodosylbenzoic acid (36.45 g, 138 mmol) was added in portions over 1 h. The mixture was allowed to warm to ambient temperature over 4 h then stirred at ambient temperature for 20 h.

The majority of solvent was removed in vacuo then the product was extracted into dichloromethane (400 mL). The extracts were washed with water (2×250 mL), dried (Na_2SO_4) and the solvent removed in vacuo to leave 2,2-dimethoxycyclopentanol **27** as a colourless oil (11.98 g) (lit.[16]).

(b) 4-(Dimethylamino)pyridine (1.0 g, 8.2 mmol) was added at 0 °C to a stirred suspension of 2,2-dimethoxycyclopentanol **27** (11.98 g, 82 mmol), (*S*)-2-*tert*-butyloxycarbonylamino-3-phenylpropionic acid (23.9 g, 90.3 mmol) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (23.6 g, 123.1 mmol) in dichloromethane (500 mL). The mixture was stirred at 0 °C for 4 h, then it was washed with water (2×300 mL) and a saturated aqueous sodium chloride solution (200 mL), dried (Na_2SO_4) and the solvent removed in vacuo to give **28** as a yellow oil (36.0 g) (lit.[16]).

(c) *para*-Toluenesulfonic acid monohydrate (1.7 g, 9.2 mmol) was added to a stirred solution of **28** (36 g, 91.6 mmol) in wet acetone (450 mL) at ambient temperature. The solution was stirred for 3 days then water (600 mL) and saturated aqueous sodium hydrogen carbonate solution (200 mL) were added, then the product was extracted into ethyl acetate (600 mL). The aqueous phase was extracted with ethyl acetate (2×400 mL) and the combined ethyl acetate solutions were washed with a saturated aqueous sodium chloride solution (2×150 mL), dried (Na_2SO_4) and the solvent removed in vacuo to give a residue (18.05 g). Flash chromatography of the residue over silica using ethyl acetate/heptane (2:1), followed by (3:1) as the eluent gave **29** as a colourless oil (18.05 g, 40% from cyclopentanone), TLC (single UV spot, $R_f = 0.25$, 30% ethyl acetate in heptane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.42 (s, 3×CH_3, 9H), 1.79–2.48 (m, (CH_2)$_3$, 6H), 3.06–3.28 (m, CH_2Ph, 2H), 4.60–5.20 (m, C*H*O, C*H*N and N*H*, 3H), 7.17–7.36 (m, C_6H_5, 5H); analytical HPLC with main broad peak R_t = 17.9–19.2 min, HPLC-MS (main broad peak, 248.1 $[M-Boc+2H]^+$, 370.2 $[M+Na]^+$, 717.3 $[2M+Na]^+$) (lit.[16]).

4.1.19. (3*S*,4*aR*,7*aS*)-3-Benzyl-2-oxo-hexahydrocyclopenta[1,4]oxazine-4*a*-carbonitrile (30). Trifluoroacetic acid (75 mL) was added dropwise at 0 °C over 1 h to a stirred solution of **29** (17.05 g, 49.1 mmol) in dichloromethane (250 mL). The mixture was stirred at 0 °C for 75 min then the majority of solvent was removed in vacuo. Toluene (75 mL) was added to the residue then the solvent was removed in vacuo to give an oil, which was dissolved in acetonitrile (700 mL). Magnesium sulfate (29.5 g) and then sodium acetate (20.1 g) were added to the stirred solution. The resulting suspension was stirred for 1.5 h then the solids were removed by filtration and the solvents removed in vacuo to give a residue. The residue was dissolved in propan-2-ol (650 mL) then stirred under nitrogen. Trimethylsilyl cyanide (13.1 mL, 98.4 mmol) was added dropwise over 15 min and then zinc chloride (49 mL, 1 M solution in diethyl ether) was added over 40 min. The mixture was stirred for 18 h then cautiously added to a saturated aqueous sodium hydrogen carbonate solution (750 mL). The mixture was diluted with water (750 mL), extracted with diethyl ether (3×500 mL), washed with a saturated aqueous sodium chloride solution (350 mL), dried ($MgSO_4$) and the solvents removed in vacuo to give a brown oil (10.05 g). Flash chromatography of the residue over silica using ethyl acetate/heptane (1:4), followed by (2:3) as the eluent gave **30** as a white solid (4.54 g, 36%). TLC (single UV spot, $R_f = 0.45$, 25% ethyl acetate in heptane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.70–2.29 (m, (CH_2)$_3$ and N*H*, 7H), 2.84 (dd, J = 9.85 and 14.30 Hz, CH_2Ph, 1H), 3.52 (dd, J = 3.60 and 14.35 Hz, CH_2Ph, 1H), 3.90 (dd, J = 3.65 and 9.80 Hz, C*H*NH, 1H), 4.74 (dd, J = 6.50 and 6.80 Hz, C*H*O, 1H), 7.15–7.32 (m, C_6H_5, 5H); analytical HPLC R_t = 14.521 min, HPLC-MS (single main peak, 257.2 $[M+H]^+$, 279 $[M+Na]^+$) (lit.[16]).

4.1.20. (1*R*,2*S*)-1-Amino-2-hydroxycyclopentanecarboxylic acid (17b). (a) *tert*-Butylhypochlorite (4.0 mL, 35.4 mmol) was added dropwise under nitrogen at 0 °C over 2 min to a stirred suspension of **30** (4.53 g, 17.7 mmol) in diethyl ether (350 mL). The mixture was stirred at 0 °C for 140 min then triethylamine (7.4 mL, 53 mmol) was added dropwise over 30 min. The resulting suspension was stirred at 0 °C for 3 h then at ambient temperature for 23 h. Insoluble materials were removed by filtration and then the filtrate was concentrated in vacuo to give a residue. Flash chromatography of the residue over silica using ethyl acetate/heptane (3:7) as the eluent gave **31** (3.3 g), TLC (single UV spot, $R_f = 0.3$, 30% ethyl acetate in heptane); analytical HPLC R_t = 16.07 min.

(b) Concentrated hydrochloric acid (190 mL) was added to **31** (3.3 g) at 0 °C. The suspension was allowed to warm to ambient temperature over 2 h then stirred for a further 20 h. The reaction mixture was partitioned equally between six pressure vessels that were sealed then heated at 100 °C for 26 h then allowed to cool to ambient temperature. The reaction mixtures were recombined then washed with diethyl ether (2×200 mL) and the aqueous phase concentrated in vacuo to leave a residue. Chromatography of the residue over Dowex® 50W×4-200 ion-exchange resin using 0.01 M hydrochloric acid, water and then 1.0 M aqueous ammonium hydroxide solution gave **17b** after freeze drying, as a light brown solid (1.73 g, 67% from **30**); ^{1}H NMR (400 MHz, D_2O): δ 1.50–1.90 (m, (CH_2)$_2$, 4H), 2.16–2.25 (m, CH_2, 2H), 4.36 (t, J = 7.65 Hz, C*H*OH, 1H); ^{13}C NMR (100 MHz, D_2O): δ 21.6 ($CH_2CH_2CH_2$), 33.5 and 35.0 ($CH_2CH_2CH_2$), 69.7 (CNH_2), 77.5 (*C*HOH), 178.3 (CH_2H); HPLC-MS (single main peak, 146.1 $[M+H]^+$) (lit.[16]).

4.1.21. (1*R*,2*S*)-1-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-2-hydroxycyclopentanecarboxylic acid allyl ester (14c). (a) To a stirred solution of sodium carbonate (1.0 g, 9.7 mmol) in water/1,4-dioxan (2:1, 45 mL) was added **17b** (0.67 g, 4.6 mmol) at 0 °C. A solution of 9-fluorenylmethyl chloroformate (1.25 g, 4.85 mmol) in 1,4-dioxan (15 mL) was added dropwise over 30 min.

The resultant suspension was stirred for 75 min at 0 °C then at ambient temperature for 45 min. Water (200 mL) was added then the cloudy solution washed with chloroform (2×140 mL). Chloroform (100 mL) was added and the mixture acidified with 1 M hydrochloric acid (pH ≈ 2). The chloroform layer was separated then the aqueous layer re-extracted with chloroform (2×100 mL). The chloroform extracts (obtained from the acidified aqueous layer) were combined then dried (Na_2SO_4) and the solvent removed in vacuo to give a colourless oil to which heptane (100 mL) was added before storing at −80 °C for 16 h. The solvent was rapidly decanted from the oily residue, which was washed with heptane (5 mL) then remaining solvents removed in vacuo to give (1*R*,2*S*)-1-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2-hydroxycyclopentanecarboxylic acid as an oil (1.27 g, 75%), TLC (main UV spot, $R_f = 0.20$, together with minor UV spot, $R_f = 0.15$, 20% MeOH in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.60–2.16 (m, C*H*$_2$CH$_2$C*H*$_2$, 5H), 2.35 (m, CH$_2$CH$_2$C*H*$_2$, 1H), 4.10 (br s, O*H*, 1H), 4.24 (m, fluorenyl C*H*CH$_2$O, 1H), 4.36–4.57 (m, fluorenyl CHC*H*$_2$O and C*H*OH, 3H), 5.93 (s, N*H*, 1H), 7.28–7.33 (m, 2×fluorenyl C*H*, 2H), 7.34–7.41 (m, 2×fluorenyl C*H*, 2H), 7.54–7.62 (m, 2×fluorenyl C*H*, 2H), 7.72–7.79 (m, 2×fluorenyl C*H*, 2H), analytical HPLC $R_t = 17.17$ min (major), $R_t = 16.80$ min (minor) and HPLC-MS (main UV peak with $R_t = 7.84$ min, 368.1 $[M+H]^+$, 390.1 $[M+Na]^+$, minor UV peak with $R_t = 7.646$ min, 368.1 $[M+H]^+$, 390.1 $[M+Na]^+$). Anal. Calcd for $C_{21}H_{21}NO_5$ 0.25EtOAc: C, 65.11; H, 6.61; N, 2.86. Found: C, 64.72; H, 6.28; N, 3.22; Exact mass calcd for $C_{21}H_{22}NO_5$: 368.1492, found 368.1509, δ +4.62 ppm.

(b) A solution of (1*R*,2*S*)-1-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2-hydroxy cyclopentanecarboxylic acid (1.75 g, 4.8 mmol, prepared as above) and tricaprylmethylammonium chloride (1.93 g, 4.8 mmol) in dichloromethane (14 mL) was added to a stirred solution of sodium hydrogen carbonate (0.4 g, 4.8 mmol) in water (14 mL). Allyl bromide (1.44 mL, 16.7 mmol) was added and the biphasic mixture stirred for 20 h then diluted with water (50 mL). The mixture was extracted with dichloromethane (2×50 mL), dried ($MgSO_4$) and the solvents removed in vacuo to give a residue. Flash chromatography of the residue over silica using ethyl acetate/heptane (3:10), followed by (7:20) as the eluent gave **14c** as a colourless oil (1.32 g, 68%), TLC (single UV spot, $R_f = 0.25$, 25% ethyl acetate in heptane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.60–2.63 (m, C*H*$_2$C*H*$_2$C*H*$_2$ and O*H*, 7H), 4.8–4.27 (m, C*H*CH$_2$O, 1H), 4.29–4.48 (m, CHC*H*$_2$O and C*H*OH, 3H), 4.57–4.66 (br s, OC*H*$_2$CHCH$_2$, 2H), 5.22 (dd, $J = 1.05$ and 10.45 Hz, OCH$_2$CHC*H*$_2$, 1H), 5.29 (d, $J = 9.65$ Hz, OCH$_2$CHC*H*$_2$, 1H), 5.77 (br s, N*H*, 1H), 5.82–5.94 (m, OCH$_2$C*H*CH$_2$, 1H), 7.27–7.32 (m, 2×fluorenyl C*H*, 2H), 7.36–7.41 (m, 2×fluorenyl C*H*, 2H), 7.55–7.62 (m, 2×fluorenyl C*H*, 2H), 7.74–7.77 (m, 2×fluorenyl C*H*, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.4 (CH$_2$CH$_2$CH$_2$), 32.5, 32.3 and 34.2 (*C*$_2$CH$_2$CH$_2$), 47.6, 47.5 (CH$_2$*C*HO), 66.5 (*C*H$_2$CHO), 67.3 (OC$_2$CHCH$_2$), 76.0 (*C*CO$_2$CH$_2$), 77.8 (*C*HOH), 119.0 (OCH$_2$CH*C*H$_2$), 120.4, 125.5, 127.5, 128.1 (8×fluorenyl *C*H), 132.1 (OCH$_2$*C*HCH$_2$), 141.7, 144.2, 144.3 (4×fluorenyl quaternary *C*), 156.7 (O*C*ON), 173.6 (*C*H$_2$CH$_2$CHCH$_2$); analytical HPLC $R_t = 20.37$ min (major) and $R_t = 19.71$ min (minor) and HPLC-MS (main peak, 408.1 $[M+H]^+$, 430.1 $[M+Na]^+$; minor peak, 408.1 $[M+H]^+$, 430.1 $[M+Na]^+$). Anal. Calcd for $C_{24}H_{25}NO_5$ 0.15DCM: C, 62.25; H, 5.61; N, 2.92. Found: C, 62.08; H, 5.60; N, 3.02; Exact mass calcd for $C_{24}H_{26}NO_5$: 408.1805, found 408.1807, δ +0.03 ppm.

4.1.22. (1*R*,2*R*)-1-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-2-hydroxycyclopentanecarboxylic acid allyl ester (14d). (a) A solution of dimethyl sulfoxide (0.224 mL, 3.15 mmol) in dichloromethane (1.0 mL) was added under nitrogen to a stirred solution of oxalyl chloride (0.132 mL, 1.51 mmol) in dichloromethane (2.5 mL) at −70 °C over 20 min. The mixture was stirred for 10 min then a solution of **14c** (0.535 g, 1.31 mmol) in dichloromethane (3 mL) added over 20 min. The mixture was stirred for 10 min then triethylamine (0.92 mL, 6.57 mmol) added dropwise over 5 min. The cooling bath was removed and stirring continued for 45 min at ambient temperature. A saturated aqueous ammonium chloride solution (50 mL) was added then the product extracted into diethyl ether (2×50 mL). The combined ethereal layers were washed with water (25 mL), dried ($MgSO_4$) and the solvent was removed in vacuo to give a residue (520 mg). Flash chromatography of the residue over silica using ethyl acetate/heptane (1:3) as the eluent gave (1*R*)-1-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2-oxocyclopentanecarboxylic acid allyl ester as a colourless oil (0.43 g, 81%), TLC (single UV spot, $R_f = 0.30$, 30% ethyl acetate in heptane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.15–2.23 (m, CH$_2$C*H*$_2$CH$_2$, 2H), 2.46–2.70 (m, C*H*$_2$CH$_2$C*H*$_2$, 4H), 4.21 (t, $J = 7.15$ Hz, C*H*CH$_2$O, 1H), 4.35 (d, $J = 7.10$ Hz, CHC*H*$_2$O, 2H), 4.66 (br s, OC*H*$_2$CHCH$_2$, 2H), 5.26–5.35 (m, OCH$_2$CHC*H*$_2$, 2H), 5.80–5.91 (m, OCH$_2$C*H*CH$_2$, 1H), 6.14 (br s, N*H*, 1H), 7.27–7.35 (m, Fmoc H-*2* and H-*7*, 2H), 7.36–7.41 (m, Fmoc H-*3* and H-*6*, 2H), 7.54–7.60 (Fmoc H-*1* and H-*8*, 2H aromatic), 7.74–7.77 (Fmoc H-*4* and H-*5*, 2H aromatic); ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.2 (CH$_2$*C*H$_2$CH$_2$), 34.5 and 36.9 (*C*H$_2$CH$_2$CH$_2$), 47.4 (Fmoc C-*9*), 67.4 and 67.7 (Fmoc *C*H$_2$ and OCH$_2$CHCH$_2$), 67.85 (*C*CO$_2$CH$_2$), 119.9 (OCH$_2$CH*C*H$_2$), 120.4, 125.5, 127.5, 128.2 (8×fluorenyl *C*H), 131.1 (OCH$_2$*C*HCH$_2$), 141.7, 144.0, 144.2 (4×fluorenyl quaternary *C*), 155.25 (O*C*ON), 169.25 (*C*$_2$CH$_2$CHCH$_2$), 211.3 (C*C*OCH$_2$CH$_2$); analytical HPLC with main peak $R_t = 19.99$ min, HPLC-MS (single UV peak with $R_t = 10.13$ min, 406.1 $[M+H]^+$, 428.1 $[M+Na]^+$).

(b) Sodium borohydride (39 mg, 1.04 mmol) was added in one portion to a stirred solution of (1*R*)-1-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2-oxocyclopentanecarboxylic acid allyl ester (0.42 g, 1.04 mmol) in methanol (6 mL) at 0 °C. The mixture was stirred for 10 min then the solvents removed in vacuo to give a residue. Water (20 mL) and dichloromethane (20 mL) were added and the mixture was then acidified with 1 M hydrochloric acid (pH ≈ 1.5). The dichloromethane layer was col-

lected then the aqueous layer extracted with dichloromethane (20 mL). The combined dichloromethane layers were washed with aqueous saturated sodium chloride solution (20 mL). The aqueous saturated sodium chloride solution was extracted with dichloromethane (10 mL) then the dichloromethane layers were combined then dried ($MgSO_4$) and the solvent removed in vacuo to give a residue (420 mg). Flash chromatography of the residue over silica using ethyl acetate/heptane (3:7), followed by (7:13) as the eluent gave **14d** as a colourless oil (288 mg, 68%), TLC (single UV spot, $R_f = 0.25$, 25% ethyl acetate in heptane), analytical HPLC $R_t = 19.68$ min (major), $R_t = 20.32$ min (minor) and HPLC-MS (main UV peak with $R_t = 9.08$ min, 408.1 $[M+H]^+$, 430.0 $[M+Na]^+$; minor UV peak with $R_t = 9.451$ min, 408.1 $[M+H]^+$, 430.0 $[M+Na]^+$). *para*-Toluenesulfonic acid monohydrate (30 mg, 0.16 mmol) was added to a solution of the oil (230 mg) in toluene (12 mL) then the mixture heated at 100 °C for 75 min. The solvents were removed in vacuo to give a residue. Flash chromatography as above gave **14d** as a colourless oil (200 mg, 87%). TLC (single UV spot, $R_f = 0.25$, 25% ethyl acetate in heptane); ^1H NMR (400 MHz, $CDCl_3$): δ 1.72–2.44 (m, $CH_2CH_2CH_2$, 6H), 4.12–4.19 (m, CHOH, 1H), 4.27 (t, $J = 6.55$ Hz, fluorenyl $CHCH_2O$, 1H), 4.37–4.55 (m, fluorenyl $CHCH_2O$ and OH, 3H), 4.60–4.75 (br s, OCH_2CHCH_2, 2H), 5.25 (d, $J = 10.45$ Hz, OCH_2CHCH_2, 1H), 5.30–5.39 (m, OCH_2CHCH_2 and NH, 2H), 5.83–5.96 (m, OCH_2CHCH_2, 1H), 7.29–7.35 (Fmoc H-*2* and H-*7*, 2H), 7.39–7.44 (Fmoc H-*3* and H-*6*, 2H), 7.58–7.65 (Fmoc H-*1* and H-*8*, 2H), 7.77–7.80 (Fmoc H-*4* and H-*5*, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.9 ($CH_2CH_2CH_2$), 32.5 and 35.6 ($CH_2CH_2CH_2$), 47.5 (Fmoc C-*9*), 66.55 and 67.4 ($CHCH_2O$ and OC_2CHCH_2), 69.4 (CCO_2CH_2), 80.5 (CHOH), 119.0 (OC_2CHCH_2), 120.4 (Fmoc C-*4* and C-*5*), 125.4 (Fmoc C-*1* and C-*8*), 127.5, 127.45 (Fmoc C-*2* and C-*7*), 128.1, 128.2 (Fmoc C-*3* and C-*6*), 132.1 (OCH_2CHCH_2), 141.7 (Fmoc C-*4′* and C-*5′*), 144.0, 144.2 (Fmoc C-*1′* and C-*8′*), 156.9 (OCON), 173.0 ($CH_2CH_2CHCH_2$); analytical HPLC single UV peak with $R_t = 18.14$ min, HPLC-MS (single UV peak with $R_t = 9.14$ min, 408.1 $[M+H]^+$, 430.1 $[M+Na]^+$). Anal. Calcd for $C_{24}H_{25}NO_5$ 0.17DCM: C, 61.12; H, 5.53; N, 2.86. Found: C, 61.10; H, 5.52; N, 3.20; Exact mass calcd for $C_{24}H_{26}NO_5$: 408.1805, found 408.1823, δ +4.31 ppm.

4.1.23. (3*aR*,6*aS*)-2-Oxo-hexahydrocyclopentaoxazole-3*a*-carboxylic acid allyl ester (32). *para*-Toluenesulfonic acid monohydrate (0.49 g, 2.59 mmol) was added to a stirred solution of (1*R*,2*S*)-1-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2-hydroxycyclopentanecarboxylic acid (0.95 g, 2.59 mmol) and allyl alcohol (3 mL, 44.2 mmol) in toluene (10 mL). The solution was heated at reflux in a Dean and Stark apparatus for 15 min after which time an additional amount of allyl alcohol (3 mL, 44.2 mmol) was added. Heating at reflux was continued for 45 min then the mixture allowed to cool to ambient temperature. The products were extracted into chloroform (50 mL) then washed with saturated aqueous sodium hydrogen carbonate solution (50 mL). The aqueous layer was extracted with chloroform (20 mL) then the combined organic layers were washed with hydrochloric acid (50 mL, 0.1 M) and aqueous saturated sodium chloride solution then dried ($MgSO_4$) and the solvent removed in vacuo to give a residue (860 mg). Flash chromatography of the residue over silica using ethyl acetate/heptane (1:4), followed by (1:1) as the eluent gave (1*R*,2*R*)-1-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2-hydroxycyclopentanecarboxylic acid allyl ester (**14d**) (100 mg, 9%) and (3*aR*,6*aS*)-2-oxo-hexahydrocyclopentaoxazole-3*a*-carboxylic acid allyl ester **32** (270 mg, 49%) as a colourless oil. TLC (single non-UV active spot, $R_f = 0.1$, 25% ethyl acetate in heptane); ^1H NMR (400 MHz, $CDCl_3$): δ 1.88–2.20 (m, $CH_2CH_2CH_2$, 6H), 4.67–4.95 (m, CH_2O, 2H), 5.09–5.13 (m, H-*6a*, 1H), 5.30 (dd, $J = 10.40$, 1.10 Hz, $CH_2CH{=}CH_2$, 1H), 5.34 (dd, $J = 17.20$, 1.40 Hz, $CH_2CH{=}CH_2$, 1H), 5.81–5.98 (m, $CH_2CH{=}CH_2$, 1H), 6.30 (br s, NH, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 23.9 ($CH_2CH_2CH_2$), 34.7 and 39.5 ($CH_2CH_2CH_2$), 67.0 ($CHCH_2O$), 70.4 (C-*3a*), 85.6 (C-*6a*), 119.9 ($CH_2{=}CHCH_2$), 131.4 ($CH_2{=}CHCH_2$), 158.8 (C-*2*), 172.2 ($CH_2CH_2CH{=}CH_2$); HPLC-MS (single non-UV active peak with $R_t = 4.9$ min, 212.1 $[M+H]^+$, 423.2 $[2M+H]^+$, 445.2 $[2M+Na]^+$). Anal. Calcd for $C_{10}H_{13}NO_4$: C, 56.86; H, 6.20; N, 6.63. Found: C, 56.80; H, 6.26; N, 6.54; Exact mass calcd for $C_{10}H_{13}NO_4Na$: 234.0737, found 234.0748, δ +4.82 ppm.

4.1.24. (1*R*,2*R*)-2-*tert*-Butoxy-1-(9*H*-fluoren-9-ylmethoxycarbonylamino)cyclopentanecarboxylic acid allyl ester (14e). A stirred solution of (1*R*,2*R*)-1-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2-hydroxycyclopentanecarboxylic acid allyl ester **14d** (360 mg, 0.88 mmol) in dichloromethane (5 mL) was cooled in a pressure vessel to −70 °C then isobutylene gas (~3 mL) condensed into the solution. Concentrated sulfuric acid (25 μL) was added then the pressure vessel sealed. The mixture was stirred at ambient temperature for 20 h then cooled to −70 °C. *N*-Methylmorpholine (50 μL) was added then the unsealed pressure vessel allowed to warm to ambient temperature. The mixture was diluted with saturated aqueous sodium hydrogen carbonate solution (50 mL) and water (25 mL) then the product extracted into dichloromethane (50 mL then 2 × 25 mL). The combined dichloromethane layers were washed with saturated aqueous sodium chloride solution (25 mL), dried (Na_2SO_4) and the solvent removed in vacuo. The residue (370 mg) was purified by flash chromatography over silica gel eluting with a gradient of heptane/ethyl acetate 4:1 to 7:3. Appropriate fractions were combined and the solvents removed in vacuo to leave **14e** as a colourless oil (305 mg, 75%). TLC (single UV spot, $R_f = 0.50$, heptane/ethyl acetate 2:1), analytical HPLC $R_t = 22.623$ min, HPLC-MS (single UV peak with $R_t = 11.611$ min, 408.1 $[M-{}^tBu+2H]^+$, 486.1 $[M+Na]^+$). ^1H NMR (400 MHz, $CDCl_3$): δ 1.06 (*s*, $C(CH_3)_3$, 9H), 1.80–2.41 (m, $CH_2CH_2CH_2$, 6H), 4.21 (t, $J = 6.80$ Hz, Fmoc H-*9*, 1H), 4.26–4.50 (m, Fmoc CH_2 and H-*2*, 3H), 4.58–4.74 (br s, OCH_2CHCH_2, 1H), 5.21 (d, $J = 10.40$ Hz, OCH_2CHCH_2, 1H), 5.35 (d, $J = 17.20$ Hz, OCH_2CHCH_2, 1H), 5.84–6.01 (m,

$CH_2CH{=}CH_2$ and N*H*, 2H), 7.26–7.30 (Fmoc H-*2* and H-*7*, 2H), 7.37–7.39 (Fmoc H-*3* and H-*6*, 2H), 7.57–7.61 (Fmoc H-*1* and H-*8*, 2H), 7.70–7.77 (Fmoc H-*4* and H-*5*, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.7, 21.5 ($CH_2CH_2CH_2$), 33.0 and 33.8 ($CH_2CH_2CH_2$), 47.6 (Fmoc C-*9*), 66.5 and 66.9 (Fmoc CH_2 and OCH_2CHCH_2), 70.1 and 74.0 (CCO_2CH_2 and $OC(CH_3)_3$), 79.5 ($CHOC(CH_3)_3$), 118.5 (OCH_2CHCH_2), 120.4 (Fmoc C-*4* and C-*5*), 125.5 (Fmoc C-*1* and C-*8*), 127.5 (Fmoc C-*2* and C-*7*), 128.1 (Fmoc C-*3* and C-*6*), 132.4 (OCH_2CHCH_2), 141.7 (Fmoc C-*4′* and C-*5′*), 144.3 (Fmoc C-*1′* and C-*8′*), 155.1 (O*C*ON), 173.0 ($CO_2CH_2CHCH_2$). Anal. Calcd for $CH_{28}H_{33}NO_5$ 0.07DCM: C, 68.46; H, 6.84; N, 2.81. Found: C, 68.90; H, 7.17; N, 2.86; Exact mass calcd for $C_{28}H_{34}NO_5$: 486.2251, found 486.2254, δ +0.06 ppm.

4.1.25. (1*R*,2*R*)-1-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-2-hydroxycyclopentanecarboxylic acid (14a). Tetrakistriphenylphosphine palladium(0) (5 mg, 0.004 mmol), dichloromethane (2 mL) then phenyltrihydrosilane (55 μL, 0.44 mmol) were added consecutively to **14d** (90 mg, 0.22 mmol) under nitrogen. The mixture was stirred for 90 min then diluted with chloroform (5 mL) and then extracted with aqueous saturated sodium hydrogen carbonate solution (5 mL). The aqueous layer was then extracted with chloroform (3×5 mL), then chloroform (5 mL) added and the biphasic mixture acidified to pH = 1 using 1 M hydrochloric acid. The chloroform layer was separated then the aqueous layer re-extracted with chloroform (2×5 mL). The combined chloroform extracts (obtained from the acidified aqueous layer) were dried (Na_2SO_4) and the solvent removed in vacuo to leave the product (**14a**) as an oil (40 mg). An additional 25 mg of **14a** was obtained by extracting initial chloroform washings with aqueous saturated sodium hydrogen carbonate solution (5 mL), which was then washed with chloroform (2×5 mL) before acidifying to pH = 1 using 1 M hydrochloric acid and extracting the product into chloroform (3×5 mL) and drying with (Na_2SO_4). The two extracts were combined then used without further purification (80% total yield). Analytical HPLC R_t = 16.786 min, HPLC-MS (main UV peak with R_t = 7.760 min, 368.1 $[M+H]^+$, 390.0 $[M+Na]^+$, 757.1 $[2M+Na]^+$).

4.1.26. Attempted preparation of diazoketone 11a: formation of (1*R*,2*R*)-(7-oxo-6-oxabicyclo[3.2.0]heptyl)carbamic acid 9*H*-fluoren-9-ylmethyl ester (33). *N*-Methylmorpholine (36 μL, 0.33 mmol) and *iso*-butylchloroformate (23 μL, 0.167 mmol) in dichloromethane (0.5 mL) were added simultaneously in portions over 15 min to a stirred solution of (1*R*,2*R*)-1-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2-hydroxycyclopentanecarboxylic acid **14a** (60 mg, 0.16 mmol) in dichloromethane (1.0 mL) at −15 °C. The mixture was stirred at −15 °C for 45 min then etheral diazomethane [approximately 2 mmol generated from diazald® and sodium hydroxide in water/ethanol (l:2) at 60 °C in diethyl ether (~10 mL)] was added to the activated amino acid solution at −15 °C. The mixture was then allowed to warm to ambient temperature and stirred for 24 h. Acetic acid (0.4 mL) was added to the mixture followed by *tert*-butyl methyl ether (25 mL). The organic layer was washed with water (3×10 mL), dried (Na_2SO_4) and the solvent removed in vacuo. The residue (53 mg) was purified by flash chromatography over silica gel eluting with a gradient of heptane/ethyl acetate 7:3 to 2:1. Appropriate fractions were combined and the solvents removed in vacuo to leave (1*R*,2*R*)-(7-oxo-6-oxabicyclo[3.2.0]heptyl) carbamic acid 9*H*-fluoren-9-ylmethyl ester (**33**) as a white solid (23 mg, 41%). Analytical HPLC R_t = 19.891 min, HPLC-MS (main UV peak with R_t = 9.349 min, 372.0 $[M+Na]^+$.

4.1.27. (1*R*,2*R*)-2-*tert*-Butoxy-1-(9*H*-fluoren-9-ylmethoxycarbonylamino)cyclopentanecarboxylic acid (14b). Tetrakistriphenylphosphine palladium(0) (15 mg, 0.013 mmol), dichloromethane (5 mL) then phenyltrihydrosilane (153 μL, 1.24 mmol) were added consecutively to **14e** (288 mg, 0.62 mmol) under nitrogen. The mixture was stirred for 45 min then 0.01 M hydrochloric acid (30 mL) added and the product extracted into chloroform (1×20 mL then 1×10 mL). The combined chloroform layers were dried (Na_2SO_4) and the solvent removed in vacuo. The residue (460 mg) was purified by flash chromatography over silica gel eluting with a gradient of heptane/ethyl acetate 2:1 to 1:3. Appropriate fractions were combined and the solvents removed in vacuo to leave **14b** as a colourless oil (205 mg, 78%). TLC (single UV spot, R_f = 0.25, heptane/ethyl acetate 1:2), analytical HPLC R_t = 19.539 min, HPLC-MS (single UV peak with R_t = 9.850 min, 368.1 $[M-{}^tBu+2H]^+$, 446.1 $[M+Na]^+$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.22 (s, $C(CH_3)_3$, 9H), 1.77–2.37 (m, $CH_2CH_2CH_2$, 6H), 4.22 (t, J = 6.80 Hz, Fmoc H-*9*, 1H), 4.27–4.36 (m, Fmoc CH_2, 2H), 4.65 (br s, H-*2*, 1H), 5.39 (br s, N*H*, 1H), 7.27–7.35 (Fmoc H-*2* and H-*7*, 2H), 7.36–7.41 (Fmoc H-*3* and H-*6*, 2H), 7.56–7.61 (Fmoc H-*1* and H-*8*, 2H), 7.73–7.76 (Fmoc H-*4* and H-*5*, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.5 ($CH_2CH_2CH_2$), 28.8 ($C(CH_3)_3$), 33.7 and 35.3 ($CH_2CH_2CH_2$), 47.6 (Fmoc C-*9*), 67.2 (Fmoc CH_2), 68.8 (CCO_2CH_2), 78.6 ($CHOC(CH_3)_3$), 120.4 (Fmoc C-*4* and C-*5*), 125.5 (Fmoc C-*1* and C-*8*), 127.5 (Fmoc C-*2* and C-*7*), 128.1, 128.1 (Fmoc C-*3* and C-*6*), 141.7, 141.7 (Fmoc C-*4′* and C-*5′*), 144.0, 144.4 (Fmoc C-*1′* and C-*8′*), 156.1 (O*C*ON), 174.9 (C_2H). Anal. Calcd for $C_{25}H_{29}NO_5$ 0.05EtOAc: C, 70.08; H, 7.01; N, 3.14. Found: C, 69.89; H, 6.88; N, 3.08; Exact mass calcd for $C_{25}H_{29}NO_5Na$: 446.1938, found 446.1960, δ +4.84 ppm.

4.1.28. (1*R*,2*R*)-(2-*tert*-Butoxy-1-fluorocarbonylcyclopentyl)carbamic acid 9*H*-fluoren-9-ylmethyl ester (14f). Pyridine (53 μL, 0.66 mmol) then cyanuric fluoride (71 μL, 0.85 mmol) were added consecutively at 0 °C to a stirred solution of (1*R*,2*R*)-2-*tert*-butoxy-1-(9*H*-fluoren-9-ylmethoxycarbonylamino)-2-hydroxycyclopentanecarboxylic acid **14b** (159 mg, 0.38 mmol) in dichloromethane (5 mL) under nitrogen. The suspension was stirred for 30 min at 0 °C then for 5 h at ambient temperature.

Crushed ice (~10 mL) and ice-chilled water (10 mL) were added, then the product was extracted into dichloromethane (20 mL). The dichloromethane layer was dried ($MgSO_4$) and the solvent removed in vacuo to leave **14f** as a pale brown oil (115 mg, 72%), which was used without further purification. TLC (single UV spot, $R_f = 0.45$, heptane/ethyl acetate 2:1), analytical HPLC main UV peak with $R_t = 23.93$ min, HPLC-MS (main UV peak with $R_t = 11.439$ min, 370.1 $[M-{}^tBu+2H]^+$, 448.1 $[M+Na]^+$).

4.1.29. (3*aR*,6*aR*)-(3-Oxo-hexahydrocyclopenta[*b*]furan-3*a*-yl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester (8). (a) Ethereal diazomethane [generated from diazald® (0.94 g, ~3 mmol) addition in diethyl ether (15 mL) to sodium hydroxide (1.05 g) in water (1.5 mL)/ethanol (3.0 mL) at 60 °C] was added to a stirred solution of (1*R*,2*R*)-(2-*tert*-butoxy-1-fluorocarbonylcyclopentyl)carbamic acid 9*H*-fluoren-9-ylmethyl ester **14f** (115 mg, 0.27 mmol) in dichloromethane (2 mL) at 0 °C. The solution was stirred for 20 min at 0 °C then at ambient temperature for 20 h. Acetic acid (0.6 mL) was added then the solution was stirred for 5 min before adding *tert*-butyl methyl ether (50 mL). The ethereal layer was washed with saturated aqueous sodium hydrogen carbonate solution (40 mL) then water (2×30 mL), dried (Na_2SO_4) and the solvent removed in vacuo. The residue (130 mg) was purified by flash chromatography over silica gel eluting with a gradient of heptane/ethyl acetate 4:1 to 1:3. Appropriate fractions were combined and the solvents removed in vacuo to leave a 4:1 mixture of (5*R*,6*R*)-6-*tert*-butoxy-2-(9*H*-fluoren-9-ylmethoxy)-3-oxa-1-azaspiro[4.4]non-1-en-4-one **34** and (1*R*,2*R*)-2-*tert*-butoxy-1-(9*H*-fluoren-9-ylmethoxycarbonylamino)cyclopentanecarboxylic acid methyl ester (70 mg) (**14b** where $R^3 = OMe$) together with (1*R*,2*R*)-[2-*tert*-butoxy-1-(2-diazoacetyl)cyclopentyl]carbamic acid 9*H*-fluoren-9-ylmethyl ester **11b** (16 mg, 13%) as an oil. Data for **11b**: TLC (single UV spot, $R_f = 0.50$, heptane/ethyl acetate 2:1), analytical HPLC main UV peak with $R_t = 18.927$ min, HPLC-MS (main UV peak with $R_t = 9.130$ min, 364.1 $[M-N_2-{}^tBu+2H]^+$, 386.0 $[M-N_2-{}^tBu+H+Na]^+$.

(b) A solution of lithium chloride (15 mg, 0.36 mmol) in acetic acid: water (4:1, 1.0 mL) was added to **11b** (16 mg, 0.036 mmol). The solution was stirred for 2.5 h then chloroform (25 mL) and saturated aqueous sodium hydrogen carbonate solution (25 mL) was added. The chloroform layer washed with saturated aqueous sodium hydrogen carbonate solution (25 mL), saturated aqueous sodium chloride solution (25 mL), dried (Na_2SO_4) and the solvent removed in vacuo. The residue (16 mg) was purified by flash chromatography over silica gel eluting with a gradient of heptane/ethyl acetate 17:5 to 3:1. Appropriate fractions were combined and the solvents removed in vacuo to leave (3*aR*,6*aR*)-(3-oxo-hexahydrocyclopenta[*b*]furan-3*a*-yl)carbamic acid 9*H*-fluoren-9-ylmethyl ester **8** (11.0 mg, 83%) as a white solid. TLC (single UV spot, $R_f = 0.3$, heptane/ethyl acetate 2:1), analytical HPLC main UV peak with $R_t = 18.872$ and HPLC-MS (main UV peak with $R_t = 9.208$ min, 364.0 $[M+H]^+$, 386.0 $[M+Na]^+$); ^1H NMR (400 MHz, $CDCl_3$): δ 1.55–2.19 (m, $CH_2CH_2CH_2$, 6H), 4.15 (d, $J = 16.75$ Hz, H-*2*, 1H), 4.19 (t, $J = 6.65$ Hz, Fmoc H-*9*, 1H), 4.31 (d, $J = 16.80$ Hz, H-*2*, 1H), 4.36–4.44 (m, Fmoc CH_2, 2H), 4.74 and 4.97 (each br s, H-*6a* and N*H*, 1H), 7.29–7.36 (Fmoc H-*2* and H-*7*, 2H), 7.38–7.44 (Fmoc H-*3* and H-*6*, 2H), 7.53–7.61 (Fmoc H-*1* and H-*8*, 2H), 7.74–7.80 (Fmoc H-*4* and H-*5*, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24 ($CH_2CH_2CH_2$), 33 and 37 ($CH_2CH_2CH_2$), 48 (Fmoc C-*9*), 68 (Fmoc CH_2), 70 (C-*3a*), 72 (C-*2*), 87 (C-*6a*), 120 (Fmoc C-*4* and C-*5*), 125 (Fmoc C-*1* and C-*8*), 127 (Fmoc C-*2* and C-*7*), 128 (Fmoc C-*3* and C-*6*), 142 (Fmoc C-*4′* and C-*5′*), 144 (Fmoc C-*1′* and C-*8′*), 156 (OCON), 215 (C-*3*). Anal. Calcd for $C_{22}H_{21}NO_4$: C, 72.71; H, 5.82; N, 3.85. Found: C, 72.68; H, 5.88; N, 3.70; Exact mass calcd for $C_{22}H_{22}NO_5$: 364.1543, found 364.1531, δ −3.33 ppm.

4.2. Solid-phase chemistry

4.2.1. Preparation of (2*R*,3*S*)-(2,3-dimethyl-4-oxo-tetrahydrofuran-3-yl)carbamic acid 9*H*-fluoren-9-ylmethyl ester—linker construct (35a). 4-[(Hydrazinocarbonyl) amino]methylcyclohexanecarboxylic acid trifluoroacetate (74 mg, 0.223 mmol, 1 equiv)[27] and sodium acetate trihydrate (46 mg, 0.334 mmol, 1.5 equiv) were added to (2*R*,3*R*)-(2,3-dimethyl-4-oxotetrahydrofuran-3-yl)carbamic acid 9*H*-fluoren-9-ylmethyl ester (**6**) (78 mg, 0.223 mmol, 1 equiv), followed by ethanol (3.90 mL) and water (0.56 mL). The mixture was heated for 3 days at 86 °C then allowed to cool to ambient temperature and diluted with chloroform (50 mL). The chloroform layer was washed with dilute aqueous hydrochloric acid (pH 3, 2×30 mL), brine (25 mL), dried (Na_2SO_4) and evaporated in vacuo to give (**35a**) as a white solid (80 mg). Analytical HPLC indicated one main peak at $R_t = 16.99$ min and a minor peak at $R_t = 17.39$ min (mixture of *E*- and *Z*-isomers), HPLC-MS (main UV peaks with $R_t = 8.02$ and 8.30 min, 549 $[M+H]^+$.

4.2.2. Preparation of (2*S*,3*S*)-(2,3-dimethyl-4-oxo-tetrahydrofuran-3-yl)carbamic acid 9*H*-fluoren-9-ylmethyl ester—linker construct (36a). 4-[(Hydrazinocarbonyl) amino]methylcyclohexanecarboxylic acid trifluoroacetate (35 mg, 0.107 mmol, 7.5 equiv)[27,28] and sodium acetate trihydrate (22 mg, 0.1605 mmol, 7.5 equiv) were added to (2*S*,3*R*)-(2,3-dimethyl-4-oxotetrahydrofuran-3-yl)carbamic acid 9*H*-fluoren-9-ylmethyl ester (**7**) (8 mg, 0.0214 mmol, 1 equiv), followed by ethanol (1.86 mL) and water (0.27 mL). The mixture was heated for 2 days at 86 °C then allowed to cool to ambient temperature and diluted with chloroform (50 mL). The chloroform layer was washed with dilute aqueous hydrochloric acid (pH 3, 2×30 mL), brine (25 mL), dried (Na_2SO_4) and evaporated in vacuo to give (**36a**) as a white solid (10 mg). Analytical HPLC indicated one main peak at $R_t = 17.53$ min and a minor peak at $R_t = 17.88$ min (mixture of *E*- and *Z*-isomers),

HPLC-MS (main UV peaks with $R_t = 8.37$ and 8.98 min, 549 $[M+H]^+$.

4.2.3. Preparation of (3*aR*,6*aR*)-(3-oxo-hexahydrocyclopenta[*b*]furan-3*a*-yl)carbamic acid 9*H*-fluoren-9-ylmethyl ester—linker construct (37a). 4-[(Hydrazinocarbonyl)amino]methylcyclohexanecarboxylic acid trifluoroacetate (23.6 mg, 0.072 mmol, 1 equiv)[27] and sodium acetate trihydrate (14.6 mg, 0.107 mmol, 1.5 equiv) were added to (3*aR*,6*aR*)-(3-oxohexahydrocyclopenta[*b*]furan-3*a*-yl)carbamic acid 9*H*-fluoren-9-ylmethyl ester (**8**) (26.0 mg, 0.072 mmol, 1 equiv), followed by ethanol (1.75 mL) and water (0.25 mL). The mixture was heated for 24 h at 86 °C then allowed to cool to ambient temperature and diluted with chloroform (35 mL). The chloroform layer was washed with dilute aqueous hydrochloric acid (pH 3, 2×15 mL), brine (15 mL), dried (Na_2SO_4) and evaporated in vacuo to give (**37a**) as a colourless gum (40.8 mg). Analytical HPLC indicated one main peak at $R_t = 17.57$ min and a minor peak at $R_t = 18.08$ min (mixture of *E*- and *Z*-isomers), HPLC-MS (main UV peaks with $R_t = 8.45$ and 9.07 min, 561 $[M+H]^+$, 1121 $[2M+H]^+$.

4.3. Solid-phase protocols

Example inhibitors (**38**–**40**) were prepared from constructs (**35a**–**37a**) by solid-phase assembly techniques utilising multipins (see www.mimotopes.com).[29,32] In general, 1.2 μmol gears (GEXXOGAP) were used to provide small scale crude examples for preliminary screening, whilst 10 μmol crowns (SPMDINOF) were used for scale-up synthesis and purification of selected examples.[34] Constructs (**35a**–**37a**), 3 equiv w.r.t. solid-phase surface loading, were coupled overnight onto 1.2 μmol gears and 10 μmol crowns using standard HBTU, HOBt and NMM activation to provide loaded constructs (**35b**–**37b**). Constructs (**35b**–**37b**) were then utilised in standard rounds of washing, Fmoc deprotection and coupling, followed by acidolytic cleavage to give crude inhibitors (**38**–**40**).[10a,b] Examples derived from 10 μmol crowns were purified by semipreparative HPLC (see General methods) and appropriate fractions combined and lyophilised into pre-tared glass vials. Purified analogues were then weighed and a volume of dimethylsulfoxide added as appropriate to give 10 mM stock solutions used for general storage and inhibition assays.

Each purified analogue was analysed giving the following characterisation data:

4.3.1. (2*R*,3*R*)-*N*-[1*S*-(2,3-Dimethyl-4-oxo-tetrahydrofuran-3-ylcarbamoyl)-3-methyl butyl]-4-(4-methylpiperazin-1-yl)benzamide (38a) . HPLC $R_t = 10.73$ min (94%), HPLC-MS 445.3 $[M+H]^+$, 911.5 $[2M+Na]^+$; Exact mass calcd for $C_{24}H_{37}N_4O_4$: 445.2809, found 445.2794, δ −3.40 ppm.

4.3.2. (2*R*,3*R*)-Thiophene-3-carboxylic acid [2-cyclohexyl-1*S*-(2,3-dimethyl-4-oxo-tetrahydrofuran-3-ylcarbamoyl)ethyl]amide (38b). HPLC $R_t = 17.03$ min (84%), HPLC-MS 393.2 $[M+H]^+$, 807.3 $[2M+Na]^+$; Exact mass calcd for $C_{20}H_{29}N_2O_4S$: 393.1843, found 393.1854, δ +2.87 ppm.

4.3.3. (2*R*,3*R*)-Benzo[*b*]thiophene-2-carboxylic acid [1*S*-(2,3-dimethyl-4-oxo-tetra-hydrofuran-3-ylcarbamoyl)-3-methylbutyl]amide (38c). HPLC $R_t = 17.68$ min (93%), HPLC-MS 403.2 $[M+H]^+$, 827.3 $[2M+Na]^+$; Exact mass calcd for $C_{21}H_{26}N_2O_4SNa$: 425.1505, found 425.1521, δ +3.64 ppm.

4.3.4. (2*R*,3*R*)-3-Bromo-*N*-[1*S*-(2,3-dimethyl-4-oxo-tetrahydrofuran-3-ylcarbamoyl)-2-(4-hydroxphenyl)ethyl]benzamide (38d). HPLC $R_t = 14.90$ min (87%), HPLC-MS 475.3/477.3 $[M+H]^+$; Exact mass calcd for $C_{22}H_{24}N_2O_5Br$: 475.0863, found 475.0875, δ +2.48 ppm.

4.3.5. (2*R*,3*R*)-3-Aminomethyl-*N*-[1*S*-(2,3-dimethyl-4-oxo-tetrahydrofuran-3-ylcarbamoyl)-3-methylbutyl]benzamide (38e). HPLC $R_t = 9.81$ min (92%), HPLC-MS 376.2 $[M+H]^+$, 398.2 $[M+Na]^+$, 773.4 $[2M+Na]^+$; Exact mass calcd for $C_{20}H_{30}N_3O_4$: 376.2231, found 376.2233, δ +0.06 ppm.

4.3.6. (2*R*,3*R*)-4-*tert*-Butyl-*N*-[1*S*-(2,3-dimethyl-4-oxo-tetrahydrofuran-3-ylcarbamoyl)-2-(4-hydroxyphenyl)ethyl]-benzamide (38f). HPLC $R_t = 16.2$ min (90%), HPLC-MS 453.2 $[M+H]^+$, 475.2 $[M+Na]^+$, 927.3 $[2M+Na]^+$; Exact mass calcd for $C_{26}H_{32}N_2O_5Na$: 475.2203, found 475.2196, δ −1.58 ppm.

4.3.7. (2*S*,3*R*)-*N*-[1*S*-(2,3-Dimethyl-4-oxo-tetrahydrofuran-3-ylcarbamoyl)-3-methyl butyl]-4-(4-methylpiperazin-1-yl)benzamide (39a). HPLC $R_t = 9.90$ min (95%), HPLC-MS 445.2 $[M+H]^+$.

4.3.8. (2*S*,3*R*)-Thiophene-3-carboxylic acid [2-cyclohexyl-1*S*-(2,3-dimethyl-4-oxo-tetrahydrofuran-3-ylcarbamoyl)ethyl]amide (39b). HPLC $R_t = 16.85$ min (92%), HPLC-MS 393.2 $[M+H]^+$; Exact mass calcd for $C_{20}H_{28}N_2O_4SNa$: 415.1662, found 415.1663, δ +0.02 ppm.

4.3.9. (2*S*,3*R*)-3-Bromo-*N*-[1*S*-(2,3-dimethyl-4-oxo-tetrahydrofuran-3-ylcarbamoyl)-2-(4-hydroxphenyl)ethyl]benzamide (39d). HPLC $R_t = 14.31$ min (89%), HPLC-MS 475.1/477.1 $[M+H]^+$; Exact mass calcd for $C_{22}H_{23}N_2O_5BrNa$: 497.0683, found 497.0680, δ −0.05 ppm.

4.3.10. (2*S*,3*R*)-3-Aminomethyl-*N*-[1*S*-(2,3-dimethyl-4-oxo-tetrahydrofuran-3-ylcarbamoyl)-3-methylbutyl]benzamide (39e). HPLC $R_t = 9.56$ min (93%), HPLC-MS

376.2 $[M+H]^+$, 398.2 $[M+Na]^+$; Exact mass calcd for $C_{20}H_{30}N_3O_4$: 376.2231, found 376.2234, δ +0.08 ppm.

4.3.11. (2*S*,3*R*)-4-*tert*-Butyl-*N*-[1*S*-(2,3-dimethyl-4-oxo-tetrahydrofuran-3-ylcarbamoyl)-2-(4-hydroxyphenyl)ethyl]-benzamide (39f). HPLC $R_t = 17.13$ min (92%), HPLC-MS 453.2 $[M+H]^+$; Exact mass calcd for $C_{26}H_{32}N_2O_5Na$: 475.2203, found 475.2192, δ −2.51 ppm.

4.3.12. (3*aR*,6*aR*)-*N*-[3-Methyl-1*S*-(3-oxo-hexahydrocyclopenta[*b*]furan-3*a*-ylcarbamoyl)butyl]-4-(4-methylpiperazin-1-yl)benzamide (40a). HPLC R_t = 11.23 min (95%) HPLC-MS 457.3 $[M+H]^+$, 935.3 $[2M+Na]^+$; Exact mass calcd for $C_{25}H_{37}N_4O_4$: 457.2809, found 457.2828, δ +4.17 ppm.

4.3.13. (3*aR*,6*aR*)-Thiophene-3-carboxylic acid [2-cyclohexyl-1*S*-(3-oxo-hexahydrocyclopenta[*b*]furan-3*a*-ylcarbamoyl)ethyl]amide (40b). HPLC $R_t = 17.52$ min (93%) HPLC-MS 405.2 $[M+H]^+$, 831.3 $[2M+Na]^+$; Exact mass calcd for $C_{21}H_{28}N_2O_4SNa$: 427.1662, found 427.1681, δ +4.41 ppm.

4.3.14. (3*aR*,6*aR*)-Benzo[*b*]thiophene-2-carboxylic acid [3-methyl-1*S*-(3-oxo-hexahydrocyclopenta[*b*]furan-3*a*-ylcarbamoyl)butyl]amide (40c). HPLC $R_t = 15.02$ min (93%), HPLC-MS 415.1 $[M+H]^+$, 851.2 $[2M+Na]^+$; Exact mass calcd for $C_{22}H_{27}N_2O_4S$: 415.1686, found 415.1707, δ +4.93 ppm.

4.3.15. (3*aR*,6*aR*)-3-Bromo-*N*-[2-(4-hydroxyphenyl)-1-(3-oxo-hexahydrocyclopenta[*b*]furan-3*a*-ylcarbamoyl)-ethyl]benzamide (40d). HPLC $R_t = 15.25$–15.60 min (86%) HPLC-MS 487.1/489.1 $[M+H]^+$; Exact mass calcd for $C_{23}H_{24}N_2O_5Br$: 487.0863, found 487.0881, δ +3.68 ppm.

4.3.16. (3*aR*,6*aR*)-3-Aminomethyl-*N*-[3-methyl-1*S*-(3-oxo-hexahydrocyclopenta[*b*]furan-3*a*-ylcarbamoyl)butyl]benzamide (40e). HPLC $R_t = 10.87$ min (89%) HPLC-MS 388.2 $[M+H]^+$, 410.2 $[M+Na]^+$, 775.4 $[2M+H]^+$; Exact mass calcd for $C_{21}H_{30}N_3O_4$: 388.2231, found 388.2225, δ −0.86 ppm.

4.4. Assays for cysteinyl proteinase activity

Stock solutions of substrate or inhibitor were made up to 10 mM in 100% dimethylsulfoxide (DMSO) (Rathburns, Glasgow, UK) and diluted as appropriately required. In all cases the DMSO concentration in the assays was maintained at less than 1% (v/v). The equilibrium inhibition constants (K_i^{ss}) for each compound were measured under steady-state conditions monitoring enzyme activity as a function of inhibitor concentration. The values were calculated on the assumption of pure competitive behaviour.[35] Assay protocols were based on literature precedent (Table 1; Ref. 1 and references cited therein) and modified as required to suit local assay protocols.[10b]

4.5. Measurement of inhibitor on-rates and off-rates

The observed rates of reaction for the association of compound with enzyme (k_{on}) and for the dissociation of compound from enzyme (k_{off}) were analysed as previously described.[36] Bovine cathepsin S (Merck Biosciences) was assayed in 10 mM bis-tri-propane; pH 6.5 containing 1 mM calcium chloride, 1 mM EDTA and 5 mM 2-mercaptoethanol employing 50 μM (equal to K_M^{app}) Boc-Val-Leu-Lys-AMC (Bachem) as the substrate. For measurements of the association rates, assays were carried out by addition of various concentrations of inhibitor to assay buffer containing substrate and initiated by the addition of enzyme. For the measurements of dissociation rates, pre-incubated enzyme plus inhibitor were diluted at least 20-fold into assay buffer containing substrate. During the course of the assay less than 10% of the substrate was consumed and the observed rates corrected for substrate kinetics.

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References and notes

1. (a) Otto, H.-H.; Schirmeister, T. *Chem. Rev.* **1997**, *97*, 133; (b) Hernandez, A. A.; Roush, W. R. *Curr. Opin. Chem. Biol.* **2002**, *6*, 459; (c) Veber, D. F.; Thompson, S. K. *Curr. Opin. Drug Disc. Dev.* **2000**, *3*, 362–369; (d) Leung-Toung, R.; Li, W.; Tam, T. F.; Karimian, K. *Curr. Med. Chem.* **2002**, *9*, 979.
2. (a) Lecaille, F.; Kaleta, J.; Brömme, D. *Chem. Rev.* **2002**, *102*, 4459; (b) Chapman, H. A.; Riese, R. J.; Shi, G.-P. *Annu. Rev. Physiol.* **1997**, *59*, 63.
3. Barrett, A. J.; Rawlings, N. D.; Woessner, J. F. *Handbook of Proteolytic Enzymes*; Academic: New York, 1998.
4. For example, see: (a) Brömme, D.; Kaleta, J. *Curr. Pharm. Des.* **2002**, *8*, 1639–1658; (b) Kim, W.; Kang, K. *Expert Opin. Ther. Patents* **2002**, *12*(3), 419.
5. In mid 2002, GlaxoSmithKline announced the advancement of SB-462795, a cyclic peptidomimetic ketone inhibitor of cathepsin K, into phase I clinical trials for osteoporosis. In autumn 2002, Novartis announced the advancement of AAE-581, an inhibitor of cathepsin K, into phase I clinical trials progressing to phase IIb by autumn 2003.
6. (a) Marquis, R. W.; Ru, Y.; Zeng, J.; Lee Trout, R. E.; LoCastro, S. M.; Gribble, A. D.; Witherington, J.; Fenwick, A. E.; Garnier, B.; Tomaszek, T.; Tew, D.; Hemling, M. E.; Quinn, C. J.; Smith, W. W.; Zhao, B.; McQueney, M. S.; Janson, C. A.; D'Alessio, K.; Veber, D.

F. *J. Med. Chem.* **2001**, *44*, 725, and references cited therein; (b) Marquis, R. W.; Ru, Y.; LoCastro, S. M.; Zeng, J.; Yamashita, D. S.; Oh, H.-J.; Erhard, K. F.; Davis, L. D.; Tomaszek, T. A.; Tew, D.; Salyers, K.; Proksch, J.; Ward, K.; Smith, B.; Levy, M.; Cummings, M. D.; Haltiwanger, R. C.; Trescher, G.; Wang, B.; Hemling, M. E.; Quinn, C. J.; Cheng, H.-Y.; Lin, F.; Smith, W. W.; Janson, C. A.; Zhao, B.; McQueney, M. S.; D'Alessio, K.; Lee, C.-P.; Marzulli, A.; Dodds, R. A.; Blake, S.; Hwang, S.-M.; James, I. E.; Gress, C. J.; Bradley, B. R.; Lark, M. W.; Gowen, M.; Veber, D. F. *J. Med. Chem.* **2001**, *44*, 1380, and references cited therein.

7. Fenwick, A. E.; Gribble, A. D.; Ife, R. J.; Stevens, N.; Witherington, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 199.
8. See: Quibell, M.; Taylor, S. Patent WO 00/69855. It has been suggested that the addition of a substituent to the β-carbon (*C*-2) of a 3-amidotetrahydrofuran-4-one **2a** provides improved configurational stability at the α-carbon (*C*-3). However, any inhibitors that retain the α-proton, may still potentially undergo enolisation and hence loss of chiral integrity.
9. In-house molecular modelling was performed using WebLab ViewerPro (http://www.accelrys.com). The literature cathepsin K X-ray crystal structures 1mem (nonprime orientations) and 1au0 (prime and nonprime orientations) were used as templates to examine the general overall spatial fit and potential binding orientations of α-methyl-3-amidotetrahydrofuran-4-ones **3a** and **4a**. The existing inhibitors bound within 1mem and 1au0 were modified into the desired monocyclic compounds **3a** and **4a** by simple draw and clean commands whilst retaining as close as possible the main thread of the inhibitor backbone. A hemithioketal tetrahedral intermediate formed between the cyclic ketone carbonyl and the active site Cys^{25} was built to examine the products of both *re* and *si* stereofacial addition of the thiolate. As part of the assessment, calculations were performed on the isolated hemithioketal tetrahedral intermediate extracted from the predicted bound complex, to ascertain whether the binding model contained a reasonable energy inhibitor conformation. Conformer energy calculations were performed using CAChe (Conflex molecular mechanics engine, CAChe Group, Fujitsu). Additionally, the full modelling process considering each possible binding orientation was repeated using the literature cruzain X-ray structure 1aim and the mammalian cathepsin S X-ray structure 1ms6. Virtually identical conclusions concerning the preferred binding orientations described herein for the cathepsin K 1mem and 1au0 structures were found when substituting with the 1aim and 1ms6 structures.
10. See: (a) Quibell, M.; Ramjee, M. K. Patent WO 02/57246; (b) Quibell, M. Patent WO 02/57249.
11. Predictions of likely binding conformations were based solely upon comparison of the four possible *re*/*si*/prime-side/nonprime-side complexes for each α-stereochemistry. Major considerations in the development of a ranking for each possibility were the identification of a reasonable energy conformer for the inhibitor that retained a good hydrogen-bonding network to the proteinase along with the absence of spatial clashes. The overall goodness of fit was assessed using the scoring functions generated by the software package 'GOLD 2.1' (Cambridge Crystallographic Data Centre) performing the calculations on the tetrahedral intermediates. It is worthwhile to note that the analysis of each possibility was warranted since three out of the four possible combinations have subsequently been reported in X-ray complexes of various 3-amido-tetrahydrofuran-4-ones **2a** and 3-amidopyrrolidin-4-ones **2b**.[6b]
12. The literature cathepsin K X-ray crystal structures that show inhibitors bound in a retro mode span the whole active site from S2/S3 to S2′/S3′. These examples are therefore able to retain the major elements of the nonprime hydrogen-bonding pattern that are present in inhibitors bound in the S2 and S3 pockets only.[6b]
13. The conclusion for *R* and *S* α-methyl-3-amidopyrrolidin-4-ones may be different since here the retro binding modes, which in α-methyl-3-amidotetrahydrofuran-4-ones **3a** and **4a** generally show no obvious clash with the proteinase, will be able to span the entire active site and may retain the major elements of the nonprime hydrogen-bonding pattern.
14. The conclusion for *R* and *S* α,β-dimethyl-3-amidopyrrolidin-4-ones may be different since here the retro binding modes will have substituents that span the entire active site and may retain the major elements of the nonprime hydrogen-bonding pattern.
15. Moon, S. H.; Ohfune, Y. *J. Am. Chem. Soc.* **1994**, *116*, 7405.
16. Ohfune, Y.; Nanba, K.; Takada, I.; Kan, T.; Horikawa, M.; Nakajima, T. *Chirality* **1997**, *9*, 459–462.
17. Ohfune et al.[15] report that the prolonged exposure of a 1:4 mixture of **19**:**20** to 1 equiv TFA in 2-propanol gives an equilibrated mixture of **19**:**20** in a ratio of 9:1, which upon recrystallisation gives enantiopure **19**.
18. Seebach, D.; Aebi, J. D.; Gander-Coquoz, M.; Naef, R. *Helv. Chim. Acta* **1987**, *70*, 1194.
19. High yield preparation of the α-diazomethylketones of all four isomers of Fmoc-Thr and the *cis* isomers of Fmoc-3-hydroxyproline and Fmoc-3-hydroxypipecolic acid, with and without side chain *tert*-butyl β-hydroxyl protection, have been reported, see: (a) Quibell, M. Patent WO 02/57248; (b) Quibell, M. Patent WO 02/57270.
20. Nicolaou, K. C.; Baran, P. S.; Zong, Y.-L.; Choi, H.-S.; Fong, K. C.; He, Y.; Won, W. H. *Org. Lett.* **1999**, *1*, 883–886.
21. Friedrich-Bochnitschek, S. H.; Waldmann, H.; Kunz, H. *J. Org. Chem.* **1989**, *54*, 751.
22. Dessolin, M.; Guillerez, M.-G.; Thieriet, N.; Guibe, F.; Loffet, A. *Tetrahedron Lett.* **1995**, *36*(32), 5741.
23. Urethane Nα-protected α,α-disubstituted amino acids are known to undergo base-catalysed 4*H*-oxazol-5-one formation in order to relieve the strain imposed by the sterically conjested groups. It is interesting to speculate that diazomethane acting as a base could promote β-elimination of dibenzofulvene from the fluorenylmethyl ether of the 4*H*-oxazol-5-one, which followed by tautomerisation could give *N*-carboxyanhydride **25**.
24. Fiammengo, R.; Licini, G.; Nicotra, A.; Modena, G.; Pasquato, L.; Scrimin, P.; Broxterman, Q. B.; Kaptein, B. *J. Org. Chem.* **2001**, *66*, 5905–5910, and references cited therein.
25. For a study on the Mitsunobu inversion of 3-hydroxyproline see: Kolodziej, S. A.; Marshall, G. R. *Int. J. Pept. Prot. Res.* **1996**, *48*, 274.
26. (a) James, W. *Tetrahedron* **1999**, *55*, 4855; (b) Fenwick, A. E.; Garnier, B.; Gribble, A. D.; Ife, R. J.; Rawlings, A. D.; Witherington, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 195; (c) Huang, L.; Ellman, J. A. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2993; (d) Huang, L.; Lee, A.; Ellman, J. A. *J. Med. Chem.* **2002**, *45*, 676.
27. Murphy, A. M.; Dagnino, R.; Vallar, P. L.; Trippe, A. J.; Sherman, S. L.; Lumpkin, R. H.; Tamura, S. Y.; Webb, T. R. *J. Am. Chem. Soc.* **1992**, *114*, 3156.
28. (a) The formation of linker constructs **35a**–**37a** was significantly more difficult than for the equivalent non-α-alkylated monocycles that are formed quantitatively in $1 \rightarrow 2$ h (see Ref. 10b). We have discovered that hindered

ketones may be more readily converted to linker constructs through the use of up to 7.5 equiv linker during reflux. Excess linker may then be removed by extraction with pH 3 HCl prior to solid-phase synthesis; (b) Cleavage of non-α-alkylated monocycles and 4-acyltetrahydrofuro[3,2-*b*]pyrrol-3-one bicycles has been shown to be essentially quantitative within 2 h (see Refs. 10b and 19).

29. For a thorough description of general multipin techniques see: Grabowska, U.; Rizzo, A.; Farnell, K.; Quibell, M. *J. Comb. Chem.* **2000**, *2*(5), 475.
30. For an example of typical coupling efficiencies to α,α-dialkylated amino acids see: Fu, Y.; Hammer, R. P. *Org. Lett.* **2002**, *4*(2), 237.
31. For comparison, the equivalent analogues with achiral unsubstituted monocyclic scaffold **2a** give inhibitors with $K_i < 250$ nM against particular proteinases as follows: (a) Cat. K, CPB, cruzain; (b) Cat. S; (c) Cat. K, cruzain; (d) Cat. L; (e) All; (f) Cruzain. However, due to the α-lability of scaffold **2a** in typical inhibition assay buffers, values for the individual 3*S* and 3*R* isomers are difficult to ascertain with certainty.[7]
32. Atherton, E.; Sheppard, R. C. *Solid Phase Peptide Synthesis A Practical Approach*; IRL: Oxford, UK, 1989.
33. Meldal, M.; Breddam, K. *Anal. Biochem.* **1991**, *195*, 141.
34. In general, the quality of crude examples was >80% as judged by analytical HPLC. In addition, a comparison of the K_i data for crude and semiprep purified examples indicated that the crude analogues provided a good basis for SAR and potency analysis.
35. Cornish-Bowden, A. *Fundamentals of Enzyme Kinetics*; Portland Ltd: London, 1995.
36. (a) Morrison, J. F.; Walsh, C. T. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1988**, *61*, 201; (b) Tian, W. X.; Tsou, C. L. *Biochemistry* **1982**, *21*(5), 1028.