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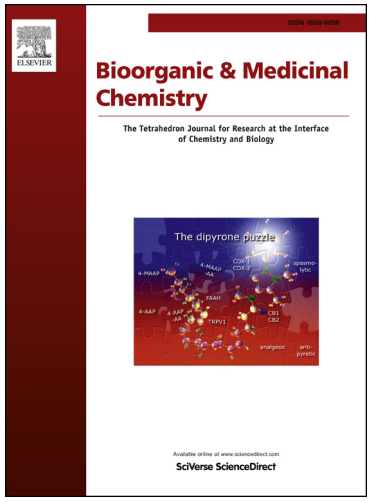

Synthesis, quorum sensing inhibition and docking studies of 1,5-dihydropyrrol-2-ones

Wai-Kean Goh, Christopher R. Gardner, Kondapalli V.G. Chandra Sekhar, Nripendra N. Biswas, Shashidhar Nizalapur, Scott A. Rice, Mark Wilcox, David StC. Black, Naresh Kumar

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Graphical Abstract

Synthesis, quorum sensing inhibition and docking studies of 1,5-dihydropyrrol-2-ones

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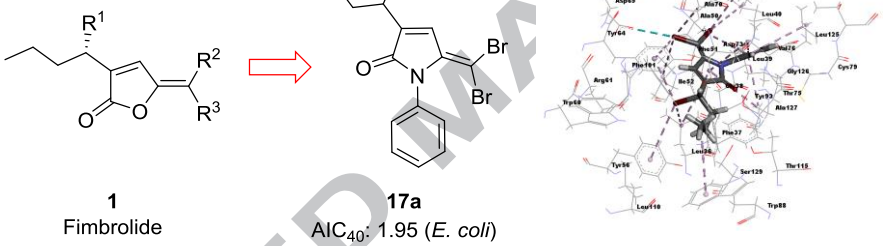
Wai-Kean Goh,[a] Christopher R. Gardner,[a] Kondapalli V. G. Chandra Sekhar,[b] Nripendra N. Biswas,[a] Shashidhar Nizalapur,[a] Scott A. Rice,[c,d] Mark Wilcox,[e] David StC. Black[a] and Naresh Kumar[a*]

[a] *School of Chemistry, UNSW Australia, Sydney, NSW 2052, Australia*
[b] *Department of Chemistry, Birla Institute of Technology & Science, Pilani, Hyderabad Campus, Hyderabad, Telangana 500078, India.*
[c] *Centre for Marine Bio-Innovation, School of Biological, Earth and Environmental Sciences, UNSW Australia, Sydney, NSW 2052, Australia.*
[d] *The Singapore Centre on Environmental Life Sciences Engineering and the School of Biological Sciences, Nanyang Technological University, Singapore.*
[e] *School of Optometry and Vision Science, UNSW Australia, Sydney, NSW 2052, Australia*



1
Fimbrolide

17a
AIC_{40}: 1.95 (*E. coli*)

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[a] *School of Chemistry, UNSW Australia, Sydney, NSW 2052, Australia*
[b] *Department of Chemistry, Birla Institute of Technology & Science, Pilani, Hyderabad Campus, Hyderabad, Telangana 500078, India.*
[c] *Centre for Marine Bio-Innovation, School of Biological, Earth and Environmental Sciences, UNSW Australia, Sydney, NSW 2052, Australia.*
[d] *The Singapore Centre on Environmental Life Sciences Engineering and the School of Biological Sciences, Nanyang Technological University, Singapore.*
[e] *School of Optometry and Vision Science, UNSW Australia, Sydney, NSW 2052, Australia*

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ABSTRACT

Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Escherichia coli* use *N*-acylated L-homoserine lactones (AHLs) as autoinducers (AIs) for quorum sensing (QS), a chief regulatory and cell-to-cell communication system. QS is responsible for social adaptation, virulence factor production, biofilm production and antibiotic resistance in bacteria. Fimbrolides, a class of halogenated furanones isolated from the red marine alga *Delisea pulchra*, have been shown to exhibit promising QS inhibitory activity against various Gram-negative and Gram-positive bacterial strains. In this work, various lactam analogues of fimbrolides *viz.*, 1,5-dihydropyrrol-2-ones, were designed and synthesized *via* an efficient lactamization protocol. All the synthesized analogues were tested for QS inhibition against the *E. coli* AHL-monitor strain JB357 *gfp* (ASV). Compound **17a** emerged as the most potent compound, followed by **9c**, with AIC_{40} values (the ratio of synthetic inhibitor to natural AHL signaling molecule that is required to lower GFP expression to 40%) of 1.95 and 19.00 respectively. Finally, the potential binding interactions between the synthesized molecules and the LasR QS receptor were studied by molecular docking. Our results indicate that 1,5-dihydropyrrol-2-ones have the ability to serve as potential leads for the further development of novel QS inhibitors as antimicrobial therapeutics.

1. Introduction

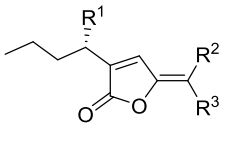
The exponential increase in the incidence of infections caused by multi-drug resistant bacteria in health-care settings has highlighted the need to identify new anti-microbial agents with an alternative extermination mechanism.[1] Over the last few decades, it has become evident that many bacteria coordinate amongst themselves or with higher organisms through intercellular communication systems, known as quorum sensing (QS) systems. QS-controlled behaviours take place when the signalling molecules reach a threshold concentration sufficient to bind to the receptor protein, leading to a change in gene expression.[2, 3] QS has been shown to regulate a myriad of phenotypes and responses such as virulence, biofilm formation, luminescence and DNA transfer.[4]

Current research on QS systems and bacterial biofilms has identified a potential approach for the development of novel antimicrobial drugs. Blocking or interfering with the communication between bacteria to prevent the production of virulence factors or biofilm formation represents an attractive strategy to combat bacterial infections. Such a non-growth inhibiting mechanism does not exert the same survival pressure on bacteria as antibiotics, and thus should result in the delayed or reduced development of drug resistance, which is a major shortcoming with current antibiotics.[5, 6]

Over fifty species of Gram-negative bacteria monitor their population densities through the release of *N*-acylated homoserine lactone (AHL)-nucleated pheromones as QS signalling molecules.[3] Several natural QS inhibitors have been discovered in marine organisms.[7] Among these, the best known are the fimbrolides (**1**) (Figure 1), isolated from the Australian marine red alga *Delisea pulchra*, which are capable of inhibiting surface biofouling by bacteria.[8] Various fimbrolides were found to act as antagonists that specifically inhibit AHL-dependent phenotypes, such as swarming in *Serratia marcescens*, biofilm formation and protease production in *Pseudomonas aeruginosa* [9-

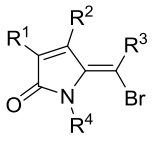
* Corresponding author. Tel.: +61 2 9385 4698; fax: +61 2 9385 6141.
E-mail address: n.kumar@unsw.edu.au (N. Kumar).

[12] and bioluminescence in *Vibrio fischeri*.[13] Brominated furanones were found to interfere with AHL-mediated QS systems by displacing AHL molecules from their cognate protein receptor.[14]



1

	R^1	R^2	R^3
a	H	H	Br
b	OAc	H	Br
c	OH	H	Br
d	H	Br	Br
e	OAc	H	Cl



2

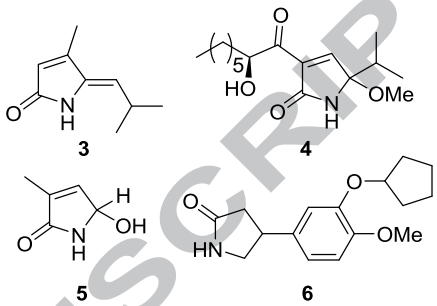
R^1 = H, alkyl
R^2 = H, Br
R^3 = H, Br
R^4 = alkyl. aryl

Figure 1: Natural fimbrolides (**1**) from *Delisea pulchra* that modulate QS in bacteria and their synthetic dihydropyrrol-2-one (**2**) analogues

The potent QS inhibitory activity of the fimbrolides has encouraged the development of synthetic analogues as novel anti-bacterial agents. Modifications to the ring may also bring significant changes in antagonist activities, some of which have not been thoroughly explored to date. A related structure to the fimbrolides containing a modification to the furanone ring are the 1,5-dihydropyrrol-2-ones (**2**) (Figure 1). Dihydropyrrol-2-ones are hydrolytically more stable compared to the corresponding lactones, potentially making them less susceptible to ring-opening reactions under physiological conditions.[15, 16]

The pyrrol-2-one ring system is a common feature of several important classes of biologically active molecules that have been the focus of many synthetic efforts. These include pulchellalactam (**3**), a CD45 protein tyrosine phosphate inhibitor,[17] γ-lactam PI-091 (**4**), a platelet aggregation inhibitor,[18] jatropham (**5**), an antitumor alkaloid,[19, 20] and more recently, rolipram (**6**), an anti-depressant (Figure 2).[15] Dihydropyrrol-2-ones have shown several pharmacological activities such as HIV integrase inhibition,[21] mineralocorticoid receptor antagonism,[22] apoptosis signal-regulating kinase 1 inhibition,[23] peptide-protein receptor inhibition,[24] vasopressin-2 receptor antagonism,[25] antitumor activity,[26] antibacterial activity[27] and are also used in Alzheimer's disease therapy.[28] Our group has previously synthesized *N*-substituted dihydropyrrol-2-one derivatives *via* the reaction of fimbrolides with different primary amines.[29] These and other dihydropyrrolones have been shown to exhibit potent QS inhibitory activity, as well as inhibition of biofilm formation.[29-31]

Numerous methods for the synthesis of pyrrol-2-ones have been reported,[16, 19, 31-33] however, these syntheses have produced molecules that lack the halogenation pattern present in fimbrolides. The uniqueness of the bromination pattern on the ring, combined with the presence of the bromomethylene group, makes this molecule more elusive to the common lactamization reactions that have been reported in the literature. Thus, the real challenge in the synthesis of fimbrolide-mimicking pyrrol-2-ones lies in preserving the bromination pattern, which is crucial for biological activity.



3 **4** **5** **6**

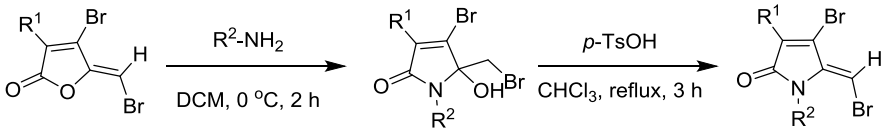
Figure 2: Medicinally active pyrrol-2-ones

Previously, our research group reported a direct route to the 5-bromomethylene dihydropyrrol-2-one ring system *via* an efficient lactone–lactam conversion.[29] Using this novel approach, the distinctive bromination pattern of the molecules remained intact. In continuation of this research, we herein report an extension of this methodology to access further bromomethylene analogues of pyrrol-2-ones. Furthermore, we also report an approach to generate dibromomethylene analogues, as well as furanones bearing substituted C3-chains, and their conversion to the corresponding dihydropyrrol-2-ones. We have utilized this procedure to generate various C3-alkylated, C4-brominated and C5-bromomethylene/dibromomethylene analogues of the dihydropyrrol-2-ones that are not accessible by other means. Further, these compounds were screened for their QS inhibition (QSI) activities against the *E. coli* AHL-monitor strain JB357 *gfp* (ASV) and their AIC_{40} values were determined. These compounds were also docked to the LasR receptor, which is the receptor responsible for AHL-mediated QS, in order to investigate their potential binding interactions with the target.

2. Results and Discussion

2.1. Chemistry

Fimbrolides **7a-c** and **15a-b** were synthesized *via* the sulfuric acid-catalyzed cyclization of brominated 2-alkyl-levulinic acids as reported previously.[34] Various combinations of halogenated furanones (**7a-c**) and amines were reacted in DCM to yield the

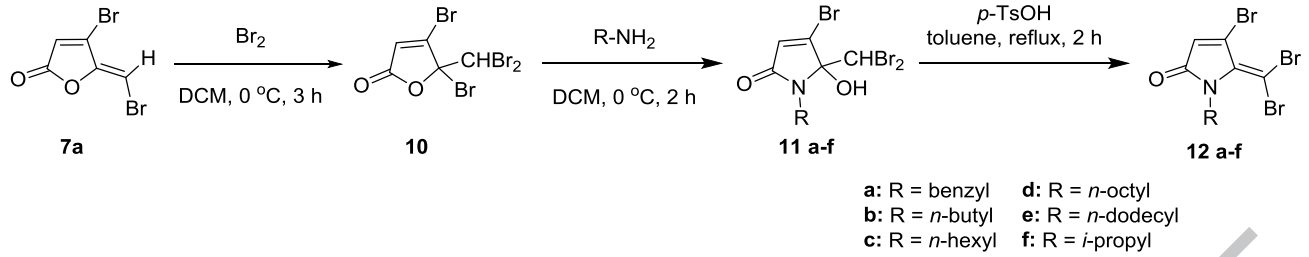


7 **8** **9**

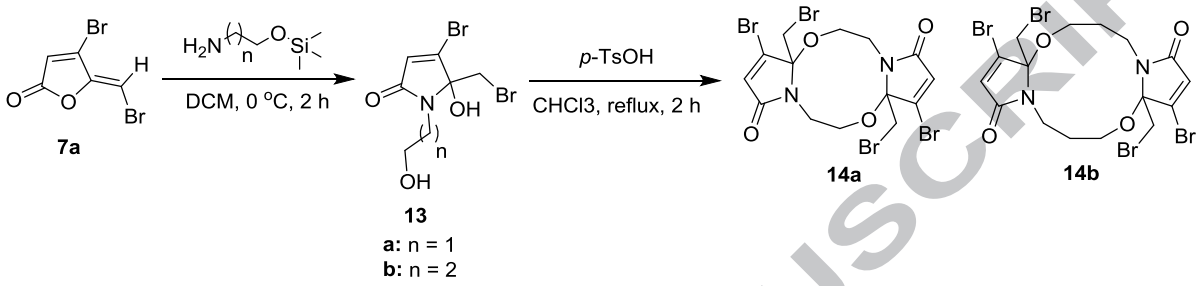
a: R^1 = H
b: R^1 = *n*-butyl
c: R^1 = *n*-hexyl

a: R^1 = H, R^2 = Ph
b: R^1 = H, R^2 = benzyl
c: R^1 = H, R^2 = *n*-butyl
d: R^1 = H, R^2 = *n*-hexyl
e: R^1 = H, R^2 = *n*-octyl
f: R^1 = H, R^2 = *n*-dodecyl
g: R^1 = H, R^2 = *i*-butyl
h: R^1 = *n*-butyl, R^2 = Ph
i: R^1 = *n*-butyl, R^2 = benzyl
j: R^1 = *n*-butyl, R^2 = *n*-butyl
k: R^1 = *n*-hexyl, R^2 = Ph
l: R^1 = *n*-hexyl, R^2 = benzyl
m: R^1 = *n*-hexyl, R^2 = *n*-butyl

Scheme 1: Synthesis of C4-bromo-C5-bromomethylene dihydropyrrol-2-ones **9a-m**.



Scheme 2: Synthesis of C4-bromo-C5-dibromomethylene dihydropyrrol-2-ones **12a-f**.



Scheme 3: Synthesis of the dimeric C4-bromo-C5-bromomethylene dihydropyrrol-2-ones **14a-b**.

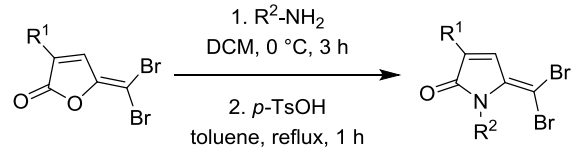
corresponding hydroxy-lactams (**8a-m**) in yields of 20-85%. The dehydration of these hydroxy-lactams was then achieved using *p*-TsOH to generate a library of the dihydropyrrol-2-one analogues (**9a-m**) in 21-96% yield (Scheme 1).[29]

The intermediate hydroxy-lactams (**8a-m**) showed a characteristic pair of doublets (δ 3.20–3.60, J = 11 Hz) belonging to the CH_2Br group at the C5 position in the ^{1}H NMR spectrum. The doublet splitting pattern of this CH_2 signal arises due to the chirality of the C5 carbon. Upon dehydration, this signal changed to a singlet (δ 6.2-6.3) integrating for one proton in the ^{1}H NMR spectra of the bromomethylene analogues (**9a-m**). The *Z*-isomer of the lactam was isolated in all cases, as demonstrated by a comparison of the chemical shift of the methylene proton with that of the corresponding parent fimbrolide.[29]

This general strategy was further extended to the dibromomethylene compounds, which contain two bromine atoms attached to the C5 exocyclic double bond. Bromination of 4-bromo-5-bromomethylene-5*H*-furan-2-one (**7a**) using bromine in DCM converted the furanone to the corresponding 5-dibromomethyl compound **10** in 78% yield. A series of the dibromomethylene compounds **12a-f** were then generated in 38-53% yield *via* the hydroxy-lactam intermediates (**11a-f**, 14-74% yield), in the same manner described for compounds **9a-m** (Scheme 2). The formation of hydroxylactams **11a-f** was confirmed by a lowering of the carbonyl stretching frequency by 50-75 cm^{-1} in the IR spectra, while the formation of compounds **12a-f** was confirmed by the disappearance of the $CHBr_2$ signal at δ 5.90-5.94 in the ^{1}H NMR spectrum.

As our group is highly interested in surface functionalization as a means of reducing biofilm formation on biomedical devices, the development of dihydropyrrol-2-ones with functionalized *N*-substituents was considered a key target. Furanone **7a** was therefore treated with trimethylsilyl-protected amines under the same conditions reported above, giving hydroxylactams **13a-b** in 50% yield (Scheme 3).[27] It is noteworthy that the acidic nature of the silica gel also resulted in deprotection of the trimethylsilyl group during chromatographic purification. Following dehydration of **13a-b**, the products were identified as the cyclic dimers **14a-b**, isolated in 61 and 34% yield, respectively. Whilst these dimers are not useful for surface modification as was our intention, these compounds are unprecedented in the literature and represent a novel class of potential QS inhibitory compounds.

The lactamization strategy was also extended to the 3-alkyl-5-dibromomethylene furanones **15a-b**, in which the C4 bromine atom is absent from the dihydropyrrol-2-one ring. A series of the dibromomethylene compounds were generated as described above, giving dihydropyrrol-2-ones **16a-g** in 56-92% yield (Scheme 4).



a: R^1 = *n*-butyl
b: R^1 = *n*-hexyl

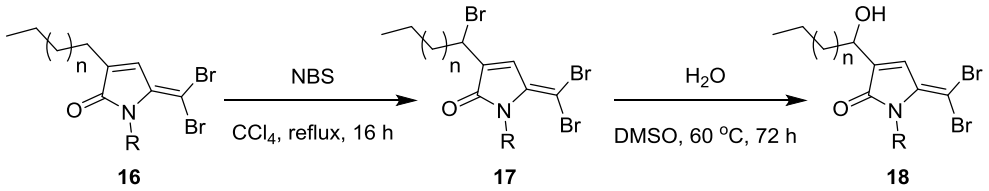
a: R^1 = *n*-butyl, R^2 = Ph
b: R^1 = *n*-butyl, R^2 = benzyl
c: R^1 = *n*-butyl, R^2 = *n*-butyl
d: R^1 = *n*-butyl, R^2 = *p*-tolyl
e: R^1 = *n*-hexyl, R^2 = Ph
f: R^1 = *n*-hexyl, R^2 = benzyl
g: R^1 = *n*-hexyl, R^2 = *n*-butyl

Scheme 4: Synthesis of C5-dibromomethylene dihydropyrrol-2-ones **16a-g**.

Since a number of the natural fimbrolides (**1**) contain a hydroxyl substituent at the α-carbon of the C3 alkyl chain, a series of hydroxy derivatives of dihydropyrrol-2-ones **16a-b/e-f** was developed in order to determine the importance of this group for their QSI activity. Firstly, bromination was achieved using *N*-bromosuccinimide in carbon tetrachloride at reflux for 24 h, affording the brominated dihydropyrrol-2-ones **17a-d** in yields of 43-92%. Heating these in a mixture of DMSO and water at 60 °C then gave hydroxylated compounds **18a-d** in 48-96% yield (Scheme 5).

2.2. Quorum sensing inhibition assay

We screened the efficacy of the dihydropyrrol-2-ones in inhibiting the QS pathway present in Gram-negative bacteria, specifically the AHL-mediated signalling pathways in *E. coli*.



a: n = 1, R = Ph
b: n = 1, R = benzyl
e: n = 3, R = Ph
f: n = 3, R = benzyl

a: n = 1, R = Ph
b: n = 1, R = benzyl
c: n = 3, R = Ph
d: n = 3, R = benzyl

Scheme 5: Synthesis of dihydropyrrol-2-ones bearing α-substituted C3-alkyl chains **18a-d**.

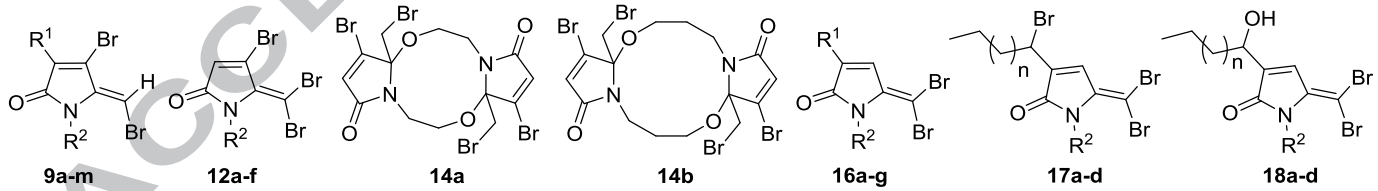
The biological screening was performed following a previously reported protocol.[35] The QS inhibition assay uses an *E. coli* AHL-monitor strain JB357 *gfp* (ASV) that expresses the green fluorescent protein (GFP) in the presence of natural AHL signalling molecule, 3-oxohexanoyl-HSL (OHHL). This induction can be blocked by the introduction of inhibitor compounds such as fimbrolides, which reduces the expression of GFP. The amount of GFP output correlates to the level of induction or repression of the AHL-dependent signalling pathways in *E. coli*.

The assay is performed by measuring the GFP output for the inhibitor compounds at various concentrations. The concentration of inhibitor required to reduce the OHHL-induced GFP expression of *luxR-PluxI-gfp* (ASV) to 40% (ID_{40}) was determined for each OHHL concentration (data not shown). This was then divided by the concentration of OHHL, giving the ratio of inhibitor (mol) to OHHL (mmol) that is required to lower GFP expression to 40% (AIC_{40}). The AIC_{40} represents the average ratio across the range of concentrations tested for each compound and OHHL ratio. A low index value indicates a more potent inhibitor, *i.e.* a lower amount of the inhibitor is needed to inhibit OHHL-induced GFP expression. All of the dihydropyrrol-2-ones were subjected to the assay and their AIC_{40} values were determined. Biological activity results are tabulated in Table 1.

The results presented in Table 1 reveal that a number of the dihydropyrrol-2-ones were able to disrupt GFP expression induced by AHL signalling molecules. If we first consider the activity of analogues **9a-m**, which each contain a C5-bromomethylene group and various substituents at the C3-position, the non-C3-alkylated compounds **9a-g** demonstrated better antagonistic activity compared to the C3-alkylated compounds **9h-m** (Table 1). Variation of the *N*-substituent did not result in any trends in activity across this class of analogue. For example, where the *N*-benzyl analogue **9b** had an AIC_{40} value of 26, the corresponding C3-alkylated derivatives **9i** and **9l** were found to have no activity. Additionally, the *N*-butyl derivative **9c** had the lowest AIC_{40} value of its class (19), as well as the second lowest value amongst all the tested compounds. Interestingly, compound **9j**, which has *n*-butyl substituents at the C3 and *N*-positions, had the highest measurable AIC_{40} value (279) of any compound.

Comparing the activity of the bromomethylene compounds (**9a-g**) to the analogous C5-dibromomethylene compounds **12a-f**, we can see that the incorporation of the additional bromine reduces the QS inhibitory activity. Where the *N*-benzyl derivative **9b** had an AIC_{40} value of 26, the corresponding dibromomethylene analogue **12a** is more than 5 fold lower in activity (AIC_{40}: 150). Similarly, where **9c** and **9e** showed activity, their respective dibromomethylene analogues **12b** and **12d** displayed no activity. The *N*-isopropyl analogue **12f** showed the greatest QS inhibitory activity amongst these dibromomethylene compounds (AIC_{40}: 65), however, this is still 2-3 fold lower activity than bromomethylene derivatives **9a-g**.

Table 1: Results of the quorum sensing inhibition assay.



Compound	AIC_{40}	Compound	AIC_{40}	Compound	AIC_{40}
9a	-	**9m**	-	**16d**	-
9b	26	**12a**	150	**16e**	248
9c	19	**12b**	-	**16f**	140
9d	-	**12c**	-	**16g**	-
9e	20	**12d**	-	**17a**	1.95
9f	-	**12e**	-	**17b**	-
9g	-	**12f**	65	**17c**	-
9h	-	**14a**	78	**17d**	-
9i	-	**14b**	112	**18a**	-
9j	279	**16a**	97	**18b**	-
9k	-	**16b**	220	**18c**	-
9l	-	**16c**	-	**18d**	-

* a '-' indicates that the compound is not active.

The C3-alkylated dibromomethylene analogues **16a-g** generally possessed low levels of QS inhibitory activity, with AIC_{40} values ≥97. Interestingly, the length of the C3-alkyl chain had resulted in different changes in activity when altering the *N*-substituent. In the case of *N*-phenyl derivatives **16a** and **16e**, increasing the length of the C3-alkyl chain from 4 carbons (**16a**) to 6 carbons (**16e**) resulted in a loss of activity (AIC_{40} values of 97 and 248, respectively). On the other hand, the same change in the C3-alkyl chains of *N*-benzyl derivatives **16b** and **16f** resulted in improved activity (AIC_{40} values of 220 and 140, respectively). Neither of the *N*-butyl derivatives (**16c** and **16g**) displayed activity, while substituting the *N*-phenyl group of **16a** with a *p*-tolyl group in **16d** resulted in a loss of activity.

It was found that the incorporation of α-substituents in the C3-alkyl chain did not affect the QS inhibitory activity of compounds **17a-c** and **18a-d**. However, α-bromo derivative **17a** was found to be the most active of all the compounds produced, with an AIC_{40} value of 1.95. It was interesting to find that the α-hydroxy compounds **18a-d**, which resemble the oxygenated fimbrolides (**1**), possessed no QS inhibitory activity.

Upon testing of the dimeric compounds **14a** and **14b**, it was gratifying to discover that they also possessed antagonistic activity, albeit 3-fold lower than the corresponding monomeric systems **9a-g**. It was initially thought that the dimers might not display significant activity as their large size may prevent them from fitting into the cavity of the LasR binding site.

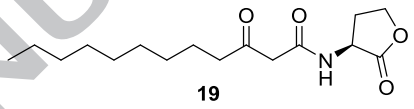
Natural fimbrolides from *D. pulchra* have AIC_{40} values of less than 1 (unpublished data). Based on the biological studies above, the dihydropyrrol-2-ones were less active than the corresponding fimbrolides. Nevertheless, the natural fimbrolides are cytotoxic to mammalian cell lines, whilst the dihydropyrrol-2-ones were shown to be non-cytotoxic and hydrolytically more stable than fimbrolides. Thus, dihydropyrrol-2-ones represent ideal candidates for further development as antagonists of bacterial signalling pathways.

2.3. Docking studies

In order to understand the nature of the interaction between the dihydropyrrol-2-ones and the QS receptor, docking studies were performed. The crystal structure of the QS signal receptor protein LasR with the agonist *N*-(3-oxododecanoyl)-L-homoserine lactone (OdDHL, **19**, Figure 3A) was used for this study (PDB code, 2UV0, resolution 1.8 Å).[36] The GOLD algorithm in the Accelrys Discovery Studio interface was used to examine the ability of the compounds to bind to LasR. The natural agonist OdDHL was docked back into the protein as a control in order to establish the reliability of the docking method (Figure 3B). The docking runs were analysed for the predicted binding interactions, including hydrogen bonding, electrostatic and hydrophobic interactions, between the compounds and the LasR receptor in the best scoring pose (Table S1, supplementary information).

Control docking of OdDHL **19** into the LasR receptor (Figure 4B) gave a pose that was highly consistent with that reported in the X-ray structure, with a root-mean-square deviation (RMSD) of 0.50 Å. The lactone head was buried within the binding pocket, with the acyl tail filling the pocket near the solvent-accessible opening. Compound 19 was found to form H-bonds with Tyr56, Trp60 and Asp73, all of which were observed in the X-ray structure. However, the H-bonding interactions with Thr75 and Ser129 that were reported in the literature were not observed.[36] This control docking gave a score of 57.78 (Table S1), which is forthwith used for comparison to the dihydropyrrol-2-one analogues.

A)



19

B)

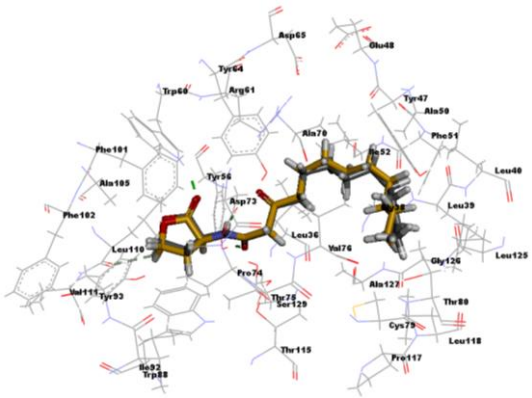
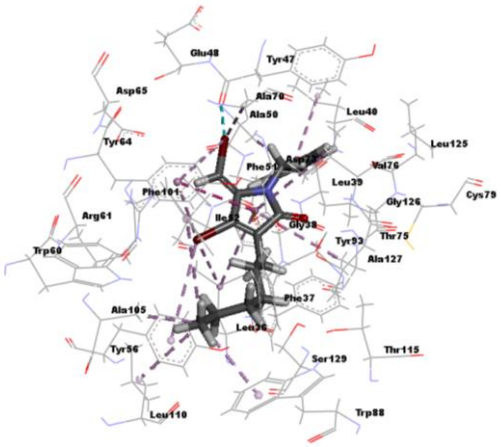


Figure 3: A) The structure of OdDHL **19**; B) an overlap of the literature and experimental docking poses of OdDHL **19**, showing the literature (X-ray) pose with yellow carbon atoms and the experimental (docked) pose with grey carbon atoms.

A)



B)

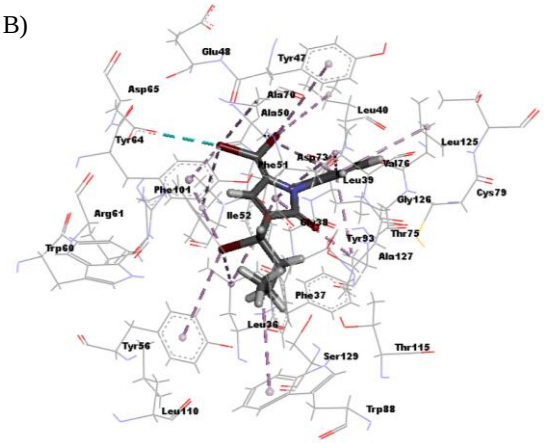


Figure 4: Docked poses of A) The inactive compound **9l**; B) The most active compound **17a** in the LasR receptor.

The docking results of dihydropyrrol-2-ones suggest that hydrogen bonding and hydrophobic interactions to amino acid residues play an important role in the binding of the compounds to the LasR receptor. Important residues for electrostatic interactions (H-bonding, halogen-bonding, π-cation) were found to include Tyr47, Asp73 and Ser129, while non-polar interactions were made with residues such as Leu36, Ala50, Ile52, Tyr64, Ala70, Val76, Trp88 and Ala127. The phenyl and alkyl groups of the tested compounds were able to form multiple hydrophobic interactions with the LasR binding pocket, leading to high docking scores that did not necessarily correlate to activity. For example, the inactive compound **9l**, which has C3-*n*-hexy and *N*-benzyl substituents, had the second highest score (68.52) and number of non-polar interactions (12), while the most active compound **17a**, which has C3-α-bromo-*n*-butyl and *N*-phenyl substituents, had the same number of non-polar interactions (12) and the twenty-third highest docking score (56.79) (Figure 4). Generally, it was observed that analogues with C3-substituents gave higher scores, with longer chains being more favourable, *i.e.* hexyl chains > butyl chains > H (*eg*. **9l** > **9i** > **9b** and **16f** > **16b**). Similarly, analogues with *N*-benzyl groups (*eg*. **9l** and **16f**) gave higher scores than those with the corresponding N-phenyl groups (*eg*. **9k** and **16e**).

Generally, there was no difference in docking scores between C5-bromomethylene compounds and C5-dibromomethylene compounds. In the case of C3-unsubstituted compounds, the C5-dibromomehtylene analogues **12a-e** gave a similar average docking score (54.49) to the C5-bromomethylene analogues **9b-f** (53.16). Similarly, in the case of C3-substituted compounds, derivatives **9h-m**, which have a C4-bromo and C5-bromomethylene substituent, gave similar average docking scores (63.44) to analogues **16a-c/e-f** (62.09), which have no C4-bromine and have a C5-dibromomethylene group. Additionally, the inclusion of C3-α-bromo (**17a-d**) or C3-α-hydroxy (**18a-d**) substituents did not significantly alter the average docking scores of these classes (62.22 and 62.73, respectively), compared to their parent compounds **16a-b/e-f** (62.08).

The investigation of the poses proposed by the modelling proved to be more illuminating than the scores. It was observed that the most active compound **17a** directed its *N*-phenyl substituent into the pocket, with the C3-alkyl group directed towards the opening (Figure 5). It was found that the active compounds with C3-substituents (**9j**, **16b**, **16e**, **16f** and **17a**) all had a conserved orientation of their C3 and *N*-substituents, with the pyrrol-2-one ring occupying a very similar position in the binding pocket (Figure 6A). On the other hand, the second most active compound **9c**, which lacks a C3-substituent, was found to have a shallower positioning of the dihydropyrrol-one moiety, closer to the position occupied by the lactone head of OdDHL **19** (Figure 6B). Similarly, active compounds without C3-substituents (**9b**, **9c**, **9e**, **12a** and **12f**) were found to conserve this shallow binding. Analogues with longer *N*-substituents directed these into the cavity, more closely mimicking the orientation of **19**.

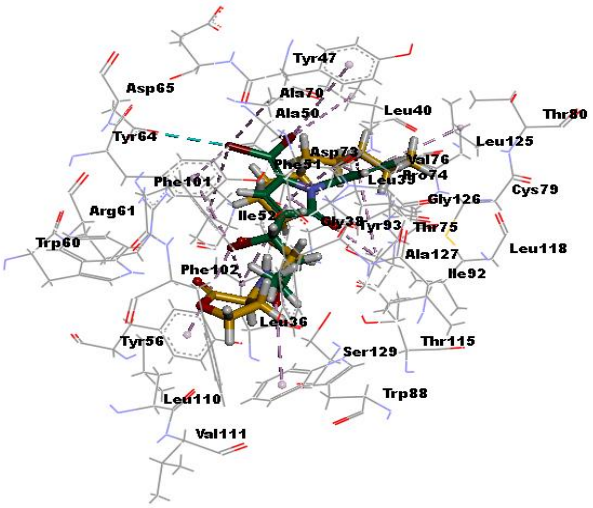


Figure 5: Overlapping poses of OdDHL**19** (yellow) and the most active compound **17a** (green) in the LasR receptor.

3. Conclusions

In the current study, we synthesized thirty six analogues of 1,5-dihydropyrrol-2-ones in moderate to good yields *via* the lactamization of fimbrolides. This two-step synthetic route was used to generate analogues with a wide variety of bromination and alkyl substitution patterns. All the novel compounds were characterized by various spectroscopic techniques and were evaluated for their QSI activity against the *E. coli* AHL-monitor strain JB357 *gfp* (ASV). Compound **17a** was found to be the most active derivative, with an AIC_{40} value of 1.95, followed by **9c** with an AIC_{40} value of 1.95. Furthermore, the compounds were docked to the LasR receptor to understand their potential binding interactions with the protein. Given the versatility of this synthesis, the direct lactamization strategy described here could be used to generate new series of QS antagonists as potential antimicrobial leads.

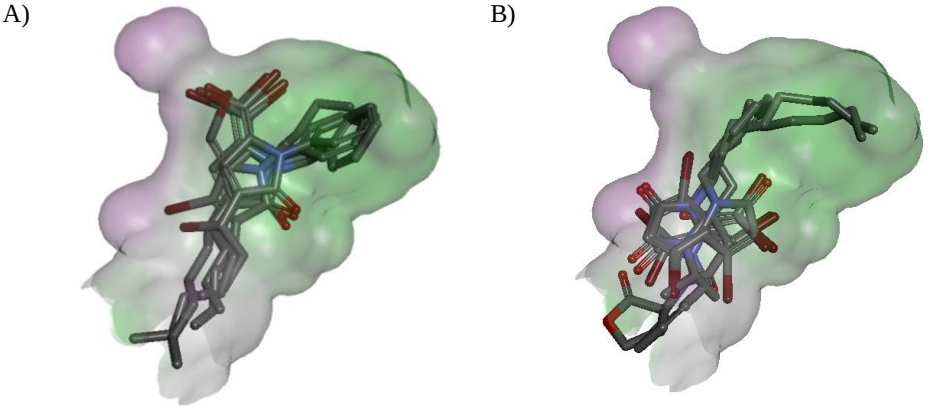


Figure 6: A) Overlapping poses of C3-substituted **9j**, **16b**, **16e**, **16f** and **17a** showing a conserved orientation (hydrogens omitted for clarity); B) Overlapping poses of C3-non-substituted **9b**, **9c**, **9e**, **12a** and **12f** with OdDHL **19**, showing a shallow dihydropyrrol-2-one positioning (hydrogens omitted for clarity).

4. Experimental Section

4.1. General Experimental

All chemical reagents were purchased from commercial sources (Alfa-Aesar and Sigma Aldrich) and used without further purification. Solvents were used from commercial sources and used as obtained. Reactions were performed using oven-dried glassware under an atmosphere of nitrogen and in anhydrous conditions (as required). Room temperature refers to the ambient temperature. Yields refer to chromatographically and spectroscopically pure compounds unless otherwise stated. Reactions were monitored by thin layer chromatography (TLC) precoated with Merck silica gel 60 F^{254}. Visualization was performed by the quenching of short or long wavelength UV fluorescence or by staining with potassium permanganate or ninhydrin solution. Flash chromatography was carried out using Grace Davison LC60A 6-35 μm silica gel. Infrared spectra were recorded using a Cary 630 FTIR spectrophotometer. Ultraviolet spectra were measured using a Cary 100 Bio UV-visible spectrophotometer in the designated solvents and data reported as wavelength (λ) in nm and absorption coefficient (ε) in $M^{-1}cm^{-1}$. High-resolution mass spectrometry was performed by the Bioanalytical Mass Spectrometry facility, UNSW. Melting points were obtained using Mel-Temp melting point apparatus and are uncorrected. Proton and Carbon NMR were recorded in designated solvents using Bruker DPX 300 or a Bruker Avance 400 spectrometer. Chemical shifts (δ) are quoted in parts per million (ppm), to the nearest 0.01ppm and internally referenced relative to the solvent nuclei. ^{1}H NMR spectral data are reported as follows: [chemical shift in ppm; multiplicity in br, broad; s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; sext, sextet; sept, septet; m, multiplet; or as a combination of these (*e.g.* dd, dt *etc*.)]; coupling constant (*J*) in hertz, integration, proton count and assignment.

4.2. Experimental Details

Compounds **9a-c**, **9h-m**, and **14a** were prepared as reported earlier.[27] Spectral characterization and melting points of these compounds matched with those reported.

4.2.1 General protocol 1: Lactamization of Fimbrolides[34]

Fimbrolides **7a-c** (2.0 mmol) were dissolved in CH_2Cl_2 (10 mL) and the solution was cooled to 0 °C. The amine (10.0 mmol, 5 equiv) in CH_2Cl_2, (10 mL) was added dropwise to the solution and the reaction mixture was maintained at 0 °C for 3 h. The reaction mixture was quenched with hydrochloric acid (2 M) and the dichloromethane layer washed with a saturated aqueous sodium bicarbonate solution followed by brine. The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. Flash column chromatography provided the hydroxy lactams **8a-m**.

*4.2.1.1 4-Bromo-5-(bromomethyl)-1-hexyl-5-hydroxy-1H-pyrrol-2-one (**8d**)*

Yield 70% as a red solid, m.p. 86-88 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.86-0.91(m, 3H, CH_3), 1.25-1.36 (m, 6H, 3 x CH_2), 1.63-1.69 (m, 2H, CH_2), 2.85 (br s, 1H, OH), 3.08-3.18 (m, 1H, NCH_2), 3.41-3.51 (m, 1H, NCH_2), 3.58 and 3.64 (each d, J_{AB} = 11.3 Hz, 1H, CH_2Br), 6.31 (s, 1H, CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9 (CH_3), 22.4 (CH_2), 26.8 (CH_2), 28.8 (CH_2), 30.6 (CH_2), 31.3 (CH_2), 39.6 (NCH_2), 92.1 (C), 128.9 (CH), 142.0 (C), 167.9 (C=O). IR (*nujol*, *v*, cm^{-1}): 3418, 2923, 2853, 1692, 1462, 1413, 1377, 1076, 843. UV (CH_3OH): λ_{max} 218.0 nm (ε 8660 $M^{-1}cm^{-1}$), 259.0 nm (ε 1480 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 375.9531 $(M+Na)^+$; $C_{11}H_{17}Br_2NO_2Na$ requires 375.9518.

*4.2.1.2 4-Bromo-5-(bromomethyl)-5-hydroxy-1-octyl-1H-pyrrol-2-one (**8e**)*

Yield 75% as a red solid, m.p. 88-90 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.87 (t, *J* = 7.1 Hz, 3H, CH_3), 1.27-1.30 (m, 8H, 4 x CH_2), 1.63-1.68 (m, 4H, 2 x CH_2), 2.92 (br s, 1H, OH), 3.07-3.17 (m, 1H, NCH_2), 3.45-3.52 (m, 1H, NCH_2), 3.56 and 3.63 (each d, J_{AB} = 11.3 Hz, 1H, CH_2Br), 6.34 (s, 1H, CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9 (CH_3), 22.5 (CH_2), 27.1 (CH_2), 28.9 (CH_2), 29.1 (2 x CH_2), 30.5 (CH_2), 31.6 (CH_2), 39.6 (NCH_2), 92.0 (C), 129.2 (CH), 141.6 (C), 167.7 (C=O). IR (*nujol*, *v*, cm^{-1}): 3431, 2923, 2852, 1699, 1670, 1462, 1412, 1376, 1075, 845. UV (CH_3OH): λ_{max} 216.0 nm (ε 3100 $M^{-1}cm^{-1}$), 266.0 nm (ε 330 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 403.9848 $(M+Na)^+$; $C_{13}H_{21}Br_2NO_2Na$ requires 403.9831.

*4.2.1.3 4-Bromo-5-(bromomethyl)-5-hydroxy-1-dodecyl-1H-pyrrol-2-one (**8f**)*

Yield 67% as a yellow solid, m.p. 141-142 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.88 (t, *J* = 7.3 Hz, CH_3), 1.25 (br s, 18H, 9 x CH_2), 1.62 (br s, 2H, CH_2), 2.89 (br s, 1H, OH), 3.56 and 3.63 (each d, J_{AB} = 11.3 Hz, 1H, CH_2Br), 3.97 (t, *J* = 7.9 Hz, 2H, NCH_2), 6.28 (s, 1H, CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9 (CH_3), 22.5 (CH_2), 26.3 (CH_2), 29.1 (CH_2), 29.2 (CH_2), 29.4 (2 x CH_2), 29.5 (2 x CH_2), 29.9 (CH_2), 31.7 (CH_2), 40.6 (CH_2), 91.9 (CHBr), 124.0 (CH), 130.9 (C), 139.7 (C), 168.7 (C=O). IR (film, *v*, cm^{-1}): 3130, 3090, 2924, 2852, 1712, 1630, 1566, 1463, 1338, 1263, 1128, 1105, 840. UV (CH_3OH): λ_{max} 222.0 nm (ε 2200 $M^{-1}cm^{-1}$), 276.0 nm (ε 10400 $M^{-1}cm^{-1}$), 317.0 nm (ε 5110 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 438.0534 $(M+H)^+$; $C_{17}H_{29}Br_2NO_2$ requires 438.0722.

*4.2.1.4 4-Bromo-5-(bromomethyl)-5-hydroxy-1-isobutyl-1H-pyrrol-2-one (**8g**)*

Yield 33% as a white solid, m.p. 136-138 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.92-0.96 (m, 6H, 2 x CH_3), 2.02-2.16 (m, 1H, $CH(CH_3)_2$), 2.85-2.92 (m, 1H, NCH_2), 3.20 (br s, 1H, OH), 3.37-3.44 (m, 1H, NCH_2), 3.56 and 3.64 (each d, J_{AB} = 11.3 Hz, 1H, CH_2Br), 6.38 (s, 1H, CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 20.2 (CH_3), 20.5 (CH_3), 27.8 ($CH(CH_3)_2$), 30.6 (CH_2Br), 47.0 (NCH_2), 92.1 (C), 129.2 (CH), 141.6 (C), 168.1 (C=O). IR (*nujol*, *v*, cm^{-1}): 3419, 3108, 2923, 2853, 1667, 1456, 1377, 1080, 736. UV (CH_3OH): λ_{max} 217.0 nm (ε 9750 $M^{-1}cm^{-1}$), 253.0 nm (ε 2390 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 325.9390 $(M+H)^+$; $C_9H_{14}Br_2NO_2$ requires 325.9393.

4.2.2 General protocol 2: Lactamization of bromo lactones[34]

Bromo lactone **10** (0.60 mmol) was dissolved in CH_2Cl_2 (10 mL) and the solution was cooled to 0 °C. The amine (3.36 mmol) in CH_2Cl_2 (10 mL) was added dropwise to the solution and the reaction mixture was maintained at 0 °C for 2 h. The reaction mixture was washed with water (10 mL), HCl (2M, 10 mL) and saturated NaCl solution (10 mL). The organic phase was separated, dried ($MgSO_4$) and the solvent evaporated *in vacuo*. Purification of the residue by silica gel chromatography (10% ethyl acetate/dichloromethane) afforded hydroxy lactams **11a-f**.

*4.2.2.1 1-Benzyl-4-bromo-5-(dibromomethyl)-5-hydroxy-1H-pyrrol-2-one (**11a**)*

Yield 53% as a white solid, m.p. 149-150 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.45 (br s, 1H, OH), 4.80 and 4.96 (each d, J_{AB} = 16.0 Hz, 1H, NCH_2), 5.93 (s, 1H, $CHBr_2$), 6.52 (s, 1H, CH), 7.27-7.42 (m, 5H, H_{aryl}). ^{13}C NMR (75 MHz, DMSO-d_6): δ 43.2 (NCH_2), 45.2 ($CHBr_2$), 91.6 (C), 127.6 (CH_{aryl}), 128.0 (2 x CH_{aryl}), 128.5 (2 x CH_{aryl}), 130.1 (CH), 136.7 (C), 140.8 (C), 168.2 (C=O). IR (*nujol*, *v*, cm^{-1}): 3439, 2923, 2854, 1667, 1455,

1428, 1402, 1361, 1246, 1142, 1047, 705. UV (CH_3OH): λ_{max} 209.0 nm (ε 18110 $M^{-1}cm^{-1}$), 257.0nm (ε 2830 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 459.8152 $(M+Na)^+$; $C_{12}H_{10}Br_3NO_2Na$ requires 459.8154.

*4.2.2.2 4-Bromo-1-butyl-5-(dibromomethyl)-5-hydroxy-1H-pyrrol-2-one (**11b**)*

Yield 71% as a brown solid, m.p. 94-96 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.95 (t, *J* = 7.5 Hz, 3H, CH_3), 1.31-1.43 (m, 2H, CH_2), 1.71-1.85 (m, 2H, CH_2), 3.42-3.68 (m, 2H, CH_2), 3.73 (br s, 1H, OH), 5.91 (s, 1H, $CHBr_2$), 6.42 (s, 1H, CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.5 (CH_3), 20.2 (CH_2), 30.7 (CH_2), 40.5 (CH_2), 45.0 ($CHBr_2$), 91.4 (C), 130.3 (CH), 140.2, 168.1 (C=O). IR (film, *v*, cm^{-1}): 202, 2958, 2871, 1688, 1405, 1370, 1243, 1120, 1037, 825. UV (CH_3OH): λ_{max} 222.0 nm (ε 7900 $M^{-1}cm^{-1}$), 253.0 nm (ε 1300 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 403.8492 $(M+H)^+$; $C_9H_{13}Br_3NO_2$ requires 403.8494.

*4.2.2.3 4-Bromo-5-(dibromomethyl)-1-hexyl-5-hydroxy-1H-pyrrol-2-one (**11c**)*

Yield 74% as a grey solid, m.p. 59-60 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.89 (t, *J* = 6.8 Hz, 3H, CH_3), 1.27-1.35 (m, 6H, 3 x CH_2), 1.72-1.87 (m, 2H, CH_2), 3.38-3.65 (m, 2H, NCH_2), 4.26 (br s, 1H, OH), 5.90 (s, 1H, $CHBr_2$), 6.39 (s, 1H, CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9 (CH_3), 22.5 (CH_2), 26.7 (CH_2), 28.6 (CH_2), 31.2 (CH_2), 40.8 (NCH_2), 44.9 ($CHBr_2$), 91.5 (C), 130.0 (CH), 140.7 (C), 168.4 (C=O). IR (film, *v*, cm^{-1}): 3302, 1666, 1454, 1243, 1123, 1039, 970, 849, 826. UV (CH_3OH): λ_{max} 220.0 nm (ε 12820 $M^{-1}cm^{-1}$), 263.0 nm (ε 1820 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 453.8650 $(M+Na)^+$; $C_{11}H_{16}Br_3NO_2Na$ requires 453.8623.

*4.2.2.4 4-Bromo-5-(dibromomethyl)-5-hydroxy-1-octyl-1H-pyrrol-2-one (**11d**)*

Yield 73% as a brown solid, m.p. 40-42 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.88 (t, *J* = 7.1 Hz, 3H, CH_3), 1.22-1.37 (m, 10H, 5 x CH_2), 1.70-1.90 (m, 2H, CH_2), 3.40-3.66 (m, 2H, NCH_2), 3.90 (br s, 1H, OH), 5.91 (s, 1H, $CHBr_2$), 6.41 (s, 1H, CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9 (CH_3), 22.5 (CH_2), 27.0 (CH_2), 28.6 (CH_2), 29.0 (CH_2), 29.1 (CH_2), 31.7 (CH_2), 40.8 (NCH_2), 45.0 ($CHBr_2$), 91.4 (C), 130.2 (CH), 140.4 (C), 168.2 (C=O). IR (film, *v*, cm^{-1}): 3238, 2924, 2853, 1673, 1608, 1455, 1406, 1372, 1243, 1122, 1038, 849, 825. UV (CH_3OH): λ_{max} 222.0 nm (ε 6270 $M^{-1}cm^{-1}$), 264.0 nm (ε 1020 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 459.9112 $(M+H)^+$; $C_{13}H_{21}Br_3NO_2$ requires 459.9122.

*4.2.2.5 4-Bromo-5-(dibromomethyl)-1-dodecyl-5-hydroxy-1H-pyrrol-2-one (**11e**)*

Yield 53% as a white solid, m.p. 62-64 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.87 (t, *J* = 6.8 Hz, 3H, CH_3), 1.13-1.37 (m, 18H, 9 x CH_2), 1.58-1.87 (m, 2H, CH_2), 3.45-3.66 (m, 2H, NCH_2), 3.73 (br s, 1H, OH), 5.91 (s, 1H, $CHBr_2$), 6.42 (s, 1H, CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9 (CH_3), 22.5 (CH_2), 27.0 (CH_2), 28.6 (CH_2), 29.1 (CH_2), 29.2 (CH_2), 29.4 (2 x CH_2), 29.5 (2 x CH_2), 31.8 (CH_2), 40.7 (NCH_2), 45.0 ($CHBr_2$), 91.4 (C), 130.3 (CH), 140.2 (C), 168.1 (C=O). IR (film, *v*, cm^{-1}): 3243, 2921, 2852, 1681, 1455, 1404, 1371, 1242, 1122, 849, 824. UV (CH_3OH): λ_{max} 219.0 nm (ε 13410 $M^{-1}cm^{-1}$), 268.0 nm (ε 3730 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 515.9746 $(M+H)^+$; $C_{17}H_{29}Br_3NO_2$ requires 515.9748.

*4.2.2.6 4-Bromo-5-(dibromomethyl)-5-hydroxy-1-isobutyl-1H-pyrrol-2-one (**11f**)*

Yield 28% as a brown solid, m.p. 139-140 °C. ^{1}H NMR (300 MHz, DMSO-d_6) : 0.92-0.98 (m, 6H, 2 x CH_3), 2.21-2.35 (m, 1H, $CH(CH_3)_2$), 3.27-3.34 (m, 1H, NCH_2), 3.47-3.55 (m, 1H, NCH_2), 3.57 (br s, 1H, OH), 5.94 (s, 1H, $CHBr_2$), 6.46 (s, 1H, CH). ^{13}C NMR (75 MHz, DMSO-d_6): 19.9 (CH_3), 20.4 (CH_3), 27.7 ($CH(CH_3)_2$), 45.1 ($CHBr_2$), 47.6 (CH_2N), 91.4 (C), 130.4 (CH), 139.9 (C), 168.3 (C=O). IR (film, *v*, cm^{-1}): 3211, 2960, 1687, 1404, 1338, 1243, 1121, 1037, 830. UV (CH_3OH): λ_{max} 290.0 nm (ε 7620 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 425.8333 $(M+Na)^+$; $C_9H_{12}Br_3NO_2Na$ requires 425.8310.

4.2.2 General protocol 3: Dehydration of hydroxy lactams[34]

Hydroxy lactam, together with *p*-TsOH (0.3 equiv) was dissolved in dry $CHCl_3$ and heated at reflux for 3 h. The mixture was concentrated *in vacuo* and purified by flash chromatography to give dihydropyrrol-2-ones.

*4.2.3.1 (Z)-4-Bromo-5-(bromomethylene)-1-hexyl-1H-pyrrol-2-one (**9d**)*

Yield 74% as a red oil. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.88 (t, *J* = 6.4 Hz, 3H, CH_3), 1.25-1.34 (m, 6H, 3 x CH_2), 1.60-1.65 (m, 2H, CH_2), 3.97 (t, *J* = 7.5 Hz, 2H, NCH_2), 6.26 (s, 1H, CH), 6.33 (s, 1H, CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.8 (CH_3), 22.4 (CH_2), 26.0 (CH_2), 29.9 (CH_2), 31.3 (CH_2), 40.6 (NCH_2), 91.9 (CHBr), 124.0 (CH), 130.9 (C), 139.7 (C), 168.7 (C=O). IR (film, *v*, cm^{-1}): 1711, 1628, 1563, 1463, 1375, 1261, 835. UV (CH_3OH): λ_{max} 222.0 nm (ε 3710 $M^{-1}cm^{-1}$), 276.0 nm (ε 15830 $M^{-1}cm^{-1}$), 316.0 nm (ε 8810 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 359.9397 $(M+Na)^+$; $C_{11}H_{15}Br_2NONa$ requires 359.9392).

*4.2.3.2 (Z)-4-Bromo-5-(bromomethylene)-1-octyl-1H-pyrrol-2-one (**9e**)*

Yield 67% as a red oil. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.87 (t, *J* = 7.2 Hz, 3H, CH_3), 1.26-1.30 (m, 8H, 4 x CH_2), 1.60-1.65 (m, 4H, 2 x CH_2), 3.96 (t, *J* =7.9 Hz, 2H, NCH_2), 6.28 (s, 1H, CH), 6.33 (s, 1H, CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9 (CH_3), 22.5 (CH_2), 26.3 (CH_2), 29.0 (CH_2), 29.1 (CH_2), 29.9 (CH_2), 31.6 (CH_2), 40.6 (NCH_2), 92.0 (CHBr), 124.0 (CH), 130.9 (C), 139.6 (C), 168.7 (C=O). IR (film, *v*, cm^{-1}): 3086, 1712, 1625, 1567, 1455, 1267, 834. UV (CH_3OH): λ_{max} 219.0 nm (ε 2810 $M^{-1}cm^{-1}$), 276.0 nm (ε 12680 $M^{-1}cm^{-1}$), 317.0 nm (ε 6280 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 387.9700 $(M+Na)^+$; $C_{13}H_{19}Br_2NONa$ requires 387.9705.

*4.2.3.3 (Z)-4-Bromo-5-(bromomethylene)-1-dodecyl-1H-pyrrol-2-one (**9f**)*

Yield 68% as a yellow solid, m.p. 42-44 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.87 (t, *J* = 7.3 Hz, CH_3), 1.25 (br s, 18H, 9 x

CH_2), 1.62 (br s, 2H, CH_2), 3.97 (t, *J* = 7.9 Hz, 2H, NCH_2), 6.25 (s, 1H, CH), 6.33 (s, 1H, CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9 (CH_3), 22.5 (CH_2), 26.3 (CH_2), 29.1 (CH_2), 29.2 (CH_2), 29.4 (2 x CH_2), 29.5 (2 x CH_2), 29.9 (CH_2), 31.7 (CH_2), 40.6 (CH_2), 91.9 (CHBr), 124.0 (CH), 130.9 (C), 139.7 (C), 168.7 (C=O). IR (film, *v*, cm^{-1}): 3130, 3090, 2924, 2852, 1712, 1630, 1566, 1463, 1338, 1263, 1128, 1105, 840. UV (CH_3OH): λ_{max} 222.0 nm (ε 2200 $M^{-1}cm^{-1}$), 276.0 nm (ε 10400 $M^{-1}cm^{-1}$), 317.0 nm (ε 5110 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 420.0534 $(M+H)^+$; $C_{17}H_{28}Br_2NO$ requires 420.0537.

*4.2.3.4 (Z)-4-Bromo-5-(bromomethylene)-1-isobutyl-1H-pyrrol-2-one (**9g**)*

Yield 96% as a brown oil. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.90 (d, *J* = 6.8 Hz, 6H), 2.02-2.12 (m, 1H, $CH(CH_3)_2$), 3.79 (d, *J* = 7.1 Hz, 2H, NCH_2), 6.29 (s, 1H, CH), 6.35 (s, 1H, CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 19.3 (2 x CH_3), 29.3 ($CH(CH_3)_2$), 47.3 (CH_2N), 92.1 (CHBr), 123.9 (CH), 130.9 (C), 139.8 (C), 169.0 (C=O). IR (film, *v*, cm^{-1}): 3085, 2960, 1709, 1625, 1566, 1448, 1382, 1315, 1263, 1102, 1018, 878, 835. UV (CH_3OH): λ_{max} 224.0 nm (ε 2590 $M^{-1}cm^{-1}$), 276.0 nm (ε 11700 $M^{-1}cm^{-1}$), 318.0 nm (ε 6470 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 329.9094 $(M+Na)^+$; $C_9H_{11}Br_2NONa$ requires 329.9100

*4.2.3.5 1-Benzyl-4-bromo-5-(dibromomethylene)-1H-pyrrol-2-one (**12a**)*

Yield 83% as a yellow solid, m.p. 125-126 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.05 (s, 2H, NCH_2), 6.43 (s, 1H, CH), 7.16-7.34 (m, 5H, H_{aryl}). ^{13}C NMR (75 MHz, DMSO-d_6): δ 44.9 (CH_2N), 93.7 (CH), 118.7 (C), 126.3 (2 x CH_{aryl}), 127.3 (CH_{aryl}), 128.5 (2 x CH_{aryl}), 130.4 (C), 136.7 (C), 138.7 (C), 164.8 (C=O). IR (film, *v*, cm^{-1}): 3080, 1712, 1621, 1570, 1453, 1434, 1355, 1249, 1108, 1072, 954. UV (CH_3OH): λ_{max} 206.0 nm (ε 10470 $M^{-1}cm^{-1}$), 293.0 nm (ε 12780 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 419.8230 $(M+H)^+$; $C_{12}H_9Br_3NO$ requires 419.8234.

*4.2.3.6 4-Bromo-1-butyl-5-(dibromomethylene)-1H-pyrrol-2-one (**12b**)*

Yield 71% as an orange solid, m.p. 66-68 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.94 (t, *J* = 7.2 Hz, 3H, CH_3), 1.29-1.41 (m, 2H, CH_2), 1.59-1.69 (m, 2H, CH_2), 4.05 (t, *J* = 7.5 Hz, 2H, NCH_2), 6.39 (s, 1H, CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.5 (CH_3), 18.5 (CH_2), 30.9 (CH_2), 40.5 (NCH_2), 91.5 (CH), 117.8 (C), 128.6 (C), 137.8 (C), 163.4 (C=O). IR (film, *v*, cm^{-1}): 3077, 2957, 2870, 1712, 1617, 1572, 1454, 1365, 1270, 1238, 1178, 1118, 1050, 931, 841, 743. UV (CH_3OH): λ_{max} 207.0 nm (ε 4420 $M^{-1}cm^{-1}$), 293.0 nm (ε 20840 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 385.8389 $(M+H)^+$; $C_9H_{11}Br_3NO$ requires 385.83908.

*4.2.3.7 4-Bromo-5-(dibromomethylene)-1-hexyl-1H-pyrrol-2-one (**12c**)*

Yield 74% as a brown solid, m.p. 40-42 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.88 (t, *J* = 7.2 Hz, 3H, CH_3), 1.27-1.35 (m, 6H, 3 x CH_2), 1.59-1.67 (m, 2H, CH_2), 4.04 (t, *J* = 7.9 Hz, 2H), 6.39 (s, 1H, CH). ^{13}C NMR: δ 13.8 (CH_3), 22.4 (CH_2), 25.9 (CH_2), 29.8 (CH_2), 31.2 (CH_2), 41.8 (NCH_2), 92.5 (CH), 118.9 (C), 129.7 (C), 138.9 (C), 164.4 (C=O). IR (film, *v*, cm^{-1}): 3083, 2954, 2928, 2856, 1716, 1620, 1572, 1455, 1365, 1253, 1058, 841, 741. UV (CH_3OH): λ_{max} 208.0 nm (ε 3900 $M^{-1}cm^{-1}$), 293.0 nm (ε 21060 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 413.8698 $(M+H)^+$; $C_{11}H_{15}Br_3NO$ requires 413.8703.

*4.2.3.8 4-Bromo-5-(dibromomethylene)-1-octyl-1H-pyrrol-2-one (**12d**)*

Yield 73% as a brown solid, m.p. 44-46 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.87(t, *J* = 7.2 Hz, 3H, CH_3), 1.26-1.31 (m, 10H, 5 x CH_2), 1.62-1.67 (m, 2H, CH_2), 4.04 (t, *J* = 7.9 Hz, 2H), 6.39 (s, 1H, CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9 (CH_3), 22.5 (CH_2), 26.3 (CH_2), 29.0 (2 x CH_2), 29.9 (CH_2), 31.6 (CH_2), 41.8 (NCH_2), 92.5 (CH), 118.9 (C), 129.7 (C), 138.9 (C), 164.4 (C=O). IR (film, *v*, cm^{-1}): 3084, 2925, 2854, 1720, 1620, 1573, 1455, 1364, 1244, 1119, 1034, 841, 741. UV (CH_3OH): λ_{max} 217.0 nm (ε 2850 $M^{-1}cm^{-1}$), 293.0 nm (ε 19210 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 441.9014 $(M+H)^+$; $C_{13}H_{19}Br_3NO$ requires 441.9016.

*4.2.3.9 4-Bromo-5-(dibromomethylene)-1-dodecyl-1H-pyrrol-2-one (**12e**)*

Yield 53% as a brown solid, m.p. 46-48 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.87 (t, *J* = 7.1 Hz, 3H, CH_3), 1.25-1.31 (m, 18H, 9 x CH_2), 1.62-1.67 (m, 2H, CH_2), 4.04 (t, *J* = 7.5 Hz, 2H, NCH_2), 6.39 (s, 1H, CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9 (CH_3), 22.5 (CH_2), 26.3 (CH_2), 29.1 (CH_2), 29.2 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.5 (2 x CH_2), 29.9 (CH_2), 31.7 (CH_2), 41.8 (NCH_2), 92.5 (CH), 118.9 (C), 129.7 (C), 138.9 (C), 164.4 (C=O). IR (film, *v*, cm^{-1}): 3085, 2923, 2852, 1720, 1621, 1573, 1464, 1364, 1246, 1050, 842, 741. UV (CH_3OH): λ_{max} 217.0 nm (ε 3210 $M^{-1}cm^{-1}$), 293.0 nm (ε 21640 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 497.9637 $(M+H)^+$; $C_{17}H_{27}Br_3NO$ requires 497.9642.

*4.2.3.10 4-Bromo-5-(dibromomethylene)-1-isobutyl-1H-pyrrol-2-one (**12f**)*

Yield 68% as a brown solid, m.p. 82-84 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.89 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 1.98-2.15 (m, 1H, C<u>H</u>$(CH_3)_2$), 3.86 (d, *J* = 7.5 Hz, 2H, NCH_2), 6.39 (s, 1H, CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 18.4 (2 x CH_3), 28.4 (<u>C</u>H$(CH_3)_2$), 47.6 (NCH_2), 91.8 (CH), 117.9 (C), 128.8 (C), 138.1 (C), 163.8 (C=O). IR (film, *v*, cm^{-1}): 3083, 2961, 1715, 1624, 1572, 1443, 1379, 1317, 1256, 1052, 910, 847, 741. UV (CH_3OH): λ_{max} 294.0 nm (ε 27110 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 407.8219 $(M+Na)^+$; $C_9H_{10}Br_3NONa$ requires 407.8205.

4.2.4 General protocol 4: One-pot lactamization[29]

Fimbrolides **15a-b** (3.3 mmol) were dissolved in CH_2Cl_2 (2 mL) and cooled to 0 °C. The amine (9.1 mmol) in CH_2Cl_2 (2 mL) was then added dropwise and the mixture stirred for 3 h at 0 °C. The mixture was washed with water (10 mL), HCl (2M, 10 mL) and saturated NaCl solution (10 mL). The organic phase was separated, dried ($MgSO_4$) and the solvent evaporated *in vacuo*. The residual solid was dissolved in toluene (10 mL) and *p*-TsOH (0.11 mmol) was added. The mixture was heated at refluxed for 1 h, the solvent was evaporated *in vacuo* and the residue purified by silica gel chromatography (2:1 dichloromethane/light petroleum) to afford lactams **16a-g.**

*4.2.4.1 1-Phenyl-3-butyl-5-(dibromomethylene)-1H-pyrrol-2-one (**16a**)*

Yield 66% as a white solid, m.p. 66-68 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.95 (t, *J* = 7.1 Hz, 3H, CH_3), 1.39 (m, 2H, CH_2), 1.58 (m, 2H, CH_2), 2.36 (m, 2H, CH_2), 7.06-7.30 (m, 6H, CH and H_{aryl}). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.6 (CH_3), 22.6 (CH_2), 26.2 (CH_2), 28.5 (CH_2), 77.7 (C), 125.9 (2 x CH_{aryl}), 126.9 (CH_{aryl}), 127.4 (2 x CH_{aryl}), 132.2 (CH), 137.7 (C), 138.7 (C), 140.3 (C), 172.0 (C=O). IR (KBr disc, *v*, cm^{-1}): 2953, 1706, 1626, 1494, 1435, 1352, 1268, 1235, 1094, 747, 721. UV (CH_3OH): λ_{max} 206.0 nm (ε 10970 $M^{-1}cm^{-1}$), 283.0 nm (ε 16200 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* .383.9557 $(M+H)^+$; $C_{15}H_{15}Br_2NO$ requires 383.9608).

*4.2.4.2 1-Benzyl-3-butyl-5-(dibromomethylene)-1H-pyrrol-2-one (**16b**)*

Yield 60% as a white solid, m.p. 56-58 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.95 (t, *J* = 7.1 Hz, 3H, CH_3), 1.39 (m, 2H,

CH_2), 1.58 (m, 2H, CH_2), 2.36 (m, 2H, CH_2), 5.26 (s, 2H, NCH_2), 7.06-7.30 (m, 6H, CH and H_{aryl}). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.6 (CH_3), 22.3 (CH_2), 25.2 (CH_2), 29.5 (CH_2), 44.1 (NCH_2), 74.7 (C), 125.9 (2 x CH_{aryl}), 126.9 (CH_{aryl}), 128.4 (2 x CH_{aryl}), 132.2 (CH), 137.7 (C), 138.7 (C), 140.3 (C), 172.0 (C=O). IR (KBr disc, *v*, cm^{-1}): 2958, 1703, 1629, 1488, 1434, 1352, 1260, 1232, 1097, 743, 721. UV (CH_3OH): λ_{max} 206.0 nm (ε 10970 $M^{-1}cm^{-1}$), 283.0 nm (ε 16200 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 397.9665 $(M+H)^+$; $C_{16}H_{17}Br_2NO$ requires 397.9678.

*4.2.4.3 1,3-Dibutyl-5-(dibromomethylene)-1H-pyrrol-2-one (**16c**)*

Yield 56% as a yellow oil. ^1H NMR (300 MHz, DMSO-d_6): δ 0.92 (m, 6H, 2 x CH_3), 1.35 (m, 4H, 2 x CH_2), 1.55 (m, 4H, 2 x CH_2), 2.31 (m, 2H, CH_2), 3.96 (t, *J* = 7.9 Hz, 2H, CH_2), 6.99 (s, 1H, CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.6 (2 x CH_3), 19.7 (CH_2), 22.3 (CH_2), 25.0 (CH_2), 29.5 (CH_2), 32.0 (CH_2), 40.6 (CH_2), 73.3 (C), 131.7 (CH), 138.7 (C), 140.6 (C), 171.9 (C=O). IR (film, *v*, cm^{-1}): 2957, 2871, 1701, 1586, 1455, 1361, 1194, 1168, 1134, 1047, 829. UV (CH_3OH): λ_{max} 205.0 nm (ε 9270 $M^{-1}cm^{-1}$), 284.0 nm (ε 20630 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 363.9798 $(M+H)^+$; $C_{13}H_{19}Br_2NO$ requires 363.9856.

*4.2.4.4 3-Butyl-5-(dibromomethylene)-1-p-tolyl-1H-pyrrol-2-one (**16d**)*

Yield 92% as a white solid, m.p. 76-78 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 0.95 (t, *J* = 7.1 Hz, 3H, CH_3), 1.39 (m, 2H, CH_2), 1.54 (m, 2H, CH_2), 2.37 (m, 5H, CH_2 and CH_3), 7.20 (m, 5H, CH and H_{aryl}). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.7 (CH_3), 21.1 (CH_3), 22.3 (CH_2), 25.2 (CH_2), 29.5 (CH_2), 75.8 (C), 129.0 (2 x CH_{aryl}), 129.4 (2 x CH_{aryl}), 131.9 (CH), 132.3 (C), 138.3 (C), 138.9 (C), 140.3 (C), 171.8 (C=O). IR (*nujol*, *v*, cm^{-1}): 2922, 2855, 1693, 1590, 1514, 1463, 1362, 1190, 1130, 1078, 857, 836, 817, 775, 741, 607. UV (CH_3OH): λ_{max} 205.0 nm (ε 16690 $M^{-1}cm^{-1}$), 303.0 nm (ε 14280 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 397.9615 $(M+H)^+$; $C_{16}H_{17}Br_2NO$ requires 397.9677).

*4.2.4.5 5-(Dibromomethylene)-3-hexyl-1-phenyl-1H-pyrrol-2-one (**16e**)*

Yield 68% as a colourless oil. ^1H NMR (300 MHz, DMSO-d_6): δ 0.89 (m, 3H, CH_3), 1.32 (m, 6H, 3 x CH_2), 1.57 (m, 2H, CH_2), 2.34 (m, 2H, CH_2), 7.16-7.42 (m, 6H, CH and H_{aryl}). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9 (CH_3), 22.4 (CH_2), 25.6 (CH_2), 27.3 (CH_2), 28.8 (CH_2), 31.4 (CH_2), 76.2 (C), 128.3 (CH_{aryl}), 128.8 (2 x CH_{aryl}), 129.3 (2 x CH_{aryl}), 132.0 (CH), 135.0 (C), 138.9 (C), 140.2 (C), 171.6 (C=O). IR (KBr disc, *v*, cm^{-1}): 2925, 2854, 1692, 1598, 1500, 1444, 1191, 1127, 1081, 743,676. UV (CH_3OH): λ_{max} 204.0 nm (ε 19690 $M^{-1}cm^{-1}$), 309.0 nm (ε 19590 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 411.9827 $(M+H)^+$; $C_{17}H_{19}Br_2NO$ requires 411.9833.

*4.2.4.6 1-Benzyl-5-(dibromomethylene)-3-hexyl-1H-pyrrol-2-one (**16f**)*

Yield 72% as a yellow oil. ^1H NMR (300 MHz, DMSO-d_6): δ 0.88 (t, *J* = 7.1 Hz, 3H, CH_3), 1.32 (m, 6H, 3 x CH_2),1.56 (m, 2H, CH_2), 2.38 (m, 2H, CH_2), 5.26 (s, 2H, NCH_2), 7.08-7.33 (m, 6H, CH and H_{aryl}). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9 (CH_3), 22.4 (CH_2), 25.5 (CH_2), 27.4 (CH_2), 27.4 (CH_2), 28.8 (CH_2), 31.4 (CH_2), 44.1(NCH_2), 74.8 (C), 125.9 (2 x CH_{aryl}), 126.9 ($_{CHaryl}$), 128.4 (2 x CH_{aryl}), 132.2 (CH), 137.7 (C), 138.7 (C), 140.3 (C), 172.0 (C=O). IR (film, *v*, cm^{-1}): 2922, 2854, 1696, 1592, 1495, 1452, 1353, 1144, 1086, 976, 844, 829, 708. UV (CH_3OH): λ_{max} 206.0 nm (ε 10970 $M^{-1}cm^{-1}$), 283.0 nm (ε 16200 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 425.9986 $(M+H)^+$; $C_{18}H_{21}Br_2NO$ requires 425.9996.

*4.2.4.7 1-Butyl-5-(dibromomethylene)-3-hexyl-1H-pyrrol-2-one (**16g**)*

Yield 64% as a yellow oil. ^1H NMR (300 MHz, DMSO-d_6): δ 0.90 (m, 6H, 2 x CH_3), 1.30 (m, 6H, 3 x CH_2), 1.54 (m, 6H, 3 x CH_2), 2.30 (m, 2H, CH_2), 3.96 (t, *J* = 7.5 Hz, 2H, CH_2), 6.98 (s, 1H, CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.6 (CH_3), 13.9 (CH_3), 19.7 (CH_2), 22.3 (CH_2), 25.3 (CH_2), 27.3 (CH_2), 28.8 (CH_2), 31.3 (CH_2), 32.0 (CH_2), 40.6 (CH_2), 73.3 (C), 131.7 (CH), 138.7 (C), 140.6 (C), 171.9 (C=O). IR (film, *v*, cm^{-1}): 2956, 2858, 1704, 1586, 1456, 1360, 1194, 1134, 1057, 828, 740. UV (CH_3OH): λ_{max} 205.0 nm (ε 9270 $M^{-1}cm^{-1}$), 284.0 nm (ε 20630 $M^{-1}cm^{-1}$). Mass spectrum: HRMS (ESI): *m/z* 392.0123 $(M+H)^+$; $C_{15}H_{23}Br_2NO$ requires 392.0148.

4.2.5 General protocol 5: α-Bromination of Lactams

A mixture of lactam **16a-b/e-f** (2.28 mmol) and *N*-bromosuccinimide (2.80 mmol) in carbon tetrachloride (30 mL) was heated at reflux for 16 h. The solvent was evaporated *in vacuo* and the residue was purified by silica gel chromatography (1:1 dichloromethane/light petroleum) to afford the α-brominated lactams **17a-d**.

*4.2.5.1 4-Bromo-3-(1-bromobutyl)-5-(dibromomethylene)-1-phenyl-1H-pyrrol-2-one (**17a**)*

Yield 74% as a red oil. ^1H NMR (300 MHz, DMSO-d_6): δ 0.89-0.92 (m, 3H, CH_3), 1.31-1.67 (m, 4H, 2 x CH_2), 5.35 (t, *J* = 7.9 Hz, 1H, CHBr), 7.36-7.58 (m, 5H, H_{aryl}). ^{13}C NMR: δ 14.3 (CH_3), 22.2 (CH_2), 30.8 (CH_2), 43.1 (CHBr), 73.2 (=CBr_2), 126.7 (2 x CH_{aryl}), 128.8 (2 x CH_{aryl}), 129.2 (2 x CH_{aryl}), 133.8 (C), 138.4 (C), 143.3 (C), 173.8 (C=O). IR (film, *v*, cm^{-1}): 3080, 1715, 1594, 1490, 1454, 1380, 1296, 1260, 1139, 809, 746, 692. UV (CH_3OH): λ_{max} 205.0 nm (ε 6340 $M^{-1}cm^{-1}$), 294.0 nm (ε 14030 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 563.7572 $(M+Na)^+$; $C_{15}H_{13}Br_4NONa$ requires 563.7645.

*4.2.5.2 (Z)-1-Benzyl-4-bromo-3-(1-bromobutyl)-5-(bromomethylene)-1H-pyrrol-2-one (**17b**)*

Yield 92% as a light brown solid, m.p. 80-82 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 0.95 (t, *J* = 7.2 Hz, 3H, CH_3), 1.30-1.50 (m, 2H, CH_2), 2.27-2.45 (m, 2H, CH_2), 4.95 (t, *J* = 7.9 Hz, 1H, CHBr), 5.29 (s, 2H, NCH_2), 6.36 (s, 1H, =CHBr), 7.13-7.44 (m, 5H, H_{aryl}). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.1 (CH_3), 21.4 (CH_2), 37.3 (CH_2), 42.8 (CHBr), 44.0 (NCH_2), 93.4 (=CHBr), 126.2 (2 x CH_{aryl}), 127.1 (CH_{aryl}), 128.2 (C), 128.5 (2 x CH_{aryl}), 133.2 (C), 137.1 (C), 138.2 (C), 166.8 (C=O). IR (film, *v*, cm^{-1}): 3083, 3031, 2959, 2930, 2871, 1712, 1622, 1496, 1454, 1434, 1384, 1356, 1333, 1240 1173, 923, 887. UV (CH_3OH): λ_{max} 294.0 nm (ε 26310 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 475.8857 $(M+H)^+$; $C_{16}H_{17}Br_3NO$ requires 475.8860.

*4.2.5.3 (Z)-4-Bromo-3-(1-bromohexyl)-5-(bromomethylene)-1-phenyl-1H-pyrrol-2-one (**17c**)*

90% as a red oil. ^1H NMR (300 MHz, DMSO-d_6): δ 0.82-0.91 (m, 3H, CH_3), 1.25-1.62 (m, 6H, 3 x CH_2), 2.31-2.46 (m, 2H, CH_2) 4.95 (t, *J* = 7.9 Hz, 1H, CHBr), 6.45 (s, 1H, C=CHBr), 7.29-7.49 (m, 5H, H_{aryl}). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.8 (CH_3), 22.2 (CH_2), 27.8 (CH_2), 30.8 (CH_2), 35.3 (CH_2), 43.1 (CHBr), 94.2 (=CHBr), 128.7 (2 x CH_{aryl}), 128.8 (2 x CH_{aryl}), 129.2 (CH_{aryl}), 133.1 (C), 133.8 (C), 138.4 (C), 146.9 (C), 166.5 (C=O). IR (film, *v*, cm^{-1}): 3084, 1716, 1595, 1499, 1454, 1382, 1299, 1263, 1139, 809, 746, 692. UV (CH_3OH): λ_{max} 205.0 nm (ε 6340 $M^{-1}cm^{-1}$), 294.0 nm (ε 14030 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 513.8825 $(M+Na)^+$; $C_{17}H_{18}Br_3NONa$ requires 513.8810.

*4.2.5.3 4-Bromo-3-(1-bromohexyl)-5-(dibromomethylene)-1-benzyl-1H-pyrrol-2-one (**17d**)*

Yield 43% as a red oil. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.82-0.91 (m, 3H, CH_3), 1.25-1.77 (m, 8H, 4 x CH_2), 2.39-2.56 (m, 2H, CH_2), 4.95 (t, *J* = 7.9 Hz, 1H, CHBr), 7.29-7.49 (m, 5H, H_{aryl}). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.8 (CH_3), 22.2 (CH_2), 27.8 (CH_2), 30.8 (CH_2), 35.3 (CH_2), 43.1 (CHBr), 46.2 (NCH_2), 84.2 (=CBr_2), 126.7 (2 x CH_{aryl}), 128.8 (2 x CH_{aryl}), 129.2 (2 x CH_{aryl}), 133.8 (C), 138.4 (C), 144.3 (C), 169.8 (C=O). IR (film, *v*, cm^{-1}): 3080, 1715, 1594, 1490, 1454, 1380, 1296, 1260, 1139, 809, 746, 692. UV (CH_3OH): λ_{max} 205.0 nm (ε 6340 $M^{-1}cm^{-1}$), 294.0 nm (ε 14030 $M^{-1}cm^{-1}$). Mass spectrum: HRMS (ESI): *m/z* 605.6872 $(M+Na)^+$; $C_{18}H_{19}Br_4NONa$ requires 605.6889.

4.2.6 General protocol 6: α-Hydroxylation of α-bromo lactams

Lactams **17a-d** (0.28 mmol) were dissolved in DMSO (5 mL) and 5 drops of H_2O were added. The mixture was heated to 60 °C and stirred for 3 days before quenching with H_2O (20 mL). The mixture was extracted with dichloromethane (20 mL). The solvent was evaporated *in vacuo* and the residue was purified by silica gel chromatography (dichloromethane) to afford the α-hydroxy lactams **18a-d**.

*4.2.6.1 4-Bromo-5-(dibromomethylene)-3-(1-hydroxybutyl)-1-phenyl-1Hpyrrol-2-one (**18a**)*

The compound **15d** was prepared in a manner similar to that described for **15a**. Yield 95% as a yellow oil. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.88-0.90 (m, 3H, CH_3), 1.33-1.49 (m, 4H, 2 x CH_2), 4.13 (br s, 1H, OH), 4.98 (br s, 1H, CHOH), 7.28-7.53 (m, 5H, H_{aryl}). ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.1 (CH_3), 20.9 (CH_2), 25.0 (CH_2), 68.9 (CHOH), 114.2 (=CBr), 124.7 (C), 126.8 (CH_{aryl}), 127.9 (2 x CH_{aryl}), 128.1 (2 x CH_{aryl}), 132.9 (C), 134.6 (C), 137.6 (C), 167.3 (C=O). IR (film, v, cm^{-1}): 3466, 3082, 1700, 1596, 1497, 1383, 1299, 1264, 1114, 809, 747, 693. UV (CH_3OH): λ_{max} 205.0 nm (ε 10980 $M^{-1}cm^{-1}$), 285.0 nm (ε 12110 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 499.8671 $(M+Na)^+$; $C_{15}H_{14}Br_3NO_2Na$ requires 499.8687.

*4.2.6.2 (Z)-1-Benzyl-4-bromo-5-(bromomethylene)-3-(1-hydroxybutyl)-1Hpyrrol-2-one (**18b**)*

Yield 67% as a yellow solid, m.p. 50-52 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.96 (t, *J* = 7.2 Hz, 3H, CH_3), 1.33-1.56 (m, 2H, CH_2), 1.67-1.94 (m, 2H, CH_2), 3.31 (br s, 1H, OH), 4.61 (t, *J* = 6.8 Hz, 1H, C<u>H</u>OH), 5.27 (s, 2H, NCH_2), 6.33 (s, 1H, =CHBr), 7.13-7.34 (m, 5H, H_{aryl}). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.7 (CH_3), 18.6 (CH_2), 38.7 (CH_2), 43.8 (NCH_2), 68.6 (CHOH), 93.3 (CHBr), 125.4 (C), 126.2 (2 x CH_{aryl}), 127.2 (CH_{aryl}), 128.5 (2 x CH_{aryl}), 134.7 (C), 137.0 (C), 138.2 (C), 169.0 (C=O). IR (film, *v*, cm^{-1}): 3465, 3084, 3031, 2958, 2930, 2870, 1693, 1626, 1496, 1454, 1435, 1387, 1356, 1333, 1248, 1104, 1073, 1030, 954, 887, 849. UV (CH_3OH): λ_{max} 292.0 nm (ε 11860 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 413.9701 $(M+H)^+$; $C_{16}H_{18}Br_2NO_2$ requires 413.9703.

*4.2.6.3 (Z)-4-Bromo-5-(bromomethylene)-3-(1-hydroxyhexyl)-1-phenyl-1Hpyrrol-2-one (**18c**)*

Yield 48% as a yellow oil. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.90-0.92 (m, 3H, CH_3), 1.25-1.54 (m, 6H, 3 x CH_2), 1.71-1.94 (m, 2H, CH_2), 3.32 (br s, 1H, OH), 4.61 (br s, 1H, C<u>H</u>OH), 6.42 (s, 1H, C=CHBr), 7.28-7.53 (m, 5H, H_{aryl}). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.8 (CH_3), 22.4 (CH_2), 25.0 (CH_2), 31.3 (CH_2), 36.6 (CH_2), 68.9 (CHOH), 94.2 (=CHBr), 125.6 (C), 128.8 (CH_{aryl}), 128.9 (2 x CH_{aryl}), 129.1 (2 x CH_{aryl}), 133.6 (C), 134.6 (C), 138.3 (C), 168.6 (C=O). IR (film, *v*, cm^{-1}): 3466, 3082, 1700, 1596, 1497, 1383, 1299, 1264, 1114, 809, 747, 693. UV (CH_3OH): λ_{max} 205.0 nm (ε 10980 $M^{-1}cm^{-1}$), 285.0 nm (ε 12110 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 451.9659 $(M+Na)^+$; $C_{17}H_{19}Br_2NO_2Na$ requires 451.9654.

*4.2.6.4 4-Bromo-5-(dibromomethylene)-3-(1-hydroxyhexyl)-1-benzyl-1Hpyrrol-2-one (**18d**)*

Yield 96% as a yellow oil. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.88-0.90 (m, 3H, CH_3), 1.23-1.69 (m, 8H, 4 x CH_2), 2.73-2.91 (m, 2H, CH_2), 4.02 (br s, 1H, OH), 4.61 (br s, 1H, C<u>H</u>OH), 7.28-7.53 (m, 5H, H_{aryl}). ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.1 (CH_3), 22.4 (CH_2), 25.0 (CH_2), 31.3 (CH_2), 36.6 (CH_2), 47.3 (CH_2), 68.9 (CHOH), 114.2 (=CBr), 125.6 (C), 128.8 (CH_{aryl}), 128.9 (2 x CH_{aryl}), 129.1 (2 x CH_{aryl}), 133.6 (C), 134.6 (C), 138.3 (C), 168.6 (C=O). IR (film, *v*, cm^{-1}): 3466, 3082, 1700, 1596, 1497, 1383, 1299, 1264, 1114, 809, 747, 693. UV (CH_3OH): λ_{max} 205.0 nm (ε 10980 $M^{-1}cm^{-1}$), 285.0 nm (ε 12110 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 543.6837 $(M+Na)^+$; $C_{18}H_{20}Br_3NO_2Na$ requires 543.6844.

4.2.7 Synthesis of other compounds

*4.2.7.1 4,5-Dibromo-5-(dibromomethyl)furan-2-one (**10**)*

Fimbrolide **7a** (2.00 g, 7.88 mmol) was dissolved in CH_2Cl_2 (10 mL) and cooled to 0 °C. Bromine (3.31 g, 20.7 mmol) in CH_2Cl_2 (10 mL) was then added dropwise and the mixture stirred for 3 h at 0 °C. After quenching with a saturated sodium metabisulphite solution, the mixture was extracted with CH_2Cl_2 (30 mL). The organic phase was separated and washed with saturated NaCl solution and dried ($MgSO_4$). The solvent was evaporated *in vacuo* and the crude pale yellow solid was recrystallized from light petroleum to afford the title compound **10** as a white solid. Yield 78 %, m.p. 130-131 °C ^{1}H NMR (300 MHz, DMSO-d_6): δ 5.93 (s, 1H, $CHBr_2$), 6.55 (s, 1H CH). ^{13}C NMR (75 MHz, DMSO-d_6): 42.9 ($CHBr_2$), 93.3 (C), 122.8(CH), 150.5 (C), 164.9 (C=O). IR (*nujol*, *v*, cm^{-1}): 3109, 2923, 2853, 1799, 1597, 1462, 1377, 1237, 1159, 1075, 926, 886, 723. UV (CH_3OH): λ_{max} 238.0 nm (ε 7230 $M^{-1}cm^{-1}$).

*4.2.7.2 1,7-Dibromo-6a,12a-bis-bromomethyl-4,5,10,11-tetrahydro-6aH,12aH-6,12-dioxa-3a,9a-diaza-dicyclopenta [a,f]cyclodecene-3,9-dione (**14a**)*

Fimbrolide **7a** (1.00 g, 3.94 mmol) was dissolved in CH_2Cl_2 (5mL) and cooled to 0 °C. Trimethylsilanyl-ethylamine (2.10 g, 15.6 mmol) in CH_2Cl_2 (10 mL) was then added dropwise and the mixture stirred for 2 h at 0 °C. The mixture was concentrated *in vacuo* and purified by silica gel chromatography (ethyl acetate). The solid (**13a**) was dissolved in dry $CHCl_3$ (10 mL) and *p*-TsOH (0.02 g, 0.11 mmol) was added. The mixture was heated at reflux for 2 h, the solvent was evaporated *in vacuo* and the residue was purified by silica gel chromatography (ethyl acetate) to afford **14a**. Yield 61% as a white solid, m.p. 80-86 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.35-3.44 (m, 1H, NCH2), 3.54 and 3.63 (each d, J_{AB} = 11.3 Hz, 1H, CH_2Br), 3.86-3.94 (m, 1H, NCH_2), 4.07-4.13 (m, 1H, CH_2O), 4.19-4.24 (m, 1H, CH_2O), 6.34 (s, 1H, CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 31.2 (CH_2Br), 44.4 (NCH_2), 69.9 (CH_2O), 101.1 (C), 130.1 (CH), 144.7 (C), 174.9 (C=O). IR (*nujol*, *v*, cm^{-1}): 1715, 1592, 1464, 1317, 1260, 1037, 877, 695. UV (CH_3OH): λ_{max} 223.0 nm (ε 31130 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 590.7759 $(M+H)^+$; $C_{14}H_{15}Br_4N_2O_4$ requires 590.7765.

*4.2.7.3 1,7-Dibromo-7a,14a-bis-bromomethyl-5,6,12,13-tetrahydro-7aH,11H,14aH-7,14- dioxa-3a,10a-diaza-dicyclopenta[a,g]cyclodecene-3,10-dione (**14b**)*

Fimbrolide **7a** (0.402 g, 1.57 mmol) was dissolved in CH_2Cl_2 (5mL) and cooled to 0 °C. Trimethylsilanyl-propylamine (0.84 g,

5.73 mmol) in CH_2Cl_2 (10 mL) was then added dropwise and the mixture stirred for 2 h at 0 °C. The mixture was concentrated *in vacuo* and purified by silica gel chromatography (ethyl acetate). The crude oil (**13b**) was dissolved in dry $CHCl_3$ (10 mL) and *p*-TsOH (0.01 g, 0.06 mmol) was added. The mixture was heated at reflux for 2 h, the solvent was evaporated *in vacuo* and the residue was purified by silica gel chromatography (ethyl acetate) to afford **14b**. Yield 34% as a yellow solid, m.p. 126-128 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.58-1.86 (m, 2H, CH_2), 2.99-3.10 (m, 1H, NCH_2), 3.56 and 3.95 (each d, J_{AB} = 11.3 Hz, 1H, CH_2Br), 3.95-3.98 (m, 2H, CH_2O), 4.30-4.36 (m, 1H, NCH_2), 6.46 (s, 1H, CH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 24.4 (CH_2), 27.1 (CH_2Br), 35.0 (NCH_2), 62.5 (CH_2O), 90.5 (C), 129.8 (CH), 141.4 (C), 167.5 (C=O). IR (*nujol*, *v*, cm^{-1}): 1702, 1605, 1456, 1400, 1282, 1043, 928, 859, 641. UV (CH_3OH): λ_{max} 219.0 nm (ε 18860 $M^{-1}cm^{-1}$), 255.0 nm (ε 3530 $M^{-1}cm^{-1}$). HRMS (ESI): *m/z* 640.7842 $(M+Na)^+$; $C_{16}H_{18}Br_4N_2O_4Na$ requires 640.7881.

4.3 AHL quorum sensing assay

The AHL-mediated quorum sensing assay utilizes a reporter strain that expresses GFP in the presence of AHL signals. The assay is performed by measuring GFP output in the presence of the compound to be tested and comparing the output to a vehicle control. By using multiple samples at varying concentrations of compound and natural AHL, an inhibition index of compound activity can be generated. The inhibition index used in the present study is the ratio of compound (mol):AHL (mmol) required to reduce GFP expression to 40% of the control. The inhibition index is termed AIC_{40}. Lower values of AIC_{40} represent better inhibitors of the AHL QS system. The reporter strain of bacterium used in this assay is *E. coli* into which the *Vibrio fischeri* luxRI system has been engineered. A GFP gene is fused to the QS-controlled luxI promoter as previously described.[37, 38] Determination of the activity of compounds using the *E. coli*-based luxRI construct was performed as follows:

4.4 Inhibition kinetics

A sample of OHHL in culture media is prepared in triplicate wells of a 96 well culture plate to give a final concentration of 10, 20, 50, or 100 nM. A solution of dihydropyrrol-2-one at an appropriate concentration (0, 0.16, 0.31, 0.63, 1.25, 2.5, 5, 10 mg/mL) is then added. An aliquot (100 µL) of diluted Lux monitor is added to each well and the plate is incubated for 2 hours at 37 °C. The level of GFP green fluorescence is then determined using a Wallac 1420 Victor III plate reader. The fluorescence in each well is then determined relative to the solvent control (100%) and a plot of OHHL concentration vs. dihydropyrrol-2-one concentration is generated and the ID_{40} (dose required to reduce GFP fluorescence to 40%) determined for each compound. The ID_{40} is plotted against the OHHL concentration and a line of best fit generated, with intercepts set as the origin. The gradient of this line represents the AIC_{40} value, *i.e*. the ratio of inhibitor (mol) to OHHL (mmol) required to reduce GFP expression to 40%.

4.5 Docking

The compounds were docked into the LasR receptor (PDB code, 2UV0, resolution 1.8 Å) using GOLD (Cambridge Crystallography Data Centre, UK) in its implementation through the Discovery Studio (Accelrys) interface. Hydrogens were added to all ligands and the receptor prior to performing the docking runs. All ligands were also minimised under the CHARMm forcefield. The binding pocket was defined as the binding site of the agonist OdDHL in the crystal structure. The number of docking runs was set to 10, the "Detect Cavity" and "Early Termination" options were set to be "False". All other parameters were left at their default values. Gold scores, hydrogen bonds, and π-interactions of the ligands were analysed for the pose with the highest Gold score.

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