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Synthesis and stability of small molecule probes for *Pseudomonas aeruginosa* quorum sensing modulation†

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Received 19th August 2004, Accepted 13th September 2004
First published as an Advance Article on the web 13th October 2004

The human pathogen *Pseudomonas aeruginosa* uses *N*-butyryl-L-homoserine lactone (BHL) and *N*-(3-oxododecanyl)-L-homoserine lactone (OdDHL) as small molecule intercellular signals in a phenomenon known as quorum sensing (QS). QS modulators are effective at attenuating *P. aeruginosa* virulence; therefore, they are a potential new class of antibacterial agent. The lactone in BHL and OdDHL is hydrolysed under physiological conditions. The hydrolysis proceeds at a rate faster than racemisation of the α-chiral centre. Non-hydrolysable, non-racemic analogues (small molecule probes) were designed and synthesised, replacing the lactone with a ketone. OdDHL analogues were found to be relatively unstable to decomposition unless they were difluorinated between the β-keto amide. Stability studies on a non-hydrolysable, cyclohexanone analogue indicated that racemisation of the α-chiral centre was relatively slow. This analogue was assayed to show that the L-isomer is likely to be responsible for the QS autoinducing activity in *P. aeruginosa* and *Serratia* strain ATCC39006.

Introduction

Bacteria can communicate intercellularly by synthesising and detecting the presence of specific small molecules; this phenomenon is known as quorum sensing (QS).[1] QS is used by diverse bacteria as a way of coordinating multiple physiological processes in step with bacterial cell population density. Many Gram-negative bacteria, such as *Pseudomonas aeruginosa*, use *N*-acyl homoserine lactones (AHL) as their small molecule intercellular signal.[2] We have reported previously that the lactone in AHL is hydrolysed non-enzymatically under physiological conditions.[3] Therefore, we investigated the preparation of non-hydrolysable AHL analogues, by replacing the lactone with a ketone (Fig. 1).[4] This paper describes our asymmetric synthesis of cyclic ketone analogues and an investigation into their stability under physiological conditions.

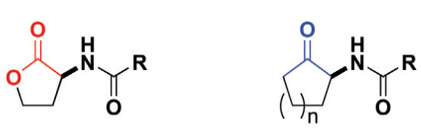
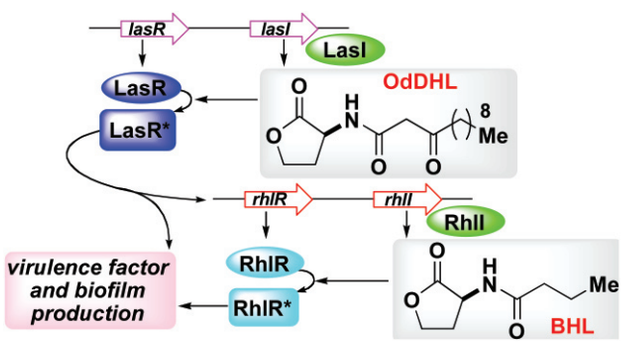


Fig. 1 *N*-acyl homoserine lactone (N-AHL) structure and non-hydrolysable ketone analogues.

P. aeruginosa is one of the leading pathogens in a range of life threatening nosocomial infections and the primary cause of respiratory deterioration and mortality in cystic fibrosis patients.[5] The capacity for rapid drug-resistance development and biofilm formation makes this organism particularly difficult to eradicate, either by antibiotics or the host defence system.[6] *P. aeruginosa* has a variety of resistance mechanisms, ranging from broad-spectrum (tetracycline, fluoroquinolones, imipenem, aminoglycosides) multi-drug efflux pumps to antibiotic-modifying enzymes (aminoglycosides, β-lactams).[7] QS enables the organism to express specific genes in a coordinated fashion leading to a rapid and full blown virulence cascade. *P. aeruginosa* QS is mediated by two small molecules with autoinductive potential: *N*-butyryl-L-homoserine lactone (BHL) and *N*-(3-oxododecanyl)-L-homoserine lactone (OdDHL) shown in Scheme 1. As the bacterial population cell density increases, the autoinducers reach a threshold concentration that triggers virulence factor production and an attack of the host. Since mutants with defects in the QS system show attenuated virulence and production of undifferentiated biofilms, the QS system constitutes a novel and exciting chemotherapeutic target. Most significantly, attenuation of *P. aeruginosa* virulence, in a mouse model of *P. aeruginosa* lung infection, has been reported using a synthetic furanone (based on a natural furanone from algae) QS inhibitor.[8] This observation is the first published data proving that QS modulators are, indeed, effective for treating infections, and, therefore, they represent a potential new class of antibacterial agent that could be used to attenuate virulence.



Scheme 1 The las and rhl QS signalling pathways in *P. aeruginosa*.

In *P. aeruginosa*, the QS system is hierarchical, involving two signalling pathways acting sequentially (Scheme 1). Briefly, the enzyme LasI produces OdDHL, which binds to and activates a LuxR homolog called LasR. LuxR homologs are ligand-activated transcriptional regulators. Activated LasR (labelled as LasR*) turns on transcription of a subset of virulence genes (*e.g.* leading to production of rhamnolipids, alkaline protease, LasA, LasB, pyocyanin, cyanide, the Xcp secretory pathway, RpoS) and of a gene encoding another LuxR homolog, *rhlR*.[9] RhlR binds the second signalling molecule BHL, (produced by RhlI) and turns on the expression of another set of virulence genes.

† Electronic Supplementary Information (ESI) available: Full experimental data of stability experiments, spectra of compounds, and assay data. See http://www.rsc.org/suppdata/ob/b4/b412802h

DOI: 10.1039/b412802h

However, the working model shown in Scheme 1 is a simplification, since there are other QS regulators in *P. aeruginosa* such as *Pseudomonas* Quinolone Signal (PQS)[10] and a third LuxR homologue QscR.[11] These regulators add an additional layer of complexity to the analysis and understanding of small molecule mediated regulation of gene expression in *P. aeruginosa*.

Earlier, we reported that the AHL signalling molecule from the plant pathogen *Erwinia carotovora* was non-enzymatically turned over by hydrolysis of the lactone moiety under physiological conditions.[3] The rate of hydrolysis was found to be dependent on the pH of the medium and temperature. This study encouraged us to make non-hydrolysable analogues by replacing the lactone ring oxygen with a methylene group to make a cyclic ketone. While we were studying these structures Smith *et al.* reported the biological activity of racemic cyclic ketone analogues **1a**–**b** and **2a**–**b** (Fig. 2).[4] The compounds' notable biological activity could be rationalised by the crystal structures of TraR (a LuxR homolog from *Agrobacterium tumefaciens*) bound to its cognate AHL.[12] In these structures the only directional bond formed between the protein and the homoserine lactone ring is from the side-chain of Trp57 to the ring carbonyl oxygen lone pair. Therefore, the other oxygen of the lactone appears not to be directly involved in receptor binding.

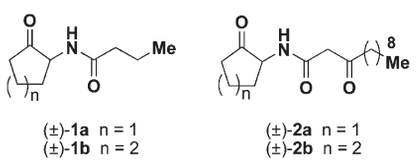


Fig. 2 Racemic cyclic ketone AHL analogues.[4]

Results and discussion

This account describes our efforts in synthesising various AHL cyclic ketone analogues in non-racemic form (Fig. 3), and an assessment of their stability under conditions similar to the growth media. Carbocycle sizes of 5, 6 and 7-members were chosen to explore systematically the structure–activity relationship with respect to changes to the homoserine lactone moiety. The difluorinated OdDHL derivatives **3a**–**c** were selected as synthetic targets rather than non-fluorinated derivatives **2**, as they were found to be more stable to decomposition under physiological conditions (*vide infra*); therefore, **4** was required as a control.[13] Natural AHL small molecules are L-form because they are biosynthesised from (*S*)-adenosyl-L-methionine where the α-chiral centre is not disturbed.[14] It has been shown by Ikeda *et al.* that although the L-isomers of AHL are essential for autoinducer activity, the D-isomers are neither agonists nor antagonists.[15] We hypothesised that the L-enantiomer of the cyclic ketone analogues should be the active enantiomer. The asymmetric synthesis of the cyclic ketones was expected to be challenging because of the extra sensitivity of the α-chiral centre of an amino-ketone to racemisation, compared to an amino–lactone. It was also important to justify the asymmetric synthesis by showing that the compounds would not racemise at a significant rate at physiological temperature and pH.

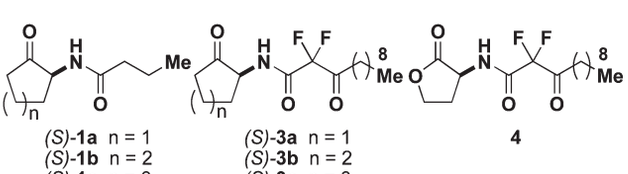


Fig. 3 (*S*)-enantiomer cyclic ketone targets of the present study.

Small molecule stability

P. aeruginosa strains, such as PAO1, can proliferate in a range of pH conditions, and can be grown and assayed readily at 37 °C in many rich media such as Luria–Bertani (LB) broth (an aqueous solution of tryptone, yeast extract and NaCl), which, typically, is not buffered. The starting pH of LB is pH 7.0, but as bacteria grow they metabolise weak carbon acids preferentially, and therefore the pH increases, so that after 48 hours of PAO1 growth the pH was approximately pH 8.5. The pH stability studies described herein were conducted with buffered solutions, therefore the studies under-estimate the stability of the small molecules under physiological conditions. NMR samples were prepared in a solution of 10% v/v $CD_3S(O)CD_3$ in deuterated aqueous inorganic buffer (pD 7.3),[16] incubated at 37 °C and then monitored at regular intervals. BHL was monitored by ^{1}H NMR to measure the rate of hydrolysis of the lactone ring (Fig. 4). The half-life of BHL under these conditions is approximately 1 day. At 30 °C the half-life of BHL is approximately 2 days. Racemisation of the chiral centre, as monitored by deuterium exchange, was less than 5% over a week; therefore, it can be concluded that AHL hydrolyse much faster than they racemise. OdDHL has a slower rate of hydrolysis, with a half-life of approximately 2 days (37 °C), consistent with an earlier report.[17] However, due to difficulties with solubility, 50% v/v $CD_3S(O)CD_3$ in deuterated aqueous buffer was required, which may account, at least in part, for the rate difference. Again, racemisation of the chiral centre was less than 5% over a week.

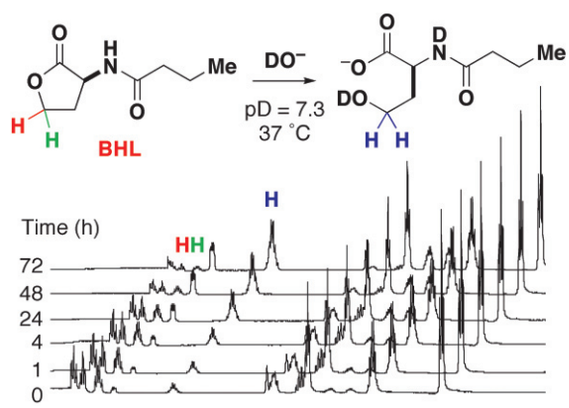


Fig. 4 Hydrolysis of BHL.

The rate of deuteration of the α-chiral centre of the amino-ketone in **1b** was monitored by ^{1}H NMR. This rate is approximately equivalent to the rate of racemisation of the α-amino position in the enantiopure amino ketone (*S*)-**1b**. The half-life of α-protonated **1b** at 37 °C was approximately 36 h (Fig. 5). At 30 °C the half-life of α-protonated **1b** is much longer; in fact, less than 20% deuteration occurred over a week. The amino–cyclopentanone **1a** would be expected to have a longer half-life than **1b** because of a kinetically less favourable enolisation, due to less σ_{C-H}–$\pi^*_{C=O}$ orbital overlap in the ground state conformation and increased torsional strain in the transition state.

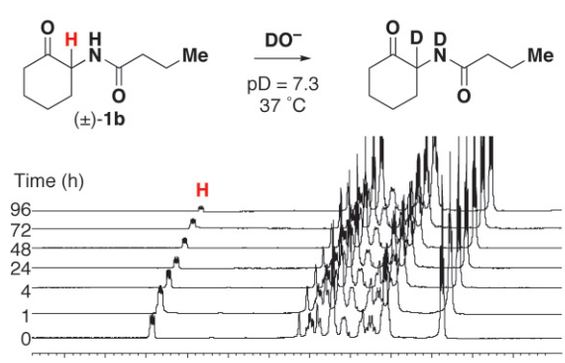


Fig. 5 Deuteration of (±)-**1b**.

Under physiological conditions **2b** was found to be very unstable to decomposition. Although the compound could be synthesised and fully characterised, when a solution of the compound was mixed with aqueous buffer (pH 7.0, room temperature) the mixture decomposed completely within 3 hours. At 37 °C, the bioassay temperature, and at higher pH the decomposition rate increased. Thin layer chromatography (TLC) indicated that at least five other compounds had been produced; several spots quenched the UV fluorescence of the TLC plate (silica gel 60 F_{254}) indicating the formation of aromatic/conjugated products. It appears likely that these compounds decomposed in the bioassays to generate a mixture of constituents responsible for the reported activity. The analysis of **2b** decomposition products by liquid chromatography-mass spectrometry (LCMS) identified compounds probably resulting from cyclisation of an enolate onto the cyclohexanone, leading to hydroxypyrrole products, and higher molecular weight products, the result of oligomerization. After several hours at room temperature in aqueous buffer (pH 7.0) the low molecular weight products disappeared and a red–brown, non-miscible oil resulted. When *P. aeruginosa* (PAO1) was treated with **1b** or **2b** (500 μM) in our standard assay conditions (37 °C, LB media, 48 h), TLC of the supernatant detected **1b**, but not **2b**. It therefore appears likely that the biological activity reported for **2b** was due to decomposition products.[4] Unfortunately, since these decomposition products were also unstable it was not possible to identify the active component. In light of these results we decided to synthesise the fluorinated analogues **3a**–**c**, which did not decompose under physiological conditions.

Synthesis

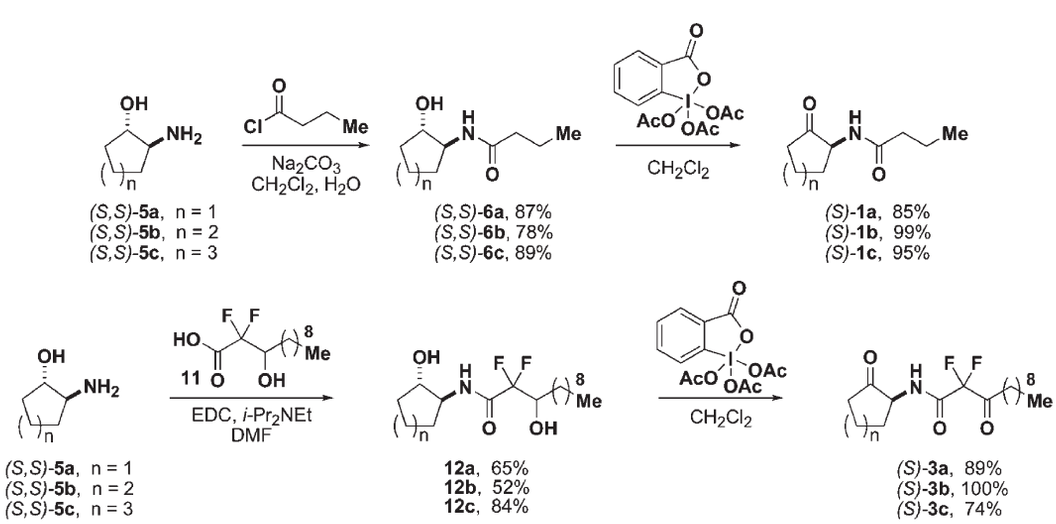
In order to synthesise the non-racemic derivatives shown in Fig. 3 it is vital to unveil the sensitive α-amino ketone only at the end of the synthesis under mild, neutral conditions. Aubé and coworkers have synthesised successfully enantiomerically enriched *N*-*tert*-butoxycarbonyl-2-aminocycloalkanones by the mild oxidation of the corresponding enantiomerically pure alcohol.[18] The same strategy was adopted in this work. Enantiomerically pure *trans*-cyclopentyl, cyclohexyl and cycloheptyl amino alcohols (**5a–c**) were synthesised using either a modified Overman and Sugai procedure[19] or the catalytic asymmetric method described by Jacobsen *et al.*[20] The Overman and Sugai procedure involves the aminolysis of a cycloalkene oxide with (*S*)-α-methylbenzylamine followed by hydrogenolysis. In our experience this procedure was straightforward and high yielding (>40% over two steps), especially when the hydrogenolysis was conducted slowly at room temperature.[21] The method of Jacobsen *et al.* worked well for the highly crystalline cyclohexyl amino alcohol.

The amino groups of the enantiopure amino alcohols **5a**–**c** were acylated selectively with one equivalent of butyryl chloride in the presence of Na_2CO_3 under two phase conditions to give (*S*,*S*)-**6a**–**c** (Scheme 2). Both Dess–Martin periodinane (DMP) and pyridinium chlorochromate (PCC) oxidation of the alcohols delivered successfully the expected amino ketones (*S*)-**1a**–**c**, which required rapid purification on silica to avoid epimerisation. The enantiopurity of (*S*)-**1b** was determined by reduction of the ketone with diisobutylaluminium hydride (DIBAL). The *trans*-diastereomer was purified using chromatography and the optical rotation measured. The optical rotation of the alcohol indicated the high enantiomeric ratio of 95 : 5 ($[a]_D^{25}$ −31.8 *versus* −28.7 [*c* 1.2 in $CHCl_3$]).[22]

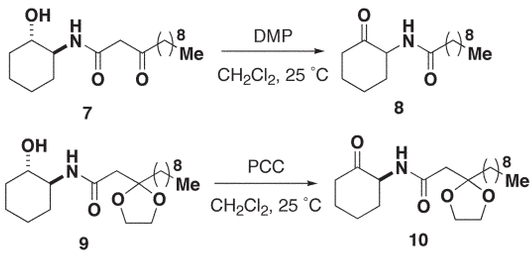
The synthesis of non-hydrolysable OdDHL analogues **2a** and **2b** proved to be more difficult than expected, due to the incompatibility of the 1,3-dicarbonyl group to oxidising conditions. Oxidation of **7** using DMP or PCC resulted in decomposition products only, including mainly **8** (Scheme 3), which was isolated pure in 20% yield. Its formation resulted possibly from the oxidation between the dicarbonyl to give the tricarbonyl, followed by sequential rearrangements with loss of CO_2. This could be avoided by protection of the ketone in **7** as a ketal (**9**). Subsequent oxidation of **9** to ketone **10** (58%), and deprotection with TFA gave **2b**, which could be purified by chromatography and characterised (60%).[4]

Synthesis of enantiopure non-hydrolysable OdDHL analogues **3a–c** required the selective coupling of enantiopure amino alcohols with 2,2-difluoro-3-hydroxydodecanoic acid (**11**), followed by oxidation of the resultant diastereomeric diols **12a–c** (Scheme 2).[13] The non-racemic α-amino ketones **3a**–**c**, once again, required rapid purification on silica to avoid epimerisation; however, the route proved reliable. All α-amino ketones (**1a**–**c** and **3a**–**c**) were stored dry at −20 °C. Under these conditions there was no detectable decomposition after several months (^{1}H NMR, TLC, $[a]_D^{25}$). The detailed biological activity of all these analogues will be described elsewhere.[23]

Phenotypic screening with *P. aeruginosa*. All natural AHL small molecules reported to date are L-form,[14] and Ikeda *et al.* have shown that, while the L-isomer is essential for autoinducer activity, the D-isomers are neither agonists nor antagonists.[15] We hypothesised that the L-enantiomer of the cyclic ketone analogues should be the active enantiomer, and therefore compared the biological activity of (*S*)-**1b** with (*R*)-**1b**. Although the enantiomerically-enriched (er *ca.* 95 : 5) α-amino ketones would be epimerised slowly under assay conditions, there should be a detectable difference in activity. A pigment production phenotypic assay was used to test this hypothesis with a mutant of *P. aeruginosa* (PAO-JP2), which cannot biosynthesise BHL or OdDHL. The green–blue pigmentation is a complex mixture of compounds, including both pyoverdin and pyocyanin, which are under QS control, although not in



Scheme 2 Synthesis of non-hydrolysable BHL and OdDHL analogues.



Scheme 3 Oxidation of alcohol **7** failed to give the required ketone (**2b**), but instead resulted in decomposition products such as **8**; however, protected derivative **9** could be oxidised successfully to **10**.

a simplistic sense, since multiple (non-QS) regulatory inputs are involved also. Nevertheless, under controlled conditions the visual, colourimetric output made the phenotypic assay a good choice. Pigment production requires both BHL and OdDHL (both *ca.* 10 μM). Addition of both autoinducers to the mutant strain PAO-JP2 and incubation for 48 hours at 37 °C restores the wild-type phenotype of PAO1. Substitution of BHL with either (*S*)-**1b** or (*R*)-**1b** revealed a difference in the production of pigmentation as shown in Fig. 6. Compound (*S*)-**1b** induced pigmentation at concentrations as low as 407 nM, whereas enantiomer (*R*)-**1b** required approximately an order of magnitude higher concentration, indicating that (*S*)-**1b** (L-isomer) is the active enantiomer. In order to confirm the result with PAO1-JP2 we also conducted a more sensitive bioassay involving *Serratia* strain ATCC39006.

Phenotypic screening with *Serratia* strain ATCC39006. The racemisation of **1b** occurs at a much slower rate at 30 °C *versus* 37 °C, the standard incubation temperature for *P. aeruginosa* (*vide supra*). *Serratia* 39006 is a Gram-negative motile rod that is grown at 30 °C, and uses BHL to produce the QS-controlled, red pigment prodigiosin. A screen was run to assess the ability of (*S*)-**1b** to produce the pigment, *versus* (*R*)-**1b** (Fig. 7). Each well contained a petone–glycerol medium containing decreasing amounts of either (*S*)-**1b** or (*R*)-**1b**. Aliquots of an overnight culture of the *Serratia* 39006 *smaI* mutant, which cannot synthesise BHL, were spotted onto the surface of each well. The plates were incubated overnight at a temperature of 30 °C. The intensity of red colouration of the spots reflects the level of prodigiosin biosynthesis. It appears that the natural ligand BHL is the most potent, but note that the halo size for (*R*)-**1b** is smaller than for (*S*)-**1b**, and that pigmentation is less intense. Under these assay conditions the (*S*)-enantiomer of **1b** activates pigment synthesis at around 45 nM, whereas for a similar level of pigment production the concentration for (*R*)-**1b** was around 11 μM. This indicates that the (*S*)-enantiomer is indeed the active component. This result is consistent with that of Ikeda *et al.*[15]

Conclusions

In summary, we have synthesised non-hydrolysable (cyclopentanone, cyclohexanone and cycloheptanone) analogues of BHL and OdDHL in non-racemic form and conducted stability studies. The lactone in BHL and OdDHL was hydrolysed under conditions similar to physiological media at a significant rate, faster than racemisation of the α-chiral centre. Stability studies on the non-hydrolysable, cyclohexanone analogue **1b** indicated that racemisation of the α-chiral centre was relatively slow. Both enantiomers of this analogue were assayed to show that the (*S*)-enantiomer (L-isomer) is likely to be responsible for the QS autoinducing activity in *P. aeruginosa* and *Serratia* 39006.

Experimental

Except as otherwise indicated, reactions were carried out under argon with dry, freshly distilled solvents. Dry tetrahydrofuran was dispensed from a delivery system which passes the solvent

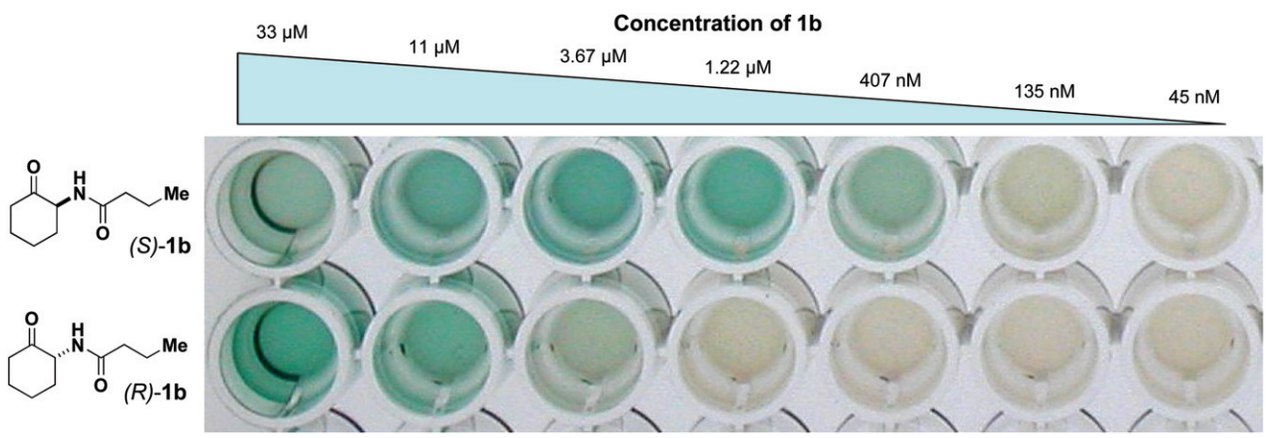


Fig. 6 *P. aeruginosa* strain PAO-JP2 pigmentation assay. All wells contain PAO-JP2 in an alanine–glucose salts medium, 20 μM OdDHL, and the indicated decreasing concentration of (*S*)-**1b** or (*R*)-**1b**. The intensity of green colouration in each well reflects the level of pigment biosynthesis.

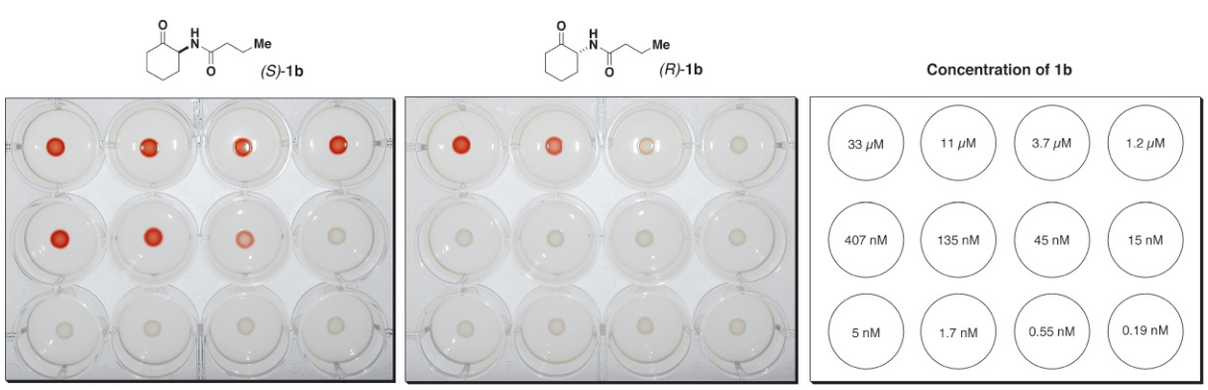


Fig. 7 *Serratia* strain ATCC39006 *smaI* mutant prodigiosin assay. All wells contain *Serratia* 39006 *smaI* mutant in a petone–glycerol medium, and the indicated decreasing concentration of (*S*)-**1b** or (*R*)-**1b**. The intensity of red colouration of the spots reflects the level of prodigiosin biosynthesis.

through packed columns of dry neutral alumina and Q5 reactant. Dichloromethane was distilled from calcium hydride. All other reagents were purified in accordance with the instructions in ref 25 or used as obtained from commercial sources.

Yields refer to chromatographically and spectroscopically pure compounds. All reactions were monitored by thin layer chromatography using glass plates precoated with Merck silica gel 60 F_{254} or aluminum oxide 60 F_{254}. Visualization was by the quenching of UV fluorescence (λ_{max} = 254 nm) or by staining with ceric ammonium molybdate or potassium permanganate or Dragendorff's reagent (0.08% w/v bismuth subnitrate and 2% w/v KI in 3 M aq. AcOH). Retention factors (R_f) are quoted to 0.01. Melting points were obtained using a Mel-Temp II melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 343 polarimeter using a sodium lamp (λ 589 nm, D-line); $[\alpha]_{\lambda}^{T^{\circ}C}$ values are reported in units of 10^{-1} deg cm^2 g^{-1}, and concentration (c) in units of g per 100 ml. Microanalyses were performed by the University of Cambridge Microanalytical Laboratory in the Department of Chemistry, and are quoted to the nearest 0.1% for all elements except for hydrogen, which is quoted to the nearest 0.05%. Reported atomic percentages are within the error limits of ± 0.3%. Infrared spectra were recorded neat on a diamond/ZeSe plate using a Perkin-Elmer Spectrum One FT-IR Universal ATR sampling accessory spectrometer with internal referencing. Absorption maxima (ν_{max}) are reported in wavenumbers (cm^{-1}) and the following abbreviations are used: w, weak; m, medium; s, strong; br, broad. Proton magnetic resonance spectra were recorded on Bruker Ultrashield 400 or 500 (See: http://wwwmethods.ch.cam.ac.uk/meth/nmr.html). Proton assignments are supported by ^{1}H–^{1}H spectra where necessary. Chemical shifts (δ_H) are quoted in ppm and are referenced to the residual non-deuterated solvent peak. Coupling constants (J) are reported in Hertz to the nearest 0.5 Hz. Data are reported as follows: chemical shift, integration, multiplicity [br, broad; s, singlet; d, doublet; t, triplet; q, quartet; qui, quintet; sept, septet; m, multiplet; or as a combination of these (*e.g.* dd, dt, *etc.*)], coupling constant(s) and assignment. Diastereotopic protons are assigned as X and X′, where the ′ indicates the lower field proton. Carbon magnetic resonance spectra were recorded on Bruker Ultrashield 500 spectrometers. Carbon spectra assignments are supported by DEPT editing and where necessary ^{13}C–^{1}H (HMQC) correlations. Chemical shifts (δ_C) are quoted in ppm to the nearest 0.01 ppm, and are referenced to the deuterated solvent. Fluorine magnetic resonance spectra (^{19}F) were recorded on a DPX 400 MHz spectrometer. Chemical shifts (δ_P) are quoted in ppm to the nearest 0.01 ppm and are referenced to CF_3CH_2OH (external). Low resolution mass spectra were obtained with Kratos MS890MS (CI/EI), and Micromass Q-TOF (APCI/ES) spectrometers. Only molecular ions, fractions from molecular ions and other major peaks are reported. High resolution mass measurements were performed by the University of Cambridge Mass Spectrometry Laboratory in the Department of Chemistry, and reported mass values are within the error limits of ± 5 ppm mass units.

(1*S*,2*S*)-2-Hydroxycyclopentylamine hydrochloride [(*S*,*S*)-5a]

(1*S*,2*S*)-2-Hydroxycyclopentylamine hydrochloride [(*S*,*S*)-**5a**] was prepared by the Aubé modification of the Overman and Sugai procedure, followed by dissolving the amino alcohol in the minimum quantity of EtOH and diluting it with Et_2O until the solution started to become cloudy. Gaseous HCl was bubbled through the solution until no more precipitate was formed. The mixture was filtered and dried under suction, then high vacuum, to give (*S*,*S*)-**5a** as an off-white solid; $[\alpha]_D^{25}$ +29.3 (*c* 0.8 in EtOH); lit.[21] $[\alpha]_D^{26}$ +29.7 (*c* 1.95 in EtOH).

(1*S*,2*S*)-2-Hydroxycyclohexylamine hydrochloride [(*S*,*S*)-5b]

(1*S*,2*S*)-2-Hydroxycyclohexylamine hydrochloride [(*S*,*S*)-**5b**] was prepared following the procedure of Jacobsen *et al.* to give an off-white solid; (free base) $[\alpha]_D^{25}$ +48.8 (*c* 0.19 in MeOH); lit.[20] $[\alpha]_D^{25}$ +48.2 (*c* 1.0 in MeOH).

(1*R*,2*R*)-2-Hydroxycyclohexylamine hydrochloride [(*R*,*R*)-5b]

(1*R*,2*R*)-2-Hydroxycyclohexylamine hydrochloride [(*R*,*R*)-**5b**] was prepared by the Aubé modification of the Overman and Sugai procedure to give an off-white solid; (free base) $[\alpha]_D^{25}$ −35.0 (*c* 0.20 in MeOH).

(1*S*,2*S*)-2-Hydroxycycloheptylamine hydrochloride [(*S*,*S*)-5c]

(1*S*,2*S*)-2-Hydroxycycloheptylamine hydrochloride [(*S*,*S*)-**5c**] was prepared by the Aubé procedure, followed by dissolving the amino alcohol in the minimum quantity of EtOH and diluting it with Et_2O until the solution started to become cloudy. Gaseous HCl was bubbled through the solution until no more precipitate was formed. The mixture was filtered and dried under suction, then high vacuum, to give (*S*,*S*)-**5b** as an off-white solid; $[\alpha]_D^{25}$ +20.9 (*c* 0.32 in EtOH); lit.[18] (free base) $[\alpha]_D$ +17 (*c* 1.01 in EtOH).

(1*S*)-*N*-(2-Oxocyclopentyl)butyramide [(*S*)-1a]

(1*S*,2*S*)-2-Hydroxycyclopentylamine hydrochloride [(*S*,*S*)-**5a**], 303 mg, 2.2 mmol) and Na_2CO_3 (700 mg, 6.6 mmol) were partitioned between CH_2Cl_2 (5 ml) and H_2O (5 ml). Butyryl chloride (0.21 ml, 2.0 mmol) was added and the mixture was stirred vigorously at room temperature for 1 h. The two-phase mixture was separated and the aqueous layer extracted twice with CH_2Cl_2. The organic layers were combined, washed (brine), dried ($MgSO_4$), filtered, concentrated *in vacuo* and chromatographed using EtOAc as an eluent to give (*1S,2S*)-*N*-(*2-hydroxycyclohexyl*)*butyramide* (*S*,*S*)-**6a** as a colourless oil that later solidified (294 mg, 87%); R_f 0.26 (SiO_2; EtOAc); ν_{max} (neat) cm^{-1} 3280s, 2962s, 2874s, 1626vs (amide), 1543vs (amide), 1455m, 1370m, 1288m, 1210m, 1111m, 1092m, 1051m; δ_H (400 MHz, $CDCl_3$) 0.94 (3H, t, *J*7.5, CH_3), 1.35–1.44 (1H, m), 1.59–1.82 (5H, m), 1.96–2.13 (2H, m), 2.15 (2H, t, *J*5, NCOCH_2), 3.77–3.84 (1H, m, C*H*OH), 3.93 (1H, apparent q, *J*7, C*H*N), 4.62 (1H, br s, O*H*), 5.74 (1H, br s, N*H*); δ_C (100 MHz, $CDCl_3$) 13.62, 19.09, 21.15, 30.12, 32.44, 38.11, 60.60, 79.55, 175.33; *m/z* (EI) 171 (M$^+$, 30%), 128 (45), 114 (60), 100 (90), 88 (100), 71 (95); *m/z* (APCI) 172 (MH$^+$, 100%); HRMS found 171.1264, $C_9H_{17}NO_2$ (M$^+$) requires 171.1259 (Δ = 2.9 ppm). Alcohol (*S*,*S*)-**6a** (16.2 mg, 94.6 μmol) was dissolved in anhydrous CH_2Cl_2 (2 ml) and Dess–Martin periodinane (80 mg, 189 μmol) was added. After the mixture was stirred for 1 h at room temperature under Ar, it was washed with H_2O, dried ($MgSO_4$), filtered, concentrated *in vacuo* and rapidly chromatographed using EtOAc as an eluent to give (*1S*)-*N*-(*2-oxocyclopentyl*)*butyramide* [(*S*)-**1a**] as a colourless oil (13.7 mg, 85%); $[\alpha]_D^{25}$ +15.6 (*c* 0.5 in EtOH); R_f 0.38 (SiO_2; EtOAc); ν_{max} (neat) cm^{-1} 2966m, 1752s (ketone), 1647s (amide), 1547s (amide); δ_H (400 MHz, $CDCl_3$) 0.95 (3H, t, *J*8, CH_3), 1.53–1.63 (1H, m), 1.66 (2H, apparent q, *J*8, CH_2Me), 1.82–1.92 (1H, m), 2.01–2.09 (1H, m), 2.13–2.23 (1H, m), 2.20 (2H, t, *J*8, NCOCH_2), 2.37–2.44 (1H, m), 2.64–2.71 (1H, m), 4.08–4.14 (1H, m, C*H*N), 5.82 (1H, br s, N*H*); δ_C (100 MHz, $CDCl_3$) 14.08, 18.45, 19.39, 30.62, 35.26, 38.67, 58.53, 173.72, 215.65; *m/z* (EI) 169 (M$^+$, 35%), 113 (55), 69 (100); HRMS found 169.1102, $C_9H_{15}NO_2$ (M$^+$) requires 169.1103 (Δ = 0.6 ppm).

(1*S*)-*N*-(2-Oxocyclohexyl)butyramide [(*S*)-1b]

(1*S*,2*S*)-2-Hydroxycyclohexylamine hydrochloride [(*S*,*S*)-**5b**], 334 mg, 2.2 mmol) and Na_2CO_3 (700 mg, 6.6 mmol) were partitioned between CH_2Cl_2 (5 ml) and H_2O (5 ml). Butyryl chloride (0.21 ml, 2.0 mmol) was added and the mixture was stirred vigorously at room temperature for 1 h. The two-phase mixture was separated and the aqueous layer extracted twice with CH_2Cl_2. The organic layers were combined, washed (brine), dried ($MgSO_4$), filtered, concentrated *in vacuo* and

chromatographed using EtOAc as an eluent to give (*1S*,*2S*)-*N*-(*2-hydroxycyclohexyl*)*butyramide* [(*S*,*S*)-**6b**] as a white solid (289 mg, 78%); $[\alpha]_D^{25}$ −31.8 (*c* 1.2 in $CHCl_3$); R_f 0.19 (SiO_2; EtOAc); mp 97–98 °C; ν_{max} (neat) cm^{-1} 3294s, 2928s, 2855s, 1632vs (amide), 1563vs (amide), 1454s, 1347s, 1085s, 1071s; δ_H (400 MHz, $CDCl_3$) 0.95 (3H, t, *J*7.5, C*H*$_3$), 1.13–1.36 (4H, m), 1.65–1.68 (2H, m, C*H*$_2$Me), 1.68–1.75 (2H, m), 1.90–1.96 (1H, m), 2.04–2.08 (1H, m), 2.18 (2H, t, *J*7.5, NCOC*H*$_2$), 3.26–3.33 (1H, m), 3.55 (1H, d, *J*5, O*H*), 3.59–3.67 (1H, m), 5.38 (1H, br s, N*H*); δ_C (100 MHz, $CDCl_3$) 14.05, 19.56, 24.40, 24.99, 31.94, 34.90, 39.04, 56.13, 75.96, 175.42; *m*/*z* (EI) 185 (M^+, 5%), 114 (65), 98 (60), 88 (100), 71 (55); *m*/*z* (APCI) 186 (MH^+, 100%); HRMS found 185.1416, $C_{10}H_{19}NO_2$ (M^+) requires 185.1416 (Δ = 0.01 ppm). Alcohol (*S*,*S*)-**6b** (10.0 mg, 53.9 μmol) was dissolved in anhydrous CH_2Cl_2 (1 ml) and Dess–Martin periodinane (46 mg, 108 μmol) was added. After the mixture was stirred for 1 h at room temperature under Ar, it was washed with H_2O, dried ($MgSO_4$), filtered, concentrated *in vacuo* and rapidly chromatographed using EtOAc as an eluent to give (*1S*)-*N*-(*2-oxocyclohexyl*)*butyramide* [(*S*)-**1b**] as a white solid (9.8 mg, 99%); mp 50–51 °C; $[\alpha]_D^{25}$ +60.4 (*c* 2.3 in $CHCl_3$); R_f 0.47 (SiO_2; EtOAc); ν_{max} (neat) cm^{-1} 3300m, 2935m, 2869m, 1719s (ketone), 1643vs (amide), 1535s (amide), 1450m, 1127s; δ_H (400 MHz, $CDCl_3$) 0.93 (3H, t, *J*7.5, C*H*$_3$), 1.27–1.38 (1H, m), 1.56–1.70 (1H, m), 1.65 (2H, apparent q, *J*7, C*H*$_2$Me), 1.73–1.88 (2H, m), 2.10–2.17 (1H, m), 2.18 (2H, t, *J*7, NCOC*H*$_2$), 2.34–2.43 (1H, m), 2.49–2.55 (1H, m), 2.64-2.70 (1H, m), 4.44–4.51 (1H, m, C*H*N), 6.39 (1H, br s, N*H*); δ_C (100 MHz, $CDCl_3$) 14.05, 19.47, 24.42, 28.48, 36.01, 38.93, 41.55, 58.40, 173.06, 208.35; *m*/*z* (EI) 183 (M^+, 55%), 88 (100), 71 (65); *m*/*z* (APCI) 184 (MH^+, 100%); HRMS found 183.1250, $C_{10}H_{17}NO_2$ (M^+) requires 183.1259 (Δ = 4.9 ppm).

(1*R*)-*N*-(2-Oxocyclohexyl)butyramide [(*R*)-1b]

Synthesis identical to the synthesis of [(*S*)-**1b**]. [(*R*,*R*)-**6b**]: $[\alpha]_D^{25}$ +36.9 (*c* 0.44 in $CHCl_3$). [(*R*)-**1b**]: $[\alpha]_D^{25}$ −58.8 (*c* 0.40 in $CHCl_3$).

(1*S*)-*N*-(2-Oxocycloheptyl)butyramide [(*S*)-1c]

(1*S*,2*S*)-2-Hydroxycycloheptylamine [(*S*,*S*)-**5c**], 10.0 mg, 60.4 μmol) and Na_2CO_3 (19.2 mg, 181 μmol) were partitioned between CH_2Cl_2 (1 ml) and H_2O (1 ml). Butyryl chloride (6.2 μl, 60.4 μmol) was added and the mixture was stirred vigorously at room temperature for 1 h. The two-phase mixture was separated and the aqueous layer extracted twice with CH_2Cl_2. The organic layers were combined, washed (brine), dried ($MgSO_4$), filtered, concentrated *in vacuo* and chromatographed using EtOAc as an eluent to give (*1S*,*2S*)-*N*-(*2-hydroxycycloheptyl*)-*butyramide* [(*S*,*S*)-**6c**] as a colourless oil (10.7 mg, 89%); R_f 0.32 (SiO_2; EtOAc); ν_{max} (neat) cm^{-1} 3300m, 2926s, 1637s (amide), 1542s (amide), 1461m; δ_H (400 MHz, $CDCl_3$) 0.95 (3H, t, *J*7, C*H*$_3$), 1.19–1.28 (2H, m), 1.45–1.78 (10H, m), 2.18 (2H, t, *J*7.5, NCOC*H*$_2$), 3.56–3.62 (1H, m, C*H*OH), 3.76–3.83 (1H, m, C*H*N), 4.04 (1H, d, *J*3, O*H*), 5.55 (1H, br s, N*H*); δ_C (125 MHz, $CDCl_3$) 13.68, 19.16, 22.91, 24.91, 29.70, 31.46, 34.05, 38.64, 59.26, 78.97, 174.62; *m*/*z* (APCI) 200 (MH^+, 100%). Alcohol (*S*,*S*)-**6c** (3.5 mg, 17.6 μmol) was dissolved in anhydrous CH_2Cl_2 (1 ml) and Dess–Martin periodinane (11.2 mg, 26.3 μmol) was added. After the reaction mixture was left to stir for 1 h at room temperature it was washed with H_2O, dried ($MgSO_4$), filtered, concentrated *in vacuo* and rapidly chromatographed (hexane : EtOAc; 1 : 1) to give (*1S*)-*N*-(*2-oxocycloheptyl*)*butyramide* [(*S*)-**1c**] as a white paste (3.3 mg, 95%); $[\alpha]_D^{25}$ +32.1 (*c* 0.39 in $CHCl_3$); R_f 0.25 (SiO_2; hexane : EtOAc; 1 : 1); ν_{max} (neat) cm^{-1} 2929s, 1713s (ketone), 1647vs (amide), 1535s (amide); δ_H (600 MHz, $CDCl_3$) 0.94 (3H, t, *J*8, C*H*$_3$), 1.22–1.28 (2H, m), 1.41–1.47 (1H, m), 1.67 (2H, apparent q, *J*8, C*H*$_2$Me), 1.73–1.88 (3H, m), 1.92–1.98 (1H, m), 2.08–2.14 (1H, m), 2.19 (2H, t, *J*7.5, NCOC*H*$_2$), 2.42–2.49 (1H, m), 2.63–2.70 (1H, m), 4.47–4.71 (1H, m, C*H*N), 6.58 (1H, br s, N*H*); δ_C (150 MHz, $CDCl_3$) 13.65, 19.02, 23.06, 27.57, 28.97, 32.88, 38.49, 41.53, 58.75, 172.21, 210.38; *m*/*z* (APCI) 198 (MH^+, 100%); HRMS found 198.1499, $C_{11}H_{20}NO_2$ (MH^+) requires 198.1497 (Δ = 1.0 ppm).

N-Butyryl–L-homoserine lactone (BHL)

(*S*)-(−)-α-Amino-γ-butyrolactone hydrobromide (401 mg, 2.2 mmol), and Na_2CO_3 (466 mg, 4.4 mmol) were partitioned between CH_2Cl_2 (5 ml) and H_2O (5 ml). Butyryl chloride (0.21 ml, 2.0 mmol) was added and the mixture was stirred vigorously at room temperature for 1 h. The two-phase mixture was separated and the aqueous layer extracted twice with CH_2Cl_2. The organic layers were combined, washed (brine), dried ($MgSO_4$), filtered, concentrated *in vacuo* and rapidly chromatographed using EtOAc as an eluent to give *N-butyryl-L-homoserine lactone* (**BHL**) as a white solid (298 mg, 87%); $[\alpha]_D^{25}$ +8.85 (*c* 2.3 in $CHCl_3$); R_f 0.34 (SiO_2; EtOAc); mp 127–129 °C (lit.[24] 83 °C); ν_{max} (neat) cm^{-1} 3308m, 2959m, 2872m, 1774s (lactone), 1644s (amide), 1547s (amide), 1361m, 1227m, 1171s, 1014s, 998m, 948m, 658m; δ_H (500 MHz, $CDCl_3$) 0.89 (3H, t, *J*7, C*H*$_3$), 1.57–1.65 (2H, m, C*H*$_2$Me), 2.06–2.14 (1H, m, OCH$_2$C*H*H′), 2.17 (2H, t, *J*7, COC*H*$_2$), 2.68–2.76 (1H, m, OCH$_2$CH*H*′), 4.19–4.25 (1H, m, OC*H*H′), 4.39 (1H, apparent t, *J*9, OCH*H*′), 4.51–4.57 (1H, m, C*H*N), 6.34 (1H, br s, N*H*); δ_C (125 MHz, $CDCl_3$) 13.63 (*C*H$_3$), 18.86, 30.27, 37.96, 49.05 (*C*HN), 66.07 (O*C*H$_2$), 173.64 (*C*=O), 175.74 (*C*=O); *m*/*z* (ESI) 194 (MNa^+, 100%); HRMS found 194.0785, $C_8H_{13}NO_3Na$ (MNa^+) requires 194.0793 (Δ = 4.1 ppm).

2,2-Difluoro-3-hydroxydodecanoic acid (11)[13]

Ethylbromodifluoroacetate (3.20 ml, 25.0 mmol) was slowly added to a stirred mixture of decanal (1.88 ml, 10.0 mmol) and activated zinc dust (2.46 g, 37.5 mmol) in anhydrous THF (50 ml) and room temperature under Ar. [**Caution: exotherm**] The reaction mixture was stirred vigourously for 3 h (TLC) and then a saturated aqueous solution of NH_4Cl was added (30 ml). The mixture was filtered through Celite and the precipitate washed with EtOAc. The two phase solution was separated and the aqueous layer was extracted with EtOAc. The organic layers were combined, washed (brine), dried ($MgSO_4$), filtered, concentrated *in vacuo* and chromatographed (hexane : EtOAc; 17 : 3) to give *2,2-difluoro-3-hydroxydodecanoic acid ethyl ester* as a colourless oil (2.79 g, 99%); R_f 0.30 (SiO_2; hexane : EtOAc; 17 : 3; $KMnO_4$); ν_{max} (neat) cm^{-1} 3478w, 2926s, 2856s, 1760vs (ester), 1466m, 1374m, 1314s, 1092vs, 855m, 783m, 722m; δ_H (400 MHz, $CDCl_3$) 0.88 (3H, t, *J*7, CH$_2$CH$_2$C*H*$_3$), 1.25–1.40 (14H, m, C*H*$_2$), 1.36 (3H, t, *J*8, OCH$_2$C*H*$_3$), 1.49–1.72 (2H, m, CHOHC*H*$_2$), 2.13 (1H, d, *J*7, O*H*), 3.97–4.08 (1H, m, C*H*OH), 4.37 (2H, q, *J*8, OC*H*$_2$CH$_3$); δ_C (100 MHz, $CDCl_3$) 14.12, 14.30, 23.04, 25.59, 29.65, 29.67, 29.80, 32.25, 63.37 (OCH$_2$*C*H$_3$), 72.18 (t, *J*25, *C*HOH), 115.05 (t, *J*255, *C*F$_2$), 164.11 (t, *J*28, *C*=O); *m*/*z* (ESI) 303 (MNa^+, 100%); HRMS found 303.1751, $C_{14}H_{26}F_2O_3Na$ (MNa^+) requires 303.1748 (Δ = 1.0 ppm). The ester (3.52 g, 12.6 mmol) was stirred in a solution of NaOH (552 mg, 13.8 mmol) in H_2O (5 ml) for 8 h (TLC) at room temperature, extracted with Et_2O, and then acidified by the addition of 3 M aqueous HCl (until pH = 1). The aqueous solution was extracted three times with EtOAc and the organic extracts were combined, washed (brine), dried ($MgSO_4$), filtered and concentrated *in vacuo* to give *2,2-difluoro-3-hydroxydodecanoic acid* (**11**) as a white solid (3.06 g, 96%); mp 59–60 °C [hexane]; ν_{max} (neat) cm^{-1} 3349w, 3245w, 2952m, 2921s, 2855s, 1759m (monomer acid), 1727s (dimer acid), 1472s, 1391m, 1198s, 1149s, 1126vs, 1102vs, 1047s, 765s, 721m, 693s; δ_H (400 MHz, $CDCl_3$) 0.88 (3H, t, *J*7, C*H*$_3$), 1.22–1.42 (14H, m, C*H*$_2$), 1.54–1.78 (2H, m, CHOHC*H*$_2$), 4.04–4.12 (1H, m, C*H*OH), 7.07 (2H, br s, O*H*); δ_C (100 MHz, $CDCl_3$) 14.45, 23.04, 25.49, 29.65, 29.79, 29.88, 32.25, 72.34 (t, *J*29, *C*HOH), 114.83 (t, *J*253, *C*F$_2$), 166.86 (t, *J*30, *C*=O); *m*/*z* (ESI) 275 (MNa^+,

100%); HRMS found 275.1430, $C_{12}H_{22}F_2O_3Na$ (MNa^+) requires 275.1435 (Δ = 1.8 ppm).

(1′*S*)-*N*-(2′-Oxocyclopentyl)-2,2-difluoro-3-oxododecanamide [(*S*)-3a]

(1*S*,2*S*)-2-Hydroxycyclopentylamine hydrochloride [(*S*,*S*)-**5a**], 275 mg, 2.0 mmol), acid **11** (606 mg, 2.4 mmol) and EDC (460 mg, 2.4 mmol) were dissolved in CH_2Cl_2 (10 ml) and *i*-Pr_2NEt (0.87 ml, 5.0 mmol) was added and the mixture was stirred at room temperature for 18 h. The mixture was partitioned between EtOAc and 3 M aqueous HCl and separated. The aqueous layer was extracted three times with EtOAc. The organic layers were combined, washed (brine), dried ($MgSO_4$), filtered, concentrated *in vacuo* and chromatographed (hexane : EtOAc; 1 : 1) to give both diastereomers of **12a** as a white solid (433 mg, 65%); R_f 0.32 and 0.27 (SiO_2; hexane : EtOAc; 1 : 1); mp 98–100 °C; ν_{max} (neat) cm^{-1} 3291s, 2918s, 2851s, 1676vs (amide), 1558s (amide), 1467m, 1092s, 1076s, 1046m, 697s; *m*/*z* (APCI) 336 (MH^+, 100%); *m*/*z* (ESI) 358 (MNa^+, 100%); HRMS found 358.2177, $C_{17}H_{31}F_2NO_3Na$ (MNa^+) requires 358.2170 (Δ = 2.0 ppm). Diastereomeric alcohols **12a** (100.1 mg, 298 μmol) were dissolved in anhydrous CH_2Cl_2 (5 ml) and Dess–Martin periodinane (380 mg, 895 μmol) was added. After the mixture was stirred for 1 h at room temperature under Ar, it was washed with H_2O, dried ($MgSO_4$), filtered, concentrated *in vacuo* and rapidly chromatographed (hexane : EtOAc; 7 : 3) to give (*1′S*)-*N*-(*2′-oxocyclopentyl*)-*2,2-difluoro-3-oxododecanamide* [(*S*)-**3a**] as a colourless oil (88.0 mg, 89%); $[a]_D^{25}$ +5.2 (*c* 0.5 in EtOH); R_f 0.31 (SiO_2; hexane : EtOAc; 7 : 3); ν_{max} (neat) cm^{-1} 2924s, 2855m, 1750vs (ketone), 1689vs (amide), 1539m (amide), 1129s, 910s, 733s; δ_H (400 MHz, $CDCl_3$) 0.87 (3H, t, *J*7, C*H*), 1.22–1.31 (12H, m), 1.58–1.68 (3H, m), 1.84–1.96 (1H, m), 2.08–2.27 (2H, m), 2.42–2.49 (1H, m), 2.67–2.74 (1H, m), 2.77 (2H, t, *J*7, CF_2COCH_2), 4.08–4.14 (1H, m, C*H*N), 6.78 (1H, br s, N*H*); δ_C (100 MHz, $CDCl_3$) 14.46, 18.44, 22.81, 23.03, 29.59, 29.62, 29.72, 32.22, 34.97, 37.84, 58.25, 112.64 (t, *J*264, *C*F_2), 162.10 (t, *J*27), 198.70 (t, *J*27), 213.18; δ_F (376 MHz, $CDCl_3$) −114.29 to −115.74 (ABq, *J*268, CF_2); *m*/*z* (APCI) 332 (MH^+, 100%); *m*/*z* (ESI) 354 (MNa^+, 100%); HRMS found 354.1849, $C_{17}H_{27}F_2NO_3Na$ (MNa^+) requires 354.1857 (Δ = 2.3 ppm).

(1′*S*)-*N*-(2′-Oxocyclohexyl)-2,2-difluoro-3-oxododecanamide [(*S*)-3b]

(1*S*,2*S*)-2-Hydroxycyclohexylamine hydrochloride [(*S*,*S*)-**5b**], 303 mg, 2.0 mmol), acid **11** (606 mg, 2.4 mmol) and EDC (460 mg, 2.4 mmol) were dissolved in CH_2Cl_2 (10 ml) and *i*-Pr_2NEt (0.87 ml, 5.0 mmol) was added and the mixture was stirred at room temperature for 18 h. The mixture was partitioned between EtOAc and 3 M aqueous HCl and separated. The aqueous layer was extracted three times with EtOAc. The organic layers were combined, washed (brine), dried ($MgSO_4$), filtered, concentrated *in vacuo* and chromatographed (hexane : EtOAc; 1 : 1) to give both diastereomers of **12b** as a white solid (364 mg, 52%); R_f 0.43 and 0.36 (SiO_2; hexane : EtOAc; 1 : 1); mp 126–128 °C; ν_{max} (neat) cm^{-1} 3299m, 2921s, 2852m, 1672vs (amide), 1562s (amide), 1468m, 1450m, 1229m, 1207m, 1194m, 1160m, 1129m, 1081s, 1044s, 1033s, 792m, 722s, 703s; *m*/*z* (APCI) 350 (MH^+, 100%); *m*/*z* (ESI) 372 (MNa^+, 100%); HRMS found 372.2336, $C_{18}H_{33}F_2NO_3Na$ (MNa^+) requires 372.2326 (Δ = 2.6 ppm). Diastereomeric alcohols **12b** (121.5 mg, 348 μmol) were dissolved in anhydrous CH_2Cl_2 (10 ml) and Dess–Martin periodinane (442 mg, 1.04 mmol) was added. After the mixture was stirred for 3 h at room temperature under Ar, it was washed with H_2O, dried ($MgSO_4$), filtered, concentrated *in vacuo* and rapidly chromatographed (hexane : EtOAc; 8 : 2) to give (*1′S*)-*N*-(*2′-oxocyclohexyl*)-*2,2-difluoro-3-oxododecanamide* [(*S*)-**3b**] as a white solid (120 mg, 100%); $[a]_D^{25}$ +14.5 (*c* 0.66 in EtOH); R_f 0.31 (SiO_2; hexane : EtOAc; 8 : 2); mp 40–42 °C; ν_{max} (neat) cm^{-1} 3337m (NH), 2918s, 2851m, 1745s (ketone), 1719s (ketone), 1682vs (amide), 1553m (amide), 1129s, 1157s, 1107s, 1077s; δ_H (400 MHz, $CDCl_3$) 0.87 (3H, t, *J*7, CH_3), 1.20–1.32 (12H, m), 1.36–1.46 (1H, m, C*H*H′CHN), 1.57–1.64 (2H, m $CF_2COCH_2CH_2$), 1.64–1.73 (1H, m), 1.74–1.86 (1H, m), 1.88–1.95 (1H, m), 2.13–2.19 (1H, m), 2.36–2.44 (1H, m), 2.55–2.61 (1H, m), 2.66–2.73 (1H, m, CH*H*′CHN), 2.75 (2H, t, *J*7.5, CF_2COCH_2), 4.38–4.46 (1H, m, C*H*N), 7.42 (1H, br s, N*H*); δ_C (100 MHz, $CDCl_3$) 14.43, 23.01, 24.21, 28.19, 29.58, 29.61, 29.71, 32.20, 34.85, 37.78, 41.22, 58.46, 109.45 (t, *J*264, *C*F_2), 161.33 (t, *J*27, *C*=O amide), 198.67 (t, *J*27, *C*=O ketone), 206.07 (*C*=O ketone); δ_F (376 MHz, $CDCl_3$) −114.41 to −115.86 (ABq, *J*268, CF_2); *m*/*z* (APCI) 346 (MH^+, 100%); *m*/*z* (ESI) 354 (MNa^+, 100%); HRMS found 368.2013, $C_{18}H_{29}F_2NO_3Na$ (MNa^+) requires 368.2013 (Δ = 0.1 ppm).

(1′*S*)-*N*-(2′-Oxocycloheptyl)-2,2-difluoro-3-oxododecanamide [(*S*)-3c]

(1*S*,2*S*)-2-Hydroxycycloheptylamine hydrochloride [(*S*,*S*)-**5c**], 16.6 mg, 100 μmol), acid **11** (30.4 mg, 120 μmol) and EDC (23.0 mg, 120 μmol) were dissolved in DMF (2 ml) and *i*-Pr_2NEt (44 μl, 250 μmol) was added and the mixture was stirred at room temperature for 18 h. The mixture was concentrated *in vacuo* and chromatographed (hexane : EtOAc; 1 : 1) to give both diastereomers of **12c** as a white solid (30.5 mg, 84%); R_f 0.43 and 0.38 (SiO_2; hexane : EtOAc; 1 : 1); *m*/*z* (APCI) 364 (MH^+, 100%); *m*/*z* (ESI) 386 (MNa^+, 100%); HRMS found 386.2489, $C_{19}H_{35}F_2NO_3Na$ (MNa^+) requires 386.2483 (Δ = 1.6 ppm). Diastereomeric alcohols **12c** (18 mg, 50 μmol) were dissolved in anhydrous CH_2Cl_2 (2 ml) and Dess–Martin periodinane (63 mg, 149 μmol) was added. After the mixture was stirred for 3 h at room temperature, it was washed with brine, dried ($MgSO_4$), filtered, concentrated *in vacuo* and rapidly chromatographed (hexane : EtOAc; 3 : 1) to give (*1′S*)-*N*-(*2′-oxocycloheptyl*)-*2,2-difluoro-3-oxododecanamide* [(*S*)-**3c**] as a white solid (13.2 mg, 74%); $[a]_D^{25}$ +17.9 (*c* 0.66 in EtOH); R_f 0.43 (SiO_2; hexane : EtOAc; 2 : 1); mp 59–60 °C; ν_{max} (neat) cm^{-1} 3390m (NH), 2935m, 1748s (ketone), 1723s (ketone), 1692vs (amide), 1514s (amide), 1124s; δ_H (600 MHz, $CDCl_3$) 0.87 (3H, t, *J*7, CH_3), 1.21–1.33 (12H, m), 1.46–1.65 (4H, m), 1.68–1.78 (2H, m), 1.80–1.98 (2H, m), 2.08–2.15 (1H, m), 2.47–2.55 (1H, m), 2.64–2.72 (1H, m), 2.75 (2H, t, *J*7, CF_2COCH_2), 4.62–4.66 (1H, m, C*H*N), 7.57 (1H, br s, N*H*); δ_C (150 MHz, $CDCl_3$) 14.04, 22.38, 22.61, 22.92, 27.50, 28.76, 28.83, 29.17, 29.21, 29.30, 31.80, 32.03, 37.44, 41.27, 58.99, 109.02 (t, *J*264, *C*F_2), 160.58 (t, *J*27, *C*=O amide), 198.41 (t, *J*27, *C*=O ketone), 208.19 (*C*=O ketone); *m*/*z* (APCI) 360 (MH^+, 100%); *m*/*z* (ESI) 382 (MNa^+, 100%); HRMS found 382.2161, $C_{18}H_{29}F_2NO_3Na$ (MNa^+) requires 382.2170 (Δ = 2.4 ppm).

(*S*)-2,2-Difluoro-3-oxododecanyl homoserine lactone [(*S*)-4][13]

(*S*)-(−)-α-Amino-γ-butyrolactone hydrobromide (109 mg, 0.6 mmol), acid **11** (126 mg, 0.5 mmol) and EDC (115 mg, 0.6 mmol) were dissolved in DMF (5 ml) and *i*-Pr_2NEt (0.22 ml, 1.3 mmol) was added and the mixture was stirred at room temperature overnight. The mixture was partitioned between EtOAc and 1 M aqueous HCl and separated. The aqueous layer was extracted three times with EtOAc. The organic layers were combined, washed (brine), dried ($MgSO_4$), filtered, concentrated *in vacuo* and chromatographed (hexane : EtOAc; 1 : 1) to give both hydroxy epimers as a white solid (94.2 mg, 56%); R_f 0.24 (SiO_2; hexane : EtOAc; 1 : 1); ν_{max} (neat) cm^{-1} 3294m, 2919s, 2851m, 1777s (lactone), 1682vs (amide), 1553s (amide), 1467m, 1383m, 1171s, 1091s, 1014s; *m*/*z* (APCI) 336 (MH^+, 100%); *m*/*z* (ESI) 358 (MNa^+, 100%); HRMS found 358.1815, $C_{16}H_{27}F_2NO_4Na$ (MNa^+) requires 358.1806 (Δ = 2.5 ppm). The diastereomeric alcohols (90.1 mg, 298 μmol) were dissolved in anhydrous CH_2Cl_2 (5 ml) and Dess–Martin periodinane (171 mg, 403 μmol) was added. After the mixture was stirred for 1 h at room temperature under Ar, it was washed with H_2O, dried ($MgSO_4$), filtered, concentrated *in*

vacuo and rapidly chromatographed (hexane : EtOAc; 6 : 4) to give (*S*)-*2,2-difluoro-3-oxododecanyl homoserine lactone* [(*S*)-**4**] as a white solid (81.3 mg, 91%); $[\alpha]_D^{25}$ −2.5 (*c* 1.0 in $CHCl_3$); R_f 0.29 (SiO_2; hexane : EtOAc; 6 : 4); mp 98–100 °C; ν_{max} (neat) cm^{-1} 3310m, 2922s, 2854m, 1775vs (lactone), 1753s (ketone), 1694s (amide), 1678s (amide), 1541s (amide), 1385m, 1227m, 1183s, 1157m, 1133m, 1012s; δ_H (400 MHz, $CDCl_3$) 0.89 (3H, t, *J*7, CH_3), 1.22–1.34 (12H, m), 1.57–1.66 (2H, m, $CF_2COCH_2CH_2$), 2.24–2.36 (1H, m, OCH_2CHH'), 2.75–2.84 (1H, m, OCH_2CHH'), 2.77 (2H, t, *J*7, CF_2COCH_2), 4.28–4.35 (1H, m, OC*H*H′), 4.49 (1H, apparent t, *J*8, OCH*H*′), 4.59–4.64 (1H, m, C*H*N), 7.28 (1H, br s, N*H*); δ_C (100 MHz, $CDCl_3$) 14.45 (CH_3), 22.78 ($CF_2COCH_2CH_2$), 23.02, 29.15 (OCH_2CH_2), 29.59, 29.62, 32.21, 37.77 (CF_2COCH_2), 49.62 (*C*HN), 66.45 (OCH_2), 109.31 (t, *J*264, *C*F_2), 162.37 (t, *J*28, *C*=O amide), 174.27 (*C*=O lactone), 198.62 (t, *J*27, *C*=O ketone); *m*/*z* (ESI) 356 (MNa^+, 100%); HRMS found 356.1648, $C_{16}H_{25}F_2NO_4Na$ (MNa^+) requires 356.1649 (Δ = 0.5 ppm).

Acknowledgements

D. Fox is thanked for his insightful comments and suggestions on this article. Marianne Chen is acknowledged for her efforts on the synthesis of **2b**. Barbara Iglewski (U. Rochester, USA) is thanked for generously supplying the *P. aeruginosa* strains PAO1 and PAO-JP2. We are grateful for financial support from the BBSRC (inc. Grant #: P19162) and to Pfizer for a summer studentship (FGG).

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