Amino Acids (2013) 45:1157–1168
DOI 10.1007/s00726-013-1571-4

ORIGINAL ARTICLE

The synthesis and biological testing of bacilysin analogues

Keith Robertson · Cormac D. Murphy · Francesca Paradisi

Received: 30 April 2013 / Accepted: 23 July 2013 / Published online: 6 August 2013

Abstract A series of compounds based on the structure of bacilysin were synthesised and tested for antibacterial activity. The key steps in the syntheses are the coupling of an iodide to a diketopiperazine (DKP) and mono-lactim ether scaffold, respectively. The diastereoselectivity of the coupling reactions was dependant on the scaffold, with selectivity for DKP of about 4:1 and mono-lactim ether exceeding 98:2. Subsequent elaboration of the compounds to give open chain dipeptides and DKPs that mimic the structure of bacilysin but substitute the epoxy ketone for a saturated or unsaturated ketone is described. Overall yield from coupling to final product was between 5 and 21 %, with the yield of the saturated products notably higher. The open chain dipeptides demonstrated moderate antibacterial and antifungal activity.

Keywords Anticapsin · *Bacillus subtilis* · Dipeptide · Diketopiperazine

Introduction

Infectious diseases remain one of the leading causes of death worldwide even with the widespread availability of antimicrobial drugs (Mathers et al. 2009). This is due in no small part to the growing prevalence of pathogens that are resistant to many of the current library of antibiotics (Davies and Davies 2010). In spite of this, the developmental pipelines of the major pharmaceutical companies contain few antimicrobials (Spellberg et al. 2004; So et al. 2011). Classical screening programmes assaying for bioactivity have high re-discovery rates, thus alternative approaches for the development of new antibiotics are required.

The dipeptide bacilysin (**1**) (originally termed bacillin and occasionally known as tetaine) is one of the simplest known antibiotics and is composed of alanine and the non-proteinogenic amino acid anticapsin (Fig. 1). It was first isolated by Foster and Woodruff (1946) from the soil bacterium *Bacillus subtilis* and is active against a range of bacteria and fungi. Bacilysin enters the cell via dipeptide transport systems and is subsequently cleaved by host peptidases to release the active agent anticapsin (Kenig et al. 1976; Chmara et al. 1982), which is a competitive irreversible inhibitor of glucosamine-6-phosphate (GlcN-6-P) synthase (Perry and Abraham 1979; Kenig et al. 1976; Chmara 1985; Chmara et al. 1984). One possible mechanism of inhibition is shown in Scheme 1 and suggests that opening of the epoxide is preceded by nucleophilic attack by a cysteine residue on the active site the carbonyl of anticapsin. The importance of the ketone moiety of anticapsin was demonstrated by inhibition studies with non-stereospecific analogues of bacilysin and anticapsin lacking the ketone moiety (Chmara et al. 1982), which were shown to be much less effective inhibitors of GlcN-6-P synthase than the natural compounds. Furthermore, studies in aqueous environments have shown that epoxyketones such as anticapsin tend to exist in equilibrium between the ketone and the hydrated hemiacetal which would tend to

K. Robertson · F. Paradisi (✉)
School of Chemistry and Chemical Biology, Centre for Synthesis and Chemical Biology, University College Dublin, Dublin, Ireland
e-mail: Francesca.paradisi@ucd.ie

C. D. Murphy (✉)
School of Biomolecular and Biomedical Science, Centre for Synthesis and Chemical Biology, University College Dublin, Dublin, Ireland
e-mail: Cormac.d.murphy@ucd.ie

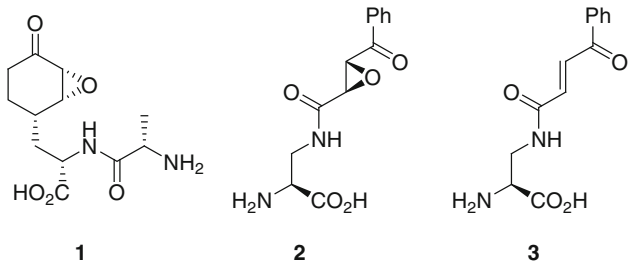
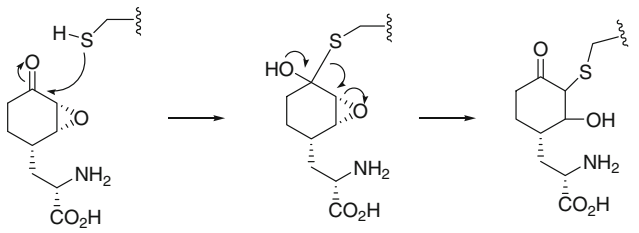


Fig. 1 The structure of bacilysin (**1**) and N^3-oxoacyl derivatives of 1-2,3-diaminopropanoic acid (**2** and **3**), known inhibitors of GlcN-6-P synthase



Scheme 1 One possible mechanism of anticapsin inhibition of GlcN-6-P synthase by covalently bonding to the sulphydryl group in the glutamine binding site of GlcN-6-P synthase

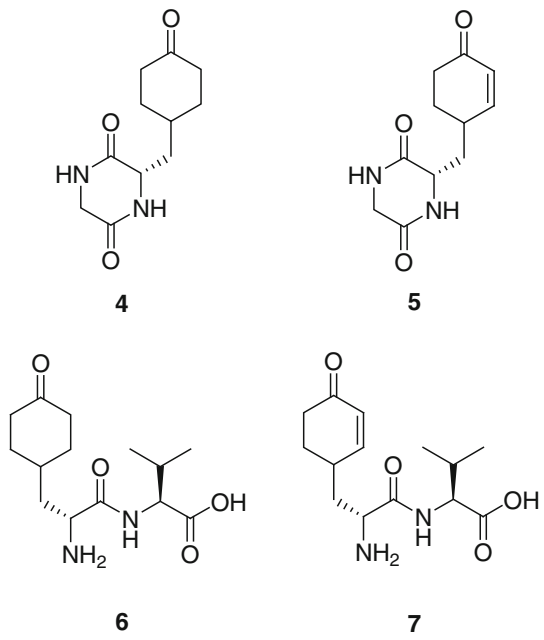


Fig. 2 The bacilysin analogues synthesised in this study

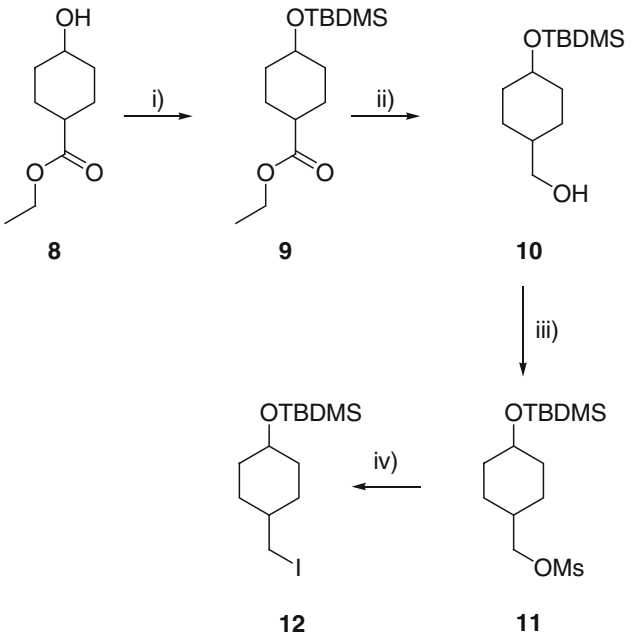
lower the electrophilicity of the epoxyketone and favour the proposed mechanism (Wipf et al. 1998; Baldwin et al. 1995). The lack of GlcN-6-P brought about by the inhibition of GlcN-6-P synthase is tolerated by mammals, at least in the short term, but is sufficiently detrimental to microbes that a number of anti-microbial compounds targeting the enzyme have been reported (Fig. 1) (Wojciechowski et al. 2005; Jedrzejczak et al. 2012; Bearne and Blouin 2000; Gonzalez-Ibarra et al. 2010; Bates et al. 1966).

Given the antimicrobial potential of bacilysin and related compounds we report here a new synthetic strategy for the production of bacilysin analogues (Fig. 2) and their antimicrobial activities. Such analogues might be useful to further explore the mechanism of GlcN-6-P synthase inhibition, since no enzymological studies have yet been conducted to investigate the importance of the epoxide.

Results and discussion

There are a number of synthetic routes to bacilysin and anticapsin available in the literature, with the first enantioselective versions published independently by Wild (1994) and Baldwin et al. (1995). Our initial synthesis planned to make use of a masked α,β–unsaturated alkylating agent in conjunction with either a diketopiperazine (DKP) or mono-lactim ether (MLE) depending on the desired product. The initial strategy was to prepare this alkylating agent via a Diels–Alder reaction of a Danishefsky-type diene with acrolein, which would be activated to the iodide in a Finkelstein reaction. However, the iodination of (4-(tert-butyldimethylsilyloxy)-2-methoxycyclohex-3-enyl) methyl methanesulfonate in refluxing acetone furnished 4-methylenecyclohex-2-enone instead of the expected iodide (Lestini et al. 2012). An alternative strategy was designed utilising a saturated alkylating agent similar to the synthesis described by Wild (1994) (Scheme 2). Ethyl 4-hydroxycyclohexanecarboxylate **8** as a mixture of *cis* and *trans* isomers was commercially available; the hydroxyl group was silylated using tert-butyldimethylsilyl triflate and triethylamine in THF. Cleavage of the ester to give a hydroxyl group was accomplished using lithium aluminium hydride in THF at 0 °C. The exposed hydroxyl group of **10** was subsequently activated to the iodide **12**, via the mesylate. This was accomplished in good yield and thus the alkylating agent was available on a multi-gram scale for use in further synthesis.

The MLE was synthesised as previously reported (Paradisi et al. 2001); however, to allow for milder deprotecting conditions of the amide, *para*-methoxybenzyl chloride instead of benzyl chloride was employed in the first step (Scheme 3). The cleavage of the benzyl group could be performed under Birch conditions which would reduce the unsaturated ketone. Indeed, as was the case for similar syntheses (Wild 1994; Baldwin et al. 1995), it was observed that basic conditions promoted a 1,4-intramolecular addition of the amide onto the enone system.

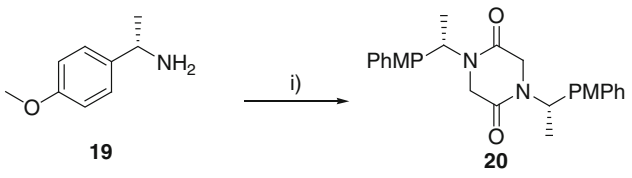


Scheme 2 *i* TBDMSOTf, Et_3N, CH_2Cl_2, 0 °C *ii* $LiAlH_4$, THF, 0 °C *iii* MsCl, Et_3N, CH_2Cl_2 *iv* NaI, acetone

By contrast, the *para*-methoxybenzyl protecting group was easily cleaved with cerium ammonium nitrate which left the enone moiety intact. Despite special care taken both in the work-up and in subsequent steps to avoid basic conditions, the yield was lower than expected and may be partially justified by the propensity of the enone system to undergo addition.

The DKP was synthesised in a one pot reaction as previously described by O'Reilly et al. (2009) (Scheme 4).

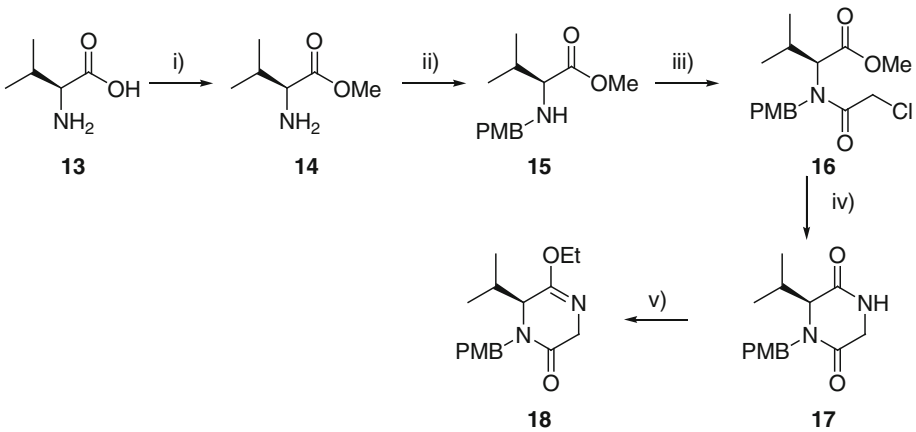
Again *para*-methoxy benzyl-methyl amine was used instead of the classic benzyl-methyl amine. The coupling of **12** to either **18** or **20** was accomplished by treating with lithium hexamethyldisilyl azide at −10 °C in THF and subsequently alkylating with **12** at −78 °C (Scheme 5). As expected, the newly generated chiral centre of the MLE

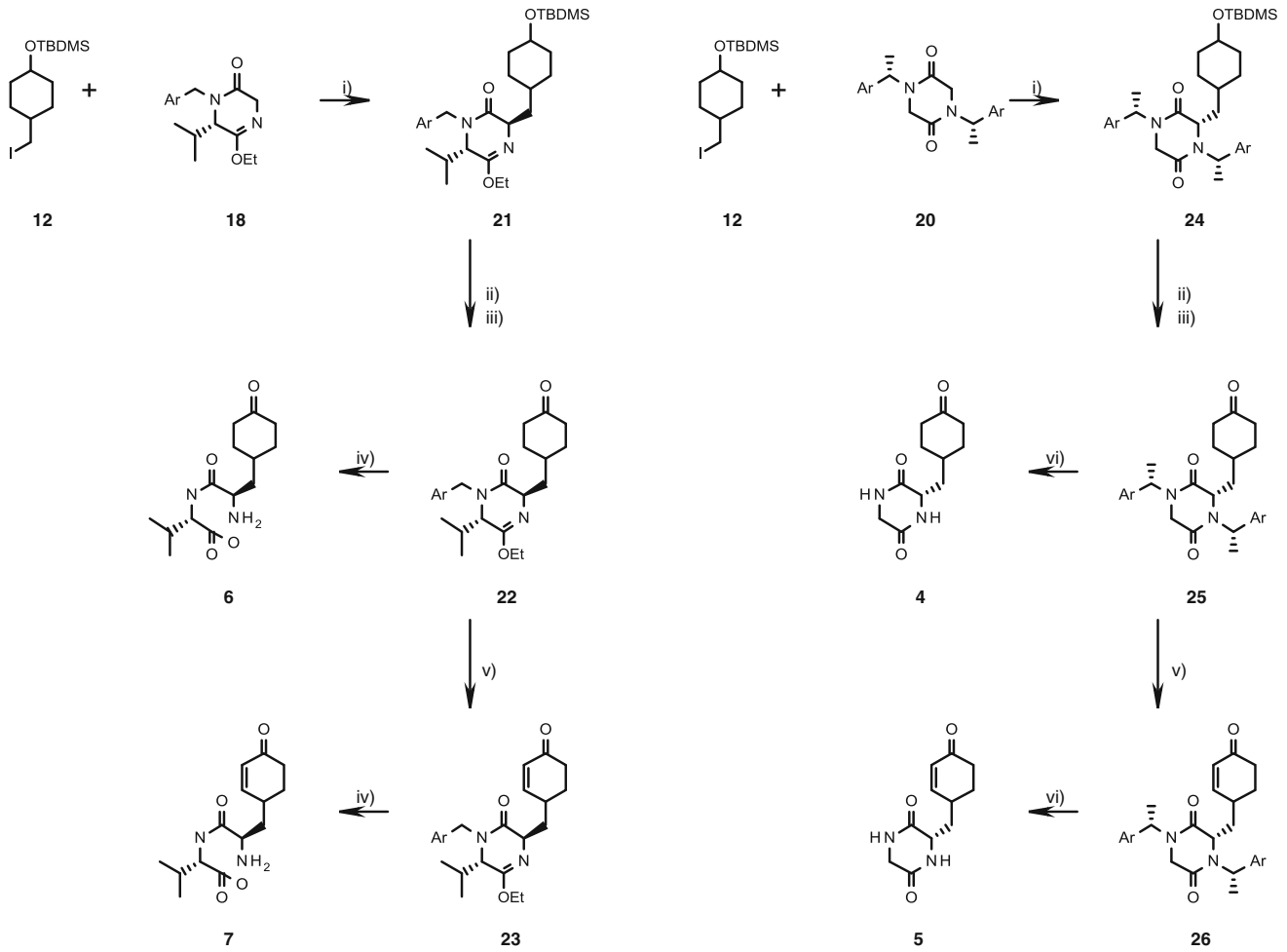


Scheme 4 *i* (a) Chloroacetyl chloride, CH_2Cl_2, $NaOH_{(aq)}$ 0 °C (b) Triethylbenzylammonium chloride

was *trans* to the iso-propyl group in a 98:2 ratio (R:S). The DKP gave the alkylated group in an *S* configuration with a ratio of 81:19. These values are comparable to those reported in the literature (O'Reilly et al. 2009; Davies et al. 2007). The silyl protecting group of the DKP adduct was cleaved with acetic acid; however, for the MLE adduct TBAF was used to avoid the cleavage of the lactim bond, which occurs under mild acidic conditions. The exposed alcohol moiety was then oxidised under Swern conditions to give the ketones **22** and **25** in good yield. The *para*-methoxy benzyl protecting groups were cleaved using CAN in acetonitrile/water. The best results for this deprotection were obtained with the minimal requirements of two equivalents of CAN, as excess unreacted reagent proved slightly difficult to purify from the product. Purification after deprotection yielded **4** in the case of the DKP adduct, whereas to generate the dipeptide **6,** a partial purification had to be followed by a mild acidic hydrolysis and then a more stringent purification. The unsaturated ketone moiety was generated in both cases starting from **22** or **25** by use of an iodic acid-dimethylsulphoxide complex as described by Nicolaou et al. (2002). The advantage of this technique was that it allowed the enone system to be generated in a single step from the ketone, albeit racemically. **23** and **26** were then subjected to the deprotection and purification or deprotection, partial purification, acidic hydrolysis and purification as outlined previously to give **5** and **7**, both as racemic mixtures. The yields were significantly lower than for the saturated analogues, probably as a

Scheme 3 *i* $SOCl_2$, MeOH, 0 °C *ii* PMBCl, pyridine, CH_2Cl_2, 0 °C *iii* $ClCOCH_2Cl$, Et_3N, CH_2Cl_2, 0 °C *iv* NH_3, EtOH *v* Et_3OBF_4, CH_2Cl_2





Scheme 5 *i* (a) **18/20**, LHMDS, THF, −10 °C (b) **12**, THF, −78 °C *ii* TBAF, THF or CH_3CO_2H, THF, H_2O *iii* Oxalyl chloride, DMSO, Et_3N, CH_2Cl_2, −78 °C *iv* (a) CAN, CH_3CN, H_2O (b) 0.5 M HCl, acetone, 40 °C *v* HIO_3, DMSO, cyclohexene, 50 °C *vi* CAN, CH_3CN, H_2O

result of the aforementioned intramolecular addition of the amide onto the enone system, which could be avoided by reducing the unsaturated ketone to the vinyl alcohol before the deprotection step and then oxidising it back to the enone as one of the final steps (Wild 1994; Baldwin et al. 1995). However, this would have added an additional two steps to the overall route and in this case the drop in yield was tolerated.

Initial screening for antimicrobial activity of **4**–**7** was carried out using the Kirby Bauer disc diffusion assay against standard laboratory strains of *Staphylococcus aureus*, *Escherichia coli*, *Saccharomyces cerevisiae* and *Candida albicans*. The open chain dipeptides **6** and **7** showed activity (zones of clearing, 6–8 mm) against both strains of yeast and to a lesser extent against the Gram positive *S. aureus*, but not the Gram negative *E. coli*. However, the DKPs **4** and **5** showed no sign of activity against any of the microbes. Additional testing against a range of antibiotic-resistant bacteria and clinical isolates was conducted, but it was found that the minimum inhibitory concentrations for all compounds were greater than 3.2 μg mL^{-1} and no effect was observed on the growth of the bacteria. Although no authentic bacilysin was available for direct comparison, previous bioassay employing comparable methods (Kenig and Abraham 1976) indicates that **6** and **7** are weaker antimicrobial agents than the natural product.

Conclusion

Novel bacilysin analogues were prepared via coupling of an iodinated alkalyating reagent with either a DKP or mono-lactim ether. The antimicrobial activity of the open chain analogues was modest, suggesting that the epoxide moiety is significant in the inhibition of GlcN-6-P synthase. The DKPs had no antimicrobial activity possibly because of poor uptake into cells and/or incomplete hydrolysis of the compounds to release the anticapsin-like warhead. Nevertheless, the synthetic strategy might be employed to

generate further bacilysin-like compounds with improved biological properties.

Experimental

All chemicals were purchased from Sigma-Aldrich unless otherwise stated. Dry solvents were obtained from a Puresol Grubbs Speciality Chemicals and Stream. Where necessary reactions were performed under a nitrogen atmosphere, using oven-dried glassware. Oxygen-free nitrogen was obtained from BOC gases and used without further drying.

^{1}H and ^{13}C-NMR spectra were recorded on a Varian NMR System 500 MHz, Varian NMR System 400 MHz or Varian NMR System 300 MHz System spectrometer. High resolution mass spectra were obtained using a Waters/Micromass instrument. Removal of solvent under reduced pressure refers to the removal of solvent on a Büchi rotary evaporator with an integrated vacuum pump. Thin-layer chromatography (TLC) was carried out on Merck aluminium backed 60 F254 silica gel. Purifications were carried out using forced flow chromatography on Merck silica gel 60 (0.040–0.063 nm) or Merck aluminium oxide, 90, standardised.

Ethyl 4-(*tert*-butyldimethylsilyloxy)cyclohexanecarboxylate(mixture of *trans* and *cis* isomers) (**9**)

8 (1.62 mL, 10 mmol) was dissolved in dry CH_2Cl_2 (35 mL) under nitrogen with activated 4 Å molecular sieves. The stirred solution was cooled to 0 °C and triethyl amine (2.8 mL, 20 mmol) was added followed by *tert*-butyldimethylsilyl triflate (2.52 mL, 11 mmol) dropwise. The mixture was allowed to warm to room temperature and stirred for 12 h when TLC showed consumption of starting material. The reaction was filtered and diluted with CH_2Cl_2 (30 mL). The organic layer was washed with saturated $NaHCO_3$ solution (25 mL), water (25 mL), brine (25 mL) and dried with Na_2SO_4, and solvent was removed under reduced pressure. The resulting yellow residue was purified on silica using 4:1 cyclohexane:EtOAc as an eluent yielding 2.72 g ethyl 4-(tert-butyldimethylsilyloxy)cyclohexanecarboxylate as a pale yellow oil (95 % yield).

Major isomer: ^{1}H NMR (500 MHz, Chloroform-d) δ 4.17–4.07 (m, 2H, overlap with minor isomer $C\underline{H}_2CH_3$), 3.89 (p, $J = 2.1$ Hz, 1H, $C\underline{H}OSi$), 2.32–2.25 (m, 1H, $C\underline{H}CO_2$), 1.99–1.87 (m, 3H, overlap with minor isomer), 1.69–1.61 (m, 3H, overlap with minor isomer), 1.54–1.41 (m, 2H), 1.26–1.24 (m, 3H, $CH_2C\underline{H}_3$ overlap with minor isomer), 0.89 (s, 9H, $SiC(CH_3)_3$), 0.03 (s, 6H $Si(CH_3)_2$). ^{13}C NMR (126 MHz, Chloroform-d) δ 175.9 ($\underline{C}O_2Et$), 66.9 ($H\underline{C}OTBDMS$), 60.2 ($O\underline{C}H_2CH_3$), 42.3 ($\underline{C}HCO_2Et$), 33.0 (($\underline{C}H_2)_2CH$(OTBDMS), 26.0 ($SiC(\underline{C}H_3)_3$), 23.6 (($\underline{C}H_2)_2CH(CH_2OEt)$), 18.3 ($Si\underline{C}(CH_3)_3$), 14.4 ($CH_2\underline{C}H_3$), −4.7 ($Si(\underline{C}H_3)_2$). HR-MS: *m/z* Calcd for $C_{15}H_{30}O_3NaSi$ $(M + Na)^+$: 309.1862; found 309.1876.

Minor isomer: ^{1}H NMR (500 MHz, Chloroform-d) δ 4.12–4.07 (m, 2H, overlap with major isomer, $C\underline{H}_2CH_3$), 3.60–3.53 (m, 1H, $C\underline{H}OSi$), 2.25–2.18 (m, 1H, $C\underline{H}CO_2$), 1.99–1.86 (m, 3H, overlap with major isomer), 1.69–1.61 (m, 3H, overlap with major isomer), 1.52–1.42 (m, 2H, overlap with major isomer), 1.24–1.22 (m, 3H, overlap with major isomer), 0.88 (s, 9H, $SiC(CH_3)_3$), 0.05 (s, 6H, $Si(CH_3)_2$). ^{13}C NMR (126 MHz, Chloroform-d) δ 175.8 9 ($\underline{C}O_2Et$), 70.7 ($H\underline{C}OTBDMS$), 60.3 ($O\underline{C}H_2CH_3$), 42.5 ($\underline{C}HCO_2Et$), 35.0 (($\underline{C}H_2)_2CH$(OTBDMS), 27.4 (($\underline{C}H_2)_2CH(CH_2OEt)$), 26.0 ($SiC(\underline{C}H_3)_3$), 18.3 ($Si\underline{C}(CH_3)_3$), 14.4 ($CH_2\underline{C}H_3$), −4.5 ($Si(\underline{C}H_3)_2$).

(4-(*Tert*-butyldimethylsilyloxy)cyclohexyl)methanol (**10**)

9 (2.72 g, 9.5 mmol) was dissolved in dry THF (22 mL) under nitrogen and cooled to 0 °C. Lithium aluminium hydride (0.43 g, 11.3 mmol) was added in portions. The mixture was allowed to warm to room temperature and stirred for 5 h when TLC showed completion of reaction. The reaction mixture was quenched by careful, slow addition of ice cold water (5 mL). The mixture was acidified using 1 M HCl to pH 2. The mixture was filtered and the layers separated. The aqueous layer was extracted a further three times with CH_2Cl_2 (35 mL). The combined organic layer was washed with saturated $NaHCO_3$ solution (20 mL), water (20 mL) and brine (20 mL). The organic layer was then dried with $MgSO_4$ and solvent was removed under reduced pressure to give **10** as a pale yellow oil in 94 % yield (2.18 g).

Major isomer: ^{1}H NMR (500 MHz, Chloroform-d) δ 3.90 (m, 1H, $C\underline{H}OSi$), 3.12 (d, $J = 5.7$ Hz, 2H, $C\underline{H}_2OH$), 1.89–0.90 (9H, aliphatic H, overlap with minor isomer, 0.89 (s, 9H, $(C\underline{H}_3)_3CSi$), 0.03 (s, 6H, $(C\underline{H}_3)_2Si$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 68.4($\underline{C}HOTBDMS$), 67.1 ($\underline{C}H_2OH$), 39.7 ($\underline{C}HCH_2OH$), 33.0 (($\underline{C}H_2)_2CH$(OTBDMS), 26.0 ($SiC(\underline{C}H_3)_3$), 23.6, 18.3 ($Si\underline{C}(CH_3)_3$), −4.7 ($Si(\underline{C}H_3)_2$). HR-MS: *m/z* Calcd for $C_{13}H_{28}O_2NaSi$ $(M + Na)^+$: 267.1756; found: 267.1760.

Minor isomer: ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.56–3.49 (m, 1H, $C\underline{H}OSi$), 3.44 (d, $J = 6.3$ Hz, 2H, $C\underline{H}_2OH$), 1.92–0.92 (m, 9H, aliphatic H, overlap with major isomer), 0.88 (s, 9H, $(C\underline{H}_3)_3CSi$, overlap with major isomer), 0.03 (s, 6H, $(C\underline{H}_3)_2Si$, overlap with major isomer). ^{13}C NMR (126 MHz, $CDCl_3$) δ 71.9 ($\underline{C}HOTBDMS$), 68.3 ($\underline{C}H_2OH$), 39.8 ($\underline{C}HCH_2OH$), 35.5 (($\underline{C}H_2)_2CH$(OTBDMS), 27.9 (($\underline{C}H_2)_2CH(CH_2OH)$), 26.1 ($SiC(\underline{C}H_3)_3$), 18.4 ($Si\underline{C}(CH_3)_3$), −4.4 ($Si(\underline{C}H_3)_2$).

(4-(*Tert*-butyldimethylsilyloxy)cyclohexyl)methyl methanesulfonate (**11**)

10 (2.18 g, 8.9 mmol) was dissolved in dry CH_2Cl_2 (20 mL) under nitrogen with activated 4 Å molecular sieves. The solution was cooled to 0 °C and triethyl amine (3.12 mL, 22.25 mmol) was added. Methanesulphonyl chloride (0.8 mL, 9.8 mmol) was added dropwise to the mixture. The reaction was allowed to warm to room temperature and stirred for 72 h when TLC showed disappearance of starting material. The reaction was worked up by filtering to remove molecular sieves and washing the reaction mixture with saturated $NaHCO_3$ solution (20 mL), water (20 mL) and brine (20 mL). The organic layer was then dried with $MgSO_4$ and solvent was removed under reduced pressure to give 2.71 g (94 % yield) of **11** as a pale yellow oil. No further purification was necessary.

Major isomer: ^{1}H NMR (500 MHz, Chloroform-d) δ 4.04 (d, $J = 6.9$ Hz, 2H, C$\underline{H}_2$OMs), 3.99–3.96 (m, 1H, C$\underline{H}$OTBDMS), 2.99 (s, 3H, SC$\underline{H}_3$), 1.93–1.78 (m, 2H), 1.72–1.62 (m, 2H), 1.48–1.43 (m, 3H), 1.31 (qd, 13.6, 3.9 Hz, 1H), 1.07 (qd, $J = 13.5$, 3.6 Hz, 1H), 0.89 (s, 9H, SiC(C$\underline{H}_3$)$_3$), 0.03 (s, 6H, Si(C$\underline{H}_3$)$_2$). ^{13}C NMR (126 MHz, Chloroform-d) δ 74.7 (C$\underline{H}$OTBDMS), 66.4 (C$\underline{H}_2$OMs), 37.4 (C$\underline{H}$CH$_2$OMs), 36.6 ((C$\underline{H}_2$)$_2$CHOTBDMS), 32.6 ((C$\underline{H}_2$)$_2$CHCH$_2$OMs), 26.0 (SiC(C$\underline{H}_3$)$_3$), 23.2 (SC$\underline{H}_3$), 18.2 (SiC(C$\underline{H}_3$)$_3$), −4.7 (Si(C$\underline{H}_3$)$_2$). HR-MS: *m/z* Calcd for $C_{14}H_{30}O_4NaSSi$ $(M + Na)^+$: 345.1532; found 345.1543.

Minor isomer: ^{1}H NMR (500 MHz, Chloroform-d) δ 4.03 (d, $J = 6.6$ Hz, 2H), 3.53 (m, 1H), 4.04 (s, 3H, overlap with major isomer), 1.95–1.03 (m, 9H, overlap with major isomer), 0.88 (s, 9H), 0.05 (s, 6H).

Tert-butyl(4-(iodomethyl)cyclohexyloxy) dimethylsilane (**12**)

11 (2.71 g, 8.4 mmol) and sodium iodide (3.15 g, 21 mmol) were dissolved in acetone (30 mL) and refluxed for 12 h when TLC showed disappearance of starting material. The reaction was worked up by removal of the solvent under reduced pressure; the residue was dissolved in a mixture of CH_2Cl_2 (35 mL) and water (35 mL). The layers were separated and the aqueous layer was extracted a further three times with CH_2Cl_2 (15 mL). The organic layers were combined, washed with brine (25 mL) dried with $MgSO_4$ and solvent was removed under reduced pressure to give a yellow oil. The crude mixture was purified on silica using a 5:1 mixture of cyclohexane: ethyl acetate as an eluent to give **12** as a clear non-viscous oil in 83 % yield (2.47 g).

^{1}H NMR (500 MHz, Chloroform-d) δ 3.92–3.87 (m, 1H, C$\underline{H}$CH$_2$I), 3.12 (d, $J = 5.7$ Hz, 2H, C$\underline{H}_2$I), 1.68–1.58 (m, 4H), 1.51–1.41 (m, 4H), 0.89 (s, 9H, (C$\underline{H}_3$)$_3$C), 0.03 (s, 6H, Si(C$\underline{H}_3$)$_2$). ^{13}C NMR (126 MHz, Chloroform-d) δ 66.4, 39.7, 33.1, 27.4, 26.0, 18.2, 15.6, −4.7 (Si(CH$_3$)(C$\underline{H}_3$), (Si(C$\underline{H}_3$)(CH$_3$)). Elemental analysis for $C_{13}H_{27}IOSi$ required: C 44.1, H 7.7; found: C 44.0, H 7.5.

(*S*)-Valine methyl ester (**14**)

L-Valine (**13**) (11.9 g, 0.1 mol) was dissolved in dry methanol (100 mL) and cooled to 0 °C. To the stirred solution, thionyl chloride (21 mL, 0.29 mol) was added slowly and the mixture was stirred until disappearance of the starting material was observed by TLC. The reaction was worked up by removal of solvent under reduced pressure and washed with Et_2O (five times) to give the hydrochloride salt as a white solid. (14.9 g, 89 % yield). This was dissolved in a mixture of $NaHCO_3$ solution (75 mL) and EtOAc (75 mL). The layers were separated and the aqueous layer was extracted with EtOAc (50 mL) twice more and the combined organic layer was washed with brine (50 mL), dried over Na_2SO_4 filtered and solvent was removed under high vacuum directly before the next step to minimise decomposition.

^{1}H NMR (500 MHz, DMSO-d6) δ 8.39 (br s, 3H, (NH$_3$), 3.86 (d, $J = 4.9$ Hz, 1H, C$\underline{H}$CHCH$_3$), 3.76 (s, 3H, OCH$_3$), 2.20–2.13 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 0.98 (d, $J = 6.9$ Hz, 3H, C$\underline{H}_3$CHCH$_3$), 0.94 (d, $J = 6.8$ Hz, 3H, CH$_3$CHC$\underline{H}_3$). ^{13}C NMR (126 MHz, DMSO) δ 169.3 (MeO$\underline{C}$=O), 57.3 (C$\underline{H}$CH(CH$_3$)$_2$), 52.6 (OCH$_3$), 29.3 (C$\underline{H}$(CH$_3$)$_2$), 18.2 (C$\underline{H}_3$CHCH$_3$), 17.6 (CH$_3$CHC$\underline{H}_3$). HR-MS: *m/z* Calcd for $C_6H_{14}O_2N$ $(M + H)^+$: 132.1025, found: 132.1031.

(*S*)-Methyl 2-(4-methoxybenzylamino)-3-methylbutanoate (**15**)

14 (4.37 g, 33.2 mmol) was dissolved in dry CH_2Cl_2 (40 mL) and cooled to 0 °C. Pyridine (2.95 mL, 36.5 mmol) was added and the solution was stirred for 5 min. *Para*-methoxybenzylchloride (4.9 mL, 36.5 mmol) was added slowly and the mixture was allowed to warm to room temperature and stirred for a further 12 h. The reaction was worked up by the addition of water (50 mL) and the layers were separated and the aqueous layer was extracted with CH_2Cl_2 (40 mL) a further two times. The combined organic layer was washed with brine (40 mL) dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified on silica gel using ethyl acetate: cyclohexane as an eluent to give the pure product in 80 % yield (6.67 g).

^{1}H NMR (500 MHz, Chloroform-d) δ 7.25 (d, $J = 8.6$ Hz, 2H, Ar$\underline{H}$), 6.85 (d, $J = 8.7$ Hz, 2H, Ar$\underline{H}$), 3.79 (s, 3H, OC$\underline{H}_3$), 3.76 (d, $J = 12.7$ Hz, 1H, ArCH$\underline{H}$), 3.72 (s, 3H, OC$\underline{H}_3$), 3.52 (d, $J = 12.8$ Hz, 1H, ArC$\underline{H}$H), 3.01 (d, $J = 6.2$ Hz, 1H, NHC$\underline{H}$C=O), 1.91 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 1.70 (s, 1H, N$\underline{H}$), 0.94 (d, $J = 6.8$ Hz, 3H,

$CHC\underline{H}_3$), 0.92 (d, $J = 6.7$ Hz, 3H, $CHC\underline{H}_3$). ^{13}C NMR (126 MHz, Chloroform-d) δ 175.9 ($O\underline{C}$=O), 158.8 (Ar), 132.4 (Ar), 129.5 (Ar), 113.8 (Ar), 66.6 ($NH\underline{C}H$), 55.4 ($O\underline{C}H_3$), 52.1 ($\underline{C}H_2$), 51.5 ($O\underline{C}H_3$), 31.8 ($\underline{C}H(CH_3)_2$), 19.4 ($CH(\underline{C}H_3)(CH_3)$), 18.81 ($CH(CH_3)(\underline{C}H_3)$). HR-MS: *m/z* Calcd for $C_{14}H_{21}O_3NNa$ $(M + Na)^+$: 274.1419; found: 274.1408. $[\alpha]_D^{20} = -39^\circ (c = 0.96, CHCl_3)$.

(*S*)-Methyl 2-(2-chloro-*N*-(4-methoxybenzyl)acetamido)-3-methylbutanoate (**16**)

15 (2.94 g, 11.7 mmol) was dissolved in dry CH_2Cl_2 (25 mL) and cooled to 0 °C. Triethyl amine (2 mL, 14.1 mmol) was added to the stirred solution, chloroacetyl chloride (1.1 mL, 14.1 mmol) was added dropwise and the reaction was stirred for additional 12 h. The reaction was worked up by the addition of water (30 mL), the layers were separated and the aqueous layer was extracted with (40 mL) a further two times. The combined organic layer was washed with brine (40 mL) dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified on silica gel using ethyl acetate: cyclohexane as an eluent to give the pure product in 89 % yield (3.4 g).

^1H NMR (400 MHz, Chloroform-d) δ 7.07 (d, $J = 8.2$ Hz, 2H, ArH), 6.86 (d, $J = 8.2$ Hz, 2H, ArH), 4.77 (d, $J = 10.3$ Hz, 1H, NCH), 4.64 (s, 2H, CH_2Ar), 3.78 (s, 3H, OCH_3), 3.48 (s, 2H, CH_2Cl), 2.44–2.29 (m, 1H, $C\underline{H}(CH_3)_2$), 0.98 (d, $J = 6.4$ Hz, 3H, $CH_3CHC\underline{H}_3$), 0.92 (d, $J = 6.7$ Hz, 3H, $C\underline{H}_3CHCH_3$). ^{13}C NMR (100 MHz, Chloroform-d) δ 171.2 (O=$\underline{C}$OMe), 162.2 (O=$\underline{C}CH_2Cl$), 159.5 ($\underline{Ar}OMe$), 129.4 (Ar), 128.7 (Ar), 114.9 (Ar), 64.3 ($\underline{C}HCH(CH_3)_2$), 56.0 ($ArO\underline{C}H_3$), 51.7 ($O{=}CO\underline{C}H_3$), 46.4 ($\underline{C}H_2Ar$), 40.9 ($\underline{C}H_2Cl$), 28.3 ($CH_3\underline{C}HCH_3$), 19.5 ($CH_3CH\underline{C}H_3$), 19.0 ($CH_3CH\underline{C}H_3$). HR-MS: *m/z* Calcd for $C_{16}H_{22}O_4NClNa$ $(M + Na)^+$: 350.1135; found 350.1135. $[\alpha]_D^{20} = -44.6^\circ (c = 0.98, CHCl_3)$.

(*S*)-6-Isopropyl-1-(4-methoxybenzyl)piperazine-2,5-dione (**17**)

16 (3.63 g, 11.1 mmol) was stirred in a saturated solution of ammonia in ethanol (100 mL) until starting material was consumed (observed by TLC, 10 h). Ethanolic ammonia was removed under reduced pressure and the residue was washed with ice cold Et_2O. The precipitate was purified on silica using 4:1 cyclohexane: EtOAc as an eluent, and 2.99 g of product was recovered as a white solid. (97 % yield).

^1H NMR (500 MHz, Chloroform-d) δ 7.16 (d, $J = 8.6$ Hz, 2H, $Ar\underline{H}$), 6.86 (d, $J = 8.7$ Hz, 2H, $Ar\underline{H}$), 6.00 (s, 1H $N\underline{H}$), 5.37 (d, $J = 14.7$ Hz, 1H, $NHCH\underline{H}C$=O), 4.13 (d, $J = 17.5$ Hz, 1H, $NCH\underline{H}Ar$), 3.96 (dd, $J = 17.4$, 4.3 Hz, 1H, $NC\underline{H}HAr$), 3.86 (d, $J = 14.5$ Hz, 1H, $NHCH\underline{H}C$=O), 3.80 (s, 3H, $OC\underline{H}_3$), 3.67 (d, $J = 4.9$ Hz, 1H, $C\underline{H}CH(CH_3)_2$), 2.25 (m, 1H, $C\underline{H}(CH_3)_2$), 1.12 (d, $J = 6.9$ Hz, 3H, $CH(C\underline{H}_3)(CH_3)$), 1.04 (d, $J = 6.9$ Hz, 3H, $CH(CH_3)(C\underline{H}_3)$). ^{13}C NMR (126 MHz, Chloroform-d) δ 167.5 ($NH\underline{C}$=O), 164.5 ($N\underline{C}$=O), 159.6 (ArO), 129.7 (Ar), 127.7 (Ar), 114.5 ($ArCH_2$), 64.5 ($\underline{C}HCH(CH_3)_2$), 55.5 ($O\underline{C}H_3$), 48.0 ($N\underline{C}H_2Ar$), 45.6 (O=C$\underline{C}$HHN), 32.1 ($\underline{C}H(CH_3)_2$), 20.0 ($CH\underline{C}H_3$), 17.9 ($CH\underline{C}H_3$). HR-MS: *m/z* Calcd for $C_{15}H_{20}N_2O_3Na$ $(M + Na)^+$: 299.1372; found 299.1380. $[\alpha]_D^{20} = -14.2^\circ (c = 1.02, CHCl_3)$.

(*S*)-5-Ethoxy-6-isopropyl-1-(4-methoxybenzyl)-1,6-dihydropyrazin-2(3H)-one (**18**)

An oven-dried three-necked flask with a stopcock sidearm was equipped with a condenser under nitrogen. Boron fluoride etherate (1.41 mL, 11.2 mmol) was dissolved in the flask in dry Et_2O (50 mL). To the stirred solution epichlorohydrin (0.57 mL, 7.01 mmol) was added at a rate to maintain boiling. The reaction mixture was refluxed for a further hour and then allowed to stand at room temperature overnight. Pale yellow solid had formed which was collected by filtering off the solvent under nitrogen and washed a further three times with dry diethyl ether (3 × 35 mL). **17** (1.55 g, 5.6 mmol) dissolved in CH_2Cl_2 (50 mL) was added and the reaction was stirred for a further 12 h. The reaction was worked up by addition of aqueous $KHCO_3$ solution (40 mL). The layers were separated and the aqueous layer was extracted a further two times with CH_2Cl_2 (50 mL). The combined organic layer was washed with brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The resulting orange residue was purified by flash chromatography on silica using 1:1 EtOAc: cyclohexane as an eluent. Product was recovered as a light orange, viscous oil (1.28 g, 60 % yield).

^1H NMR (400 MHz, Chloroform-d) δ 7.20–7.10 (m, 2H, $Ar\underline{H}$), 6.86 (d, $J = 8.6$ Hz, 2H, $Ar\underline{H}$), 5.39 (d, $J = 14.8$ Hz, 1H, O=CC$\underline{H}$HN), 4.16 (dd, $J = 17.4$, 1.3 Hz, 2H, $NC\underline{H}_2Ar$), 4.13–4.08 (m, 2H, $C\underline{H}_2CH_3$), 3.84 (d, $J = 14.9$ Hz, 1H, O=CCH$\underline{H}$N), 3.79 (s, 3H, $OC\underline{H}_3$), 3.66 (m, 1H, $C\underline{H}CH(CH_3)_2$), 2.24–2.14 (m, 1H, $C\underline{H}(CH_3)_2$), 1.23 (t, $J = 7.1$ Hz, 3H, $CH_2C\underline{H}_3$), 1.03 (d, $J = 7.0$ Hz, 3H, $CHC\underline{H}_3$), 0.91 (d, $J = 6.9$ Hz, 3H, $CHC\underline{H}_3$). ^{13}C NMR (101 MHz, Chloroform-d) δ 168.2 ($\underline{C}$=O), 160.6 (N=$\underline{C}$O, 159.3 (ArO), 129.7 (Ar), 128.1 (Ar), 114.3 (Ar), 61.5 ($\underline{C}H_2CH_3$), 61.2 ($\underline{C}HCH(CH_3)_2$), 55.4 ($O\underline{C}H_3$), 51.1 ($N\underline{C}H_2Ar$), 46.6 (O=C$\underline{C}$HHN), 31.9 ($\underline{C}H(CH_3)_2$), 20.1 ($CH\underline{C}H_3$), 17.6($CH\underline{C}H_3$), 14.4 ($CH_2\underline{C}H_3$). HR-MS: *m/z*

Calcd for $C_{17}H_{25}N_2O_3$ $(M + H)^+$: 305.1865; found: 305.1852. $[\alpha]_D^{20} = -29.5°(c = 1.02, CHCl_3)$.

1,4-*Bis*((*S*)-1-(4-methoxyphenyl)ethyl)piperazine-2, 5-dione (**20**)

(*S*)-1-(4-Methoxyphenyl)ethanamine (**19**) (40 mmol, 5.9 mL) was dissolved in dichloromethane (80 mL) and cooled to 0 °C. To the stirred solution, 50 % (w/v) sodium hydroxide solution (320 mmol, 25.6 mL) was added slowly. Chloroacetyl chloride (40 mmol, 3.2 mL) was added slowly to the stirred mixture. The mixture was stirred for 12 h when TLC showed that the amine had been consumed. *N*-benzyl-*N,N,N*-triethylethanaminium chloride (4 mmol, 1.02 g) was added portion-wise over a 48 h period. Work-up was performed by separating the organic and aqueous layers and evaporating the organic layer under reduced pressure. The residue was dissolved in EtOAc (75 mL), washed with 1 M HCl (25 mL), water (35 mL), brine (35 mL) dried over $MgSO_4$ and evaporating under reduced pressure. Purification was carried out on silica using 7:3 cyclohexane: EtOAc as an eluent. Product was recovered as an off-white solid (5.32 g, 13.9 mmol, 69 % yield).

^{1}H NMR (500 MHz, Chloroform-d) δ 7.18 (d, $J = 8.7$ Hz, 4H, ArH), 6.86 (d, $J = 8.7$ Hz, 4H, ArH), 5.89 (q, $J = 7.1$ Hz, 2H ArCHN), 3.85–3.78 (m, 8H, overlap of C=OCH$_2$N and OCH$_3$), 3.50 (d, $J = 16.4$ Hz, 2H C=OCH$_2$N), 1.51 (d, $J = 7.1$ Hz, 6H, CH$_3$). ^{13}C NMR (126 MHz, Chloroform-d) δ 163.9 (C=O), 159.5 (ArOMe), 130.4 (Ar), 128.8 (Ar), 114.3 (Ar), 55.5 (CHCH$_3$), 49.9 (OCH$_3$), 44.7 (NCH$_2$C=O), 15.5 (CHCH$_3$). HR-MS: *m/z* Calcd for $C_{22}H_{26}N_2O_4Na$ $(M + Na)^+$: 405.1790; found: 405.1786.

(3*R*,6*S*)-5-Ethoxy-6-isopropyl-1-(4-methoxybenzyl)-3-((4-oxocyclohexyl)methyl)-1,6-dihydropyrazin-2(3H)-one (**22**)

18 (1 g, 3.3 mmol) was placed in an oven-dried two-necked flask and dissolved in dry THF (20 mL). The solution was cooled to 0 °C and 4.29 mL of 1 M LHMDS solution in THF were added slowly. The reaction was stirred for 30 min during which time the solution developed a noticeably brighter orange colour. The solution was cooled to −78 °C with a liquid nitrogen/EtOAc bath and a solution of **12** (1.17 g, 3.3 mmol) in dry THF was added dropwise. The mixture was stirred for 2 h, then the cold bath was removed and the mixture was allowed to reach room temperature. The reaction was worked up by pouring the reaction mixture into a mixture of deionised water (100 mL) and EtOAc (100 mL). The layers were separated and the aqueous layer was extracted two more time with EtOAc (50 mL). The combined organic layer was washed with brine (50 mL), dried over Na_2SO_4 and the solvent was removed under vacuum to give crude **21** as a brown residue. Crude **21** (1.16 g, 2.17 mmol) was immediately dissolved in THF (10 mL). A 1 M solution of TBAF in THF (5.24 mL, 5.24 mmol) was added and the mixture was stirred at room temperature for 8 h when TLC showed consumption of the starting material. The solvent was removed under vacuum and the residue was dissolved in CH_2Cl_2 (50 mL) and washed with deionised water (50 mL) and the layers were separated. The aqueous layer was extracted a further two times with CH_2Cl_2 (50 mL) and the combined organic layer was washed with brine (35 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure to give an orange oil. Oxalyl chloride (0.22 mL, 2.6 mmol) was dissolved in dry CH_2Cl_2 (12 mL) at −78 °C under nitrogen, dry DMSO (0.41 mL, 5.75 mmol) was slowly added and the reaction mixture was stirred for 5 min. The orange oil which had been dried thoroughly on a high vacuum pump was dissolved in CH_2Cl_2 (5 mL) and added slowly to the reaction mixture and was stirred for a further hour, taking care to keep the temperature at −78 °C. Triethylamine (1.47 mL, 10.43 mmol) was added and the reaction was allowed to warm to room temperature. The mixture was diluted with CH_2Cl_2 (50 mL) and poured into aqueous $NaHCO_3$ solution (35 mL). The layers were separated and the aqueous layer was extracted a further two times with CH_2Cl_2 (35 mL). The combined organic layer was washed with aqueous $NaHCO_3$ solution (35 mL), water (35 mL), brine (35 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure to give an orange oil. Purification was carried out on silica gel using 1:1 cyclohexane, ethyl acetate as an eluent, and 530 mg of product was recovered as a light orange viscous oil (39 % combined yield).

^{1}H NMR (300 MHz, Chloroform-d) δ 7.13 (d, $J = 8.2$ Hz, 2H, ArH), 6.85 (d, $J = 8.1$ Hz, 2H, ArH), 5.38 (d, $J = 14.7$ Hz, 1H, ArCHHN), 4.19–3.99 (m, 4H), 3.92–3.75 (m, 4H), 3.73–3.50 (m, 1H, CHCH(CH$_3$)$_2$), 2.47–1.94 (m, 8H), 1.85–1.68 (m, 1H, NCH(CH$_2$CH)), 1.53–1.35 (m, 2H), 1.26 (m, 4H), 1.05 (d, $J = 6.9$ Hz, 3H, CH(CH$_3$)(CH$_3$)), 0.92 (d, $J = 6.6$ Hz, 3H, CH(CH$_3$)(CH$_3$)). ^{13}C NMR (126 MHz, Chloroform-d) δ 212.0 (C=O), 171.8 (O=CN), 159.5 (OC=N), 156.3 (ArOMe), 130.5 (Ar), 128.2 (Ar), 114.2 (Ar). 61.5 (CHCH(CH$_3$)$_2$), 61.0 (O=CCHN=C), 55.4 (OCH$_3$), 50.7 (OCH$_2$CH$_3$), 46.3 (CH$_2$Ar), 40.5 (CH$_2$C=OCH$_2$), 40.3 (CH$_2$C=OCH$_2$), 34.0 (CH$_2$CH$_2$C=O), 33.2 (CH$_2$CH$_2$C=O), 32.0 (CH(CH$_3$)$_2$) 31.7 (O=CCH(CH$_2$CHC$_5$H$_8$O), 20.1 (CH$_3$)CH(CH$_3$)), 17.6 (CH$_3$)CH(CH$_3$)), 14.4 (OCH$_2$CH$_3$). HR-MS: *m/z* Calcd for $C_{24}H_{34}N_2O_4Na$ $(M + Na)^+$: 437.2416; found: 437.2417. $[\alpha]_D^{20} = -26.4°(c = 0.99, CHCl_3)$.

1,4-*Bis*((*S*)-1-(4-methoxyphenyl)ethyl)-3-((4-oxocyclohexyl)methyl)piperazine-2,5-dione (**25**)

20 (1.29 g, 3.39 mmol) was placed in an oven-dried two-necked flask and dissolved in dry THF (12.5 mL) under nitrogen. The solution was cooled to 0 °C and 4.1 mL of 1 M LHMDS solution in THF were added slowly. The reaction was stirred for 30 min during which time the solution developed a noticeably brighter orange colour. The solution was cooled to −78 °C with a liquid nitrogen/EtOAc bath and a solution of **12** (1.2 g, 3.39 mmol) in dry THF (10 mL) was added dropwise. The mixture was stirred for 10 h then the cold bath was removed and the mixture was warmed to room temperature. The reaction was worked up by pouring the reaction mixture into a mixture of deionised water (50 mL) and EtOAc (50 mL). The layers were separated and the aqueous layer was extracted two more time with EtOAc (40 mL). The combined organic layer was washed with brine (40 mL), dried over Na_2SO_4 and the solvent was removed under vacuum to give crude **24** as a yellow oil; 1.32 g (2.06 mmol) of this was dissolved in a mixture of acetic acid, THF and deionised water (16:4:4 mL). The mixture was stirred for 10 h at room temperature when TLC showed consumption of starting material. The solvent was removed under high vacuum and the residue was dissolved in a mixture of deionised water (50 mL) and EtOAc (50 mL). The layers were separated and the aqueous layer was extracted two more times with EtOAc (40 mL). The combined organic layer was washed with brine (40 mL), dried over Na_2SO_4 and the solvent was removed under vacuum to give the crude alcohol as a yellow oil. Oxalyl chloride (0.21 mL, 2.4 mmol) was dissolved in dry CH_2Cl_2 (7 mL) and cooled to −78 °C using an ethyl acetate/liquid nitrogen bath. To the stirred solution, DMSO (0.38 mL, 5.3 mmol) was added slowly. The mixture was stirred for a further 15 min and the dried crude product (0.87 g, 2 mmol) was added dropwise as a solution in CH_2Cl_2 (5 mL). The mixture was stirred for a further hour maintaining the temperature at −78 °C. Triethylamine (1.3 mL, 9.62 mmol) was added and the solution was allowed to warm to −10 °C. The mixture was stirred for a further 4 h and the colour had darkened from yellow to brown. The mixture was diluted with CH_2Cl_2, filtered to remove sieves and washed with saturated ammonium chloride solution, water and brine. The organic phase was dried with $MgSO_4$ and the solvent was removed under reduced pressure. The resulting brown residue was purified on silica using 3:2 EtOAc:cyclohexane as an eluent to give the product in a diastereomeric ratio of 0.81–0.19. Combined overall yield for both isomers was 651 mg (39 %). The analysis for the major isomer (527 mg) is reported here.

^{1}H NMR (500 MHz, Chloroform-d) δ 7.11 (m, 4H, ArH), 6.88 (d, $J = 8.5$ Hz, 4H, ArH), 5.77 (dq, $J = 30.7$, 7.1 Hz, 2H, CH_3CH), 3.88 (d, $J = 17.1$ Hz, 1H, C=OCHHN), 3.82 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.75 (dd, $J = 11.9$, 3.7 Hz, 1H, C=OCH($C_7H_{11}O$)N), 3.63 (d, $J = 17.0$ Hz, 1H, C=OCHHN), 2.36 (dd, $J = 10.0$, 4.7 Hz, 4H, CH_2C=OCH_2), 2.33–2.27 (m, 1H), 1.96 (m, 2H, CHHCH_2C=OCH_2CHH), 1.83 (m, 1H, CHH(C_6H_9O)), 1.63 (m, CHH(C_6H_9O)), 1.56 (d, $J = 7.1$ Hz, 3H, CH_3CH), 1.48 (d, $J = 7.1$ Hz, 3H, CH_3CH), 1.45–1.31 (m, 2H, CHHCH_2C=OCH_2CHH). ^{13}C NMR (126 MHz, Chloroform-d) δ 211.2 (C=O), 166.7 (O=CN), 165.4 (O=CN), 159.5 (ArOMe), 159.5 (ArOMe), 131.2 (CHAr), 130.9 (CHAr), 128.4 (ArH), 128.3 (ArH), 114.4 (ArH), 114.3 (ArH), 55.5 (OCH_3), 55.4 (OCH_3), 55.3 (NCHC=O), 51.5 ($CHCH_3$), 49.7 ($CHCH_3$), 44.6 (NCH_2C=O), 40.8 (CH_2C=OCH_2), 40.5 (CH_2C=OCH_2), 39.9 (CH_2(C_6H_9O)), 34.0 (CH_2CH_2C=O), 33.0 (CH_2(CHC_5H_8O)), 31.8 (CH_2CH_2C=O), 17.7 ($CHCH_3$), 15.9 ($CHCH_3$). HR-MS: *m/z* Calcd for: C_{29}–$H_{36}N_2O_5Na$ $(M + Na)^+$: 515.2522; found 515.2523. $[\alpha]_D^{20} = -214.2^\circ (c = 1.12, CHCl_3)$.

(3*R*,6*S*)-5-Ethoxy-6-isopropyl-1-(4-methoxybenzyl)-3-((4-oxocyclohex-2-enyl)methyl)-1,6-dihydropyrazin-2(3H)-one (**23**)

A 1 M solution of iodic acid in DMSO was made up and heated at 80 °C in the absence of light for 1 h; 0.36 mL of this solution was added to a solution of **22** (75 mg, 0.18 mmol) in DMSO (0.2 mL) and cyclohexene (0.1 mL). The mixture was heated at 50 °C for 8 h in the absence of light, when TLC showed consumption of starting material. Work-up was performed by pouring the mixture into a mixture of EtOAc (25 mL) and deionised water (25 mL). The layers were separated and the aqueous layer was extracted with EtOAc (20 mL) a further five times. The combined organic layer was washed with brine (25 mL) dried over Na_2SO_4 and the solvent was removed under vacuum. The crude product was purified on silica using a 95:5 mixture of EtOAc:MeOH as an eluent, recovering 50 mg of product as a yellow oil (67 % crude yield). NMR data showed that the product was contaminated with ~15 % unknown contaminant that could not be separated. The new stereocentre at the γ carbon was a 50:50 mixture of *R* and *S*, the diastereomers could not be separated by silica chromatography and the ^{1}H NMR reported here refers to the mixture of diastereomers.

^{1}H NMR (300 MHz, Chloroform-d) δ 7.21–7.09 (m, 2H, ArH), 6.91–6.80 (m, 3H), 6.02–5.94 (m, 1H, O=CCH=CH), 5.41–5.35 (m, 1H, ArCHHN), 4.23–3.99 (m, 4H), 3.95–3.56 (m, 4H), 2.86–2.78 (m, 2H, O=CCH_2CH_2), 2.48–1.93 (m, 3H), 1.84–1.67 (m, 1H, NCH(CH_2CH)), 1.53–1.35 (m, 2H),

1.31–1.20 (m, 3H), 1.10–1.01 (m, J = 3H, $CH(CH_3)(C\underline{H}_3)$), 0.97–0.93 (m, 3H, $CH(C\underline{H}_3)(CH_3)$). ^{13}C NMR (126 MHz, Chloroform-d), diastereomer 1: δ 198.9 (C=O), 173.5 (O=CN), 159.6 (OC=N), 156.3 (ArOMe), 142.6 (O=CCH=$\underline{C}$H), 129.7 (Ar), 128.7 (Ar), 124.7 (O=C$\underline{C}$H=CH), 115.9 (Ar). 62.3 ($\underline{C}$HCH$(CH_3)_2$), 61.1 (O=C$\underline{C}$HN=C), 55.4 (OCH_3), 50.8 ($O\underline{C}H_2CH_3$), 46.5 (CH_2Ar), 40.8 ($\underline{C}H_2$C=OCH=CH), 36.7 ($\underline{C}H_2(C_6H_7O)$), 33.8 ($\underline{C}H(C_5H_6O)$), 30.3 ($CH_2CH_2$C=O), 27,1 ($\underline{C}H(CH_3)_2$), 20.0 ($\underline{C}H_3$)CH$(CH_3)$), 18.0 (CH_3)CH($\underline{C}H_3$)), 14.0 ($OCH_2\underline{C}H_3$); diastereomer 2: δ 198.8 (C=O), 173.4 (O=CN), 159.6 (OC=N), 156.3 (ArOMe), 139.4 (O=CCH=$\underline{C}$H), 129.6 (Ar), 128.7 (Ar), 123.9 (O=C$\underline{C}$H=CH), 114.2 (Ar), 61.6 ($\underline{C}$HCH$(CH_3)_2$), 60.9 (O=C$\underline{C}$HN=C), 55.4 (OCH_3), 50.8 ($O\underline{C}H_2CH_3$), 46.4 (CH_2Ar), 40.8 ($\underline{C}H_2$C=OCH=CH), 36.6 ($\underline{C}H_2(C_6H_7O)$), 33.8 ($\underline{C}H(C_5H_6O)$), 30.3 ($CH_2CH_2$C=O), 27.1 ($\underline{C}H(CH_3)_2$), 20.0 (($\underline{C}H_3$)CH$(CH_3)$), 18.0 (CH_3)CH($\underline{C}H_3$)), 14.0 ($OCH_2\underline{C}H_3$). HR-MS: m/z Calcd for $C_{24}H_{32}N_2O_4Na$ $(M + Na)^+$: 437.2261; found: 437.2254.

(*S*)-1,4-Bis((S)-1-(4-methoxyphenyl)ethyl)-3-((4-oxocyclohex-2-enyl)methyl) piperazine-2,5-dione (**26**)

A 1 M solution of iodic acid in DMSO was made up and heated at 80 °C in the absence of light for 1 h (caution: explosion hazard); 0.4 mL of this solution was added to a solution of **25** (100 mg, 0.2 mmol) in DMSO (0.25 mL) and cyclohexene (0.15 mL). The mixture was heated at 50 °C for 7 h in the absence of light, when TLC showed consumption of starting material. Work-up was performed by pouring the mixture into a mixture of EtOAc (25 mL) and deionised water (25 mL). The layers were separated and the aqueous layer was extracted with EtOAc (20 mL) a further five times. The combined organic layer was washed with brine (25 mL) dried over Na_2SO_4 and the solvent was removed under vacuum. The crude product was purified on silica using a 95:5 mixture of EtOAc:MeOH as an eluent. Product (79 mg) was recovered as a pale yellow oil. NMR data showed that the product was contaminated with $\sim$10 % unknown contaminant that could not be separated. The new stereocentre at the γ carbon was a 50:50 mixture of *R* and *S*; the diastereomers could not be separated by silica gel chromatography.

^{1}H NMR (400 MHz, Chloroform-d), diastereomer 1: δ 7.15–7.06 (m, 4H, overlap), 6.93 (dd, J = 10.6, 3.5 Hz, 1H), 6.90–6.86 (m, 4H, overlap), 6.00–5.97 (m, 1H), 5.85–5.68 (m, 2H), 3.89 (d, J = 17.2 Hz, 1H), 3.82 (s, 3H), 3.81 (s, 3H, overlap), 3.79–3.74 (m, 1H, overlap), 3.63 (d, J = 17.2 Hz, 1H, overlap), 2.52–2.34 (m, 2H, overlap), 2.15–1.64 (m, 5H, overlap), 1.56 (d, J = 7.2 Hz, 3H), 1.46 (d, J = 7.1 Hz, 3H); diastereomer 2: δ 7.15–7.06 (m, 4H, overlap), 6.90–6.86 (m, 4H), 6.68 (dd, J = 10.1, 2.6 Hz, 1H), 6.02 (broad d, J = 10.3 Hz, 1H), 5.85–5.68 (m, 2H, overlap), 3.87 (d, J = 17.2 Hz, 1H), 3.82 (s, 3H, overlap), 3.81 (s, 3H, overlap), 3.79–3.74 (m, 1H, overlap), 3.63 (d, J = 17.2 Hz, 1H), 2.52–2.34 (m, 2H, overlap), 2.12–1.64 (m, 5H, overlap), 1.56 (d, J = 7.2 Hz, 3H, overlap), 1.41 (d, J = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-d), diastereomer 1: δ 199.2 (C=O), 166.2 (NC=O), 165.3 (NC=O), 159.6 (ArOMe), 159.5 (ArOMe), 153.2 ($\underline{C}$H=CHC=O), 130.9 (Ar), 130.6 (Ar), 129.9 (CH=$\underline{C}$HC=O), 128.4 (Ar), 128.3 (Ar), 114.5 (Ar), 114.3 (Ar), 55.4 (OCH_3), 55.4 (OCH_3), 55.1 (N$\underline{C}$HC=O), 51.8 ($\underline{C}CH_3$), 51.6 ($\underline{C}CH_3$), 44.5 (N$\underline{C}H_2$C=O), 38.6 (NCH$\underline{C}H_2(C_6H_7O)$), 36.7 ($CH_2\underline{C}H_2$C=O), 33.2 (DKPC$H_2\underline{C}$H), 29.8 (C=OC$H_2\underline{C}H_2$), 17.8 (CH$\underline{C}H_3$), 15.9 (CH$\underline{C}H_3$); diastereomer 2: δ 198.9 (C=O), 166.1 (NC=O), 165.2 (NC=O), 159.5 (ArOMe), 159.5 (ArOMe), 151.7 ($\underline{C}$H=CHC=O), 129.7 (CH=$\underline{C}$HC=O), 130.8 (Ar), 130.6 (Ar), 128.3 (Ar), 128.3 (Ar), 114.4 (Ar), 114.3(Ar), 55.4 (OCH_3), 55.4 (OCH_3), 54.8 (N$\underline{C}$HC=O), 49.8 ($\underline{C}CH_3$), 49.7 ($\underline{C}CH_3$), 44.5 (N$\underline{C}H_2$C=O), 38.6 (NCH$\underline{C}H_2(C_6H_7O)$), 36.4 ($CH_2\underline{C}H_2$C=O), 32.5 (DKPC$H_2\underline{C}$H), 29.8 (C=OC$H_2\underline{C}H_2$), 17.7 (CH$\underline{C}H_3$), 14.2 (CH$\underline{C}H_3$). HR-MS: m/z Calcd for: C_{29}–$H_{34}N_2O_5Na$ $(M + Na)^+$: 513.2365; found 513.2359.

S-3-((4-Oxocyclohexyl)methyl)piperazine-2,5-dione (**4**)

25 (70 mg, 0.14 mmol) was dissolved in acetonitrile/water (3 mL: 2 mL), and cerium ammonium nitrate was added (311 mg, 0.57 mmol). The mixture was stirred at room temperature until TLC showed consumption of starting material (4 h). The reaction mixture was purified directly on silica using 4:1 CH_2Cl_2:MeOH as an eluent. The product (20.8 mg, 66 % yield) was recovered as a white solid.

^{1}H NMR (500 MHz, deuterium oxide) δ 4.25–4.18 (m, 1H, C=OC$\underline{H}(CH_2C_6H_9O)$, overlaps with peak at 4.22), 4.22 (d J = 18.0 Hz, 1H, C=OC$\underline{H}$HNH, overlaps with multiplet at 4.25–4.18) 4.06 (d, J = 18.2 Hz, 1H, C=OC$\underline{H}$HNH), 2.63–2.50 (m, 2H, C$\underline{H}$HC=OC$\underline{H}$H), 2.51–2.39 (m, 2H, CH$\underline{H}$C=OCH$\underline{H}$), 2.22–1.85 (m, 5H), 1.65–1.50 (m, 2H). ^{13}C NMR (126 MHz, deuterium oxide) δ 219.9 (C=O), 171.5 (HN$\underline{C}$=O), 169.2 (HN$\underline{C}$=O), 53.6 (HN$\underline{C}$HC=O), 44.3 (HN$\underline{C}H_2$C=O), 40.6 ($\underline{C}H_2$C=OCH_2), 40.4 (CH_2C=O$\underline{C}H_2$), 39.1 ($CH_2\underline{C}H(C_5H_8O)$, 32.9, 32.0, 31.6. HR-MS: m/z Calcd for: $C_{11}H_{14}N_2O_3Na$ $(M + Na)^+$: 247.1061; found 247.1059.

(3*S*)-3-((4-Oxocyclohex-2-enyl)methyl)piperazine-2,5-dione (**5**)

26 (72 mg, 0.15 mmol) was dissolved in acetonitrile/water (2 mL: 0.5 mL) and cerium ammonium nitrate was added (321 mg, 0.59 mmol). The mixture was stirred at room

temperature until TLC showed consumption of starting material (5 h). The solvent was removed under high vacuum and the residue was purified directly on silica using 9:1 CH_2Cl_2:MeOH as an eluent. The product (9.4 mg, 28 % yield) was recovered as an off-white solid; the diastereomers could not be separated.

^{1}H NMR (500 MHz, deuterium oxide), diastereomer 1: δ 7.15–7.06 (m, 1H, O=CCH=C<u>H</u>), 6.10–6.02 (m, 1H, O=CC<u>H</u>=CH), 4.27–4.15 (m, 2H), 4.10–4.01 (m, 1H, C=OC<u>H</u>HNH), 2.71–2.35 (m, 3H), 2.23–2.15 (m, 1H, C<u>H</u>HCH$_2$C=O), 1.99–1.90 (m, 2H, C<u>H</u>$_2$C$_6$H$_7$O), 1.79–1.72 (m, 1H, CH<u>H</u>CH$_2$C=O); diastereomer 2: δ 7.08–7.00 (m, 1H, O=CCH=C<u>H</u>), 6.05–5.99 (m, 1H, O=CC<u>H</u>=CH), 4.27–4.15 (m, 2H, overlap), 4.04–3.98 (m, 1H, C=OC<u>H</u>HNH), 2.71–2.35 (m, 3H, overlap), 2.23–2.15 (m, 1H, C<u>H</u>HCH$_2$C=O, overlap), 1.93–1.88 (m, 2H, C<u>H</u>$_2$C$_6$H$_7$O, overlap), 1.75–1.69 (m, 1H, CH<u>H</u>CH$_2$C=O, overlap). ^{13}C NMR (126 MHz, deuterium oxide), diastereomer 1: δ 199.3 (O=C), 171.0 (O=<u>C</u>N), 169.1 (O=<u>C</u>N), 153.5 (O=CCH=<u>C</u>H), 130.0 (O=CCH=<u>C</u>H), 53.5 (O=<u>C</u>HN), 43.7 (O=<u>C</u>H$_2$N), 41.0 ((NCH<u>C</u>H$_2$(C$_6$H$_7$O)), 36.9 (O=C<u>C</u>H$_2$CH$_2$), 32.5 (CH$_2$<u>C</u>HC$_5$H$_6$O), 28.5 (O=CCH$_2$<u>C</u>H$_2$); diastereomer 2: δ 199.0 (O=C), 170.9 (O=CN), 168.7 (O=CN), 153.4, (O=CCH=<u>C</u>H), 130.0 (O=CCH=<u>C</u>H), 53.3 (O=<u>C</u>HN), 43.6 (O=<u>C</u>H$_2$N), 41.0 ((NCH<u>C</u>H$_2$(C$_6$H$_7$O)), 36.8 (O=C<u>C</u>H$_2$CH$_2$), 32.5 (CH$_2$<u>C</u>HC$_5$H$_6$O), 28.4 (O=CCH$_2$<u>C</u>H$_2$). HR-MS: *m/z* Calcd for: $C_{11}H_{14}N_2O_3Na$ $(M + Na)^+$: 245.0902; found 245.0910.

(*S*)-2-((*R*)-2-Amino-3-(4-oxocyclohexyl)propanamido)-3-methylbutanoic acid (**6**)

22 (120 mg, 0.29 mmol) was dissolved in a mixture of acetonitrile and deionised water (4:1 mL) cerium (IV) ammonium nitrate (323 mg, 0.59 mmol) was added and the mixture was stirred at room temperature until TLC showed consumption of starting material (3.5 h). The solvent was removed under high vacuum and the residue was partially purified using silica with 10 % MeOH in CH_2Cl_2 as an eluent. The residue was dissolved in 1 M hydrochloric acid (3 mL) and the mixture was stirred at 45 °C for 9 h monitoring by TLC. The solvent was removed under high vacuum and dissolved in water (10 mL) and EtOAc (10 mL). The layers were separated and the aqueous layer was washed with EtOAc (5 mL) two more times. The product as the hydrochloride salt was recovered as an off-white solid after drying the aqueous layer under high vacuum. (40 mg, 43 % yield).

^{1}H NMR (500 MHz, deuterium oxide) δ 4.40–4.32 (m, 1 H, NH$_2$<u>C</u>H), 4.02 (d, $J = 7.5$ Hz, 1H, <u>C</u>HCH(CH$_3$)$_2$), 2.79–2.61 (m, 5H), 2.36–2.25 (m, 2H, CH<u>C</u>H$_2$(C$_6$H$_9$)), 2.09–1.83 (m, 5H), 1.03 (d, $J = 7.1$ Hz, 3H, (CH$_3$)CH(<u>C</u>H$_3$)), 0.91 (d, $J = 6.9$ Hz, 3H, (CH$_3$)CH(<u>C</u>H$_3$)). ^{13}C NMR (126 MHz, deuterium oxide) δ 211.5 (CH$_2$<u>C</u>=OCH$_2$), 178.1 (HOC=O), 177.0 (HNC=O), 60.0 (<u>C</u>H*iso*Pr), 52.5 (H$_2$N<u>C</u>HC=O), 38.3 (<u>C</u>H$_2$C=OCH$_2$), 37.8 (CH$_2$C=O<u>C</u>H$_2$), 36.3 (CH<u>C</u>H$_2$C$_6$H$_9$O), 32.2 (CH$_3$<u>C</u>HCH$_3$), 30.6 (<u>C</u>H$_2$CH$_2$C=OCH$_2$<u>C</u>H$_2$), 29.9 (CH$_2$<u>C</u>HC$_5$H$_8$O), 18.2 (<u>C</u>H$_3$CHCH$_3$), 17.5 (CH$_3$CH<u>C</u>H$_3$). HR-MS: *m/z* Calcd for $C_{14}H_{25}N_2O_4$: 285.1815; found: 285.1821.

(*S*)-2-((*R*)-2-Amino-3-(4-oxocyclohex-2-enyl)propanamido)-3-methylbutanoic acid (**7**)

23 (79 mg, 0.19 mmol) was dissolved in a mixture of acetonitrile and deionised water (2:0.5 mL) cerium (IV) ammonium nitrate (208 mg, 0.38 mmol) was added and the mixture was stirred at room temperature until TLC showed consumption of starting material (4 h). The solvent was removed under high vacuum and the residue was partially purified using silica with 10 % MeOH in CH_2Cl_2 as an eluent. The residue was dissolved in 1 M hydrochloric acid (2.5 mL) and the mixture was stirred at 45 °C for 9 h. The solvent was removed under high vacuum and dissolved in water (10 mL) and EtOAc (10 mL). The layers were separated and the aqueous layer washed with EtOAc (5 mL) two more times. The product as the hydrochloride salt was recovered as an off-white solid after drying the aqueous layer under high vacuum. (11.5 mg, 19 % yield). The mixture of diastereomers could not be separated and the ^{1}H NMR reported here refers to the mixture.

^{1}H NMR (500 MHz, Deuterium oxide) δ: 6.99–6.93 (m, 1H, O=CCH=C<u>H</u>), 6.07–6.03 (m, 1H, O=CC<u>H</u>=CH), 4.42–4.33 (m, 1 H, NH$_2$<u>C</u>H), 4.09–3.99 (m, 1H, <u>C</u>HCH(CH$_3$)$_2$), 2.78–2.62 (m, 3H), 2.36–2.25 (m, 2H, CH<u>C</u>H$_2$(C$_6$H$_9$)), 2.07–1.85 (m, 5H), 1.05–1.00 (m, 3H, (CH$_3$)CH(<u>C</u>H$_3$)), 0.96–0.89 (m, 3H, (CH$_3$)CH(<u>C</u>H$_3$)). ^{13}C NMR (126 MHz, D_2O), diastereomer 1: δ: 198.9 (CH$_2$<u>C</u>=OCH$_2$), 178.1 (HOC=O), 177.0 (HNC=O), 151.5 (O=CCH=CH), 123.7 (O=C<u>C</u>H=CH), 60.0 (<u>C</u>H*iso*Pr), 52.7 (H$_2$N<u>C</u>HC=O), 38.3 (CH$_2$<u>C</u>H$_2$C=O), 36.7 (CH<u>C</u>H$_2$C$_6$H$_9$O), 31.4 (CH$_3$<u>C</u>HCH$_3$), 30.6 (<u>C</u>H$_2$CH$_2$C=O), 29.9 (CH$_2$<u>C</u>HC$_5$H$_8$O), 17.5 (<u>C</u>H$_3$CHCH$_3$), 15.3 (<u>C</u>H$_3$CHCH$_3$); diastereomer 2: δ 198.8 (CH$_2$<u>C</u>=OCH$_2$), 178.1 (HOC=O), 176.5.0 (HNC=O), 151.3 (O=CCH=<u>C</u>H), 123.4 (O=C<u>C</u>H=CH), 59.8 (<u>C</u>H*iso*Pr), 52.5 (H$_2$N<u>C</u>HC=O), 38.1 (<u>C</u>H$_2$C=OCH$_2$), 36.3 (CH<u>C</u>H$_2$C$_6$H$_9$O), 31.2 (CH$_3$<u>C</u>HCH$_3$), 30.3 (<u>C</u>H$_2$CH$_2$C=O), 29.9 (CH$_2$<u>C</u>HC$_5$H$_8$O), 18.1 (<u>C</u>H$_3$CHCH$_3$), 17.5 (<u>C</u>H$_3$CHCH$_3$). HR-MS: *m/z* Calcd for $C_{14}H_{23}N_2O_4$: 285.1658; found: 285.1652.

Antibacterial testing

Non-resistant strains of *S. aureus*, *E. coli*, *S. cerevisiae* and *C. albicans* (all obtained from the Microbiology Culture Collection, UCD School of Biomolecular and Biomedical

Science) were employed to test compounds **4**–**7** via the disc diffusion assay. The compounds were prepared in sterile water (20 mg mL^{-1}) and 25 µL was added to sterile discs, which were placed onto inoculated agar plates.

Antibiotic-resistant strains of *E. coli* DSM1103, methicillin resistant *S. aureus* ATCC 43300 plus in-house clinical isolates of *E. coli* and MRSA were also used to assess the antibacterial activity of **4**–**7**. Serial dilutions of the test compounds were prepared in Mueller–Hinton broth (MHB) and aliquots (100 µL) transferred to wells of a microtitre plate. Bacterial cultures were incubated at 37 °C overnight, diluted × 1,000 in phosphate buffered saline and 5 µL of this was used to inoculate wells containing the test compounds. The plate was then incubated at 37 °C for 18 h using an Ominolog® automated incubator (Biolog Inc. CA 94545, USA).

Acknowledgments This projected was funded under the Irish Government's Programme for Research Training in Third Level Institutes (PRTLI). The authors thank Dr Maria Martins of the UCD Centre for Food Safety for conducting the microtitre plate assays.

Conflict of interest The authors declare that they have no conflict of interest.

References

Baldwin JE, Adlington RM, Mitchell MB (1995) Stereocontrolled enantiospecific synthesis of anticapsin. Tetrahedron 51:5193–5206

Bates CJ, Adams WR, Handschumacher RE (1966) Control of formation of uridine diphospho-*n*-acetyl-hexosamine and glycoprotein synthesis in rat liver. J Biol Chem 241:1705–1712

Bearne SL, Blouin C (2000) Inhibition of *Escherichia coli* glucosamine-6 phosphate synthase by reactive intermediate analogues—the role of the 2-amino function in catalysis. J Biol Chem 275:135–140

Chmara H (1985) Inhibition of glucosamine synthase by bacilysin and anticapsin. J Gen Microbiol 131:265–271

Chmara H, Milewski S, Dzieduszycka M, Smulkowski M, Sawlewicz P, Borowski E (1982) Epoxypeptides—a novel group of metabolic-inhibitors in prokaryotic and eukaryotic microorganisms. Drugs Exp Clin Res 8:11–20

Chmara H, Zahner H, Borowski E (1984) Anticapsin, an active-site directed irreversible inhibitor of glucosamine-6-phosphate synthetase from *Escherichia coli*. J Antibiot 37:1038–1043

Davies J, Davies D (2010) Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 74:417–433

Davies SG, Garner AC, Ouzman JVA, Roberts PM, Smith AD, Snow EJ, Thomson JE, Tamayo JA, Vickers RJ (2007) Diastereoselective synthesis of quaternary alpha-amino acids from diketopiperazine templates. Org Biomol Chem 5:2138–2147

Foster JW, Woodruff HB (1946) Bacillin, a new antibiotic substance from a soil isolate of *Bacillus subtilis*. J Bacteriol 51:363–369

Gonzalez-Ibarra J, Milewski S, Villagomez-Castor JC, Cano-Canchola C, Lopez-Romero E (2010) *Sporothrix schenckii*: purification and partial biochemical characterization of glucosamine-6-phosphate synthase, a potential antifungal target. Med Mycol 48:110–121

Jedrzejczak R, Wojciechowski M, Andruszkiewicz R, Sowinski P, Kot-Wasik A, Milewski S (2012) Inactivation of glucosamine-6-phosphate synthase by N3-oxoacyl derivatives of L-2,3-diaminopropanoic acid. ChemBioChem 13:85–96

Kenig M, Abraham EP (1976) Antimicrobial activities and antagonists of bacilysin and anticapsin. J Gen Microbiol 94:37–45

Kenig M, Vandamme E, Abraham EP (1976) Mode of action of bacilysin and anticapsin and biochemical properties of bacilysin-resistant mutants. J Gen Microbiol 94:46–54

Lestini E, Robertson K, Murphy CD, Paradisi F (2012) Alternative mild route to the synthesis of 4-methylenecyclohex-2-enone, a key moiety of the anticancer compounds ottelione A and B. Synth Commun 42(12):1864–1876

Mathers CD, Boerma T, Fat DM (2009) Global and regional causes of death. Br Med Bull 92(1):7–32

Nicolaou KC, Montagnon T, Baran PS (2002) HIO_3 and I_2O_5: mild and selective alternative reagents to IBX for the dehydrogenation of aldehydes and ketones. Angewandte Chemie-Int Edition 41(8):1386–1389

O'Reilly E, Lestini E, Baiducci D, Paradisi F (2009) One-step diketopiperazine synthesis using phase transfer catalysis. Tetrahedron Lett 50(15):1748–1750

Paradisi F, Porzi G, Sandri S (2001) A new stereocontrolled synthesis of uncommon tripeptides derived from 2,6-diaminopimelic acid (2,6-DAP). Tetrahedron Asymmetry 12(23):3319–3324. doi:10.1016/s0957-4166(02)00002-2

Perry D, Abraham EP (1979) Transport and metabolism of bacilysin and other peptides by suspensions of *Staphylococcus aureus*. J Gen Microbiol 115:213–221

So AD, Gupta N, Brahmachari SK, Chopra I, Munos B, Nathan C, Outterson K, Paccaud JP, Payne DJ, Peeling RW, Spigelman M, Weigelt J (2011) Towards new business models for R&D for novel antibiotics. Drug Resist Updates 14:88–94

Spellberg B, Powers JH, Brass EP, Miller LG, Edwards JE (2004) Trends in antimicrobial drug development: implications for the future. Clin Infect Dis 38(9):1279–1286

Wild H (1994) Enantioselective total synthesis of the antifungal natural-products chlorotetaine, bacilysin, and anticapsin and of related-compounds—revision of the relative configuration. J Org Chem 59(10):2748–2761

Wipf P, Jeger P, Kim Y (1998) Thiophilic ring-opening and rearrangement reactions of epoxyketone natural products. Bioorg Med Chem Lett 8(4):351–356

Wojciechowski M, Milewski S, Mazerski J, Borowski E (2005) Glucosamine-6-phosphate synthase, a novel target for antifungal agents. Molecular modelling studies in drug design. Acta Biochim Pol 52(3):647–653