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## Substituted Lactam and Cyclic Azahemiacetals Modulate *Pseudomonas aeruginosa* Quorum Sensing

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### Abstract

Quorum sensing (QS) is a population-dependent signaling process bacteria use to control multiple processes including virulence that is critical for establishing infection. The most common QS signaling molecule used by Gram-negative bacteria are acylhomoserine lactones. The development of non-native acylhomoserine lactone (AHL) ligands has emerged as a promising new strategy to inhibit QS in Gram-negative bacteria. In this work, we have synthesized a set of optically pure  $\gamma$ -lactams and their reduced cyclic azahemiacetal analogues, bearing the additional alkylthiomethyl substituent, and evaluated their effect on the AHL-dependent *Pseudomonas aeruginosa las* and *rhl* QS pathways. The concentration of these ligands and the simple structural modification such as the length of the alkylthio substituent has notable effect on activity. The  $\gamma$ -lactam derivatives with nonylthio or dodecylthio chains acted as inhibitors of *las* signaling with moderate potency. The cyclic azahemiacetal with shorter propylthio or hexylthio substituent was found to strongly inhibit both *las* and *rhl* signaling at higher concentrations while the propylthio analogue strongly stimulated the *las* QS system at lower concentrations.

### Keywords

Azahemiacetals; Azasugars; *Pseudomonas aeruginosa*; Quorum sensing

## INTRODUCTION

Quorum sensing (QS) is a type of bacterial cell-to-cell signaling pathway mediated through the production, release and detection of the small signaling molecules called autoinducers (AIs).<sup>1</sup> Such communication allows bacterial control of crucial functions in united communities for enhancement of symbiosis, virulence, antibiotic production, biofilm

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Supporting Information Available: General experimental methods, synthetic procedures and characterization data for compounds **7c–d**, **8c–d**, **10c–d**, **18**, **20**, **22** and **24**. This material is free *via* the Internet.

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formation, and many other processes. The recent increase in prevalence of bacterial strains resistant to antibiotics emphasizes the need for the development of a new generation of antibacterial agents. As QS is utilized by number of pathogenic bacteria to direct virulence and biofilm formation, inhibitors/modulators of QS may serve as tools to study or intercept such community behaviors and might be beneficial as antibacterial agents.<sup>2</sup> The most common QS signaling molecule used by Gram-negative bacteria are acylhomoserine lactones (AHLs), which are detected by their cognate regulator (R) proteins.<sup>1</sup>

*Pseudomonas aeruginosa* is an important human opportunistic pathogen affecting immunocompromised individuals, cancer patients, burn victims, cystic fibrosis patients and patients with impaired lung function. It uses two AHL systems called, *las* and *rhl* to mediate QS. LasI/R synthesizes and detects *N*-(3-oxo-dodecanoyl)-L-homoserine lactone (3-oxo-C<sub>12</sub>-AHL) while RhlI/R synthesizes and detects *N*-butanoyl-L-homoserine lactone (C<sub>4</sub>-AHL) (Figure 1a). In addition, *P. aeruginosa* has a third QS-dependent pathway, *Pseudomonas* quinolone signal (PQS) that uses 2-heptyl-3-hydroxy-4-quinolone as an autoinducer.<sup>3</sup> Although certain genes appear to be regulated by one pathway, for example regulation of genes involved in rhamnolipid synthesis by the *rhl* pathway,<sup>4</sup> there is much overlap and crosstalk between the pathways and what was once thought to be hierarchical regulation, with *las* activating *rhl*, is now known to be much more complex.<sup>5</sup> Accumulated evidences clearly indicate the importance of *P. aeruginosa* QS in disease.<sup>6</sup>

Over two decades, several small molecules have been identified by many research groups as inhibitors of the AHL:R protein complex.<sup>7-9</sup> These are mostly AHL-based structures with moderate changes on the acyl side chain and amide linkage. Some of the most potent inhibitors prepared by Geske and Blackwell (**1a** and **2**, Figure 1b).<sup>10</sup> Recently, Meijler and co-workers designed a ligand, **3**, which covalently modified LasR.<sup>11</sup> Since AHL is the pharmacophore present in the natural substrates, AHL-based inhibitors are likely to modulate R protein activation.

Studies of structural features other than the AHL scaffold as tools to understand the R type protein interaction with AHLs are limited,<sup>12</sup> although the recent X-ray crystal structure of LasR with its native ligand and triphenyl mimics ought to facilitate the rational design of QS inhibitors.<sup>14,15</sup> Only a few examples of inhibitors with the altered lactone ring structure of AHL have been reported.<sup>12,13,16,17</sup> For example, Smith *et al.* reported 3-oxo-C<sub>12</sub>-(2-aminocyclohexanone) (**4**, Figure 1c) as a strong antagonist of LasR system,<sup>12</sup> while Muh *et al.* identified two LasR inhibitors having a phenyl and tetrazole ring (*e.g.*, **5**), with IC<sub>50</sub> in nM range.<sup>13</sup> It is noteworthy that in the LuxR system  $\gamma$ -thiolactone analogue **1b** showed inhibition while the corresponding  $\epsilon$ -lactam (caprolactam) analogue was reported to lack LuxR binding.<sup>18</sup> To explore further effects of non-native AHL scaffold on QS, we have designed novel lactam ligands. Here, we report optically pure  $\gamma$ -lactams and cyclic azahemiacetals, bearing alkylthiomethyl substituents with different carbon chain lengths (C<sub>3</sub>-C<sub>12</sub>), which are capable of either inhibiting or, in some cases, inducing *P. aeruginosa* QS pathways. The lactam ring was chosen because it is a more stable isoster of lactone ring present in AHL inhibitors. Moreover, the  $\gamma$ -lactam and cyclic azahemiacetal ligands were further modified in a such way that they resemble *S*-ribosyl-L-homocysteine, which is known to regulate QS through the LuxS-mediated biosynthesis of AI-2,<sup>1,19-21</sup> in which the ribose oxygen is replaced with a nitrogen atom and the homocysteine unit is substituted with a simple alkylthiol chain. Although, *P. aeruginosa* does neither harbor LuxS nor produce AI-2, AI-2 does alter *P. aeruginosa* gene expression.<sup>22</sup>

## RESULTS AND DISCUSSION

### Design and synthesis

The (*S*)-5-(bromomethyl)pyrrolidin-2-one (**6**), a key substrate for the synthesis of  $\gamma$ -lactam analogue **7** was conveniently prepared from L-pyroglutamic acid.<sup>23</sup> Displacement of bromide in **6** with sodium propanethiolate produced 5(*S*)-(propylthiomethyl)pyrrolidin-2-one (**7a**, 96%; Scheme 1). Since it was previously demonstrated that the length of the side chain is crucial for determining the agonistic and antagonistic activity,<sup>24</sup> lactams containing C6, C9 and C12 alkylthio chain lengths (**7b–d**) were also analogously prepared. A set of the cyclic azahemiacetals (*N,O*-acetals or hemiaminals) **10** with a hydroxyl group instead of a carbonyl oxygen at C2 was synthesized as well. Thus, although, attempted reduction of lactams **7** with LiEt<sub>3</sub>H was unsuccessful, reduction<sup>25</sup> of the *N*-Boc protected lactams **8a–d** proceeded smoothly to afford azahemiacetals **9a–d**. Subsequent deprotection with trifluoroacetic acid afforded 5(*S*)-(alkylthiomethyl)pyrrolidin-2-ols **10a–d** as a mixture of isomers in equilibrium<sup>25–28</sup> with the corresponding imines **11a–d** in addition to the open form aldehydes **12a–d**. Structure of the hemiaminal **10a** was additionally confirmed by conversion to the corresponding *O*-benzyloxime derivative **13a** with benzyloxyamine hydrochloride in anhydrous pyridine.

To increase the polarity/solubility of the lactam and azahemiacetal analogues in the testing media, we also prepared aza analogues with hydroxyl groups at C3 and C4. The *N*-Boc protected 1,4-dideoxy-1,4-imino-D-ribitol (**14**),<sup>29–31</sup> conveniently prepared from D-gulonic- $\gamma$ -lactone, served as a suitable starting material for the synthesis of dihydroxy  $\gamma$ -lactams **17** and **18**. Thus, mesylation of ribitol **14** followed by the selective oxidation<sup>32</sup> of the resulting **15** afforded **16**. Displacement of the mesylate **16** with sodium hexane- and nonanethiolate produced 5-alkylthiomethyl lactams **17** and **18** in high yields which were deprotected with TFA to give 5(*S*)-(hexyl- or nonylthiomethyl)-3,4-dihydroxypyrrrolidin-2-ones **19** and **20** (Scheme 2). Reduction of 5-alkylthiomethyl lactams **17** and **18** with LiEt<sub>3</sub>H afforded cyclic azahemiacetals **21** and **22**. Subsequent deprotection with TFA provided 5(*S*)-(alkylthiomethyl)-3,4-dihydroxypyrrrolidin-2-ols **23** and **24** as a complex mixture of azahemiacetals existing in equilibrium with dehydrated form (imine) as well as with open aldehyde and dimeric forms, as reported for such class of 4-azaribofuranoses.<sup>25,28,33</sup> These azahemiacetals can be considered as aza analogues of *S*-ribosyl-L-homocysteine<sup>28</sup> (SRH) in which (a) ribose oxygen is replaced by nitrogen atom and (b) the homocysteine moiety is substituted with *n*-alkylthiols with different length of carbon chain.

### Screening against *las* signaling

To determine the effect of the lactams (**7**, **19**, and **20**) and the cyclic azahemiacetal derivatives (**10**, **23** and **24**) on the *P. aeruginosa las* AHL-mediated pathway, a *las*-dependent  $\beta$ -galactosidase reporter ( $P_{lasI-lacZ}$ ) was expressed with *lasR* in *E. coli*.<sup>34,35</sup> Almost no activity was observed in absence of exogenous AHL demonstrating the AHL-dependence of the system ( $0.21 \pm 0.31$  Miller units). As expected, and in agreement with published data,<sup>34,35</sup> addition of exogenous 3-oxo-C<sub>12</sub>-AHL activated the  $P_{lasI-lacZ}$  ( $55.4 \pm 1.5$  Miller units). The lactams and their azahemiacetal counterparts were screened at a concentration of 0.2 to 1.2 mM for their activity against the *las* system (Table 1). At 0.29 mM concentration, the propylthio lactam **7a** slightly but insignificantly inhibited LasR activity and its corresponding azahemiacetal **10a** significantly stimulated (approximately by 2.3-fold) *las* reporter activity (*p*-value <0.01). This was dependent upon the addition of 2  $\mu$ M of 3-oxo-C<sub>12</sub>-AHL as **10a** did not stimulate  $\beta$ -galactosidase activity in the absence of exogenous AHL (data not shown). At the concentration of 0.57 mM, the azahemiacetal **10a** was found to enhance *las* reporter activity by 15% (*p*-value <0.01). However, *las* reporter activity was inhibited by 69% (*p*-value <0.05) and 86% (*p*-value <0.01) at 0.87 and 1.14

mM, respectively. In contrast, the lactam analogue **7a** inhibited *las* activity at all concentrations tested. Cell growth was not inhibited by the addition of lactam and azahemiacetal compounds at the tested concentrations (data not shown).

Among other lactam analogues tested, the percent inhibition of *las* promoter activity increased in a concentration dependent manner (Table 1). Inhibition potency also increased as the alkylthio chain length increased. Specifically, nonylthio lactam **7c** and dodecylthio lactam **7d** were found to possess greatest inhibition at all concentrations tested. At the lowest concentration tested (0.19 and 0.17 mM, respectively), nonylthio lactam **7c** modestly inhibited while dodecylthio lactam **7d** significantly inhibited *las* activity (48%; *p*-value <0.01). On the contrary, among the cyclic azahemiacetals analogues, no general trend was observed between chain length and percent inhibition. Unlike the propylthio azahemiacetal **10a**, hexylthio azahemiacetal **10b** did not significantly stimulate QS at the lowest concentration tested (0.23 mM) but inhibited *las* activity 100% at all higher concentrations used (*p*-value <0.01). In comparison, azahemiacetals containing nonyl side chain **10c** and dodecyl chain **10d** showed moderate but significant inhibitory activity (Table 1).

The ribolactam analogues **19** and **20** and their cyclic azahemiacetal counterparts **23** and **24** were found to significantly inhibit *las* activity at all concentrations tested but only with moderate potency. The azahemiacetals (**23** and **24**) appears slightly more active (Table 1).

### Screening against the *rhl* pathway

To determine the effect of the lactams (**7**, **19**, and **20**) and the cyclic azahemiacetal derivatives (**10**, **23** and **24**) on the *P. aeruginosa rhl* AHL-mediated pathways, a *rhl*-dependent  $\beta$ -galactosidase reporter ( $P_{rhlA}$ -*lacZ*) was expressed with *rhlR* in *E. coli*.<sup>4</sup> As expected, and in agreement with published data,<sup>4</sup> exogenous  $C_4$ -AHL activated the *rhl*  $\beta$ -galactosidase reporter ( $266 \pm 20$  Miller units). There was minimal activity in the absence of  $C_4$ -AHL ( $0.12 \pm 2.72$  Miller units). In general, except for the cyclic azahemiacetals with shorter alkylthio chain **10a** and **10b**, the rest either had no effect or stimulated *rhl* expression (Table 1). The lactam analogues **7a**, **7c** and **7d** did not modulate *rhl* activity, while hexylthio lactam **7b** significantly stimulated (*p*-value <0.01) *rhl* QS activities at higher concentrations. In contrast to lactams, cyclic azahemiacetals with shorter alkylthio chain **10a** (at higher concentration) and **10b** (at all concentration) significantly inhibited *rhl* activity, while analogues **10c** and **10d** with longer alkyl chain were inactive. The hexylthio azahemiacetal **10b** completely inhibited *rhl* signaling at concentrations of 0.46 mM and higher (*p*-value <0.02). The strong inhibition observed with propylthio **10a** and hexylthio **10b** azahemiacetal analogues having side chain lengths similar to  $C_4$ -AHL is in agreement with the structure activity relationship reported for various synthetic AHL mimetics targeting RhlR.<sup>36</sup>

The hexyl-ribolactam analogue **19** significantly stimulated (*p*-value <0.01) *rhl* activity, while its cyclic azahemiacetal counterpart **23** had no effect (Table 1). The nonyl-azahemiacetal analogue **24** had significant stimulatory activity at 0.34 mM (*p*-value <0.05; Table 1).

## CONCLUSIONS

We have designed and synthesized a set of optically pure  $\gamma$ -lactams with alkylthiomethyl substitution at carbon  $\gamma$  and their *N,O*-acetal counterparts. These ligands were evaluated for their effect on *P. aeruginosa* AHL-dependent *las* and *rhl* QS pathways isolated in *E. coli*. Lactam analogues **7** showed selectivity between two QS systems, acting as inhibitors against *las* signaling and weak activators against *rhl* signaling, possibly due to differences in the active sites of their cognate R proteins or transport of the native signaling molecule. Antagonism of *las* activity increased with the length of the alkylthio chain. Interestingly, the

cyclic azahemiacetal derivative with shorter propylthio chain (**10a**) significantly stimulated *las* signaling at lower concentrations while strongly inhibiting both QS systems at higher concentrations. At least with **10a**, the stimulation of the *las* system only occurs in the presence of exogenous AHL. It is possible that heterodimeric LasR is more active as compared to homodimers. The ribolactam (**19** and **20**) and cyclic azahemiacetal (**24**) analogs inhibited *las* and stimulated *rhl* moderately. Of all the compounds tested, the 5-(hexylthiomethyl)pyrrolidin-2-ol (**10b**) appears most potent inhibitor against both *las* and *rhl* systems. The mechanism of inhibition is still unknown. Since the effect tested utilized a whole cell assay, the QS inhibition could occur at multiple steps in the pathway. For example, the compound could affect the import of the natural ligand, compete with the natural ligand for binding to the regulator, LasR or RhlR, or alter binding of the QS regulator to the promoter. Alternatively, it is possible that the compounds with longer side chains affect the membrane and that the *las* pathway is more sensitive to these changes. Future experiments can address these possibilities. For example, quantification of extracellular AHL would reveal if the compounds affect AHL import. If this were the mechanism of action, it is expected that there would be less extracellular AHL in the absence of compound than in the presence. If the compound competes for binding to the regulator, inhibition should be overcome by increased AHL concentration. Given the central role the *las* and *rhl* QS pathways play in *P. aeruginosa* virulence, inhibitors such as the ones described here, have significant potential as therapeutics.

## METHODS

General experimental methods are described in Supporting Information Section. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were determined with solutions in  $\text{CDCl}_3$  unless otherwise noted

### 5-(Propylthiomethyl)pyrrolidin-2-one [7a(5S)]. Procedure A

Propanethiol (50  $\mu\text{L}$ , 42 mg, 0.55 mmol) was added dropwise to a stirred suspension of NaH (35 mg, 0.875 mmol, 60%/mineral oil) in dry DMF (1 mL) under Ar atmosphere at  $0^\circ\text{C}$ . After 10 min (till gas evolution has ceased), solution of compound **6**<sup>23</sup> [(5S), 82 mg, 0.46 mmol] in dry DMF (1 mL) was added dropwise, and after 15 min the reaction mixture was allowed to warm to ambient temperature. After 12 h the resulting mixture was quenched with water at  $0^\circ\text{C}$ , volatiles were evaporated, and the residue was column chromatographed (EtOAc  $\rightarrow$  10% MeOH/EtOAc) to give **7a(5S)** (77 mg, 96%) as a colorless oil:  $^1\text{H}$  NMR  $\delta$  0.98 (t,  $J = 7.3$  Hz, 3H), 1.60 (sx,  $J = 7.3$  Hz, 2H), 1.76–1.87 (m, 1H), 2.25–2.34 (m, 1H), 2.34–2.45 (m, 2H), 2.52 (t,  $J = 7.3$  Hz, 2H), 2.54 (dd,  $J = 7.7$ , 13.2 Hz, 1H), 2.68 (dd,  $J = 5.5$ , 13.2 Hz, 1H), 3.80 ('quint',  $J = 5.5$  Hz, 1H), 6.73 (br. s, 1H);  $^{13}\text{C}$  NMR  $\delta$  13.4, 23.1, 26.6, 30.2, 34.7, 38.6, 53.9, 178.0; MS (APCI)  $m/z$  174 ( $\text{MH}^+$ ). HRMS (AP-ESI)  $m/z$  calcd for  $\text{C}_8\text{H}_{15}\text{NNaOS}$  [ $\text{M}+\text{Na}$ ] $^+$  196.0772; found 196.0779.

### 5-(Hexylthiomethyl)pyrrolidin-2-one [7b(5S)]

Treatment of **6**<sup>22</sup> [(5S), 823 mg, 4.62 mmol] in dry DMF (6 mL) with a thiolate solution in dry DMF (6 mL) generated from hexanethiol (682  $\mu\text{L}$ , 573 mg, 4.86 mmol), and NaH (204 mg, 5.09 mmol, 60%/mineral oil) by Procedure A [column chromatography (80% EtOAc/hexane  $\rightarrow$  5% MeOH/EtOAc)] gave **7b(5S)** (932 mg, 94%) as a colorless oil:  $[\alpha]_{\text{D}} = +40.7$  ( $c = 0.03$ ,  $\text{CHCl}_3$ );  $^1\text{H}$  NMR  $\delta$  0.90 (t,  $J = 7.0$  Hz, 3H), 1.24–1.33 (m, 4H), 1.33–1.42 (m, 2H), 1.58 ('quint',  $J = 7.4$  Hz, 2H), 1.78–1.87 (m, 1H), 2.27–2.46 (m, 3H) 2.53 (dd,  $J = 8.0$ , 13.4 Hz, 1H), 2.54 (t,  $J = 7.3$  Hz, 2H), 2.70 (dd,  $J = 5.3$ , 13.2 Hz, 1H), 3.81 ('quint',  $J = 6.6$  Hz, 1H), 6.47 (br. s, 1H);  $^{13}\text{C}$  NMR  $\delta$  14.0, 22.5, 26.8, 28.5, 29.7, 30.1, 31.4, 32.7, 38.7, 53.8, 177.7; MS (ESI)  $m/z$  216 (100,  $\text{MH}^+$ ); HRMS (TOF MS-ESI)  $m/z$  calcd for  $\text{C}_{11}\text{H}_{21}\text{NOSNa}$  [ $\text{M}+\text{Na}$ ] $^+$  238.1236; found 238.1252.



***N*-tert-Butoxycarbonyl-5-(propylthiomethyl)pyrrolidin-2-one [8a(5S)]. Procedure B**

DMAP (114 mg, 0.93 mmol), and (Boc)<sub>2</sub>O (398 mg, 1.82 mmol) were added to a stirred solution of compound **7a** (77 mg, 0.445 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) at ambient temperature under Ar atmosphere. After 48 h, the reaction mixture was quenched with H<sub>2</sub>O (5 mL) and partitioned between CH<sub>2</sub>Cl<sub>2</sub>/NaHCO<sub>3</sub>/H<sub>2</sub>O. The organic layer was washed (brine), dried (MgSO<sub>4</sub>) and evaporated. The residue was column chromatographed (30 → 40% EtOAc/hexane) to give **8a** (5S) (107 mg, 88%) as a colorless oil: <sup>1</sup>H NMR δ 0.95 (t, *J* = 7.3 Hz, 3H), 1.50 (s, 9H), 1.58 (sx, *J* = 7.3 Hz, 2H), 1.96–2.04 (m, 1H), 2.06–2.17 (m, 1H), 2.40 (ddd, *J* = 2.6, 9.6, 17.9 Hz, 1H), 2.50 (“dt”, *J* = 4.9, 7.3 Hz, 2H), 2.58–2.67 (m, 1H), 2.60 (dd, *J* = 9.2, 13.5 Hz, 1H), 2.86 (ddd, *J* = 0.5, 2.8, 13.5 Hz, 1H), 4.20–4.27 (m, 1H); <sup>13</sup>C NMR δ 13.3, 21.9, 23.1, 28.0, 31.2, 34.8, 35.4, 57.5, 83.1, 149.8, 174.2; MS (ESI) *m/z* 274 (10, MH<sup>+</sup>), 215 (100, [MH-59]<sup>+</sup>).

***N*-tert-Butoxycarbonyl-5-(hexylthiomethyl)pyrrolidin-2-one [8b(5S)]**

Treatment of **7b** (311 mg, 1.45 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (6 mL) with DMAP (185 mg, 1.52 mmol), and (Boc)<sub>2</sub>O (746 mg, 3.42 mmol) by procedure B [column chromatography (20 → 40% EtOAc/hexane)] gave **8b**(5S) (429 mg, 94%) as a colorless oil: <sup>1</sup>H NMR δ 0.89 (t, *J* = 7.0 Hz, 3H), 1.25–1.33 (m, 4H), 1.34–1.42 (m, 2H), 1.55 (s, 9H), 1.59 (‘quint’, *J* = 7.4 Hz, 2H), 2.01–2.08 (m, 1H), 2.10–2.21 (m, 1H), 2.45 (ddd, *J* = 2.5, 9.6, 17.9 Hz, 1H), 2.56 (‘dt’, *J* = 2.9, 7.3 Hz, 2H), 2.62–2.72 (m, 1H), 2.63 (dd, *J* = 9.3, 13.5 Hz, 1H), 2.91 (dd, *J* = 2.7, 13.5 Hz, 1H), 4.24–4.31 (m, 1H); <sup>13</sup>C NMR δ 14.0, 22.0, 22.5, 28.1, 28.4, 29.8, 31.2, 31.4, 32.9, 35.5, 57.5, 83.1, 149.8, 174.1; MS (ESI) *m/z* 315 (15, M<sup>+</sup>), 256 (100, [M-59]<sup>+</sup>); HRMS (TOF MS-ESI) *m/z* calcd for C<sub>16</sub>H<sub>29</sub>NO<sub>3</sub>Na [M+Na]<sup>+</sup> 338.1760; found 338.1752.

**5-(Propylthiomethyl)pyrrolidin-2-ol [10a(5S)]. Step a. Procedure C**

LiEt<sub>3</sub>BH (1M soln in THF, 0.98 mL, 0.98 mmol) was added to a stirred solution of **8a** (107 mg, 0.39 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (3 mL) at –78 °C under N<sub>2</sub> atmosphere. After 30 min, the reaction mixture was quenched with MeOH (4 mL) and was allowed to warm to ambient temperature. Volatiles were evaporated and the residue was partitioned (EtOAc/NaHCO<sub>3</sub>/H<sub>2</sub>O), washed (brine) and dried (MgSO<sub>4</sub>). The resulting oil was chromatographed (30 → 40% EtOAc/hexane) to give *N*-tert-butoxycarbonyl-5-(propylthiomethyl)pyrrolidin-2-ol [**9a**(5S); 104 mg, 96%] as a colorless oil of the mixture of anomers/rotamers: MS (ESI) *m/z* 274 (10, [M-1]<sup>+</sup>), 258 (100, [M-17]<sup>+</sup>). **Step b. Procedure D.** Compound **9a** (104 mg, 0.37 mmol) in TFA (4.0 mL) was stirred at room temperature for 2 h. Volatiles were evaporated to give **10a** (62 mg, 96%) as a light yellow oil of a mixture of isomers accompanied by ~25% of the aldehyde **12a** [<sup>1</sup>H NMR δ 8.89 (s, ~0.25H); and <sup>13</sup>C NMR δ 180.8]; MS (ESI) *m/z* 158 (100, [M-17]<sup>+</sup>).

A solution of crude **10a** (5S; 8 mg, 0.046 mmol) and *O*-benzylhydroxylamine hydrochloride (48 mg, 0.3 mmol) in anhydrous pyridine (1 mL) was stirred under an atmosphere of nitrogen at room temperature for 12 h. Pyridine was evaporated to afford 4-amino-5-(propylthio)pentanal *O*-benzyloxime [**13a**(4S)] of sufficient purity (~90%) for spectroscopic characterization together with the excess of BnONH<sub>2</sub> used: MS (ESI) *m/z* 281 (60, MH<sup>+</sup>), 158 (100, [M-BnONH]<sup>+</sup>), (APCI) *m/z* 281 (100, MH<sup>+</sup>).

**5-(Hexylthiomethyl)pyrrolidin-2-ol [10b(5S)]**

**Step a.** Treatment of **8b** (178 mg, 0.56 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (3 mL) with LiEt<sub>3</sub>BH (1M soln in THF, 1.41 mL, 1.41 mmol), by procedure C [quenched with MeOH (4 mL) at low temp., column chromatography (30 → 40% EtOAc/hexane)] gave *N*-tert-butoxycarbonyl-5-(hexylthiomethyl)pyrrolidin-2-ol [**9b**(5S); 170 mg, 95%] as a colorless oil of a mixture of isomers: MS (ESI) *m/z* 316 (100, [M-1]<sup>+</sup>), 300 (20, [M-17]<sup>+</sup>); HRMS (TOF MS-ESI) *m/z*



calcd for  $C_{16}H_{31}NO_3SNa$   $[M+Na]^+$  340.1926; found 340.1955. *Step b.* Compound **9b** (38.5 mg, 0.12 mmol) in TFA (0.8 mL) was stirred at 0 °C (ice-bath) for 3 h. The reaction mixture was diluted with excess of ice-cold  $CH_2Cl_2$  and neutralized with solid  $NaHCO_3$ . Resulting mixture was stirred for 20 min at ambient temperature and was decanted. The residual slurry was extracted with fresh portion of  $CH_2Cl_2$  and the combined extracts were dried ( $Na_2SO_4$ ) and concentrated to give crude **10b** (25 mg) as a colorless oil. Crude product was column chromatographed to give first (0 → 0.25% MeOH/ $CHCl_3$ ) anomeric mixture of azahemiacetals **10b** ( $\alpha/\beta$ , 9:20; 2.0 mg, 8%) as a colorless oil:  $^1H$  NMR  $\delta$  0.89 (t,  $J$  = 7.0 Hz, 4.35H), 1.26–1.45 (m, 8.7H), 1.47–1.72 (m, 3.9H), 1.75–1.85 (m, 0.45H), 1.92–2.07 (m, 3.45H), 2.10–2.23 (m, 0.9H), 2.46 (dd,  $J$  = 9.5, 13.0 Hz, 1H), 2.52–2.65 (m, 2.9H), 2.93–3.02 (m, 0.9H), 3.23 (dd,  $J$  = 2.5, 13.0 Hz, 1H), 3.60–3.68 (m, 1H), 3.67–3.75 (m, 0.45H), 4.04–4.10 (m, 1H), 4.20–4.27 (m, 0.45H); MS (ESI)  $m/z$  200 (100,  $[M-17]^+$ ). Further elution (0.25 → 0.5% MeOH/ $CHCl_3$ ) gave imine **11b** (5.8 mg, 22%) as a colorless oil:  $^1H$  NMR  $\delta$  0.91 (t,  $J$  = 7.0 Hz, 3H), 1.27–1.46 (m, 7H), 1.55–1.65 (m, 2H), 2.02–2.11 (m, 1H), 2.49–2.63 (m, 5H), 2.95 (dd,  $J$  = 5.3, 12.8 Hz, 1H), 4.21–4.29 (m, 1H), 7.63 (br t,  $J$  = 1.1 Hz, 1H);  $^{13}C$  NMR  $\delta$  14.0, 22.5, 26.1, 28.6, 29.8, 31.4, 33.0, 37.0, 38.1, 72.9, 167.0; MS (ESI)  $m/z$  200 (100,  $MH^+$ ).

*Note:* The composition of crude products after *step b* depends strongly on the work up conditions. For example, the reaction mixture contained also ~22% of the aldehyde **12b** [ $^1H$  NMR  $\delta$  8.91 (s, ~0.22H)] at pH lower than 7.

#### 4-Amino-*N*-(*tert*-butoxycarbonyl)-4-deoxy-2,3-*O*-isopropylidene-5-*O*-methanesulfonyl-D-ribose-1,4-lactam (**16**)

*Step a.* Triethylamine (93  $\mu$ L, mg, 67 mg, 0.66 mmol) and MsCl (25  $\mu$ L, 38 mg, 0.33 mmol) were added dropwise to stirred solution of **14**<sup>30</sup> (60 mg, 0.22 mmole) in anhydrous  $CH_2Cl_2$  (6 mL) at 0 °C (ice-bath). After 5 min, ice-bath was removed and the reaction mixture was allowed to stir at ambient temperature for 30 min. The reaction mixture was quenched with saturated  $NaHCO_3/H_2O$  and was extracted with  $CH_2Cl_2$ . The organic layer was washed (brine), dried ( $MgSO_4$ ) and evaporated to give 1-amino-1,4-anhydro-*N*-*tert*-butoxycarbonyl-1-deoxy-2,3-*O*-isopropylidene-5-*O*-methanesulfonyl-D-ribose **15** (73 mg, 96%) as a mixture (~3:2) of two rotamers of sufficient purity to be directly used for next step:  $^1H$  NMR  $\delta$  1.28 (s, 3,  $CH_3$ ), 1.42 (s, 12H, *t*-Bu,  $CH_3$ ), 2.96 (s, 1.2, Ms), 2.98 (s, 1.8, Ms), 3.39 (dd,  $J$  = 12.5, 4.8 Hz, 0.4H), 3.46 (dd,  $J$  = 12.5, 4.8 Hz, 0.6H), 3.69 (d,  $J$  = 12.5 Hz, 0.6H), 3.82 (d,  $J$  = 12.5 Hz, 0.4H), 4.10–4.14 (m, 0.4H), 4.22–4.30 (m, 1H), 4.22–4.29 (m, 1.4H), 4.45 (dd,  $J$  = 10.1, 4.1 Hz, 0.6H), 4.65 ('d',  $J$  = 5.9 Hz, 1H); 4.72 ('t',  $J$  = 5.3 Hz, 1H);  $^{13}C$  NMR (major)  $\delta$  24.9, 26.9, 29.6, 37.1, 52.5, 62.4, 68.9, 79.2, 80.4, 81.7, 112.1, 154.2;  $^{13}C$  NMR (minor)  $\delta$  24.9, 26.9, 29.6, 37.5, 53.1, 62.6, 68.6, 78.5, 80.6, 82.5, 112.1, 153.6; MS (APCI)  $m/z$  352 (10,  $MH^+$ ), 252 (100,  $[MH_2-Boc]^+$ ). *Step b.*  $RuO_2 \times H_2O$  (8.5 mg, 0.064 mmol) was added to a stirred solution of  $NaIO_4$  (172 mg, 0.96 mmol) in  $H_2O$  (1 mL) at ambient temperature. After 5 min, a solution of **15** (80 mg, 0.32 mmol) in EtOAc (1 mL) was added dropwise and the reaction mixture was continued to stir for 12 h.  $H_2O$  (20 mL) and EtOAc (20 mL) were added and the separated aqueous layer was furthermore extracted with EtOAc ( $2 \times 20$  mL). The combined organic layers were washed (brine), dried ( $MgSO_4$ ) and evaporated. The residue was column chromatographed (EtOAc) to give **16** (78 mg, 95%) as a colorless oil:  $^1H$  NMR  $\delta$  1.37 (s, 3H), 1.44 (s, 3H), 1.54 (s, 9H), 3.01 (s, 3H), 4.39–4.43 ('m', 2H), 4.58 (d,  $J$  = 5.45 Hz, 1H), 4.64 (dd,  $J$  = 11.2, 3.1 Hz, 1H), 4.70 (d,  $J$  = 5.45 Hz, 1H);  $^{13}C$  NMR  $\delta$  25.6, 27.0, 28.0, 37.7, 59.2, 67.0, 74.5, 77.5, 84.7, 112.8, 149.7, 170.2; MS (APCI)  $m/z$  298 (100,  $[MH_2-Boc+MeOH]^+$ ).

#### 4-Amino-*N*-(*tert*-butoxycarbonyl)-4-deoxy-5-*S*-hexyl-2,3-*O*-isopropylidene-5-thio-*D*-ribo-1,4-lactam (**17**)

Treatment of **16** (60 mg, 0.16 mmol) in dry DMF (0.5 mL) with sodium hexathiolate [generated from hexanethiol (46.8  $\mu$ L, 0.33 mmol)/NaH (14 mg, 0.35 mmol, 60%/mineral oil) in dry DMF (0.5 mL)] by Procedure A [column chromatography (5%  $\rightarrow$  10% MeOH/EtOAc)] gave **17** (25 mg, 40%) as a colorless oil and *N*-Boc deprotected **17** (24 mg, 38%) as a white crystalline solid. Compound **17** had:  $^1\text{H NMR}$   $\delta$  0.81 (t,  $J$  = 7.0 Hz, 3H), 1.16–1.27 (m, 6H), 1.30 (s, 3H), 1.39 (s, 3H), 1.44–1.59 (m, 11H), 2.36–2.50 (m, 2H), 2.76 (dd,  $J$  = 6.2, 14.4 Hz, 1H), 2.82 (dd,  $J$  = 2.7, 14.4 Hz, 1H), 4.31 (dd,  $J$  = 2.7, 6.2 Hz, 1H), 4.38 (d,  $J$  = 5.5 Hz, 1H), 4.78 (d,  $J$  = 5.5 Hz, 1H);  $^{13}\text{C NMR}$   $\delta$  14.0, 22.5, 25.5, 27.0, 28.0, 28.3, 29.6, 31.3, 33.7, 33.9, 60.8, 76.1, 77.6, 83.9, 112.3, 149.8, 171.0; MS (APCI)  $m/z$  288 (100,  $[\text{MH}_2\text{-Boc}]^+$ ). *N*-Boc deprotected **14** had:  $^1\text{H NMR}$   $\delta$  0.88 (t,  $J$  = 7.0 Hz, 3H), 1.25–1.36 (m, 6H), 1.38 (s, 3H), 1.48 (s, 3H), 1.56–1.62 (m, 2H), 2.52–2.75 (m, 3H), 2.73 (dd,  $J$  = 5.9, 13.4 Hz, 1H), 3.81 (t,  $J$  = 6.1 Hz, 1H), 4.50 (d,  $J$  = 5.9 Hz, 1H), 4.69 (d,  $J$  = 5.9 Hz, 1H), 5.94 (s, 1H);  $^{13}\text{C NMR}$   $\delta$  14.0, 29.7, 22.5, 28.5, 31.4, 25.6, 26.9, 33.2, 33.7, 58.0, 76.6, 79.2, 112.7, 173.2; MS (APCI)  $m/z$  288 (100,  $\text{MH}^+$ ).

#### 4-Amino-4-deoxy-5-*S*-hexyl-5-thio-*D*-ribo-1,4-lactam (**19**)

TFA/H<sub>2</sub>O (1 mL, 9:1) was added to **17** or *N*-Boc deprotected **17** (22 mg, 0.07 mmol) and the resulting solution was stirred at 0 °C for 3 h. Evaporation of volatiles gave light yellow oil that was column chromatographed (5  $\rightarrow$  10% MeOH/EtOAc) to give **19** (12 mg, 63%) as a colorless oil:  $^1\text{H NMR}$   $\delta$  0.87 (t,  $J$  = 7.0 Hz, 3H), 1.24–1.39 (m, 6H), 1.52–1.59 (m, 2H), 2.50–2.55 (m, 3H), 2.73 (dd,  $J$  = 5.5, 13.6 Hz, 1H), 3.71 (t,  $J$  = 6.4 Hz, 1H), 4.21 (d,  $J$  = 5.0 Hz, 1H), 4.44 (d,  $J$  = 5.0 Hz, 1H), 7.11 (s, 1H);  $^{13}\text{C NMR}$   $\delta$  14.0, 29.6, 14.1, 22.5, 31.4, 32.7, 35.3, 59.9, 69.8, 71.8, 176.0; MS (APCI)  $m/z$  248 (100,  $\text{MH}^+$ ); HRMS (TOF MS-ESI)  $m/z$  calcd for C<sub>11</sub>H<sub>21</sub>NO<sub>3</sub>SNa  $[\text{M}+\text{Na}]^+$  270.1134; found 270.1137.

#### 4-Amino-4-deoxy-5-*S*-hexyl-5-thio- $\alpha/\beta$ -*D*-ribofuranose (**23**)

Treatment of **17** (40 mg, 0.1 mmol) in THF (1 mL) with LiEt<sub>3</sub>BH (1M/THF, 0.26 mL, 0.26 mmol), by procedure C [column chromatography (10  $\rightarrow$  20% EtOAc/hexane)] gave 4-amino-*N*-(*tert*-butoxycarbonyl)-4-deoxy-5-*S*-hexyl-3,4-*O*-isopropylidene-5-thio- $\alpha/\beta$ -*D*-ribofuranose **21** (39 mg, 97%) as a colorless oil of the mixture of isomers: MS (ESI)  $m/z$  389 (100,  $\text{M}^+$ ); HRMS (TOF MS-ESI)  $m/z$  calcd for C<sub>19</sub>H<sub>35</sub>NO<sub>5</sub>SNa  $[\text{M}+\text{Na}]^+$  412.2128; found 412.2117. Deprotection of **21** (39 mg, 0.1 mmol) with TFA/H<sub>2</sub>O (0.9:0.1 mL) by Procedure D gave a light yellow oil that was column chromatographed (5  $\rightarrow$  10% MeOH/EtOAc) to give **23** (22 mg, 88%) as a light yellow oil.  $^1\text{H NMR}$  showed a mixture of isomers accompanied by the open aldehyde form. MS (APCI)  $m/z$  230 (40,  $\text{MH}^+$ ), 232 (100,  $[\text{M}-17]^+$ ).

#### Anti-Quorum Sensing Assay ( $\beta$ -Galactosidase Assay)

An overnight (O/N) culture of *Escherichia coli* DH5 $\alpha$  harboring the plasmids pSC11, which contains a P<sub>lasI</sub>-*lacZ* translational fusion,<sup>34</sup> and pJN105L, which contains a P<sub>BAD</sub>-*lasR* expression plasmid<sup>35</sup> grown in LB media (10 g tryptone, 5 g yeast extract, 5 g sodium chloride per liter) supplemented with ampicillin (100  $\mu$ g/ml) and gentamycin (15  $\mu$ g/ml), was diluted to an OD<sub>600</sub> of 0.150. At this time, arabinose (0.2 % w/v), *N*-3-(oxododecanoyl)homoserine lactone (3-oxo-C<sub>12</sub>-AHL; 2  $\mu$ M), and either the compound under analysis or solvent (DMSO), was added to the culture (1.5 mL). A negative control containing only solvent, antibiotic, and arabinose (0.2 % w/v) without 3-oxo-C<sub>12</sub>-AHL was also assayed (data not shown). The cultures were incubated with shaking for three hours at 37 °C.

The conditions for the *rhl* biomonitor *Escherichia coli* DH5 $\alpha$  harboring pECP61.5 plasmid, which contains P<sub>*tac*</sub>-*rhlR* and P<sub>*rhlA*</sub>-*lacZ*<sup>4</sup> were essentially same except that the LB medium was only supplemented with ampicillin (100  $\mu$ g/ml), the O/N culture was diluted to an OD<sub>600</sub> of 0.150, induced with 1 mM IPTG, 2  $\mu$ M C<sub>4</sub>-HSL and the compounds or the controls added when the OD<sub>600</sub> reached 1.0. A negative control containing only solvent, antibiotic and IPTG (1 mM) without C<sub>4</sub>-AHL was also assayed (data not shown). After incubation at 37°C for 4 hours with shaking,  $\beta$ -galactosidase activity was assayed as described previously.<sup>37</sup> Miller units were calculated as described.<sup>38</sup> Assays were repeated at least twice. For each biological replicate, experimental triplicates were performed and the average percent activity calculated by dividing the average Miller units from the samples containing compound or extract by the average Miller units from the sample containing solvent and multiplying by 100. Significance of inhibition was determined using a paired two-tailed Student *t*-test.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

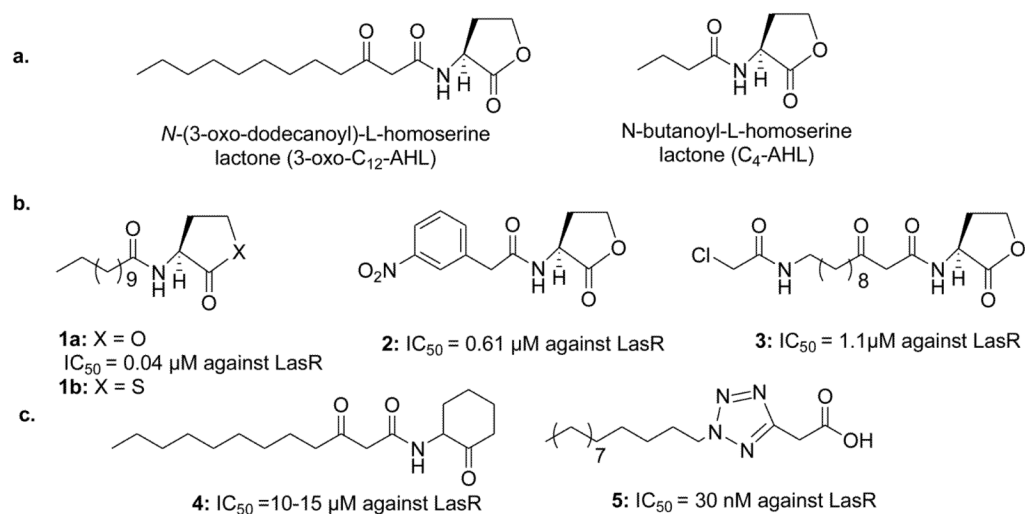
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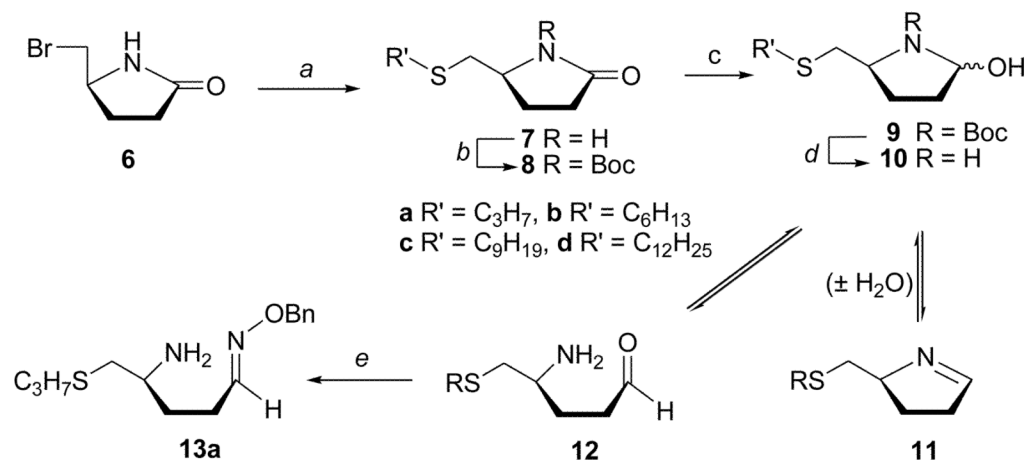
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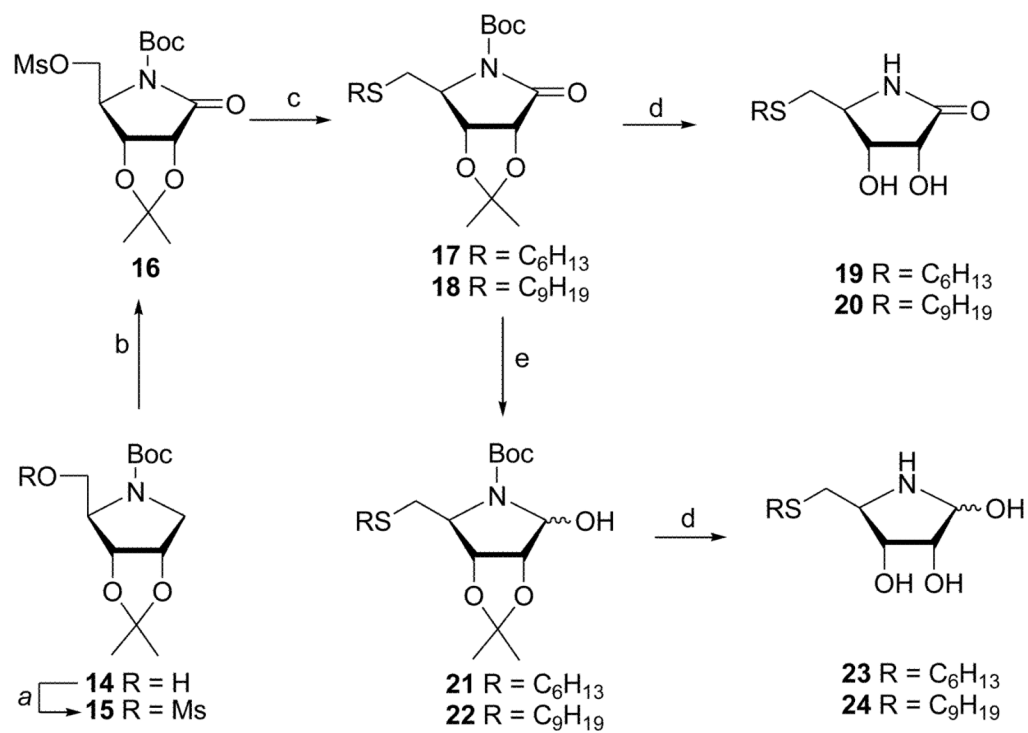
**Figure 1.**

**(a)** AHL-based signal molecules in *P. aeruginosa*. **(b)** Examples of potent synthetic QS inhibitors in various Gram-negative bacteria along with their reported IC<sub>50</sub> values (tested in the same reporter strain used in the current studies).<sup>10,11</sup> **(c)** Examples of QS inhibitors with a modified AHL scaffold tested in different reporter assays.<sup>12,13</sup>

**Scheme 1.**

Reagents and conditions: (a)  $\text{R}'\text{SH}/\text{NaH}/\text{DMF}$ ; (b)  $(\text{Boc})_2\text{O}/\text{DMAP}/\text{CH}_2\text{Cl}_2$ ; (c)  $\text{LiEt}_3\text{BH}/\text{THF}/\text{CH}_2\text{Cl}_2/-78\text{ }^\circ\text{C}$ ; (d)  $\text{TFA}/\text{rt}$ ; (e)  $\text{BnONH}_2/\text{pyr}$ .



**Scheme 2.**

Reagents and conditions: (a)  $MsCl/NEt_3/CH_2Cl_2/rt$ ; (b)  $NaIO_4/hydrated\ RuO_2/EtOAc/H_2O/rt$ ; (c)  $RSR'/NaH/DMF$ ; (d)  $TFA/H_2O$ ; (e)  $LiEt_3BH/THF/CH_2Cl_2/-78\ ^\circ C$ .

Table 1

Effect of lactam and cyclic azahemiacetal derivatives on *las* and *rhl* systems.

| Compound         | Activity <sup>d</sup> (%) ± Standard Deviation <sup>b</sup> |                              | Compound   | Activity <sup>d</sup> (%) ± Standard Deviation <sup>b</sup> |                              |                              |
|------------------|---|------------------------------|------------|---|------------------------------|------------------------------|
|                  | Concentration (mM)  | <i>las</i>                   |            | <i>Rhl</i>  | Concentration (mM)           | <i>las</i>                   |
| AHL <sup>c</sup> | 0.002   | 100 ± 0                      |            | 100 ± 0 <sup>c</sup>  |                              |                              |
| <b>7a</b>        | 0.29  | 90 ± 19                      | <b>10a</b> | 0.29  | <b>228<sup>†</sup> ± 39</b>  | <b>121<sup>*</sup> ± 6</b>   |
|                  | 0.58  | 72 ± 17                      |            | 0.57  | 115 ± 50                     | <b>138<sup>*</sup> ± 3</b>   |
|                  | 0.87  | 51 ± 7                       |            | 0.86  | <b>31<sup>*</sup> ± 20</b>   | <b>41<sup>†</sup> ± 1</b>    |
|                  | 1.16  | 43 ± 9                       |            | 1.14  | <b>11<sup>*</sup> ± 7</b>    | <b>9<sup>†</sup> ± 1</b>     |
| <b>7b</b>        | 0.23  | 106 ± 17                     | <b>10b</b> | 0.23  | 109 ± 4                      | 26 ± 0.2                     |
|                  | 0.47  | 87 ± 12                      |            | 0.46  | <b>0<sup>†</sup> ± 0</b>     | <b>0.2<sup>†</sup> ± 0.3</b> |
|                  | 0.70  | <b>74<sup>*</sup> ± 5</b>    |            | 0.69  | <b>0<sup>†</sup> ± 0</b>     | <b>0<sup>†</sup> ± 0</b>     |
|                  | 0.93  | <b>61<sup>*</sup> ± 9</b>    |            | 0.92  | <b>0<sup>†</sup> ± 0</b>     | <b>0<sup>*</sup> ± 0</b>     |
| <b>7c</b>        | 0.19  | <b>72<sup>*</sup> ± 7</b>    | <b>10c</b> | 0.19  | 74 ± 9                       | 93 ± 12                      |
|                  | 0.39  | <b>26<sup>*</sup> ± 8</b>    |            | 0.39  | 65 ± 17                      | 80 ± 13                      |
|                  | 0.58  | <b>18<sup>†</sup> ± 1</b>    |            | 0.58  | <b>57<sup>†</sup> ± 4</b>    | 100 ± 11                     |
|                  | 0.78  | <b>6<sup>†</sup> ± 3</b>     |            | 0.77  | <b>47<sup>†</sup> ± 21</b>   | 99 ± 3                       |
| <b>7d</b>        | 0.17  | <b>52<sup>†</sup> ± 11</b>   | <b>10d</b> | 0.17  | <b>72<sup>†</sup> ± 3</b>    | 98 ± 13                      |
|                  | 0.33  | <b>32<sup>†</sup> ± 3</b>    |            | 0.33  | <b>56<sup>†</sup> ± 4</b>    | 93 ± 27                      |
|                  | 0.50  | <b>18<sup>†</sup> ± 2</b>    |            | 0.50  | <b>45<sup>†</sup> ± 4</b>    | 91 ± 8                       |
|                  | 0.67  | <b>11<sup>†</sup> ± 2</b>    |            | 0.66  | <b>34<sup>†</sup> ± 7</b>    | 96 ± 5                       |
| <b>19</b>        | 0.20  | <b>96<sup>†</sup> ± 0.14</b> | <b>23</b>  | 0.20  | <b>84<sup>†</sup> ± 0.7</b>  | 94 ± 8                       |
|                  | 0.40  | <b>78<sup>†</sup> ± 0.08</b> |            | 0.40  | <b>68<sup>†</sup> ± 0.44</b> | 105 ± 3                      |
|                  | 0.61  | <b>79<sup>†</sup> ± 0.5</b>  |            | 0.60  | <b>51<sup>†</sup> ± 0.37</b> | 109 ± 12                     |
|                  | 0.81  | <b>68<sup>†</sup> ± 0.6</b>  |            | 0.80  | <b>42<sup>†</sup> ± 0.36</b> | 116 ± 18                     |
| <b>20</b>        | 0.17  | 93 ± 0.5                     | <b>24</b>  | 0.17  | <b>78<sup>†</sup> ± 0.32</b> | 98 ± 5                       |
|                  | 0.35  | <b>87<sup>†</sup> ± 6</b>    |            | 0.34  | <b>78<sup>†</sup> ± 1</b>    | <b>127<sup>*</sup> ± 14</b>  |

| Compound | Concentration (mM) | Activity <sup>a</sup> (%) ± Standard Deviation <sup>b</sup> |                  | Compound | Concentration (mM) | Activity <sup>a</sup> (%) ± Standard Deviation <sup>b</sup> |            |
|----------|--------------------|---|------------------|----------|--------------------|---|------------|
|          |                    | <i>las</i>  | <i>Rhl</i>       |          |                    | <i>las</i>  | <i>rhl</i> |
|          | 0.52               | 80 ± 7  | <b>115* ± 11</b> |          | 0.52               | <b>71* ± 0.4</b>  | 116 ± 9    |
|          | 0.69               | <b>63* ± 7</b>  | <b>135* ± 5</b>  |          | 0.69               | <b>53* ± 0.5</b>  | 121 ± 14   |

<sup>a</sup>The *P. aeruginosa* P*las*-*lacZ* and P*rhlA*-*lacZ* expressions in *E. coli* tested at the indicated concentrations (0.2–1.2 mM) against 2 μM of 3-oxo-C12-AHL or 2 μM of C4-AHL, respectively. The units are presented as percent activity relative to the DMSO treated control.

<sup>b</sup>\* *p*-value < 0.05, † *p*-value < 0.02, ‡ *p*-value < 0.01 according to a paired, two-tailed Student *t*-test. Statistically significant values are in bold.

<sup>c</sup>The exogenous AHLs added to the *las* and *rhl* systems are 3-oxo-C12-AHL and C4-AHL, respectively. The 100% *las* and *rhl* activities refer to 55.4 ± 1.5 and 266 ± 20 Miller units, respectively.