A concise stereoselective synthesis of orthogonally protected lanthionine and β-methyllanthionine†

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Lantibiotics such as nisin are active against most Gram-positive bacteria and constitute an important class of antibacterial agents. These ribosomally synthesized peptides contain either one or both of the unusual amino acids *meso*-lanthionine (*m*-Lan) or β-methyllanthionine (β-MeLan). Nucleophilic ring opening of sulfamidates allows facile preparation of stereochemically pure derivatives of *m*-Lan and β-MeLan with orthogonal protection for solid phase synthesis of lantibiotic analogues.

Introduction

Lantibiotics are ribosomally synthesized antimicrobial peptides that have extremely potent activity against a broad spectrum of Gram-positive bacteria, including food pathogens (*e.g. Listeria monocytogenes*) and organisms that exhibit resistance to conventional antibiotics (*e.g.* MRSA and VRE).[1] Consequently, lantibiotics have emerged as a promising new class of antibacterial agents. Lantibiotics such as nisin,[2] lacticin 3147[3] and gallidermin[4] are characterized by the presence of the unusual amino acids *meso*-lanthionine (*m*-Lan) (**1**) and β-methyllanthionine ((2*S*,3*S*,6*R*)-β-MeLan) (**2**) (Fig. 1). *m*-Lan and β-MeLan arise due to the action of a series of post-translational modification enzymes.

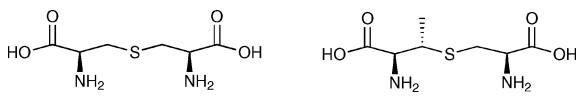


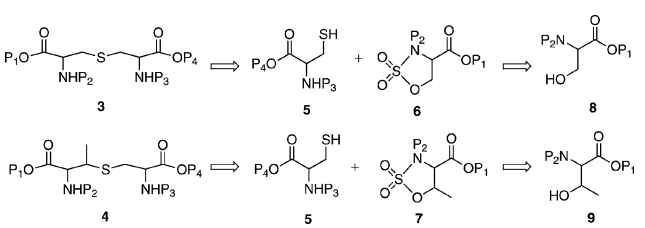
Fig. 1 *meso*-Lanthionine (*m*-Lan) (**1**) and β-methyllanthionine ((2*S*,3*S*,6*R*)-β-MeLan) (**2**).

In particular, dehydration of serine or threonine residues in the original linear peptide sequence introduces dehydroalanine or dehydrobutyrine residues respectively. A cyclase enzyme then mediates the stereoselective Michael addition of a nearby cysteine residue onto the dehydro residue, thereby leading to the formation of *m*-Lan or β-MeLan bridges within the lantibiotic peptide.[5] In an attempt to further enhance the bioactivity and stability of natural lantibiotics such as nisin, we are currently pursuing the solid phase synthesis of analogues of these peptides. In order to achieve this goal, facile access to orthogonally protected derivatives of both *m*-Lan and β-MeLan is necessary.

To date, nisin A is the only lantibiotic that has been prepared by total synthesis.[6] However, since this monumental synthesis was reported by Shiba and co-workers, several stereoselective methods for the preparation of orthogonally protected derivatives of *m*-Lan have been developed and reported.[7,8] Furthermore, these *m*-Lan derivatives have been successfully applied to the solid phase synthesis of fragments of natural lantibiotics, such as Tabor's elegant synthesis of the C ring of nisin.[8a] However, the additional stereocentre present at the C-3 position makes the stereoselective synthesis of orthogonally protected derivatives of β-MeLan more challenging. Consequently, to the best of our knowledge, there have been only two previously reported syntheses of linear orthogonally protected derivatives of β-MeLan.[9] The best procedure requires about seven synthetic steps, and further use in lantibiotic synthesis would necessitate removal of a benzyl carbamate (Cbz) and a benzyl ester (Bn) in the presence of a sulfur atom. As orthogonally protected derivatives of amino acids **1** and **2** would be key components in solid phase peptide synthesis of lantibiotics, it seemed useful to develop an alternative synthetic route to these compounds. A key objective was to develop an efficient methodology that utilizes protecting groups that could be selectively removed under mild conditions.

Results and discussion

Sulfamidates are useful synthetic building blocks that can be opened stereo- and regioselectively by a variety of nucleophiles.[10] Hence, it would appear that a suitably protected cysteine **5** could attack a cyclic sulfamidate (**6** or **7**) derived from a protected serine **8** or threonine **9** to give orthogonally protected derivatives of of *m*-Lan or β-MeLan (Scheme 1). However, attack of sulfur nucleophiles on sulfamidates can be plagued by undesired elimination reactions[11] unless the α-position is blocked.[10c] A fully unprotected threonine-derived sulfamidate has been successfully opened in water with a thio-sugar,[12] but attack of cysteine-derived nucleophiles on protected serine and threonine sulfamidates is relatively unexplored.[10c] In the present study, we investigate the



Scheme 1

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use of cyclic sulfamidates to prepare orthogonally protected derivatives of lanthionine **3** and β-methyllanthionine **4** (Scheme 1).

Although *N*-alloc and allyl ester protecting groups on *m*-Lan have previously been shown to allow sequential construction of lanthionine rings during standard peptide synthesis,[8] they are incompatible with the oxidation conditions required to prepare the cyclic sulfamidates from their corresponding sulfamidites.[13] The alkene moieties in these groups are also prone to add aliphatic thiols in Markovnikov fashion. Hence, a *p*-methoxybenzyl (PMB) group was chosen for the nitrogen protection, as it can be removed using mild reaction conditions and has been successfully used in the preparation of D-*allo*-threonine- and L-serine-derived cyclic sulfamidates.[12] Similarly, methyl ester protection of the acid functionality of an amino acid has been shown to be compatible with the preparation of cyclic sulfamidates.[11,13] In addition, the required amino acid methyl ester starting materials, **10a–e**, are commercially available, and D-*allo*-Thr-OMe·HCl **10e** can be prepared in quantitative yield using acetyl chloride/MeOH.

The PMB protecting group was introduced to compounds **10a–e** using a reductive amination procedure to afford the bis-protected amino acids **11a–e** in good to excellent yields (65–89%) (Fig. 2). The conversion of compounds **11a–e** to their corresponding cyclic sulfamidates **12a–e** was achieved in good yields (74–78%) by using a slight modification of the procedure detailed by Cohen and Halcomb[12] (Fig. 2).

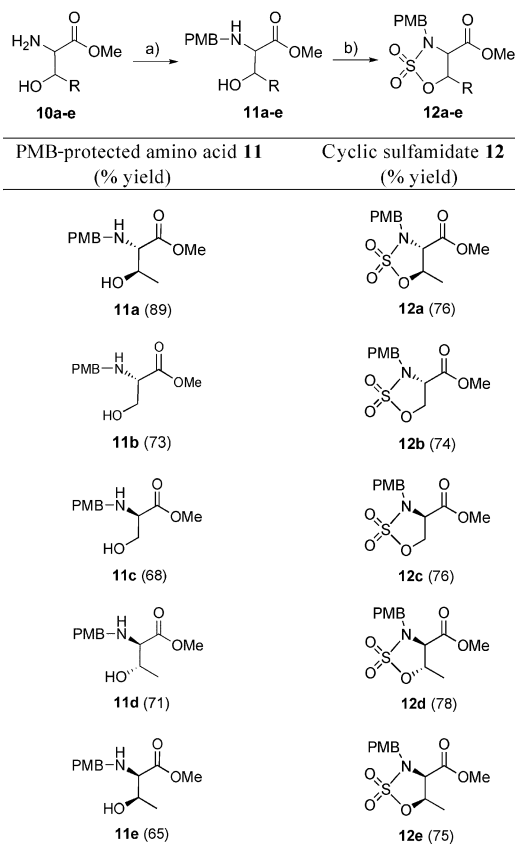

Fig. 2 Preparation of cyclic sulfamidates. *Reagents and conditions*: a) $NaCNBH_3$, *p*-anisaldehyde, acetic acid, MeOH, 0 °C to rt, 16 h; b) (i) $SOCl_2$, pyridine, CH_2Cl_2 −78 °C to rt, (ii) $RuCl_3{\cdot}3H_2O$, $NaIO_4$, H_2O–MeCN (1 : 1), 0 °C to rt.

The ring opening of a cyclic sulfamidate with a thiol nucleophile requires the use of a base. However, the presence of an acidic α-proton whose removal can initiate elimination reactions prevents the use of previously employed bases such as DBU.[10c] Therefore, we first examined the Cs_2CO_3-mediated nucleophilic ring opening of the L-Thr-derived sulfamidate **12a** with trityl thiol **13**. After acidic hydrolysis (1 M NaH_2PO_4 buffer, pH 5.4)[10b,11] of the intermediate sulfamic acid, the orthogonally protected β-methyl-L-cysteine ((2*R*,3*S*)-β-MeCys), **14** was obtained in good yield (67%; 45% overall yield from **10a**). The ring opening reaction of **12a** with Fmoc-Cys-O*t*Bu **15**[8] and commercially available Boc-Cys-OMe **16** was examined next. Fmoc-Cys-O*t*Bu **15** has been successfully used as a thiol nucleophile under basic conditions (Cs_2CO_3/DMF) in other reactions.[14] However, in our case all attempts to use **15** as a nucleophile with sulfamidates were unsuccessful and the reactions yielded only a complex mixture of products. We assume that the problems that we encountered were due to instability of the Fmoc protecting group under the basic reactions conditions utilised. Similar stability issues were reported by Smith and Goodman, who were unable to use Fmoc-Cys-OMe in the Cs_2CO_3-meditated ring opening of an α-methyl-D-serine-β-lactone.[15] However, the same reaction conditions could be successfully used in the ring opening of **12a** with Boc-Cys-OMe **16** to afford the β-MeLan derivate **17**, albeit in low yield (40%). No unreacted sulfamidate **12a** was recovered from the reaction mixture, but it was possible to confirm by electrospray mass spectrometry (ESMS) the presence of the open sulfamic acid intermediate. This result indicated a problem with the final hydrolysis stage that should liberate the *N*-PMB amino group. Fortunately, the conditions developed by Kim and So (*n*PrSH/$BF_3{\cdot}Et_2O$, CH_2Cl_2)[16] could be used to hydrolyze the intermediate sulfamic acid even in the presence of the Boc protecting group. When this system was employed in place of the phosphate buffer, the yield of **17** could be increased considerably from 40% to 79% (Fig. 3).

With the reaction conditions refined, attention refocused on the initial goal of preparing orthogonally protected derivatives of Lan and β-MeLan. In order to achieve this, a cysteine derivative with acid labile protecting groups was required. To this end, Boc-Cys-O*t*Bu **18** was prepared in a good yield (63% over two steps) from commercially available $(Boc\text{-}Cys\text{-}OH)_2$.[17] The ring openings of the L- and D-serine-derived cyclic sulfamidates **12b** and **12c** with **18** were done in DMF using Cs_2CO_3 as a base. *n*PrSH/$BF_3{\cdot}Et_2O$ hydrolyses of the intermediate sulfamic acids then furnished the orthogonally protected Lan **19a** and *m*-Lan **19b** in excellent yields (85% and 89%) (Fig. 4). The diastereomeric purity of both **19a** and **19b** were shown by ^{1}H NMR spectroscopy to be >20 : 1 (other isomers undetectable, see ESI†). Similarly, the nucleophilic ring opening of the D-threonine- and D-*allo*-threonine-derived sulfamidates **12d** and **12e** with **18** using the aforementioned reaction conditions afforded the orthogonally protected (2*S*,3*R*,6*R*)-β-MeLan **20a** (72%) and (2*S*,3*S*,6*R*)-β-MeLan **20b** (70%), respectively (Fig. 4). The diastereomeric purity of both the β-MeLan derivatives **20a** and **20b** was again determined by ^{1}H NMR spectroscopy to be >20 : 1.

Conclusions

In conclusion, we have developed a facile synthetic route to stereochemically pure and orthogonally protected lanthionines

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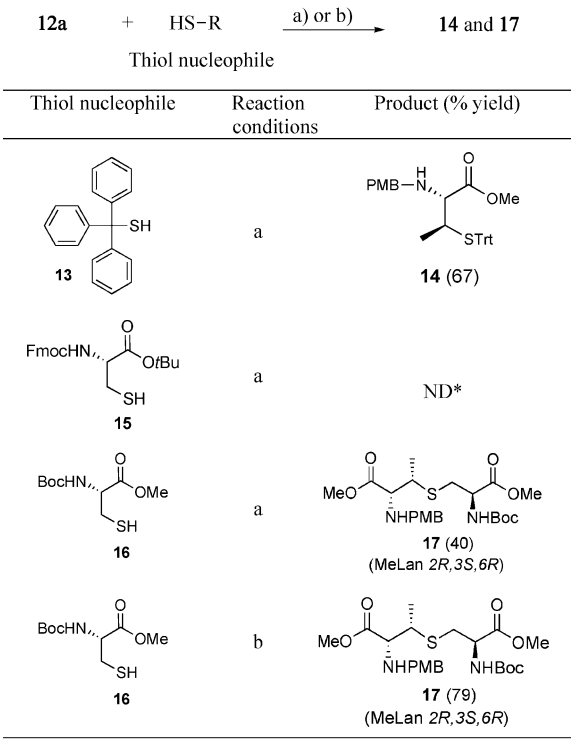

Fig. 3 Initial sulfamidate ring opening reactions. *Regents and conditions*: a) (i) Cs_2CO_3, DMF, rt, (ii) 1 M NaH_2PO_4 buffer pH 5.4, rt; b) (i) Cs_2CO_3, DMF, rt, (ii) *n*PrSH/BF_3·Et_2O, CH_2Cl_2, rt, then NH_4OH, rt. * No formation of the desired product was detected.

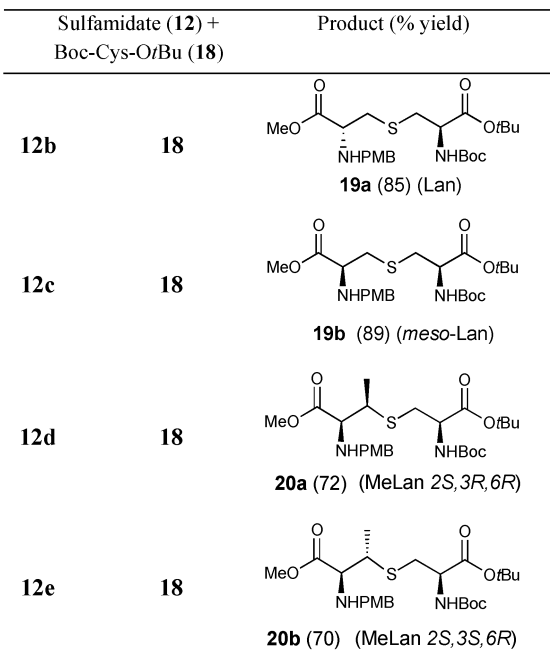

Fig. 4 Preparation of orthogonally protected Lan **19a** and **19b** and β-MeLan **20a** and **20b**. *Reagents and conditions*: a) (i) Cs_2CO_3, DMF, rt, (ii) *n*PrSH/BF_3·Et_2O, CH_2Cl_2, rt, then NH_4OH, rt.

19a and **19b** as well as β-methyllanthionines **20a** and **20b**. The methodology described is versatile enough to allow other orthogonally protected stereoisomers of both Lan and β-MeLan to be readily prepared from easily available starting materials. Studies to incorporate *m*-Lan **19b** and β-MeLan **20b** into natural lantibiotics and their synthetic analogues are ongoing.

Experimental

General

All reactions involving air- or moisture-sensitive reagents were performed in oven-dried glassware under an atmosphere of dry argon. Solvents were reagent grade and were used as supplied unless otherwise stated. For anhydrous reactions, solvents were dried according to the procedures detailed in Perrin and Armarego (D. D. Perrin and W. L. F. Armarego, *Purification of Laboratory Chemicals*, 3rd edn, Pergamon Press). All reactions were monitored by thin layer chromatography (TLC) using glass plates with UV fluorescent indicator (normal SiO_2, Merck 60 F254). Nuclear magnetic resonance (NMR) spectra were obtained on Inova Varian 300, 400 or 500 MHz spectrometers. ^{1}H NMR chemical shifts are reported in parts per million (ppm) relative to $CDCl_3$ (δ 7.27). ^{1}H NMR data are reported in the following order multiplicity (s, singlet; d, doublet; t, triplet; q, quartet and m, multiplet), number of protons, coupling constants (*J*) in Hertz and assignment. ^{13}C NMR chemical shifts are reported relative to $CDCl_3$ δ 77.0. Optical rotations were measured on a Perkin–Elmer 241 polarimeter with a microcell (10 cm, 1 mL) at ambient temperature and are reported in units of 10^{-1} deg cm^2 g^{-1}. Infrared spectra (IR) were recorded on a Nicolet Magna 750 or a 20SX FT-IR spectrometer. High-resolution mass spectra (HRMS) were obtained on a Kratos AEIMS-50 high resolution instrument.

D-*allo*-Threonine methyl ester (10e)

Acetyl chloride (6.00 mL, 83.9 mmol) was slowly added to a solution of the amino acid (1.00 g, 8.39 mmol) in MeOH (40 mL) at 0 °C. With the addition complete, the ice bath was removed and the reaction mixture was heated to reflux and left for 16 h. Upon cooling, the reaction solution was concentrated under reduced pressure to afford **10e** as a white solid, which was used without further purification (^{1}H NMR indicated a purity of >95%); δ_H(500 MHz; D_2O) 1.22 (3 H, d, *J* 6.6, CH_3), 3.78 (3 H, s, OCH_3), 4.14 (1 H, d, *J* 3.6, H-α), 4.27 (1 H, dq, *J* 6.6 and 3.6, H-β); δ_C(100 MHz; D_2O) 18.4 (CH_3), 54.3 (OCH_3), 58.7 (C-α), 66.3 (C-β), 169.0 (CO); *m*/*z* (ES+) calcd for $C_5H_{12}NO_3$ 134.0812, found 134.0812 [MH^+].

N-(*p*-Methoxybenzyl)-L-threonine methyl ester (11a)

$NaCNBH_3$ (556 mg, 8.85 mmol) and *p*-anisaldehyde (0.79 mL, 6.49 mmol) were added to a cooled (0–5 °C) solution of **10a** (1.00 g, 5.90 mmol) in MeOH (50 mL) and acetic acid (0.68 mL, 11.80 mmol). The reaction solution was stirred at 0–5 °C for 1 h and then at rt for a further 16 h. Solid $NaHCO_3$ (1.50 g, 17.86 mmol) was added and the solvent was removed under reduced pressure. The resulting white residue was partitioned between CH_2Cl_2 (75 mL) and H_2O (30 mL) and the aqueous layer was re-extracted with CH_2Cl_2 (75 mL). The organic layers were combined and the solvent removed under vacuum. After column purification (SiO_2, 2 : 1 Hex–EtOAc to 100% EtOAc), **11a** was obtained as a clear oil (1.330 g, 89%). TLC (SiO_2, 1 : 1 Hex–EtOAc)

R_f 0.36; $[\alpha]_D^{26}$ −49.41 (*c* 1.23, $CHCl_3$); ν_{max}(microscope)/cm^{-1} 3450 (br), 2953, 2837, 1735, 1612, 1514, 1462, 1373, 1249, 1199, 1108, 1035; δ_H(400 MHz; $CDCl_3$) 1.17 (3 H, d, *J* 6.0, CH_3), 3.02 (1 H, d, *J* 7.2, H-α), 3.62 (1 H, d, *J* 12.4, PMB-CH_2), 3.67 (1 H, dq, *J* 7.2 and 6.0, H-β), 3.76 (1 H, d, *J* 12.4, PMB-CH_2), 3.70 (3 H, s, OCH_3), 3.77 (3 H, s, OCH_3), 6.84-6.82 (2 H, m, Ar-H), 7.29-7.19 (2 H, m, Ar-H); δ_C(100 MHz; $CDCl_3$) 19.2 (CH_3), 51.8 (PMB-CH_2), 51.9 (OCH_3), 55.9 (OCH_3), 66.9 (C-α), 67.8 (C-β), 113.7 (Ar-C), 129.4 (Ar-C), 131.1 (Ar-C), 158.8 (Ar-C), 174.0 (CO), *m*/*z* (ES+) Calcd for $C_{13}H_{20}NO_4$ 254.1387, found 254.1385 [MH^+].

N-(*p*-Methoxybenzyl)-L-serine methyl ester (11b)

$NaCNBH_3$ (606 mg, 9.65 mmol) and *p*-anisaldehyde (0.85 mL, 7.07 mmol) were added to a cooled (0–5 °C) solution of **10b** (1.00 g, 6.43 mmol) in MeOH (50 mL) and acetic acid (0.74 mL, 12.86 mmol). The reaction solution was stirred at 0–5 °C for 1 h and then at rt for a further 16 h. Solid $NaHCO_3$ (1.62 g, 19.29 mmol) was added and the solvent was removed under reduced pressure. The resulting white residue was partitioned between CH_2Cl_2 (75 mL) and H_2O (30 mL) and the aqueous layer was re-extracted with CH_2Cl_2 (75 mL). The organic layers were combined and the solvent removed under vacuum. After column purification (SiO_2, 1 : 1 Hex–EtOAc to 100% EtOAc) **11b** was obtained as a white solid (1.123 g, 73%). TLC (SiO_2, EtOAc) R_f 0.30; $[\alpha]_D^{26}$ −41.65 (*c* 1.04, $CHCl_3$); ν_{max}(microscope)/cm^{-1} 3320 (br), 2998, 2952, 2836, 1736, 1611, 1513, 1462, 1248; δ_H(400 MHz; $CDCl_3$) 3.40 (1 H, dd, *J* 6.0 and 4.4, H-α), 3.60 (1 H, dd, *J* 10.8 and 6.0, Ser-CH_2), 3.62 (1 H, d, *J* 12.8, PMB-CH_2), 3.73 (3 H, s, OCH_3), 3.75 (1 H, dd, *J* 10.8 and 4.4, Ser-CH_2), 3.78 (3 H, s, OCH_3), 3.78 (1 H, d, *J* 12.8, PMB-CH_2), 6.87-6.83 (2 H, m, Ar-H), 7.24-7.20 (2 H, m, Ar-H); δ_C(100 MHz; $CDCl_3$) 51.5 (PMB-CH_2), 52.1 (OCH_3), 55.2 (OCH_3), 61.7 (C-α), 62.4 (C-β), 113.9 (Ar-C), 129.4 (Ar-C), 131.3 (Ar-C), 158.9 (Ar-C), 173.5 (CO); *m*/*z* (ES+) Calcd for $C_{12}H_{17}NO_4Na$ 262.1050, found 262.1052 [MNa^+].

N-(*p*-Methoxybenzyl)-D-serine methyl ester (11c)

$NaCNBH_3$ (606 mg, 9.65 mmol) and *p*-anisaldehyde (0.85 mL, 7.07 mmol) were added to a cooled (0–5 °C) solution of **10c** (1.00 g, 6.43 mmol) in MeOH (50 mL) and acetic acid (0.74 mL, 12.86 mmol). The reaction solution was stirred at 0–5 °C for 1 h and then at rt for a further 16 h. Solid $NaHCO_3$ (1.62 g, 19.29 mmol) was added and the solvent was removed under reduced pressure. The resulting white residue was partitioned between CH_2Cl_2 (75 mL) and H_2O (30 mL) and the aqueous layer was re-extracted with CH_2Cl_2 (75 mL). The organic layers were combined and the solvent removed under vacuum. After column purification (SiO_2, 2 : 1 Hex–EtOAc to 100% EtOAc) **11c** was obtained as a white solid (1.046 g, 68%). TLC (SiO_2, EtOAc) R_f 0.30; $[\alpha]_D^{26}$ +40.78 (*c* 1.39, $CHCl_3$); ν_{max}(DCM)/cm^{-1} 3327 (br), 2999, 2952, 2837, 1736, 1612, 1513, 1248, 1177, 1035; δ_H(400 MHz; $CDCl_3$) 3.40 (1 H, dd, *J* 6.0 and 4.4, H-α), 3.60 (1 H, dd, *J* 11.2 and 6.0, Ser-CH_2), 3.63 (1 H, d, *J* 12.0, PMB-CH_2), 3.79-3.70 (8H, m, 2 × OCH_3, PMB-CH_2 and Ser-CH_2), 6.86-6.82 (2 H, m, Ar-H), 7.23-7.20 (2 H, m, Ar-H); ^{13}C (100 MHz; $CDCl_3$) 51.3 (PMB-CH_2), 52.0 (OCH_3), 55.1 (OCH_3), 61.6 (C-α), 62.4 (C-β), 113.7 (Ar-C), 129.4 (Ar-C), 131.2 (Ar-C), 158.8 (Ar-C), 173.4 (CO); *m*/*z* (ES+) Calcd for $C_{12}H_{17}NO_4Na$ 262.1050, found, 262.1048 [MNa^+].

N-(*p*-Methoxybenzyl)-D-threonine methyl ester (11d)

$NaCNBH_3$ (3.80 g, 60.4 mmol) and *p*-anisaldehyde (6.40 mL, 52.4 mmol) were added to a cooled (0–5 °C) solution of **10d** (6.83 g, 40.3 mmol) in MeOH (180 mL) and acetic acid (4.60 mL, 80.4 mmol). The reaction solution was stirred at 0–5 °C for 1 h and then at rt for a further 16 h. Solid $NaHCO_3$ (10.20 g, 120.9 mmol) was added and the solvent was removed under reduced pressure. The resulting white residue was partitioned between CH_2Cl_2 (200 mL) and H_2O (120 mL) and the aqueous layer was re-extracted with CH_2Cl_2 (150 mL). The organic layers were combined and the solvent removed under vacuum. After column purification (SiO_2, 2 : 1 Hex–EtOAc to 100% EtOAc) **11d** was obtained as a clear oil (6.81 g, 71%). TLC (SiO_2, 1 : 1 Hex–EtOAc) R_f 0.35; $[\alpha]_D^{26}$ +49.71 (*c* 0.44, $CHCl_3$); ν_{max}($CHCl_3$ cast)/cm^{-1} 3445 (br), 2952, 2837, 1734, 1612, 1513, 1462, 1248, 1175; δ_H(400 MHz; $CDCl_3$) 1.18 (3 H, d, *J* 6.4, CH_3), 3.02 (1 H, d, *J* 7.6, H-α), 3.62 (1 H, d, *J* 12.8, PMB-CH_2), 3.68 (1 H, m, H-β), 3.71 (3 H, s, OCH_3), 3.77 (1 H, d, *J* 12.8, PMB-CH_2), 3.79 (3 H, s, OMe), 6.87 (2 H, m, Ar-H), 7.23-7.19 (2 H, m, Ar-H); δ_C(100 MHz; $CDCl_3$) 19.3 (CH_3), 51.9 (PMB-CH_2), 52.0 (OCH_3), 55.2 (CH_3), 66.8 (C-α), 67.9 (C-β), 113.8 (Ar-C), 129.5 (Ar-C), 131.1 (Ar-C), 158.9 (Ar-C), 174.1 (CO); *m*/*z* (ES+) Calcd for $C_{13}H_{19}NO_4Na$ 276.1206, found 276.1207 [MNa^+].

N-(*p*-Methoxybenzyl)-D-*allo*-threonine methyl ester (11e)

$NaCNBH_3$ (0.79 g, 12.59 mmol) and *p*-anisaldehyde (1.12 mL, 9.23 mmol) were added to a cooled (0–5 °C) solution of **10e** (1.42 g, 8.39 mmol) in MeOH (40 mL) and acetic acid (1.36 mL, 16.78 mmol). The reaction solution was stirred at 0–5 °C for 30 min and then at rt for a further 16 h. Solid $NaHCO_3$ (2.10 g, 25.17 mmol) was added and the solvent was removed under reduced pressure. The resulting white residue was partitioned between CH_2Cl_2 (75 mL) and H_2O (30 mL) and the aqueous layer was re-extracted with CH_2Cl_2 (50 mL). The organic layers were combined and the solvent removed under vacuum, giving **11e** as a clear oil (1.28 g, 65%). TLC (SiO_2, 1 : 1 Hex–EtOAc) R_f 0.21; $[\alpha]_D^{26}$ +42.88 (*c* 0.52, $CHCl_3$); ν_{max}(microscope)/cm^{-1} 3440 (br), 2952, 2837 1734, 1612, 1514, 1248 cm^{-1}; δ_H(400 MHz; $CDCl_3$) 1.02 (3 H, d, *J* 6.4, CH_3), 3.40 (1 H d, *J* 4.8, H-α), 3.60 (1 H, d, *J* 12.8, PMB-CH_2), 3.75 (3 H, s, OCH_3), 3.80 (3 H, s, OCH_3), 3.83 (1 H, d, *J* 12.8, PMB-CH_2), 4.00 (1 H, dq, *J* 6.4 and 4.8, H-β), 6.89-6.85 (2 H, m, Ar-H), 7.27-7.23 (2 H, m, Ar-H); δ_C(400 MHz; $CDCl_3$) 18.8 (CH_3), 51.9 (PMB-CH_2), 52.1 (OCH_3), 55.2 (OCH_3), 65.3 (C-α), 67.2 (C-β), 113.8 (Ar-C), 129.5 (Ar-C), 131.5 (Ar-C), 158.9 (Ar-C), 173.6 (CO); *m*/*z* (ES+) Calcd for $C_{13}H_{20}NO_4$ 254.1387, found 254.1388 [MH^+].

(4*S*,5*R*)-*N*-(*p*-Methoxybenzyl)-2,2-dioxo-1,2,3-oxathiazolidinone-5-methyl-4-carboxylic acid methyl ester (12a)

Pyridine (5.67 mL, 70.1 mmol) was added to a solution of the di-protected amino acid **11a** (3.55 g, 14.02 mmol) in CH_2Cl_2 (40 mL) and the reaction solution was then cooled to −78 °C. $SOCl_2$ (1.23 mL, 16.8 mmol) was added dropwise over 5 min, and the solution was left to stir at −78 °C for 5 min and allowed to warm to rt over 1 h. The reaction mixture was quenched by the addition of 1% HCl (25 mL). The aqueous layer was extracted with CH_2Cl_2 (100 mL and 50 mL), and the combined organic

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layers were washed with saturated $NaHCO_3$ (20 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a yellow residue. The residue was dissolved in MeCN (50 mL) and the solution was cooled to 0–5 °C. $RuCl_3{\cdot}3H_2O$ (220 mg, 0.85 mmol), $NaIO_4$ (3.30 g, 15.42 mmol) and H_2O (50 mL) were then added sequentially, and the reaction mixture was left to stir for 10 min at 0–5 °C and a further 10 min at rt. The reaction solution was then partioned between CH_2Cl_2 (200 mL) and saturated $NaHCO_3$ (50 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL) and the combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. Column purification over silica gel (4 : 1 to 3 : 1 Hex–EtOAc) afforded **12a** as a clear oil (3.37 g, 76%). TLC (SiO_2, 4 : 1 Hex–EtOAc) R_f 0.16; $[\alpha]_D^{26}$ −32.32 (*c* 0.54, $CHCl_3$); ν_{max}(microscope)/cm^{-1} 3000 (br), 2957, 2840, 1754, 1613, 1515, 1441, 1350, 1252, 1187; δ_H(400 MHz; $CDCl_3$) 1.49 (3 H, d, *J* 6.4, CH_3), 3.68 (1 H, d, *J* 6.4, H-α), 3.65 (3 H, s, OCH_3), 3.76 (3 H, s, OCH_3), 4.36 (2 H, s, PMB-CH_2), 4.84 (1 H, m, H-β), 6.86-6.82 (2 H, m, Ar-H), 7.28-7.24 (2 H, m, Ar-H); δ_C(100 MHz; $CDCl_3$) 19.3 (CH_3), 50.1 (PMB-CH_2), 53.0 (OCH_3), 55.2 (OCH_3), 64.8 (C-α), 77.5 (C-β), 114.1 (Ar-C), 125.2 (Ar-C), 130.7 (Ar-C), 159.8 (Ar-C), 167.9 (CO); *m*/*z* (ES+) Calcd for $C_{13}H_{17}NO_6SNa$ 338.0669, found 338.0670 MNa^+.

(4*S*)-*N*-(*p*-Methoxybenzyl)-2,2-dioxo-1,2,3-oxathiazolidinone-4-carboxylic acid methyl ester (12b)

Pyridine (1.18 mL, 14.63 mmol) was added to a solution of the di-protected amino acid **11b** (700 mg, 2.93 mmol) in CH_2Cl_2 (10 mL) and the reaction solution was then cooled to −78 °C. $SOCl_2$ (0.26 mL, 3.56 mmol) was added dropwise over 5 min, and the solution was left to stir at −78 °C for 5 min and allowed to warm to rt over 1 h. The reaction mixture was quenched by the addition of HCl (1% sol. 15 mL). The aqueous layer was extracted with CH_2Cl_2 (75 mL), and the combined organic layers were washed with saturated $NaHCO_3$ (25 mL), brine (40 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a yellow residue. The residue was dissolved in MeCN (20 mL) and the solution was cooled to 0–5 °C. $RuCl_3{\cdot}3H_2O$ (46 mg, 0.18 mmol), $NaIO_4$ (752 mg, 3.52 mmol) and H_2O (20 mL) were then added sequentially, and the reaction mixture was left to stir for 10 min at 0–5 °C and to warm to rt over 45 min. The reaction solution was then partioned between CH_2Cl_2 (100 mL) and saturated $NaHCO_3$ (50 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL) and the combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. Column purification over silica gel (2 : 1 to 1 : 1 Hex–EtOAc) afforded **12b** as a clear oil (683 mg, 74%); TLC (SiO_2, 1 : 1 Hex–EtOAc) R_f 0.33; $[\alpha]_D^{26}$ −49.76 (*c* 1.54, $CHCl_3$); ν_{max}(cast)/cm^{-1} 3004, 2957, 2840, 1754 (br), 1613, 1586, 1515, 1440, 1352 (br); δ_H(400 MHz, $CDCl_3$), 3.74 (3 H, s, OCH_3), 3.80 (3 H, s, OCH_3), 4.06 (1 H, dd, *J* 7.6 and 4.8, H-α), 4.39 (1 H, d, *J* 14.0, PMB-CH_2), 4.47 (1 H, d, *J* 14.0, PMB-CH_2), 4.58 (1 H, dd, *J* 8.8 and 7.6, Ser-CH_2), 4.64 (1 H, dd, *J* 8.8 and 4.8, Ser-CH_2), 6.90-6.86 (2 H, m, Ar-H), 7.34-7.30 (2 H, m, Ar-H), δ_C(100 MHz, $CDCl_3$) 49.8 (PMB-CH_2), 53.0 (OCH_3), 55.2 (OCH_3), 57.9 (C-α), 67.3 (C-β), 114.1 (Ar-C), 125.3 (Ar-C), 130.6 (Ar-C), 159.8 (Ar-C), 168.3 (CO); *m*/*z* (ES+) Calcd for $C_{12}H_{15}NO_6SNa$ 324.0512, found 324.0512 [MNa^+].

(4*R*)-*N*-(*p*-Methoxybenzyl)-2,2-dioxo-1,2,3-oxathiazolidinone-4-carboxylic acid methyl ester (12c)

Pyridine (1.24 mL, 15.36 mmol) was added to a solution of the di-protected amino acid **11c** (735 mg, 3.07 mmol) in CH_2Cl_2 (12 mL) and the reaction solution was then cooled to −78 °C. $SOCl_2$ (0.27 mL, 3.68 mmol) was added dropwise over 5 min, and the solution was left to stir at −78 °C for 5 min and allowed to warm to rt over 1 h. The reaction mixture was quenched by the addition of 1% HCl (25 mL). The aqueous layer was extracted with CH_2Cl_2 (100 mL) and the combined organic layers were washed with saturated $NaHCO_3$ (20 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a yellow residue. The residue was dissolved in MeCN (50 mL) and the solution was cooled to 0–5 °C. $RuCl_3{\cdot}3H_2O$ (48 mg, 0.18 mmol), $NaIO_4$ (787 mg, 3.68 mmol) and H_2O (50 mL) were then added sequentially, and the reaction mixture was left to stir for 10 min at 0–5 °C and a further 10 min at rt. The reaction solution was then partioned between CH_2Cl_2 (200 mL) and saturated $NaHCO_3$ (50 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL) and the combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. Column purification over silica gel (2 : 1 to 1 : 1 Hex–EtOAc) afforded **12c** as a clear oil (718 mg, 76%); TLC (SiO_2, 1 : 1 Hex–EtOAc) R_f 0.33; $[\alpha]_D^{26}$ +49.30 (*c* 0.29, $CHCl_3$); ν_{max}(microscope)/cm^{-1} 3005, 2958, 2840, 1750, 1612, 1515, 1441, 1350, 1251, 1185 cm^{-1}; δ_H(400 MHz, $CDCl_3$) 3.74 (3 H, s, OCH_3), 3.80 (3 H, s, OCH_3), 4.06 (1 H, dd, *J* 7.6 and 4.8, H-α), 4.40 (1 H, d, *J* 14.0, PMB-CH_2), 4.47 (1 H, d, *J* 14.0, PMB-CH_2), 4.58 (1 H, dd, *J* 8.8 and 7.6, Ser-CH_2), 4.65 (1 H, dd, *J* 8.8 and 4.8, Ser-CH_2), 6.90-6.86 (2 H, m, Ar-H), 7.34 (2 H, m, Ar-H); δ_C(100 MHz, $CDCl_3$) 49.8 (PMB-CH_2), 53.0 (OCH_3), 55.2 (OCH_3), 57.8 (C-α), 67.3 (C-β), 114.1 (Ar-C), 125.3 (Ar-C), 130.6 (Ar-C), 159.8 (Ar-C), 168.3 (CO); *m*/*z* (ES+) Calcd for $C_{12}H_{15}NO_6SNa$ 324.0512, found 324.0509 [MNa^+].

(4*R*,5*S*)-*N*-(*p*-Methoxybenzyl)-2,2-dioxo-1,2,3-oxathiazolidinone-5-methyl-4-carboxylic acid methyl ester (12d)

Pyridine (1.60 mL, 19.75 mmol) was added to a solution of the di-protected amino acid **11d** (1.00 g, 3.95 mmol) in CH_2Cl_2 (20 mL) and the reaction solution was then cooled to −78 °C. $SOCl_2$ (0.35 mL, 4.74 mmol) was added dropwise over 10 min, and the solution was left to stir at −78 °C for 10 min and allowed to warm to rt over 1 h. The reaction mixture was quenched by the addition of 1% HCl (25 mL). The aqueous layer was extracted with CH_2Cl_2 (80 mL), and the combined organic layers were washed with saturated $NaHCO_3$ (20 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a yellow residue. The residue was dissolved in MeCN (20 mL) and the solution was cooled to 0–5 °C. $RuCl_3{\cdot}3H_2O$ (62 mg, 0.24 mmol), $NaIO_4$ (0.93 g, 4.35 mmol) and H_2O (20 mL) were then added sequentially, and the reaction mixture was left to stir for 10 min at 0–5 °C and a further 10 min at rt. The reaction solution was then partitioned between CH_2Cl_2 (80 mL) and saturated $NaHCO_3$ (40 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL), and the combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. Column purification over silica gel (2 : 1 to 1 : 1 Hex–EtOAc)

afforded **12d** as a colourless oil (0.97 g, 78%). TLC (SiO_2, 2 : 1 Hex–EtOAc) R_f 0.19; $[\alpha]_D^{26}$ +29.97 (*c* 0.87, $CHCl_3$); ν_{max}(DCM, microscope)/cm^{-1} 3000, 2957, 2840, 1754, 1613, 1515, 1441, 1351, 1252, 1187; δ_H(400 MHz, $CDCl_3$) 1.53 (3 H, d, *J* 6.4, CH_3), 3.69 (3 H, s, OCH_3), 3.70 (1 H, d, *J* 6.4, H-α), 3.80 (3 H, s, OCH_3), 4.41 (2 H, s, PMB-CH_2), 4.88 (1 H, app. p, *J* 6.4, H-β), 6.90-6.86 (2 H, m, Ar-H), 7.32-7.28 (2 H, m, Ar-H); δ_C(100 MHz; $CDCl_3$) 19.3 (CH_3); 50.0 (PMB-CH_2), 53.0 (OCH_3), 55.2 (OCH_3), 64.8 (C-α), 77.5 (C-β), 114.1 (Ar-C), 130.7 (Ar-C), 159.8 (Ar-C), 167.8 (CO), *m*/*z* (ES+) Calcd for $C_{13}H_{17}NO_6SNa$ 338.0669, found 338.0671 [MNa^+].

(4*R*,5*R*)-*N*-(*p*-Methoxybenzyl)-2,2-dioxo-1,2,3-oxathiazolidinone-5-methyl-4-carboxylic acid methyl ester (12e)

Pyridine (0.57 mL, 7.07 mmol) was added to a solution of the di-protected amino acid **11e** (358 mg, 1.41 mmol) in CH_2Cl_2 (8 mL) and the reaction solution was then cooled to −78 °C. $SOCl_2$ (0.13 mL, 1.69 mmol) was added dropwise over 5 min, and the solution was left to stir at −78 °C for 5 min and allowed to warm to rt over 1 h. The reaction mixture was quenched by the addition of 1% HCl (10 mL). The aqueous layer was extracted with CH_2Cl_2 (50 mL), and the combined organic layers were washed with saturated $NaHCO_3$ (20 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a yellow residue. The residue was dissolved in MeCN (10 mL) and the solution was cooled to 0–5 °C. $RuCl_3{\cdot}3H_2O$ (22 mg, 0.08 mmol), $NaIO_4$ (787 mg, 3.68 mmol) and H_2O (10 mL) were then added sequentially, and the reaction mixture was left to stir for 15 min at 0–5 °C and a further 30 min at rt. The reaction solution was then partioned between CH_2Cl_2 (50 mL) and saturated $NaHCO_3$ (15 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 40 mL) and the combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. Column purification over silica gel (2 : 1 to 1 : 1 Hex–EtOAc) afforded **12e** as a white solid (333 mg, 75%). TLC (SiO_2, 2 : 1 Hex–EtOAc) R_f 0.21; $[\alpha]_D^{26}$ +62.59 (*c* 0.91, $CHCl_3$); ν_{max}($CHCl_3$, microscope)/cm^{-1} 2956, 2841, 1755, 1613, 1515, 1443, 1346, 1252, 1188, 1029; δ_H(400 MHz; $CDCl_3$) 1.41 (3 H, d, *J* 6.4, CH_3), 3.70 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.97 (1 H, d, *J* 6.4, H-α), 4.21 (1 H, m, *J* 14.0, PMB-CH_2), 4.38 (1 H, d, *J* 14.0, PMB-CH_2), 4.99 (1 H, app. p, *J* 6.4, H-β), 6.86-6.83 (2 H, m, Ar-H), 7.26-7.22 (2 H, m, Ar-H), δ_C(100 MHz; $CDCl_3$) 15.8 (CH_3), 48.8 (PMB-CH_2), 52.6 (OCH_3), 55.3 (OCH_3), 63.4 (C-α), 76.4 (C-β), 114.2 (Ar-C), 125.4 (Ar-C), 130.6 (Ar-C), 158.9 (Ar-C), 167.3 (CO); *m*/*z* (ES+) Calcd for $C_{13}H_{17}NO_6SNa$ 338.0669, found 338.0672 [MNa^+].

(2*R*,3*S*)-*N*-(*p*-Methoxylbenzyl)-3-tritylsulfanyl-3-methylcarboxylic acid methyl ester (14)

Trityl-SH **13** (132 mg, 0.48 mmol) was added to a solution of **12a** (100 mg, 0.32 mmol) in DMF (2 mL) at rt. Cs_2CO_3 (155 mg, 0.48 mmol) was then added and the solution was left to stir for 16 h. The reaction was added to 1 M NaH_2PO_4 buffer (20 mL), and the solution left to stir for 1 h at rt. EtOAc (25 mL) was added, and the layers separated. The aqueous layer was extracted with EtOAc (2 × 25 mL), and the combined organic extracts were dried, filtered and concentrated under reduced pressure. Column purification over silica gel (2 : 1 Hex–EtOAc) afforded **14** as a clear oil (106 mg, 67%). TLC (SiO_2, 2 : 1 Hex–EtOAc) R_f 0.35; $[\alpha]_D^{26}$ −14.90 (*c* 0.30, $CHCl_3$), ν_{max}($CHCl_3$, microscope)/cm^{-1} 3339, 3057, 2951, 2835, 1733, 1611, 1512, 1445, 1248; δ_H(400 MHz, $CDCl_3$) 0.96 (3 H, d, *J* 7.2, CH_3), 2.78 (1 H, dq, *J* 7.2 and 3.3, H-β), 2.88 (1 H, d, *J* 3.3, H-α), 3.53 (1 H, d, *J* 12.8, PMB-CH_2), 3.57 (3 H, s, OCH_3), 3.72 (1 H, d, *J* 12.8, PMB-CH_2), 3.81 (3 H, s, OCH_3), 6.89-6.86 (2 H, m, Ar-H), 7.34-7.19 (11 H, m, Ar-H), 7.55-7.52 (6 H, m, Ar-H), δ_C(100 MHz, $CDCl_3$) 16.7 (CH_3), 43.2 (C-β), 51.3 (OCH_3), 52.4 (PMB-CH_2), 55.2 (OCH_3), 64.3 (C-α), 67.5 ($C(Ar)_3$), 113.6 (Ar-C), 126.4 (Ar-C), 127.8 (Ar-C), 129.6 (Ar-C), 132.0 (Ar-C), 145.0 (Ar-C), 158.7 (Ar-C), 173.5 (CO), *m*/*z* (ES+) Calcd for $C_{32}H_{33}NO_3SNa$ 534.2073, found 534.2076 MNa^+.

3-(*S*)-[(*R*)-2-*tert*-Butoxycarbonyl-2-(*tert*-butoxycarbonylamino)-ethylsulfanyl]-(*R*)-(*p*-methoxybenzylamino)butanoic acid methyl ester (17)

Procedure 1. Boc-Cys-OMe **16** (152 mg, 0.65 mmol) was added to a solution of **12a** (136 mg, 0.43 mmol) in DMF (4 mL) at rt. Cs_2CO_3 (210 mg, 0.65 mmol) was added and the solution was left to stir for 16 h. The reaction was added to 1 M NaH_2PO_4 buffer (20 mL), and the solution was left to stir for 24 h at rt. EtOAc (25 mL) and the layers separated. The aqueous layer was extracted with EtOAc (2 × 25 mL), and the combined organic extracts were dried, filtered and concentrated under reduced pressure. Column purification over silica (2 : 1 Hex–EtOAc) afforded **17** as a colourless oil (81 mg, 40%). TLC (SiO_2, 2 : 1 Hex–EtOAc) R_f 0.31; $[\alpha]_D^{26}$ −6.71 (*c* 1.08, $CHCl_3$), ν_{max}(microscope)/cm^{-1} 33.65 (br), 2975, 2837, 1715, 1612, 1512, 1454, 1367, 1248, 1169, 1034; δ_H (400 MHz, $CDCl_3$) 1.27 (3 H, d, *J* 7.2, CH_3), 1.44 (9 H, s, $C(CH_3)_3$), 2.88 (1 H, dd, *J* 13.2 and 6.0, CH_2), 2.96 (1 H, dd, *J* 13.2 and 5.0, CH_2), 3.04 (1 H, dq, *J* 7.2 and 5.2, H-3), 3.33 (1 H, d, *J* 5.2, H-2), 3.60 (1 H, d, *J* 12.8, PMB-CH_2), 3.73 (3 H, s, OCH_3), 3.74 (3 H, s, OCH_3), 3.80 (3 H, s, OCH_3), 3.80 (1 H, d, *J* 12.8, PMB-CH_2), 4.51 (1 H, m, H-6), 5.43 (1 H, br d, *J* 6.8, NH), 6.87-6.83 (m, 2H, Ar-H), 7.26-7.23 (m, 2H, Ar-H); δ_C(100 MHz, $CDCl_3$) 17.6 (CH_3), 21.3 ($C(CH_3)_3$), 33.6 (C-5), 43.8 (C-3), 51.8 (PMB-CH_2), 51.8 (OCH_3), 52.5 (OCH_3), 53.3 (C-6), 55.2 (OCH_3), 64.5 (C-2), 80.1 ($C(CH_3)_3$), 113.7 (Ar-C), 129.5 (Ar-C), 131.5 (Ar-C), 155.1 (CO), 158.8 (Ar-C), 171.4 (CO), 173.7 (CO); *m*/*z* (ES+) Calcd for $C_{22}H_{32}N_2O_7SNa$ 493.1979, found 493.1982 [MNa^+].

Procedure 2. Boc-Cys-OMe **16** (94 mg, 0.40 mmol) was added to a solution of **12a** (105 mg, 0.33 mmol) in DMF (1.5 mL) at rt. Cs_2CO_3 (130 mg, 0.40 mmol) was added, and the solution was left to stir for 18 h. The solvent was removed under vacuum to give a thick residue. This residue was dissolved in CH_2Cl_2 (2 mL) and cooled to 0 °C. $BF_3{\cdot}Et_2O$ (0.08 mL, 0.60 mmol) was added and the reaction solution was left to stir for 30 min at 0 °C. *n*PrSH (0.05 mL, 0.60 mmol) was then added and the reaction was left to stir for a further 18 h at rt. NH_4OH solution (30% NH_3, 1 ml) was added and the resulting solution was left to stir for 30 min before CH_2Cl_2 (10 ml) and $MgSO_4$ (excess) were added. The reaction solution was filtered and the solid washed with CH_2Cl_2. The organic washes were combined, and the solvent was removed under reduced pressure to give a pale yellow oil. Column chromatography (SiO_2, 2 : 1 Hex–EtOAc) yielded **17** as a

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colourless oil (124 mg, 79%). The physical and spectral properties for **17** obtained marched those reported for Procedure 1.

3-[(*R*)-2-*tert*-Butoxycarbonyl-2-(*tert*-butoxycarbonylamino)-ethylsulfanyl]-(*R*)-(*p*-methoxybenzylamino)propionic acid methyl ester (19a)

Boc-Cys-O*t*Bu **18** (213 mg, 0.77 mmol) and Cs_2CO_3 (251 mg, 0.77 mmol) were added to a solution of **12b** (193 mg, 0.64 mmol) in DMF (2 mL). The reaction solution was left to stir at rt for 20 h. The solvent was removed under vacuum to give a thick residue. This residue was dissolved in CH_2Cl_2 (4 mL) and cooled to 0 °C. $BF_3{\cdot}Et_2O$ (0.12 mL, 0.96 mmol) was added, and the reaction solution was left to stir for 30 min. *n*PrSH (0.09 mL, 0.99 mmol) was then added, and the reaction was left to stir for a further 16 h. NH_4OH (30% NH_3, 1.5 mL) was added, and the resulting solution was left to stir 30 min before $MgSO_4$ (excess) and CH_2Cl_2 (10 ml) were added. The reaction solution was filtered and the solid washed with CH_2Cl_2. The solvent was removed under reduced pressure, and the resulting oil was purified by column chromatography (SiO_2, 2 : 1 Hex–EtOAc) to give **19a** as a colourless oil (257 mg, 85%). TLC (SiO_2, 2 : 1 Hex–EtOAc) R_f 0.28; $[\alpha]_D^{26}$ −9.18 (*c* 1.25, $CHCl_3$); ν_{max}(cast)/cm^{-1} 3363 (br), 2977, 2933, 2836, 1737, 1713, 1611, 1513, 1392, 1367, 1155, 1035; δ_H(400 MHz, $CDCl_3$) 1.44 (9 H, s, $C(CH_3)_3$), 1.46 (9 H, s, $C(CH_3)_3$), 2.85 (2 H, m, H-3), 2.95 (2 H, m, H-5), 3.45 (1 H, t, *J* 6.4, H-2), 3.65 (1 H, d, *J* 12.8, PMB-CH_2), 3.73 (3 H, s, CO_2CH_3), 3.77 (1 H, d, *J* 12.8, PMB-CH_2), 3.79 (3 H, s, PMB-OCH_3), 4.39 (1 H, m, H-6), 5.56 (1 H, br d, NH), 6.87-6.83 (2 H, m, Ar-H), 7.26-7.23 (2 H, m, Ar-H); δ_C(100 MHz, $CDCl_3$) 27.9 ($C(CH_3)_3$), 28.3 ($C(CH_3)_3$), 35.5 (C-3), 36.0 (C-5), 51.3 (PMB-CH_2), 52.0 (PMB-OCH_3), 54.2 (C-6), 55.2 (CO_2CH_3), 60.3 (C-2), 79.8 ($C(CH_3)_3$), 82.4 ($C(CH_3)_3$), 113.8 (Ar-C), 129.5 (Ar-C), 131.4 (Ar-C), 155.2 (CO), 158.8 (Ar-C), 169.8 (C-7), 173.8 (C-1); *m*/*z* (ES+) Calcd for $C_{24}H_{39}N_2O_7S$ 499.2473, found 499.2471 [MH^+].

3-[(*S*)-2-*tert*-Butoxycarbonyl-2-(*tert*-butoxycarbonylamino)-ethylsulfanyl]-(*R*)-(*p*-methoxybenzylamino)propionic acid methyl ester (19b)

Boc-Cys-O*t*Bu **18** (201 mg, 0.73 mmol) and Cs_2CO_3 (236 mg, 0.73 mmol) were added to a solution of **12c** (182 mg, 0.60 mmol) in DMF (2 mL). The reaction solution was left to stir at rt for 20 h. The solvent was removed under vacuum to give a thick residue. The residue was dissolved in CH_2Cl_2 (4 mL) and cooled to 0 °C. $BF_3{\cdot}Et_2O$ (0.11 mL, 0.91 mmol) was added, and the reaction solution was left to stir for 30 min. *n*PrSH (0.08 ml, 0.91 mmol) was then added, and the reaction was left to stir for a further 16 h. NH_4OH (30% NH_3, 1.5 mL) was added, and the resulting solution was left to stir 30 min before $MgSO_4$ (excess) and CH_2Cl_2 (10 mL) were added. The reaction solution was filtered and the solid washed with CH_2Cl_2. The solvent was removed under reduced pressure, and the resulting oil was purified by column chromatography (SiO_2, 4 : 1 to 2 : 1 Hex–EtOAc) to give **19b** as an oil (252 mg, 89%). TLC (SiO_2, 2 : 1 Hex–EtOAc) R_f 0.29; $[\alpha]_D^{26}$ +7.47 (*c* 2.16, $CHCl_3$); ν_{max}($CHCl_3$, microscope)/cm^{-1} 3360 (br), 2978, 2934, 1737, 1715, 1513, 1393, 1368, 1248, 1156, 1035; δ_H(400 MHz, $CDCl_3$) 1.44 (9 H, s, $C(CH_3)_3$), 1.46 (9 H, s, $C(CH_3)_3$), 2.84 (1 H, dd, *J* 13.2 and 6.0, H-3), 2.89 (1 H, dd, *J* 13.2 and 6.0, H-3), 2.95 (2 H, m, H-5), 3.46 (1 H, t, *J* 6.0, H-2), 3.66 (1 H, d, *J* 12.8, PMB-CH_2), 3.72 (3 H, s, CO_2CH_3), 3.78 (1 H, d, *J* 12.8, PMB-CH_2), 3.79 (3 H, s, PMB-OCH_3), 4.39 (1 H, m, H-6), 5.66 (1 H, br s, NH), 6.86-6.83 (2 H, m, Ar-H), 7.26-7.23 (2 H, m, Ar-H); δ_C(100 MHz, $CDCl_3$) 27.9 ($C(CH_3)_3$), 28.3 ($C(CH_3)_3$), 35.5 (C-3), 36.0 (C-5), 51.2 (PMB-CH_2), 51.9 (PMB-OCH_3), 54.1 (C-6), 55.2 (CO_2CH_3), 60.1 (C-2), 79.8 ($C(CH_3)_3$), 82.4 ($C(CH_3)_3$), 113.8 (Ar-C), 129.4 (Ar-C), 131.4 (Ar-C), 155.2 (CO), 158.8 (Ar-C), 169.8 (C-7), 173.8 (C-1); *m*/*z* (ES+) Calcd for $C_{24}H_{39}N_2O_7S$ 499.2473, found 499.2475 [MH^+].

3-(*R*)-[(*R*)-2-*tert*-Butoxycarbonyl-2-(*tert*-butoxycarbonylamino)-ethylsulfanyl]-(*S*)-(*p*-methoxybenzylamino)butanoic acid methyl ester (20a)

Boc-Cys-O*t*Bu **18** (322 mg, 1.16 mmol) and Cs_2CO_3 (377 mg, 1.16 mmol) were added to a solution of **12d** (304 mg, 0.96 mmol) in DMF (5 mL). The reaction solution was left to stir at rt for 20 h. The solvent was removed under vacuum to give a thick residue. The residue was dissolved in CH_2Cl_2 (6 mL), and cooled to 0 °C. $BF_3{\cdot}Et_2O$ (0.18 mL, 1.44 mmol) was added, and the reaction solution was left to stir for 30 min. *n*PrSH (0.13 mL, 1.44 mmol) was then added, and the reaction was left to stir for a further 16 h. NH_4OH (30% NH_3, 2.0 mL) was added, and the resulting solution was left to stir 30 min before $MgSO_4$ (excess) and CH_2Cl_2 (15 mL) were added. The reaction solution was filtered and the solid washed with CH_2Cl_2. The solvent was removed under reduced pressure, and the resulting oil was purified by column chromatography (SiO_2, 4 : 1 Hex–EtOAc), to give **20a** as an oil (354 mg, 72%). TLC (SiO_2, 4 : 1 Hex–EtOAc) R_f 0.15; $[\alpha]_D^{26}$ +15.19 (*c* 0.65, $CHCl_3$); ν_{max}($CHCl_3$, microscope)/cm^{-1} 3363 (br), 2978, 2933, 1735, 1716, 1513, 1368, 1248, 1155, 1035; δ_H(400 MHz, $CDCl_3$) 1.28 (3 H, d, *J* 7.2, CH_3); 1.44 (9 H, s, $C(CH_3)_3$), 1.47 (9 H, s, $C(CH_3)_3$), 2.89 (1 H, dd, *J* 13.8 and 4.8, H-5), 2.97 (1 H, dd, *J* 13.8 and 4.8, H-5), 3.07 (1 H, m, H-3), 3.33 (1 H, d, *J* 5.6, H-2), 3.62 (1 H, d, *J* 12.8, PMB-CH_2), 3.72 (3 H, s, PMB-OCH_3), 3.79 (3 H, s, CO_2CH_3), 3.81 (1 H, d, *J* 12.8, PMB-CH_2), 4.42 (1 H, m, CH), 5.69 (1 H, d, *J* 8.4, NH), 6.87-6.83 (2 H, m, Ar-H), 7.27-7.23 (2 H, m, Ar-H); δ_C(100 MHz, $CDCl_3$) 18.3 (CH_3), 27.9 ($C(CH_3)_3$), 28.3 ($C(CH_3)_3$), 34.0 (C-5), 44.4 (C-3), 51.7 (PMB-CH_2), 51.7 (PMB-OCH_3), 54.3 (C-6), 55.2 (CO_2CH_3), 64.5 (C-2), 79.7 ($C(CH_3)_3$), 82.4 ($C(CH_3)_3$), 113.7 (Ar-C), 129.5 (Ar-C), 131.5 (Ar-C), 155.3 (CO), 158.8 (Ar-C), 169.8 (CO), 173.7 (CO); *m*/*z* (ES+) Calcd for $C_{25}H_{41}N2O_7S$ 513.2629, found 513.2628 [MH^+].

3-(*S*)-[(*R*)-2-*tert*-Butoxycarbonyl-2-(*tert*-butoxycarbonylamino)-ethylsulfanyl]-(*S*)-(*p*-methoxybenzylamino)butanoic acid methyl ester (20b)

Boc-Cys-O*t*Bu **18** (178 mg, 0.64 mmol) and Cs_2CO_3 (210 mg, 0.64 mmol) were added to a solution of **12e** (169 mg, 0.54 mmol) in DMF (3 mL). The reaction solution was left to stir at rt for 20 h. The solvent was removed under vacuum to give a thick residue. The residue was dissolved in CH_2Cl_2 (4 mL) and cooled to 0 °C. $BF_3{\cdot}Et_2O$ (0.11 mL, 0.80 mmol) was added, and the reaction solution was left to stir for 30 min. *n*PrSH (0.06 ml, 0.80 mmol) was then added, and the reaction was left to stir for a further 16 h. NH_4OH (30% NH_3, 1.5 mL) was added, and the resulting solution was left to stir 30 min before $MgSO_4$ (excess) was added. The

reaction solution was filtered and the solid washed with CH_2Cl_2. The solvent was removed under reduced pressure and the resulting oil was purified by column chromatography (SiO_2, 4 : 1 to 2 : 1 Hex–EtOAc) to give **20b** as an oil (194 mg, 70%). TLC (SiO_2, 4 : 1 Hex–EtOAc) R_f 0.13; $[a]_D^{26}$ +11.02 (*c* 2.04, $CHCl_3$); ν_{max}($CHCl_3$, microscope)/cm^{-1} 3372 (br), 2978, 2934, 1737, 1715, 1513, 1456, 1513, 1368, 1248 1155; δ_H(400 MHz; $CDCl_3$) 1.31 (3 H, d, *J* 6.8, CH_3), 1.45 (9 H, s, $C(CH_3)_3$), 1.46 (9 H, s, $C(CH_3)_3$), 2.91 (1 H, dd, *J* 13.6 and 5.2, H-5), 2.96 (1 H, dd, *J* 13.6 and 4.8, H-5), 3.13 (1 H, m, H-3), 3.25 (1 H, d, *J* 5.6, H-2), 3.60 (d, 1H, *J* 13.2, PMB-CH_2), 3.74 (3 H, s, PMB-OCH_3), 3.79 (3 H, s, CO_2CH_3), 3.83 (1 H, d, *J* 13.2, PMB-CH_2), 4.37 (1 H, m, H-6), 5.49 (1 H, d, *J* 7.2, NH), 6.86-6.83 (2 H, m, Ar-H), 7.27-7.23 (2 H, m, Ar-H), δ_C(100 MHz; $CDCl_3$) 19.3 (CH_3), 28.0 ($C(CH_3)_3$), 28.3 ($C(CH_3)_3$), 33.6 (C-5), 44.0 (C-3), 51.7 (PMB-CH_2), 51.8 (PMB-OCH_3), 53.8 (C-6), 55.2 (CO_2CH_3), 64.9 (C-2), 79.8 ($C(CH_3)_3$), 82.5 ($C(CH_3)_3$), 113.7 (Ar-C), 129.7 (Ar-C), 131.7 (Ar-C), 155.2 (CO), 158.7 (Ar-C), 169.8 (CO), 173.9 (CO); *m/z* (ES+) Calcd for $C_{25}H_{41}N_2O_7S$ 513.2629, found 513.2627 [MH^+].

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