

Cite this: *RSC Adv.*, 2019, 9, 30019Received 29th July 2019
Accepted 12th September 2019

DOI: 10.1039/c9ra07244f

rsc.li/rsc-advances

Formal synthesis of (+)-lactacystin from L-serine†

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Alexandra M. Z. Slawin^b and Steven M. Allin^cA formal, stereocontrolled synthesis of lactacystin has been completed from *t*-Bu-O-L-serine, providing the key intermediate **13**, also useful for the generation of a range of C-9 analogues.

Introduction

The 20S proteasome is a large barrel-shaped protein comprised of 28 subunits.¹ The primary function of the proteasome involves the degradation of damaged proteins, a vital component of the ubiquitin proteasome pathway. Inhibition of the proteasome can lead to cell death. This property of the proteasome has made it a promising target for cancer therapeutics.²

Microbial metabolites have provided a wealth of proteasome inhibitors (Fig. 1). Lactacystin **1** was discovered in 1991 by Ōmura through extraction from the cultured broth of *Streptomyces* sp OM-6519,^{3,4} after observations that it induced differentiation of the mouse neuroblastoma cell line, a consequence of proteasome inhibition. Further studies, driven by several early efforts to prepare lactacystin and analogues, found that lactacystin undergoes cyclization to the β -lactone omuralide **2**, which inhibits the proteasome⁵ and can induce apoptosis. The potential for beta-lactone, gamma-lactam proteasome inhibitors was further highlighted by the discovery of the salinosporamides, e.g. **3**,^{6–8} and cinnabaramides, e.g. **4**.⁹ Similar lactam cores have also been discovered in the metabolite oxazolomycin¹⁰ **5**, which possesses antibiotic activity. A number of strategies to access these cores to produce natural products and analogues of high therapeutic value have been reported.¹¹

Results & discussion

Our synthetic work has focused on the development of a functionalized lactam core from glycine,^{12,13} and recently using *L*-

leucine **6** as a starting material to produce a formal synthesis of the C9-deoxyomuralide analogue, using the natural chirality of the amino acid to direct the synthesis.¹⁴ Formation of the carbon skeleton of omuralide was achieved in 4 steps (Scheme 1). Peptide coupling of PMB-protected leucine **7** to the malonic acid benzyl ester **8** provided the precursor **9** to the key Dieckmann cyclization/alkylation step. Cyclization was induced with TBAF, and subsequent addition of methyl iodide provided two diastereoisomers. The major, **10b**, was isolated and used in an acylation using Mander's reagent to give **11**, thus completing the carbon skeleton. Six further steps produced pyroglutamate **12**, which can be cyclized to give 9-deoxyomuralide in one step.

With this methodology in place, we turned our attention to a serine-derived route to lactacystin. This would provide a hydroxy group in the C9 position, which previous SAR studies have shown to be key for effective proteasome inhibition.

We envisaged that intermediate **13** could be synthesized from a suitably protected tetramic acid-like core **14** (Scheme 2). A removable benzyl ester would be used to help direct acylation of lactam **15** using Mander's reagent; **15** could in turn be formed using the cyclization/alkylation procedure previously developed. The Dieckmann cyclization precursor **16** could be synthesized from peptide coupling of a suitably protected *O*-*t*-Bu-L-serine **17**. This starting material was used due to the size of the *t*-butyl group, its tolerance towards a wide range of conditions, and because both enantiomers are commercially available.

PMB protection of the serine derivative **17** was carried out using a modification of a procedure by Vázquez using the PMB sulphite adduct **18**.¹⁵ We have previously observed¹⁴ epimerization during imine formation, and so a one-pot procedure was developed. After work up, the protected serine **19** could be used without further purification. Peptide coupling to benzyl malonic ester **8** provided the Dieckmann cyclization precursor in 49% yield over the two steps. Analysis using chiral stationary phase HPLC showed that only negligible epimerization had occurred at this point, providing **16** in 97% ee (Scheme 3).

With the Dieckmann precursor **16** in hand, we turned our attention to the tandem cyclization/alkylation step (Scheme 4).

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† Electronic supplementary information (ESI) available: Copies of ¹H and ¹³C NMR spectra. Copies of HPLC traces. ORTEP diagrams and X-ray data. CCDC 1916072–1916076. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c9ra07244f

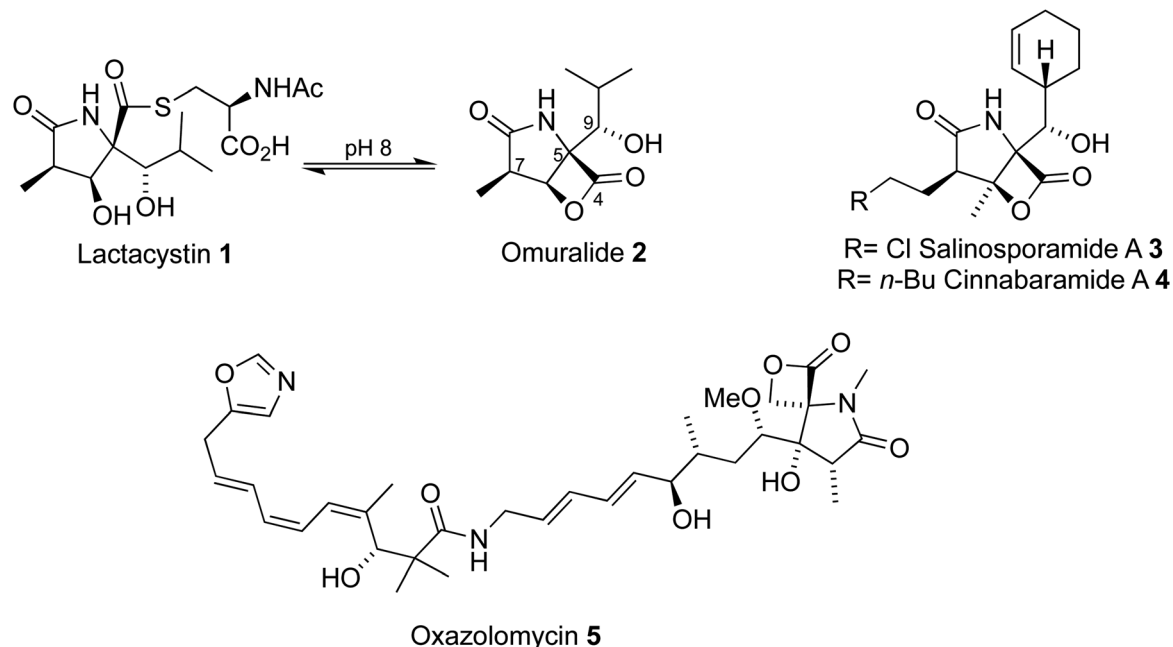
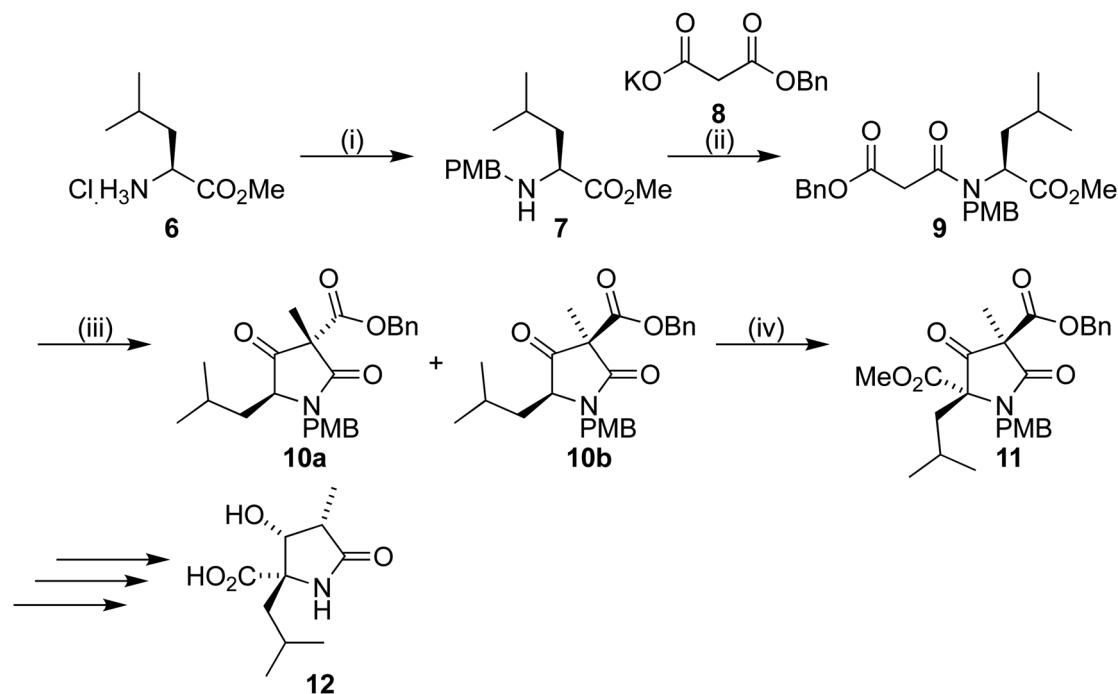


Fig. 1 Examples of proteasome inhibitors.

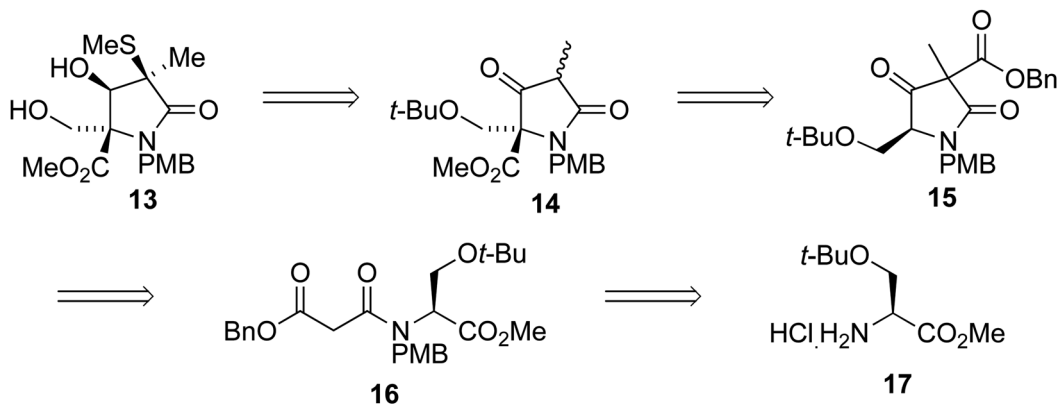
When subjected to the conditions used previously (TBAF in THF, then MeI addition), the cyclization/alkylation proceeded with good yield and provided a 10 : 1 ratio of diastereoisomers **15a** and **15b**, in favour of **15a**, where the newly added methyl group is situated on the same face of the molecule as the *t*-BuO group.

Our previous work on the leucine analogue¹⁴ showed that addition of the methyl iodide is in that case preferentially introduced opposite the isobutyl moiety (Scheme 5). This outcome was expected due to the bulky nature of the amino acid group and the assumed planarity of the intermediate. The diastereoisomers were isolated in a 1 : 2 mixture and the



Scheme 1 Reagents and conditions: (i) *p*-methoxybenzaldehyde, Et₃N, MeOH, NaBH₄, 71%; (ii) NMM, EDAC·HCl, DMAP, CH₂Cl₂, r.t., overnight, 93%; (iii) TBAF, THF, r.t., 0.5 h, then MeI, 0 °C to r.t., overnight, **10a/b** 57%; (iv) LiHMDS, DMPU, THF, −78 °C, 0.5 h, then NCCO₂Me, −78 °C, 4 h, 70%.





Scheme 2

observation was confirmed by single crystal X-ray analysis of **20**, derived from the minor diastereoisomer **10a** by PMB removal.

We were therefore surprised to find that our serine derivative afforded the diastereoisomers in a 10 : 1 mixture favouring the diastereoisomer **15a** with the methyl group *cis* to the *t*-BuO group, presumably because in forming **15b** the benzyl ester unit is forced nearer to the *t*-BuO, so raising the transition state energy of that pathway.

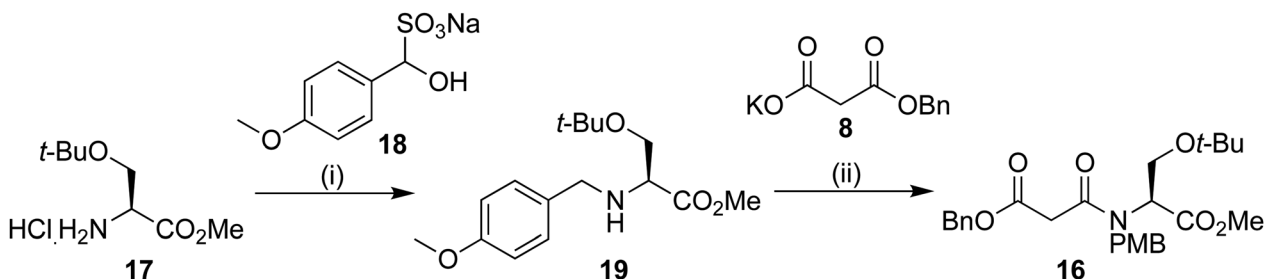
On analysis by chiral HPLC the major diastereoisomer was found to have a disappointing ee of 44%. Running the reaction at decreased temperatures provided higher ee at the expense of yield. Further optimization (Table 1), including increased reaction times, lower temperatures and the separation of the cyclization and alkylation steps, achieved ees of up to 79% and yields of 66%. The ee could be improved further through recrystallization from isopropanol, which produced 97% ee. We suspect that the decrease in ee is primarily the result of epimerization at the C5 position in the mixture of diastereoisomers **15a/b** following alkylation, leading to the (presumed) more stable diastereoisomer **15a**. Lower temperatures reduce the degree of this epimerization, leading to the decreased diastereoselectivities (3 : 1) but higher ees.

During our work with the *L*-leucine derived analogue **9**, we were able to isolate a 1 : 2 ratio of diastereoisomers **10a** and **10b** in 9% ee and 79% ee respectively (Scheme 5).¹⁴ Our results with leucine strongly indicate that the partial racemization occurs after the alkylation. If racemization occurred solely before

alkylation, the ratio of diastereoisomers in the racemic material should match the ratio of diastereoisomers in the enantiopure material. As we observed the diastereoisomers in a 1 : 2 ratio at 9% and 79% ee respectively, the vast majority of racemization must occur after alkylation. Due to the structural similarity between our *L*-leucine-derived lactams and our serine-derived lactams, it seems likely that the mode of racemization is analogous.

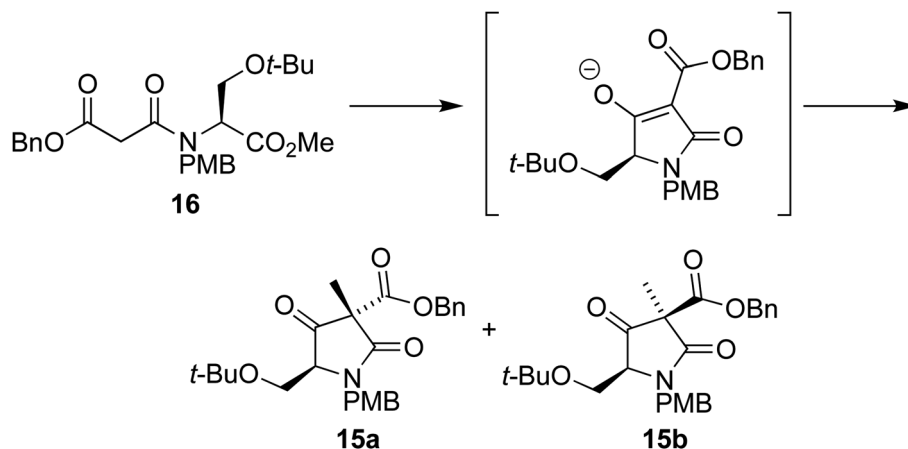
To investigate the stereoselectivity further, the benzyl ester was replaced by a methyl counterpart (Scheme 6). Coupling of **19** to the half malonic methyl ester potassium salt in an analogous procedure to that of **16** proceeded in good yield, to produce **21**. Once treated with our cyclization/alkylation procedure, NMR analysis showed that the cyclization occurred efficiently, but we found that **22a/b** decomposed if left in contact with silica gel for extended periods of time. Partial purification was therefore completed with a silica plug to produce a 3 : 1 mixture of diastereoisomers; the mixture was treated with CAN removing the PMB group (Scheme 6). The purified major isomer **23a** was obtained as a colourless crystalline solid. Analysis by single crystal X-ray diffraction showed that the methyl group was still preferentially added to the same face as the *tert*-butoxy group, allowing us to conclude that the benzyl ester was not the primary influence on diastereoselectivity in our system.

The system was further investigated by replacing the PMB group with a phenyl (Scheme 6). Chan–Lam coupling of the



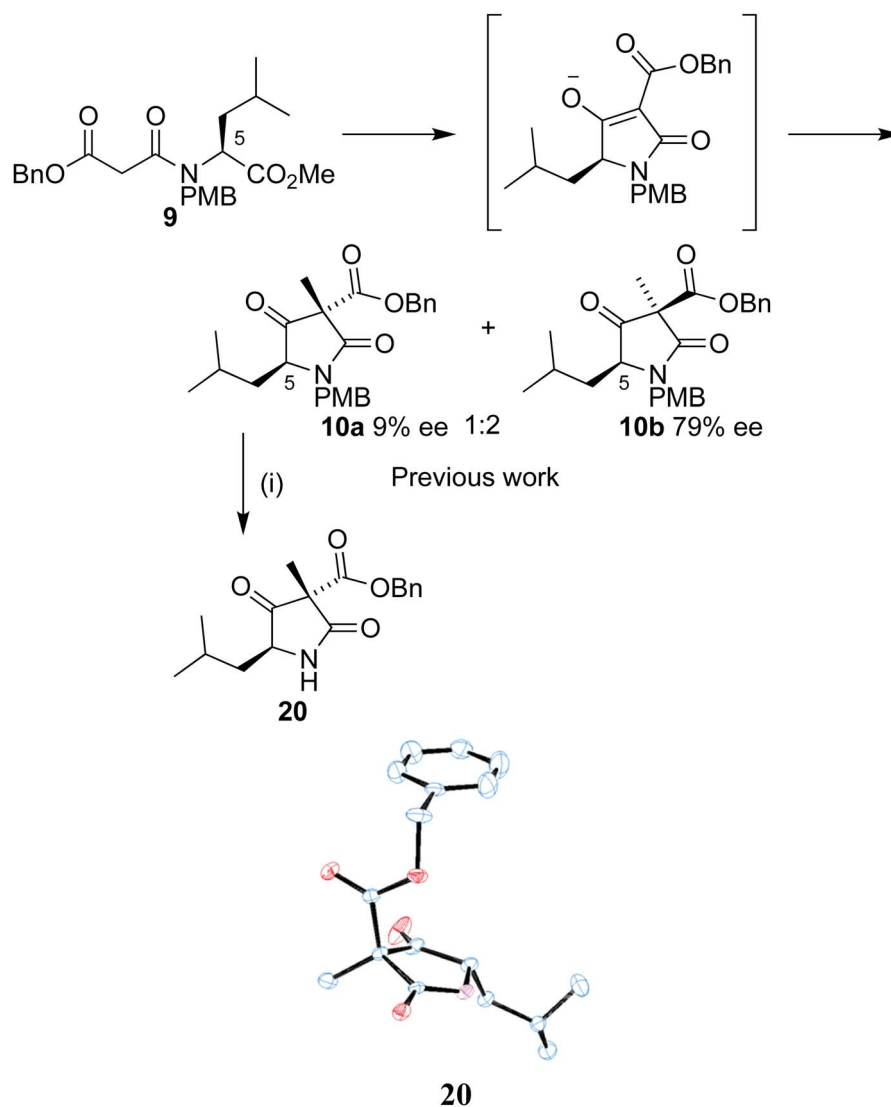
Scheme 3 Reagents and conditions: (i) **18**, NaBH₃CN, Et₃N, MeOH 0 °C 16 h (ii) **8**, EDAC·HCl, *N*-methyl morpholine, DMAP, DCM, 16 h; 49% over the 2 steps.





serine derivative with phenyl boronic acid and subsequent peptide coupling to the benzyl malonic half ester **8** produced the Dieckmann cyclization precursor **24**. Compound **24** was

subjected to our cyclization conditions. The methylated product **25** was produced in a 5 : 1 ratio of diastereoisomers according to the ^1H NMR spectrum, albeit in a low yield,



Scheme 5 Reagents and conditions: (i) CAN, 3 : 1 MeCN/H₂O, 84%.



Table 1 Optimization of the Dieckmann cyclization/alkylation giving **15a**

Entry	Conditions	Yield	Ratio of diastereoisomers 15a : 15b	ee of 15a ^a
1	TBAF, THF 2 h then MeI 2 h	62%	10 : 1	44%
2	TBAF, THF 0.5 h, MeI, 0 °C to r.t. over 16 h	42%	10 : 1	60%
3	TBAF, THF 0.5 h, MeI, −10 °C	Trace	—	76%
4	TBAF, ether, 5 m, THF MeI, −15 °C, 64 h	30%	3 : 1	76%
5	TBAF, ether, 5 m, THF MeI, −12 °C, 64 h	66%	3 : 1	79%

^a Determined by HPLC on chiral stationary phase by comparison with racemic material using Chiralpak AD-H or Knauer Eurocel 01 columns.

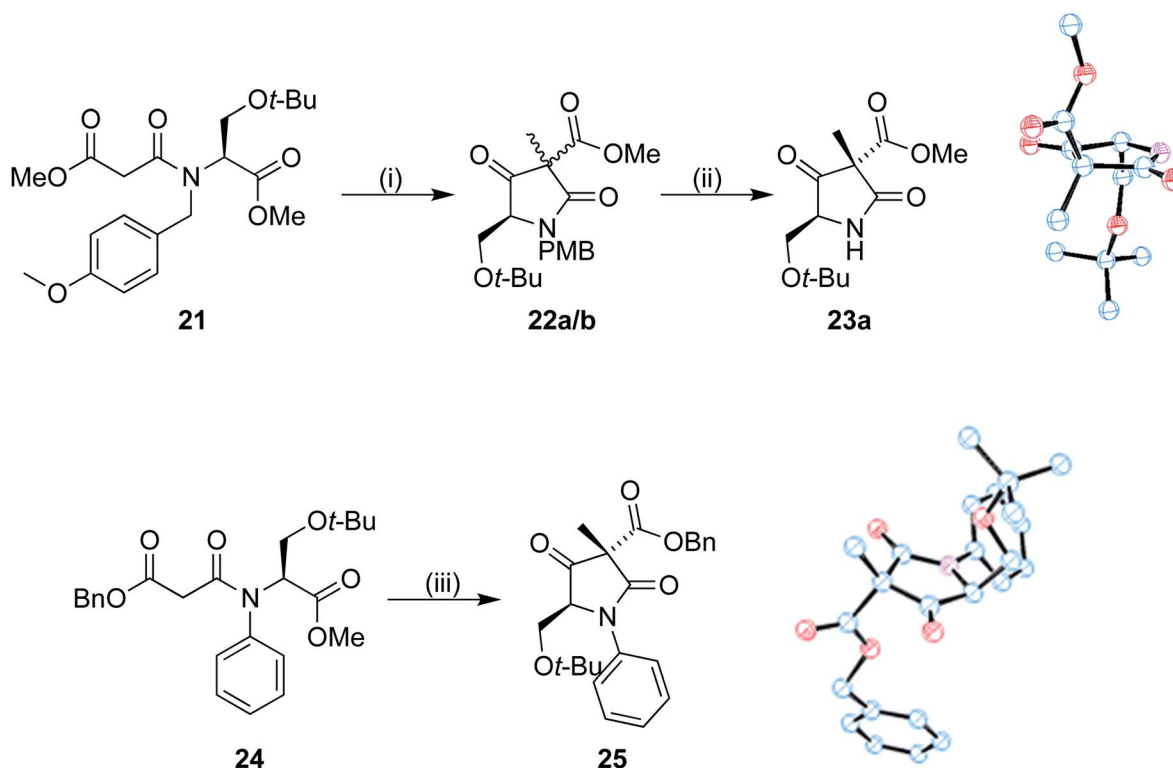
perhaps due to the conformation required for cyclization being more unfavourable. A crystal of the major diastereoisomer suitable for X-ray analysis was obtained. Once again, in the major product the methyl group had been introduced to the same face as the *tert*-butoxy.

With **15a** in hand, we sought to install the methyl ester moiety that would eventually form the beta lactone found in omuralide. Acylation using Mander's reagent at −40 °C was found to produce compound **26** in good yield with no observed *O*-acetylation. In addition, only one diastereoisomer could be observed (Scheme 7). A small drop in ee was observed during the acylation, to 69%, perhaps due to the presence of small quantities of the minor diastereomer **15b**. Using recrystallized **15a**, the ee of **26** was 84%. Single crystal X-ray analysis of **26** was carried out on both the racemic and enantiomerically pure (obtained by recrystallization) forms, the latter confirming that

the absolute stereochemistry of our C9 centre is as found in (+)-lactacystin.

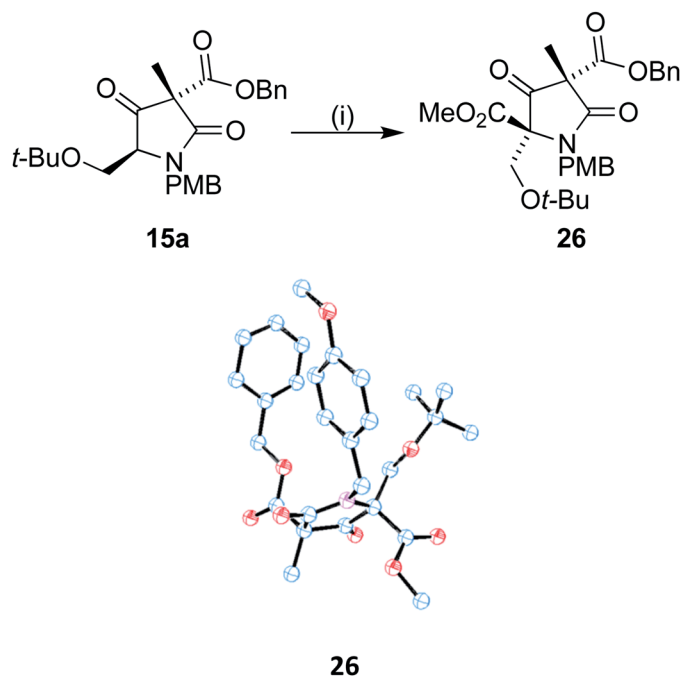
Removal of the benzyl ester from **26** by hydrogenolysis led to tetramic acid derivative **14** as a mixture of diastereoisomers. The mixture proved unstable towards silica gel and so was used in the next step without purification. On treatment with *S*-methyl *p*-toluenethiosulfonate **27**, in a similar manner to Pattenden,^{16,17} a 4 : 1 mixture of inseparable diastereoisomers **28a/b** was formed. NOESY experiments confirmed that the major observed diastereoisomer was the desired one, with the methyl thioether *trans* to the *t*-BuO group (Scheme 8).

Removal of the *t*-butyl group from **28a** would allow completion of the formal synthesis to form the Corey intermediate **29a**.¹⁸ Treatment of **28a/b** with a 1 : 1 TFA/DCM mixture led to an inseparable mixture of the diastereoisomers **29a/b** in a 2 : 1 mixture after purification by column chromatography. To our



Scheme 6 Reagents and conditions: (i) TBAF, ether, 5 m, THF, MeI, −12 °C, 58 h; 87% (ii) CAN, 3 : 1 MeCN/H₂O, 57%, (iii) TBAF, ether, 5 m, THF MeI, −12 °C, 64 h; 11%.





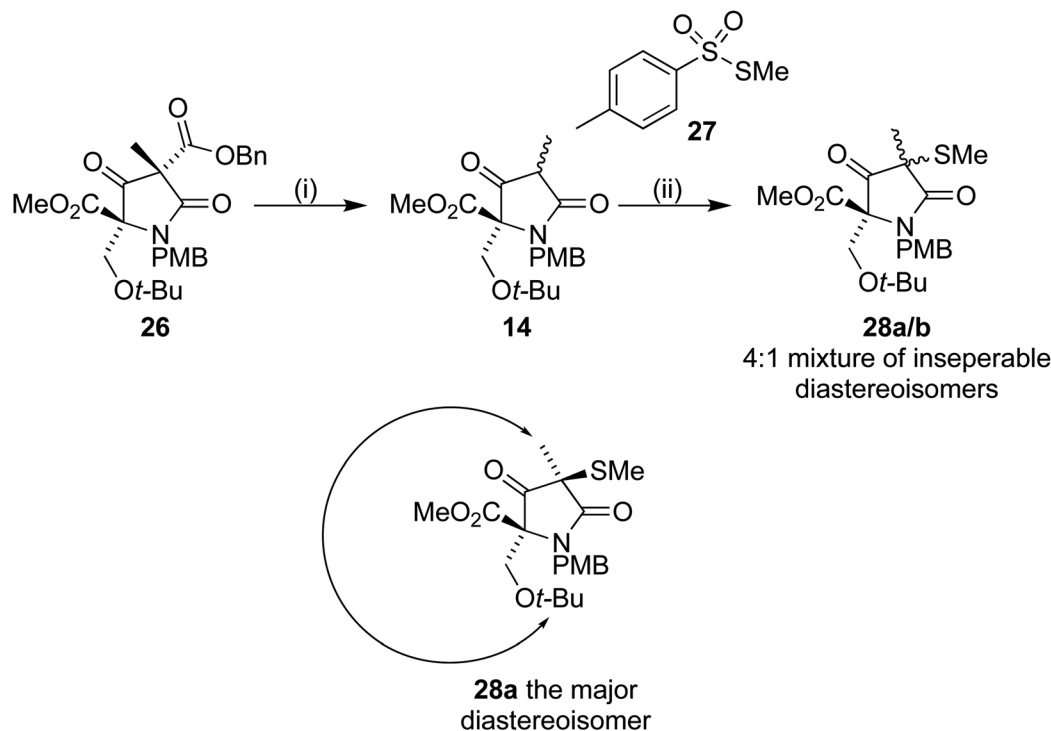
Scheme 7 Reagents and conditions: (i) LiHMDS, DMPU, THF, $-40\text{ }^{\circ}\text{C}$, 0.5 h then MeOCOCN, 3 h, $-40\text{ }^{\circ}\text{C}$; 69%.

surprise, analysis by chiral HPLC showed a drop in ee from 84% to 58% and 41% respectively for **29a** and **29b** (Scheme 9).

A mechanism for this epimerization did not seem obvious, and a simpler substrate was synthesized to probe

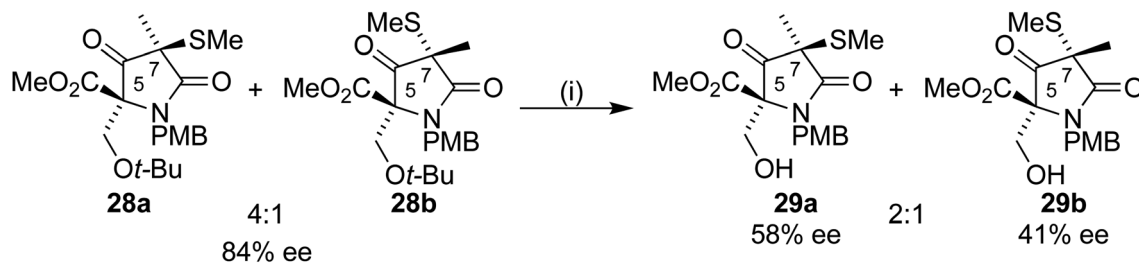
the reaction further. Use of methyl iodide as alkylating agent in place of **27** added a second methyl group to the C7 position, giving **30** (Scheme 10). This *gem*-methylated analogue of our substrate was chosen for two reasons, to eliminate a stereogenic centre, which could confirm C5 as the epimerizing centre, and to reduce the number of potentially reactive functional groups. Removal of the *t*-butyl group with TFA under the conditions used for **28a/b** gave **31** in racemic form. Further analysis revealed that the stereo-centre remained unchanged upon treatment of **30** with TFA. The racemization occurred during preparation for the purification by column chromatography, where crude **29a/b** was dissolved in DCM and adsorbed onto silica gel, perhaps a result of a retroaldol process. We were pleased to find that changing to a wet loading method, where crude **31** was loaded onto the chromatography column in the eluting solvent using petroleum ether/ethyl acetate, resulted in complete preservation of the ee.

Returning to our original system, the 4 : 1 mixture of **28a/b** was treated with TFA/DCM to produce **29a/b**. Analysis by ^1H NMR spectroscopy showed retention of the 4 : 1 ratio in the crude mixture. Silica gel was added, and the NMR spectrum obtained again, now showing a ratio of 3 : 1.3. After leaving **29a/b** in contact with the silica gel for 16 h, the ^1H NMR spectroscopy was repeated, the spectrum this time showing a 3 : 2 ratio. Despite largely preventing the epimerization, we were unable to separate the diastereoisomers **29a/b** efficiently. Because of this problem we changed the order of deprotection and reduction, aiming to produce the later Corey intermediate lactam **13** (Scheme 11).¹⁸



Scheme 8 Reagents and conditions (i) $\text{Pd}(\text{OH})_2/\text{C}$, H_2 , $35\text{ }^{\circ}\text{C}$, 16 h (ii) **27**, Et_3N , DCM, 4 h (70% over 2 steps).



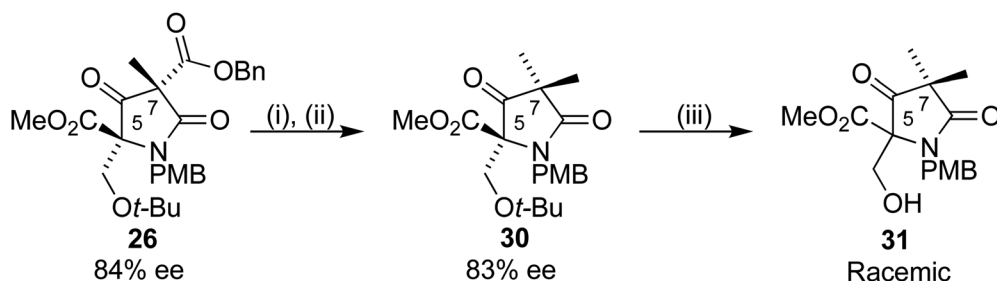


Scheme 9 Reagents and conditions: (i) TFA/DCM 1 : 1, 1.5 h; 76%.

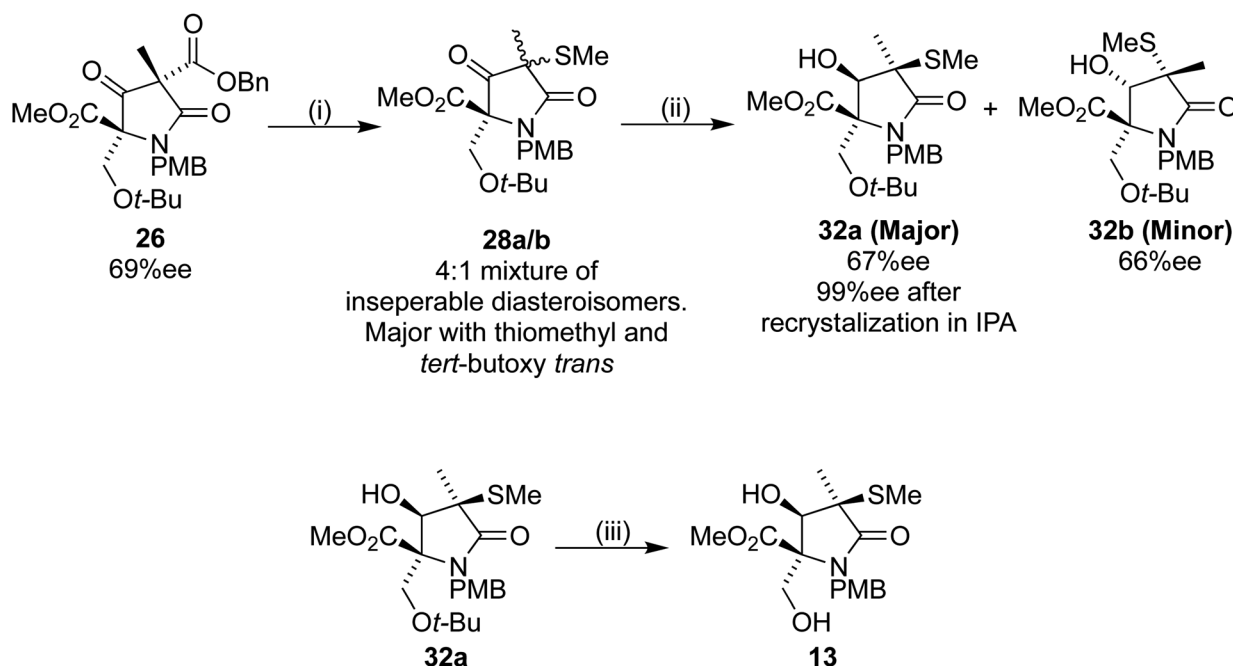
Sodium borohydride reduction of the inseparable 4 : 1 diastereoisomeric mixture **28a/b** led to two separable isomers **32a** and **32b** in a 4 : 1 ratio. Analysis of the products by HPLC using a chiral stationary phase indicated ees of 67% and 66% for **32a** and **32b** respectively. The desired first eluting diastereoisomer **32a** was recrystallized from IPA, to our delight providing **32a** with 99% ee. Treatment of the filtrate with TFA/

DCM resulted in the desired lactam **13** without further epimerization, thus completing our formal synthesis of (+)-lactacystin in six steps from **16**.

A formal synthesis of (+)-lactacystin has thus been completed in six steps from **16**. Lactam **13** has also been functionalized by Corey to give a variety of C9 analogues.¹⁹



Scheme 10 Reagents and conditions: (i) Pd(OH)₂/C, H₂, 35 °C, 16 h (ii) MeI, Et₃N, DCM, 4 h (46% over 2 steps), (iii) TFA/DCM 1 : 1 67%.



Scheme 11 Reagents and conditions (i) (1) Pd(OH)₂/C, H₂, 35 °C, 16 h, (2) **27**, Et₃N, DCM, 4 h (70% over 2 steps); (ii) NaBH₄, EtOH, 30 min, 0 °C: **32a**, 54%, **32b**, 13%; (iii) TFA/DCM 1 : 1, 75%.



Experimental detail

Benzyl (3*S*,5*S*)-3-methyl-5-(2-methylpropyl)-2,4-dioxypyrrolidine-3-carboxylate **20**

Lactam **10a** (1.2023 g, 2.83 mmol) was dissolved in a MeCN/water mixture (3 : 1, 27.6 mL). CAN (8.3 g, 14.22 mmol, 5 equiv.) was added and the solution stirred until complete consumption of the starting material had occurred (approx. 2 h). The solution was diluted with water (150 mL) and extracted with ethyl acetate (150 mL \times 3). The organic layers were combined and washed with brine (100 mL \times 2), dried (sodium sulfate), filtered, and the solvents removed under reduced pressure. The residue was purified by column chromatography using petroleum ether (40–60 °C)/ethyl acetate (8 : 2) as eluent to produce **20** as a colourless crystalline solid. (0.725 g, 84%). Mp 118–124 °C; $[\alpha]_D^{24} = +11.6$ (*c* 2.3, CHCl₃) (9% ee); ν_{\max} (thin film)/cm⁻¹: 3209, 2960, 1781, 1748, 1705; ¹H NMR (500 MHz, CDCl₃) δ_H 7.38–7.30 (m, 3H), 7.29–7.23 (m, 2H), 7.02–6.60 (m, 1H), 5.24–5.11 (m, 2H), 4.18 (dd, *J* = 9.5, 3.5 Hz, 1H), 1.78–1.68 (m, 2H), 1.53 (s, 3H), 1.43 (m, 1H), 0.96 (d, *J* = 6.2 Hz, 3H), 0.94 (d, *J* = 6.2 Hz, 3H); ¹³C NMR (126 MHz, CDCl₃) δ_C 206.7, 171.9, 165.5, 135.0, 128.8, 128.6, 127.9, 68.1, 61.4, 58.6, 41.3, 25.2, 23.2, 21.5, 15.9; HRMS (NSI-FTMS) *m/z* [M + NH₄]⁺ calcd for [C₁₇H₂₅N₂O₄]⁺ 321.1809, found 321.1812.

Sodium hydroxy(4-methoxyphenyl)methanesulfonate **18** (ref. 15)

p-Methoxybenzaldehyde (20 mL, 0.164 mol, 1.2 equiv.) was stirred in ethanol (330 mL). An aqueous solution of sodium sulfite (17.56 g, 0.139 mol in 34 mL) was added slowly, forming a cloudy precipitate. The mixture was stirred for 16 h at 30 °C, then stirred in an ice bath for 2 hours allowing further precipitation. The resulting suspension was filtered, and the collected solid washed with hexane and dried in a vacuum oven to yield the adduct sulphite **18** as a fluffy colourless solid (33.72 g, 81%). Mp 166–168 °C (decomp.) (lit²⁰ 155–157 °C (decomp.)); ν_{\max} (solid)/cm⁻¹ 3226, 1516, 1249; ¹H NMR (500 MHz, DMSO) δ_H 7.34 (d, *J* = 8.3 Hz, 2H), 6.79 (d, *J* = 8.8 Hz, 2H), 5.56 (d, *J* = 5.1 Hz, 1H), 4.87 (d, *J* = 5.0 Hz, 1H), 3.72 (s, 3H); ¹³C NMR (126 MHz, DMSO) δ_C 158.6, 131.6, 129.1, 112.6, 84.7, 55.2.

(*S*)-Methyl 3-(*tert*-butoxy)-2-((4-methoxybenzyl)amino)propanoate **19**

t-Bu-O-Serine methyl ester hydrochloride **17** (5 g, 23.61 mmol) was dissolved in methanol (250 mL). The sulfite salt **18** (9 g, 37.46 mmol, 1.6 equiv.) was added along with triethylamine (3 mL, 21.52 mmol, 0.9 equiv.). The stirred solution was cooled with an ice bath, and sodium cyanoborohydride (9.09 g, 144 mmol, 6.1 equiv.) added in small portions. The suspension was kept overnight and allowed to reach room temperature, the solvents removed, and the residue dissolved in ethyl acetate (200 mL). The organic solution was washed with equal amounts of brine, saturated sodium sulfite solution, and water, dried (magnesium sulfate), filtered, and the solvents removed under reduced pressure to give **19** (5.92 g, 85%), which was used in the next step without further purification. For analytical purposes, a portion was purified by column chromatography on silica gel using petroleum ether (40–60 °C)/ethyl acetate (9 : 1) as eluent

to yield **19** as a colourless oil. $[\alpha]_D^{24} = -23.61$ (*c* 1.05, CHCl₃) ν_{\max} (thin film)/cm⁻¹: 3434, 2974, 1743; ¹H NMR (500 MHz, CDCl₃) δ 7.25 (d, *J* = 7.5 Hz, 2H), 6.85 (d, *J* = 8.7 Hz, 2H), 3.83 (d, *J* = 12.8 Hz, 1H), 3.79 (s, 3H), 3.72 (s, 3H), 3.65 (d, *J* = 12.8 Hz, 1H), 3.60 (dd, *J* = 8.7, 5.3 Hz, 1H), 3.55 (dd, *J* = 8.7, 4.9 Hz, 1H), 3.43 (t, *J* = 5.1 Hz, 1H), 2.14 (s, 1H), 1.14 (s, 9H); ¹³C NMR (126 MHz, CDCl₃) δ 174.1, 158.8, 132.1, 129.7, 113.9, 73.3, 63.3, 61.1, 55.4, 51.8, 51.5, 27.5; HRMS (NSI-FTMS) *m/z* [M + H]⁺ calcd for [C₁₆H₂₆NO₄]⁺ 296.1856, found 296.1859.

(*S*)-Benzyl 3-((3-(*tert*-butoxy)-1-methoxy-1-oxopropan-2-yl)(4-methoxybenzyl)amino)-3-oxopropanoate **16**

Crude amine **19** (5.92 g, 20.05 mmol) was dissolved in anhydrous DCM (182 mL) in a flame-dried flask. The benzyl malonic half ester **8** (11.59 g, 49.89 mmol, 2.5 equiv.) was added along with EDAC·HCl (10.30 g, 53.72 mmol, 2.7 equiv.), DMAP (0.5 g, 4.1 mmol, 0.2 equiv.) and *N*-methyl morpholine (5.5 mL, 50 mmol, 2.5 equiv.). The solution was stirred for 16 h under an atmosphere of nitrogen. Aqueous HCl (1 M, 6 mL) was added, and the solution transferred into a separating funnel, and washed with water (200 mL) and brine (200 mL). The organic layer was dried (magnesium sulfate), filtered and the solvents removed under reduced pressure to give a yellow oil, which was purified by column chromatography on silica gel using petroleum ether (40–60 °C)/ethyl acetate (8 : 2) as eluent, to give **16** as a pale yellow oil. (5.493 g, 49% over the 2 steps) $[\alpha]_D^{24} = -23.61$ (*c* 1.05, CHCl₃) ν_{\max} (neat)/cm⁻¹: 3434, 2974, 1743; ¹H NMR (500 MHz, CDCl₃) (major conformation) δ_H 7.40–7.29 (m, 5H), 7.24 (d, *J* = 8.8 Hz, 2H), 6.86 (d, *J* = 8.7 Hz, 2H), 5.15 (s, 2H), 4.69 (s, 2H), 4.58 (dd, *J* = 7.5, 3.4 Hz, 1H), 3.90 (dd, *J* = 9.7, 7.6 Hz, 1H), 3.80–3.74 (m, 4H), 3.67 (s, 3H), 3.51 (d, *J* = 15.4 Hz, 1H), 3.43 (d, *J* = 15.4 Hz, 1H), 1.06 (s, 9H); ¹³C NMR (126 MHz, CDCl₃) δ_C 169.7, 167.3, 167.2, 159.1, 128.8, 128.6, 128.4, 128.1, 114.2, 73.4, 67.1, 60.7, 59.7, 55.4, 52.3, 52.1, 41.5, 27.3; HRMS (NSI-FTMS) *m/z* [M + H]⁺ calcd for [C₂₆H₃₄NO₇]⁺ 472.2330, found 472.2325. Determination of ee (97% ee) was carried out by HPLC using an AD-H Chiralpak column 90 : 10 hexane/IPA, 230 nm, 0.8 mL min⁻¹, 15 °C.

(3*S*,5*S*)-Benzyl 5-(*tert*-butoxymethyl)-1-(4-methoxybenzyl)-3-methyl-2,4-dioxypyrrolidine-3-carboxylate **15a** and (3*R*,5*S*)-benzyl 5-(*tert*-butoxymethyl)-1-(4-methoxybenzyl)-3-methyl-2,4-dioxypyrrolidine-3-carboxylate **15b**

Compound **16** (1.605 g, 3.4 mmol) was dissolved in ether (9.8 mL), TBAF (1 M in THF) (7 mL, 7 mmol, 2.1 equiv.) was added, and the solution stirred for 10 m. The solvents were removed under reduced pressure, and the residue placed under high vacuum for approximately 2 h. To the resulting colourless solid was added THF (9.8 mL), and the solution cooled to –12 °C. MeI (2.1 mL, 34 mmol, 10 equiv.) was added and the solution stirred for 72 h. Water (approximately 3 mL) was added and the solution was allowed to reach room temperature. The solution was dissolved in ethyl acetate (100 mL), and washed with water (100 mL) and brine (100 mL). The organic layer was dried (magnesium sulfate), filtered, and evaporated. The resulting brown oil was purified by column chromatography on silica gel using



petroleum ether (40–60 °C)/ethyl acetate (9 : 1) as the eluent, to produce **15a/b** as a colourless oil in a 3 : 1 ratio of diastereoisomers (1.0273 g, 67%) which could be partially separated. For the major, first eluting diastereoisomer **15a**: (0.491 g, 32%), $[\alpha]_D^{19.5} = -91.3$ (c 1.06, CHCl_3) (98% ee) ν_{max} (neat)/ cm^{-1} : 2934, 1781, 1749, 1698; ^1H NMR (400 MHz, CDCl_3) δ_{H} 7.39–7.33 (m, 3H), 7.28–7.23 (m, 2H), 7.01 (d, J = 8.4 Hz, 2H), 6.60 (d, J = 8.7 Hz, 2H), 5.38 (d, J = 15.1 Hz, 1H), 5.21 (d, J = 12.3 Hz, 1H), 5.10 (d, J = 12.3 Hz, 1H), 3.86 (t, J = 2.3 Hz, 1H), 3.83 (d, J = 15.1 Hz, 1H), 3.74 (s, 3H), 3.58 (dd, J = 9.9, 2.1 Hz, 1H), 3.53 (dd, J = 9.9, 2.4 Hz, 1H), 1.56 (s, 3H), 1.12 (s, 9H); ^{13}C NMR (126 MHz, CDCl_3) δ_{C} 205.0, 170.3, 166.0, 159.3, 135.0, 129.3, 128.8, 128.6, 128.4, 126.7, 114.3, 73.9, 68.2, 65.2, 58.8, 57.2, 55.4, 43.1, 27.3, 15.3; HRMS (NSI-FTMS) m/z $[\text{M} + \text{H}]^+$ calcd for $[\text{C}_{26}\text{H}_{32}\text{NO}_6]^+$ 254.2224, found 454.2218. Determined by HPLC to have 79% ee; recrystallization from IPA provided a supernatant of 98% ee. Determination of ee was carried out by HPLC using an AD-H Chiralpak column 80 : 20 hexane/IPA, 230 nm, 0.8 mL min^{-1} , 25 °C. For the partially purified, minor, second eluting diastereoisomer **15b**: (0.416 g, 35%), ^1H NMR (400 MHz, CDCl_3) δ 7.38–7.28 (m, 5H), 7.22 (d, J = 8.6 Hz, 2H), 6.82 (d, J = 8.7 Hz, 2H), 5.25–5.16 (m, 2H), 5.11 (d, J = 12.2 Hz, 1H), 4.31 (d, J = 14.3 Hz, 1H), 3.87 (dd, J = 7.2, 2.6 Hz, 1H), 3.78 (s, 3H), 3.59 (dd, J = 9.7, 2.5 Hz, 1H), 3.48 (dd, J = 9.7, 7.1 Hz, 1H), 1.52 (s, 3H), 1.02 (s, 9H). ^{13}C NMR (101 MHz, CDCl_3) δ 204.0, 169.6, 165.6, 159.4, 135.0, 130.0, 128.8, 128.7, 128.3, 128.0, 114.2, 74.0, 68.2, 65.5, 62.8, 58.5, 55.4, 44.5, 27.2, 16.7.

(2*R*,4*S*)-4-Benzyl 2-methyl 2-(*tert*-butoxymethyl)-1-(4-methoxybenzyl)-4-methyl-3,5-dioxypyrrolidine-2,4-dicarboxylate 26

Lactam **15a** (0.1125 g, 0.25 mmol), in a flame-dried flask, was dissolved in anhydrous THF (4.5 mL), cooled to –40 °C and placed under an atmosphere of nitrogen. LiHMDS (1 M in THF/ethylbenzene) (0.56 mL, 0.56 mmol, 2.2 equiv.) and DMPU (0.9 mL, 0.75 mmol, 3 equiv.) were added, and the solution allowed to stand for 0.5 h. Methyl cyanofornate (0.09 mL, 1.13 mmol, 4.5 equiv.) was added and stirring was continued for 3 h. Saturated aqueous NH_4Cl (0.2 mL) was added, and the mixture allowed to reach room temperature. Water (20 mL) was added and the mixture extracted with equal amounts of ethyl acetate twice. The combined organic extracts were washed with water (20 mL) and brine (2 × 20 mL), dried (sodium sulfate), filtered, and the solvents removed under reduced pressure. The residue was purified by column chromatography using petroleum ether (40–60 °C)/ethyl acetate (9 : 1) as eluent to yield **26** as a colourless crystalline solid (0.0881 g, 69%). Mp 90–94 °C $[\alpha]_D^{25} = +2.85$ (c 1.12, CHCl_3), ν_{max} (neat)/ cm^{-1} : 3434, 2974, 1743; ^1H NMR (500 MHz, CDCl_3) δ_{H} 7.39–7.30 (m, 5H), 7.18 (d, J = 8.7 Hz, 2H), 6.70 (d, J = 8.7 Hz, 2H), 5.24 (d, J = 12.5 Hz, 1H), 5.16 (d, J = 12.5 Hz, 1H), 4.72 (d, J = 15.3 Hz, 1H), 4.57 (d, J = 15.3 Hz, 1H), 3.83 (d, J = 9.7 Hz, 1H), 3.75 (s, 3H), 3.73 (d, J = 9.7 Hz, 1H), 3.53 (s, 3H), 1.66 (s, 3H), 0.87 (s, 9H); ^{13}C NMR (126 MHz, CDCl_3) δ_{C} 199.7, 170.7, 166.3, 165.1, 159.0, 135.1, 129.5, 128.7, 128.6, 128.5, 128.0, 113.7, 74.1, 68.1, 61.0, 57.7, 55.5, 53.4, 44.7, 26.8, 18.9; HRMS (NSI-FTMS) m/z $[\text{M} + \text{H}]^+$ calcd for $[\text{C}_{28}\text{H}_{34}\text{NO}_8]^+$ 512.2279, found 512.2271. Determination of ee was carried out

by HPLC using an AD-H Chiralpak column 90 : 10 hexane/IPA, 230 nm, 0.8 mL min^{-1} , 25 °C.

Methyl (2*S*)-2-anilino-3-*tert*-butoxypropanoate 33 (ref. 21)

Compound **17** (1.249 g, 5.9 mmol) was dissolved in anhydrous DCM (40 mL). Phenyl boronic acid (1.447 g, 11.94 mmol, 2 equiv.) was added, together with $\text{Cu}(\text{OAc})_2$ (1.2 g, 6.6 mmol, 1.1 equiv.). Triethylamine (1.65 mL, 11.8 mmol, 2 equiv.) and molecular sieve (4 Å, 4.4 g) were added, and the mixture was placed under a static atmosphere of oxygen and stirred for 3 days. Aqueous ammonium hydroxide (1 M, 40 mL) was added, and the suspension stirred for 40 min and filtered through diatomaceous earth. The organic layer was removed, and the aqueous layer extracted with DCM (50 mL). The organic layers were combined, washed with water (80 mL) and brine (80 mL), dried (magnesium sulfate), filtered, and the solvents removed under reduced pressure. The residue was purified by column chromatography using petroleum ether (40–60 °C)/ether 18 : 1 as eluent to yield **33** as a cream coloured solid (0.319 g, 22%). Recrystallization from petroleum ether gave crystals of 95% ee. Mp 52–59 °C (lit²¹ 47–50 °C); $[\alpha]_D^{22} = -13.69$ (c 1.11, CHCl_3) (95% ee) $[\alpha]_D^{24} = (\text{lit}^{21} [\alpha]_D^{24} = -10.7$ (c 1.1, CHCl_3) (71% ee)); ν_{max} (neat)/ cm^{-1} : 3398, 2975, 1751; ^1H NMR (500 MHz, CDCl_3) δ_{H} 7.20–7.14 (m, 2H), 7.75 (td, J = 7.4, 0.8 Hz, 1H), 6.64 (d, J = 8.2 Hz, 2H), 4.62 (s, 1H), 4.20 (t, J = 4.1 Hz, 1H), 3.78 (dd, J = 8.8, 4.0 Hz, 1H), 3.73 (s, 3H), 3.69 (dd, J = 8.8, 4.2 Hz, 1H), 1.17 (s, 9H); ^{13}C NMR (126 MHz, CDCl_3) δ_{C} 172.0, 146.9, 129.40, 118.5, 113.8, 73.70, 62.60, 57.4, 52.3, 27.5. Determination of ee was carried out by HPLC using a Eurocel 01 Knauer column 90 : 10 hexane/IPA, 230 nm, 0.8 mL min^{-1} , 25 °C.

(*S*)-Benzyl 3-((3-(*tert*-butoxy)-1-methoxy-1-oxopropan-2-yl)(phenyl)amino)-3-oxopropanoate 24

Amine **33** (0.2308 g, 0.91 mmol) was dissolved in anhydrous DCM (7 mL) in a flame-dried flask. The benzyl malonic half ester **8** (0.47 g, 2.02 mmol, 2.2 equiv.) was added together with EDAC·HCl (0.467 g, 2.4 mmol 2.7 equiv.), DMAP (0.021 g, 0.17 mmol, 0.19 equiv.) and *N*-methylmorpholine (0.23 mL, 2.09 mmol, 2.3 equiv.). The mixture was stirred for 16 h under a nitrogen atmosphere. Aqueous HCl (0.3 mL, 1 M solution) was added, and the solution diluted with DCM (50 mL). The solution was washed with water (50 mL) and brine (50 mL). The organic layer was dried (magnesium sulfate), filtered, and the solvents removed under reduced pressure to give a yellow oil, which was purified by column chromatography using petroleum ether (40–60 °C)/ethyl acetate (9 : 1 to 7 : 3) as eluent to yield **24** as colourless oil (0.1136 g, 71%). $[\alpha]_D^{23} = +1.48$ (c 0.54, CHCl_3) (95% ee) ν_{max} (neat)/ cm^{-1} : 3022, 2974, 1743, 1663; ^1H NMR (500 MHz, CDCl_3) δ_{H} 7.45 (s, 2H), 7.38–7.28 (m, 8H), 5.10 (s, 2H), 4.69–4.64 (m, 1H), 3.81–3.78 (m, 2H), 3.75 (s, 3H), 3.24 (d, J = 15.7 Hz, 1H), 3.20 (d, J = 15.8 Hz, 1H), 1.08 (s, 9H). ^{13}C NMR (126 MHz, CDCl_3) δ_{C} 169.8, 167.3, 166.4, 141.5, 135.6, 129.6, 129.5, 128.8, 128.6, 128.5, 128.4, 73.5, 67.1, 62.0, 59.2, 52.4, 42.2, 27.4; HRMS (NSI-FTMS) m/z $[\text{M} + \text{H}]^+$ calcd for $[\text{C}_{24}\text{H}_{30}\text{NO}_6]^+$ 428.2068, found 428.2065. Determination of ee was carried out by HPLC using an



AD-H Chiralpak column 80 : 20 hexane/IPA, 230 nm, 0.8 mL min⁻¹, 25 °C.

(3*S*,5*S*)-Benzyl 5-(*tert*-butoxymethyl)-3-methyl-2,4-dioxo-1-phenylpyrrolidine-3-carboxylate 25

The Dieckmann cyclization precursor **24** (0.104 g, 0.24 mmol) was dissolved in ether (0.67 mL), TBAF (1 M in THF), (0.5 mL, 0.5 mmol, 2.1 equiv.) added, and the solution stirred for 10 min. The solvents were removed under reduced pressure, and the residue placed under high vacuum for approximately 2 h. THF (0.67 mL) was added, and the solution cooled to -12 °C. MeI (0.07 mL, 1.12 mmol, 4.7 equiv.) was added, and the suspension stirred for 72 h. Water (0.1 mL) was added, the solution allowed to reach room temperature, ethyl acetate (30 mL) added, and the solution washed with water (30 mL) and brine (30 mL). The organic layer was dried (magnesium sulfate), filtered, and the solvents removed under reduced pressure. The resulting brown oil was purified by column chromatography using petroleum ether (40–60 °C)/ethyl acetate (9 : 1) as the eluent. Only the major diastereoisomer **25** could be isolated (0.0081 g, 8%) mp 121–124 °C [α_D^{25} = +14.2 (*c* 0.81, CHCl₃) (87% ee) ν_{\max} (neat)/cm⁻¹: 2977, 2253, 1782, 1752, 1702; ¹H NMR (500 MHz, CDCl₃) δ_H 7.44–7.23 (m, 10H), 5.18 (s, 2H), 4.57 (t, *J* = 2.1 Hz, 1H), 3.68 (dd, *J* = 9.6, 1.8 Hz, 1H), 3.39 (dd, *J* = 9.6, 2.4 Hz, 1H), 1.65 (s, 3H), 1.02 (s, 9H); ¹³C NMR (126 MHz, CDCl₃) δ_C 204.5, 169.5, 165.8, 135.8, 135.2, 129.4, 128.8, 128.5, 127.8, 127.5, 125.6, 73.9, 68.8, 68.0, 59.7, 57.9, 27.2, 15.2; HRMS (NSI-FTMS) *m/z* [M + H]⁺ calcd for [C₂₄H₂₈NO₅S]⁺ 410.1962, found 410.1956. Determination of ee (87% ee) was carried out by HPLC using an AD-H Chiralpak column 80 : 20 hexane/IPA, 230 nm, 0.8 mL min⁻¹, 25 °C.

Potassium 3-methoxy-3-oxopropanoate 34 (ref. 22)

KOH (22.06 g, 393.18 mmol, 1.2 equiv.) was dissolved in methanol (75 mL). The resulting solution was added to a solution of dimethyl malonate (38 mL, 332.5 mmol) in methanol (85 mL). A colourless precipitate was formed, which was collected by vacuum filtration and dried in a vacuum oven to provide (26.35 g, 51%) of **34** as a colourless solid. Mp: 204–209 °C (lit²³ 204–207 °C); ν_{\max} (solid)/cm⁻¹: 1726, 1595, 1368; ¹H NMR (500 MHz, D₂O) δ_H 3.75 (s, 3H), 3.34 (s, 2H); ¹³C NMR (101 MHz, D₂O) δ_C 174.8, 172.6, 53.2, 45.0.

(*S*)-Methyl 3-(*tert*-butoxy)-2-(3-methoxy-*N*-(4-methoxybenzyl)-3-oxopropanamido)propanoate 21

Amine **19** (0.5558 g, 1.88 mmol) was dissolved in anhydrous DCM (17.5 mL) in a flame-dried flask. The methyl malonic half ester **34** (0.692 g, 4.43 mmol, 2.4 equiv.) was added together with EDAC·HCl (1.06 g, 5.53 mmol, 2.9 equiv.), DMAP (0.0405 g, 0.33 mmol, 0.18 equiv.) and *N*-methylmorpholine (0.5 mL, 4.54 mmol, 2.4 equiv.). The mixture was stirred for 24 h under an atmosphere of nitrogen. Aqueous HCl (1 M, 0.5 mL) was added, and the solution washed with water (20 mL) and brine (20 mL). The organic layer was dried (magnesium sulfate), filtered, and the solvents removed under reduced pressure to give a yellow oil, which was purified by column chromatography

using petroleum ether (40–60 °C)/ethyl acetate (8 : 2) as eluent to yield the desired compound **21** as a pale-yellow oil (0.54 g, 73%). [α_D^{25} = -42.0 (*c* 1.13, CHCl₃); ν_{\max} (neat)/cm⁻¹: 2973, 1744, 1656, 1514; ¹H NMR (400 MHz, CDCl₃) δ_H 7.29 (d, *J* = 7.5 Hz, 2H), 6.91 (d, *J* = 8.7 Hz, 2H), 4.73 (s, 2H), 4.60 (dd, *J* = 7.6, 3.4 Hz, 1H), 3.93 (dd, *J* = 9.7, 7.6 Hz, 1H), 3.86–3.81 (m, 4H), 3.74 (s, 3H), 3.72 (s, 3H), 3.50 (d, *J* = 15.3 Hz, 1H), 3.40 (d, *J* = 15.3 Hz, 1H), 1.10 (s, 9H); ¹³C NMR (101 MHz, CDCl₃) δ_C 169.7, 167.8, 167.4, 159.2, 128.8, 128.1, 114.2, 73.5, 60.7, 59.8, 55.4, 52.5, 52.3, 52.2, 41.3, 27.3; HRMS (NSI-FTMS) *m/z* [M + H]⁺ calcd for [C₂₀H₃₀NO₇]⁺ 396.2017, found 396.2016.

(3*S*,5*S*)-Methyl 5-(*tert*-butoxymethyl)-1-(4-methoxybenzyl)-3-methyl-2,4-dioxopyrrolidine-3-carboxylate 22a and (3*R*,5*S*)-methyl 5-(*tert*-butoxymethyl)-1-(4-methoxybenzyl)-3-methyl-2,4-dioxopyrrolidine-3-carboxylate 22b

The Dieckmann cyclization precursor **21** (0.2219 g, 0.56 mmol) was dissolved in ether (1.6 mL). TBAF (1 M in THF), (1.6 mL, 1.6 mmol, 3 equiv.) was added, and the solution stirred for 5 min. The solvents were removed under reduced pressure, and the resulting brown oil was dissolved in THF (1.6 mL). The solution was cooled to -12 °C, MeI (0.15 mL, 2.4 mmol, 4.3 equiv.) added, and the suspension stirred for 58 h. Water (1 mL) was added, and the mixture added to a short silica gel column. The column was eluted with ethyl acetate. The 3 : 1 mixture of diastereoisomers **22a/b** was collected as a yellow oil and used onto the next step without further purification (0.1832 g, 87%). Data for the major diastereoisomer **22a**: ¹H NMR (400 MHz, CDCl₃) δ_H 7.21 (d, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 8.6 Hz, 2H), 5.35 (d, *J* = 15.0 Hz, 1H), 3.93 (d, *J* = 15.0 Hz, 1H), 3.93 (t, *J* = 2.2 Hz, 1H), 3.80 (s, 3H), 3.73 (s, 3H), 3.60 (dd, *J* = 9.8, 2.2 Hz, 1H), 3.56 (dd, *J* = 9.9, 2.4 Hz, 1H), 1.53 (s, 3H), 1.11 (s, 9H). ¹³C NMR (101 MHz, CDCl₃) δ_C 205.0, 170.5, 166.7, 159.5, 129.5, 127.2, 114.4, 73.9, 65.4, 58.7, 57.4, 55.5, 53.4, 43.4, 27.3, 15.2. Data for the minor diastereoisomer **22b**: ¹H NMR (400 MHz, CDCl₃) δ_H 7.23 (d, *J* = 6.6 Hz, 2H), 6.85 (d, *J* = 7.2 Hz, 2H), 5.27 (d, *J* = 14.6 Hz, 1H), 4.23 (d, *J* = 14.5 Hz, 1H), 3.86 (dd, *J* = 5.8, 2.7 Hz, 1H), 3.79 (s, 3H), 3.75 (s, 3H), 3.69 (m, 2H), 1.52 (s, 3H), 1.17 (s, 9H). ¹³C NMR (101 MHz, CDCl₃) δ_C 204.0, 169.0, 166.3, 159.5, 129.0, 127.8, 114.3, 74.0, 65.1, 61.3, 58.3, 55.4, 53.4, 44.2, 27.4, 17.4. Data for both diastereoisomers: ν_{\max} (neat)/cm⁻¹: 2974, 1782, 1750, 1698, 1514; HRMS (NSI-FTMS) *m/z* [M + H]⁺ calcd for [C₂₀H₂₈NO₆]⁺ 378.1911, found 378.1913.

(3*S*,5*S*)-Methyl 5-(*tert*-butoxymethyl)-3-methyl-2,4-dioxopyrrolidine-3-carboxylate 23a and (3*R*,5*S*)-methyl 5-(*tert*-butoxymethyl)-3-methyl-2,4-dioxopyrrolidine-3-carboxylate 23b

The mixture of diastereoisomers **22a/b** (0.1251 g, 0.331 mmol) was dissolved in a MeCN/H₂O mixture (3 : 1) (3.5 mL). CAN (0.9 g, 1.64 mmol, 5 equiv.) was added, and the solution stirred vigorously until consumption of the starting material had occurred (approx. 1.5 h). Ethyl acetate (25 mL) was added, and the solution washed with water (25 mL). The aqueous layer was extracted with ethyl acetate (25 mL). The



combined organic layers were washed with water (25 mL) and brine (25 mL), dried (magnesium sulfate), filtered, and the solvents removed under reduced pressure. The crude material was purified by column chromatography using petroleum ether (40–60 °C)/ethyl acetate (8 : 2–7 : 3) as the eluent to give a mixture of partially separable diastereoisomers (0.0483 g, 57% total) from which only **23a** could be obtained uncontaminated (0.01 g, 12%). Data for the major diastereoisomer **23a**: mp 111–116 °C; $[\alpha]_D^{24} = -46.2$ (c 0.91, CHCl_3); ν_{max} (neat)/ cm^{-1} : 3234, 2976, 1785, 1750, 1706; ^1H NMR (400 MHz, CDCl_3) δ_{H} 6.72 (d, J = 39.7 Hz, 1H), 4.18 (dd, J = 9.2, 3.6 Hz, 1H), 3.77–3.69 (m, 4H), 3.53 (t, J = 9.1 Hz, 1H), 1.55 (s, 3H), 1.19 (s, 9H); ^{13}C NMR (101 MHz, CDCl_3) δ_{C} 204.4, 171.4, 166.2, 74.3, 63.4, 62.4, 58.5, 53.6, 27.5, 16.7; HRMS (NSI-FTMS) m/z $[\text{M} + \text{H}]^+$ calcd for $[\text{C}_{12}\text{H}_{20}\text{NO}_5]^+$ 258.1336, found 258.1339. Data for the minor diastereoisomer **23b**: ^1H NMR (400 MHz, CDCl_3) δ_{H} 6.73–6.31 (m, 1H), 4.31 (t, J = 3.6 Hz, 1H), 3.74 (s, 3H), 3.61 (d, J = 3.6 Hz, 2H), 1.51 (s, 3H), 1.14 (s, 9H); ^{13}C NMR (101 MHz, CDCl_3) δ_{C} 205.2, 172.2, 166.3, 74.1, 63.5, 61.0, 58.6, 53.6, 27.4, 15.1.

(2R,4R)-Methyl 2-(tert-butoxymethyl)-1-(4-methoxybenzyl)-4-methyl-4-(methylthio)-3,5-dioxypyrrolidine-2-carboxylate 28a and (2R,4S)-methyl 2-(tert-butoxymethyl)-1-(4-methoxybenzyl)-4-methyl-4-(methylthio)-3,5-dioxypyrrolidine-2-carboxylate 28b

Methyl ester **26** (0.1426 g, 0.31 mmol) was dissolved in THF (0.7 mL), and the solution heated to 35 °C. A micro-spatula of $\text{Pd}(\text{OH})_2/\text{C}$ was added and the stirred solution placed under a static atmosphere of hydrogen. After 16 h the solution was filtered through diatomaceous earth and washed with DCM. The solvents were removed under reduced pressure to produce a foam, which was dissolved in anhydrous DCM (0.5 mL) in a flame-dried flask. **27** (0.1090 g, 0.53 mmol, 1.7 equiv.) was added together with triethylamine (0.05 mL, 0.35 mmol, 1.1 equiv.). The solution was stirred at 20 °C for 5 h under an atmosphere of nitrogen, and the solvents removed under reduced pressure to give a yellow oil, which was purified by column chromatography using petroleum ether (40–60 °C)/ethyl acetate (9 : 1) as the eluent to yield a 4 : 1 mixture of inseparable diastereoisomers **28a/b** as a pale-yellow oil (0.127 g, 70%). Data for the major diastereoisomer **28a**: ^1H NMR (500 MHz, CDCl_3) δ_{H} 7.26 (d, J = 8.7 Hz, 2H), 6.82 (d, J = 8.7 Hz, 2H), 4.74 (d, J = 15.2 Hz, 1H), 4.44 (d, J = 15.2 Hz, 1H), 3.89 (d, J = 9.6 Hz, 1H), 3.78 (s, 3H), 3.72 (d, J = 9.6 Hz, 1H), 3.47 (s, 3H), 2.16 (s, 3H), 1.57 (s, 3H), 1.01 (s, 9H); ^{13}C NMR (101 MHz, CDCl_3) δ_{C} 201.6, 172.5, 166.2, 159.3, 130.3, 128.4, 113.8, 76.2, 74.3, 60.3, 55.4, 52.0, 49.7, 44.1, 27.1, 18.0, 12.6. Data for the minor diastereoisomer **28b**: ^1H NMR (500 MHz, CDCl_3) δ_{C} 7.26 (d, J = 8.7 Hz, 2H), 6.82 (d, J = 8.7 Hz, 2H), 4.84 (d, J = 15.1 Hz, 1H), 4.36 (d, J = 15.1 Hz, 1H), 4.00 (d, J = 10.2 Hz, 1H), 3.86 (d, J = 10.2 Hz, 1H), 3.78 (s, 3H), 3.37 (s, 3H), 2.20 (s, 3H), 1.61 (s, 3H), 1.08 (s, 9H); ^{13}C NMR (101 MHz, CDCl_3) δ_{C} 203.4, 172.7, 166.7, 159.3, 130.4, 128.0, 113.8, 75.8, 74.4, 59.7, 55.4, 53.1, 50.7, 44.2, 27.1, 20.7, 12.9. Data for both diastereoisomers: ν_{max} (neat)/ cm^{-1} : 3020, 1741, 1699; HRMS (NSI-FTMS) m/z $[\text{M} + \text{H}]^+$ calcd for $[\text{C}_{21}\text{H}_{30}\text{NO}_6\text{S}]^+$ 424.1788, found 424.1790.

(2R,4R)-Methyl 2-(hydroxymethyl)-1-(4-methoxybenzyl)-4-methyl-4-(methylthio)-3,5-dioxypyrrolidine-2-carboxylate 29a (ref. 17) and (2R,4S)-methyl 2-(hydroxymethyl)-1-(4-methoxybenzyl)-4-methyl-4-(methylthio)-3,5-dioxypyrrolidine-2-carboxylate 29b

The 4 : 1 mixture of inseparable diastereoisomers **28a/b** (0.0297 g, 0.07 mmol) was dissolved in anhydrous DCM (0.15 mL) in a flame-dried flask, and TFA (0.15 mL) added. The solution was stirred under nitrogen for 1.5 h until TLC showed complete consumption of starting material. The solution was diluted with DCM (50 mL), and washed with water (50 mL). The organic layer was washed with aqueous NaHCO_3 (50 mL), dried (sodium sulfate), filtered, and the solvents removed under reduced pressure to give a brown residue, which was purified by column chromatography using petroleum ether (40–60 °C)/ethyl acetate (2 : 1) as eluent to yield **29a/b** as a 2 : 1 mixture of inseparable isomers (0.0195 g, 76%). Data for the major diastereomer **29a**: ^1H NMR (400 MHz, CDCl_3) δ_{H} 7.33 (d, J = 8.6 Hz, 2H), 6.86 (d, J = 8.6 Hz, 2H), 5.08 (d, J = 15.2 Hz, 1H), 4.35 (d, J = 15.2 Hz, 1H), 4.17 (d, J = 12.0 Hz, 1H), 3.79 (d, J = 12.8 Hz, 4H), 3.67 (s, 3H), 2.12 (s, 3H), 1.54 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ_{C} 199.1, 172.2, 165.7, 159.7, 129.9, 128.8, 114.6, 77.6, 61.8, 55.4, 53.4, 49.7, 44.3, 16.9, 12.4. Analysis by HPLC determined the ee to be 58% ee. Data for the minor diastereomer **29b**: ^1H NMR (400 MHz, CDCl_3) δ_{H} 7.27 (d, J = 9.7 Hz, 2H), 6.84 (d, J = 7.2 Hz, 2H), 4.68 (d, J = 15.0 Hz, 1H), 4.60 (d, J = 15.0 Hz, 1H), 4.20 (d, J = 11.8 Hz, 1H), 4.07 (d, J = 12.6 Hz, 1H), 3.77 (s, 3H), 3.42 (s, 3H), 2.17 (s, 3H), 1.61 (s, 3H); ^{13}C NMR (101 MHz, CDCl_3) δ_{C} 200.3, 172.2, 166.7, 159.6, 130.3, 127.9, 114.3, 76.6, 62.01, 55.4, 53.4, 49.5, 44.4, 18.1, 12.2. Analysis by HPLC determined the ee to be 41% ee. Data for both diastereoisomers: ν_{max} (neat)/ cm^{-1} : 3419, 3000, 2932, 1776, 1742, 1699; HRMS (NSI-FTMS) m/z $[\text{M} + \text{H}]^+$ calcd for $[\text{C}_{17}\text{H}_{22}\text{NO}_6\text{S}]^+$ 368.1162, found 368.1165. Determination of ee was carried out by HPLC using an AD-H Chiralpak column, 80 : 20 hexane/IPA, 230 nm, 0.8 mL min^{-1} , 25 °C.

(R)-Methyl 2-(tert-butoxymethyl)-1-(4-methoxybenzyl)-4,4-dimethyl-3,5-dioxypyrrolidine-2-carboxylate 30

Methyl ester **26** (0.0832 g, 0.16 mmol) was dissolved in THF (0.5 mL) and the solution heated to 35 °C. $\text{Pd}(\text{OH})_2/\text{C}$ (20% nominally) (0.604 g) was added and the solution placed under a static atmosphere of hydrogen. After 16 h the solution was filtered through diatomaceous earth and washed through with DCM. The solvents were removed under reduced pressure to produce a foam which was dissolved in DCM (0.6 mL). The solution was stirred under an atmosphere of nitrogen and triethylamine (0.04 mL, 0.29 mmol, 1.8 equiv.) added. After 15 min, MeI was added (0.04 mL 0.64 mmol, 4 equiv.), and the solution stirred for a further 5 h, diluted with DCM (50 mL), and washed with water (50 mL) and brine (50 mL). The organic layer was dried (sodium sulfate), filtered, the solvents were removed under reduced pressure, and the residue purified by column chromatography using petroleum ether (40–60 °C)/ethyl acetate (8 : 2) as eluent to produce a pink oil. The oil was dissolved in DCM and washed with a saturated sodium thiosulfate solution until the organic layer became colourless, dried (sodium sulfate), filtered, and the solvents removed under reduced



pressure to produce **30** as a colourless oil (0.0286 g, 46% over the 2 steps). $[\alpha]_D^{22} = +50$ (c 0.31, CHCl_3); ν_{max} (neat)/ cm^{-1} : 3019, 2978, 1779, 1742; ^1H NMR (500 MHz, CDCl_3) δ_{H} 7.22 (d, $J = 8.7$ Hz, 2H), 6.81 (d, $J = 8.7$ Hz, 2H), 4.87 (d, $J = 15.1$ Hz, 1H), 4.19 (d, $J = 15.1$ Hz, 1H), 3.89 (d, $J = 9.8$ Hz, 1H), 3.77 (s, 3H), 3.75 (d, $J = 9.8$ Hz, 1H), 3.31 (s, 3H), 1.34 (s, 3H), 1.28 (s, 3H), 1.05 (s, 9H); ^{13}C NMR (126 MHz, CDCl_3) δ_{C} 209.1, 177.3, 166.9, 159.3, 130.5, 128.2, 113.8, 75.8, 74.1, 58.9, 55.4, 52.8, 46.1, 43.3, 27.2, 22.2, 20.6; HRMS (NSI-FTMS) m/z $[\text{M} + \text{H}]^+$ calcd for $[\text{C}_{21}\text{H}_{30}\text{NO}_6]^+$ 392.2068, found 392.2068. Determination of ee was carried out by HPLC using an Eurocel 01 Knauer column, 95 : 5 hexane/IPA, 230 nm, 0.8 mL min^{-1} , 25 °C.

(R)-Methyl 2-(hydroxymethyl)-1-(4-methoxybenzyl)-4,4-dimethyl-3,5-dioxopyrrolidine-2-carboxylate **31**

Lactam **30** (0.0109 g, 0.027 mmol) was dissolved in dry DCM (0.1 mL), and TFA (0.1 mL) added. The solution was stirred under nitrogen for 1.5 h until TLC showed complete consumption of starting material. The solution was diluted to 50 mL with DCM and washed with water (50 mL). The organic layer was washed with a saturated NaHCO_3 solution (50 mL), dried (sodium sulfate), filtered, and the solvents removed under reduced pressure to give a colourless solid, which was purified by column chromatography using petroleum ether (40–60 °C)/ethyl acetate (2 : 1) as eluent to yield the deprotected alcohol **31** as a gum (0.0062 g, 67%). $[\alpha]_D^{24} = -7.74$ (c 0.62, CHCl_3) (86% ee) ν_{max} (neat)/ cm^{-1} : 3396, 2919, 1778, 1742, 1678; ^1H NMR (500 MHz, CDCl_3) δ_{H} 7.30 (d, $J = 8.7$ Hz, 2H), 6.86 (d, $J = 8.7$ Hz, 2H), 4.89 (d, $J = 15.1$ Hz, 1H), 4.31 (d, $J = 15.1$ Hz, 1H), 4.14 (dd, $J = 12.3$, 8.6 Hz, 1H), 3.85 (dd, $J = 12.3$, 4.4 Hz, 1H), 3.79 (s, 3H), 3.55 (s, 3H), 1.35 (s, 3H), 1.28 (s, 3H), 1.17 (dd, $J = 8.6$, 4.5 Hz, 1H); ^{13}C NMR (126 MHz, CDCl_3) δ_{C} 208.3, 177.3, 166.4, 159.6, 130.1, 128.7, 114.5, 77.3, 60.8, 55.4, 53.2, 46.1, 43.7, 22.0, 20.5; HRMS (NSI-FTMS) m/z $[\text{M} + \text{H}]^+$ calcd for $[\text{C}_{17}\text{H}_{22}\text{NO}_6]^+$ 336.1442, found 336.1443. Determination of ee (86% ee) was carried out by HPLC using an AD-H Chiralpak column, 80 : 20 hexane/IPA, 230 nm, 0.8 mL min^{-1} , 25 °C.

(2R,3R,4R)-Methyl 2-(tert-butoxymethyl)-3-hydroxy-1-(4-methoxybenzyl)-4-methyl-4-(methylthio)-5-oxopyrrolidine-2-carboxylate **32a** and (2R,3S,4S)-methyl 2-(tert-butoxymethyl)-3-hydroxy-1-(4-methoxybenzyl)-4-methyl-4-(methylthio)-5-oxopyrrolidine-2-carboxylate **32b**

The mixture of diastereoisomers **28a/b** (0.1407 g, 0.33 mmol) was dissolved in ethanol (9 mL) and the solution cooled with an ice bath. Sodium borohydride (0.0073 g, 0.19 mmol, 0.6 equiv.) was added, and the mixture stirred for 20 min. Water (40 mL) was added, and the resulting solution extracted with ethyl acetate (3 \times 40 mL). The combined organic extracts were washed with brine (120 mL), dried (sodium sulfate), filtered, and the solvents removed under reduced pressure. The residue was purified using column chromatography using petroleum ether (40–60 °C)/ethyl acetate (8 : 2–2 : 1) as eluent to provide two separable diastereoisomers as gums, **32a**, the first eluting diastereoisomer (0.0764 g, 54%) and **32b**, the second (0.0181 g, 13%). Analysis by chiral HPLC showed the ees of the

diastereoisomers to be 67% and 66% ee respectively. Diastereomer **32a** upon recrystallization from IPA gave 0.0511 g of material from the supernatant at 99% ee. Data for major, first eluting diastereoisomer **32a**: $[\alpha]_D^{23} = +6.2$ (c 0.71, CHCl_3) (99% ee) ν_{max} (neat)/ cm^{-1} : 3418, 2973, 2926, 1743, 1697; ^1H NMR (500 MHz, CDCl_3) δ_{H} 7.24 (d, $J = 8.7$ Hz, 2H), 6.81 (d, $J = 8.7$ Hz, 2H), 4.71 (d, $J = 15.2$ Hz, 1H), 4.46 (d, $J = 15.2$ Hz, 1H), 3.99–3.87 (m, 2H), 3.82 (d, $J = 9.7$ Hz, 1H), 3.78 (s, 3H), 3.65 (s, 3H), 3.44 (d, $J = 9.7$ Hz, 1H), 2.12 (s, 3H), 1.60 (s, 3H), 1.04 (s, 9H); ^{13}C NMR (126 MHz, CDCl_3) δ_{C} 173.0, 172.1, 158.9, 130.4, 129.6, 113.7, 77.9, 74.1, 70.7, 62.8, 55.4, 53.4, 52.5, 45.2, 27.2, 22.9, 12.3; HRMS (NSI-FTMS) m/z $[\text{M} + \text{H}]^+$ calcd for $[\text{C}_{21}\text{H}_{32}\text{NO}_6\text{S}]^+$ 426.1945, found 426.1942. Data for the minor, second eluting diastereomer **32b**: $[\alpha]_D^{22} = +5.26$ (c 0.38, CHCl_3) (66% ee) ν_{max} (neat)/ cm^{-1} : 3385, 3016, 2975, 1743, 1686; ^1H NMR (500 MHz, CDCl_3) δ_{H} 7.18 (d, $J = 8.6$ Hz, 2H), 6.81 (d, $J = 8.7$ Hz, 2H), 4.77 (d, $J = 15.5$ Hz, 1H), 4.43 (d, $J = 15.5$ Hz, 1H), 4.32 (s, 1H), 3.96 (d, $J = 9.6$ Hz, 1H), 3.77 (s, 3H), 3.75 (s, 1H), 3.67 (d, $J = 9.7$ Hz, 1H), 3.64 (s, 3H), 2.19 (s, 3H), 1.53 (s, 3H), 1.02 (s, 9H); ^{13}C NMR (126 MHz, CDCl_3) δ_{C} 173.8, 171.7, 158.8, 130.3, 128.9, 113.7, 78.0, 74.4, 71.6, 62.1, 55.7, 55.4, 52.7, 45.3, 27.1, 22.6, 12.7; HRMS (NSI-FTMS) m/z $[\text{M} + \text{H}]^+$ calcd for $[\text{C}_{21}\text{H}_{32}\text{NO}_6\text{S}]^+$ 426.1945, found 426.1945. Determination of ee was carried out by HPLC using an AD-H Chiralpak column, 90 : 10 hexane/IPA, 230 nm, 0.8 mL min^{-1} , 25 °C.

(2R,3R,4R)-Methyl 3-hydroxy-2-(hydroxymethyl)-1-(4-methoxybenzyl)-4-methyl-4-(methylthio)-5-oxopyrrolidine-2-carboxylate **13** (ref. 18)

Alcohol **32a** (0.0427 g, 0.1 mmol) was dissolved in anhydrous DCM (0.21 mL) in a flame-dried flask. TFA (0.21 mL) was added, and the mixture stirred under an atmosphere of nitrogen until TLC showed complete consumption of starting material (about 1.5 h). The mixture was diluted with DCM (20 mL) and washed with water (20 mL). The aqueous layer was extracted with DCM (20 mL), and the combined organic layers were washed with saturated aqueous NaHCO_3 (20 mL) and brine (20 mL), dried (sodium sulfate), filtered, and the solvents removed under reduced pressure. The residue was purified by column chromatography using petroleum ether (40–60 °C)/ethyl acetate 1 : 1 as eluent to yield **13** as a colourless solid (0.0279 g, 75%). Mp 128–130 °C (lit¹⁸ 129 °C); $[\alpha]_D^{23} = -33.84$ (c 0.13, CHCl_3) (lit¹⁸ $[\alpha]_D^{23} = -41.8$ (c 0.1, CHCl_3)) ν_{max} (neat)/ cm^{-1} : 3416, 2925, 2852, 1737, 1675; ^1H NMR (500 MHz, CDCl_3) δ_{H} 7.29 (d, $J = 8.5$ Hz, 2H), 6.85 (d, $J = 8.7$ Hz, 2H), 5.11 (d, $J = 15.3$ Hz, 1H), 4.13 (d, $J = 7.7$ Hz, 1H), 4.05 (d, $J = 15.3$ Hz, 1H), 3.85–3.77 (m, 5H), 3.76 (s, 3H), 3.67 (d, $J = 8.1$ Hz, 1H), 2.14 (s, 3H), 1.61 (s, 3H); ^{13}C NMR (126 MHz, CDCl_3) δ_{C} 173.5, 171.6, 159.5, 129.8, 129.6, 114.6, 76.8, 72.4, 62.5, 55.4, 53.4, 52.9, 44.8, 22.9, 12.4; HRMS (NSI-FTMS) m/z $[\text{M} + \text{H}]^+$ calcd for $[\text{C}_{17}\text{H}_{24}\text{NO}_6\text{S}]^+$ 370.1319, found 370.1320. Determination of ee was carried out by HPLC using an AD-H Chiralpak column, 80 : 20 hexane/IPA, 230 nm, 0.8 mL min^{-1} , 25 °C.

Conflicts of interest

There are no conflicts to declare.



Acknowledgements

This investigation has enjoyed the support of Charnwood Molecular Ltd and the University of East Anglia.

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