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# Synthesis of New 3- and 4-Substituted Analogues of Acyl Homoserine Lactone Quorum Sensing Autoinducers†

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**Abstract**—The quorum sensing mechanism in Gram-negative bacteria uses small intercellular signal molecules, *N*-acyl-homoserine lactones (AHLs), to control transcription of specific genes in relation to population density. In this communication, we describe the parallel synthesis of new AHL analogues, in which substituents have been introduced into the 3- and 4-positions of the lactone ring. These analogues have been screened for their ability to activate and inhibit a *Vibrio fischeri* LuxI/LuxR-derived quorum sensing reporter system. 

## Introduction

Many host-associated bacteria use pheromone signal molecules (autoinducers) to monitor population density and control expression of specific target genes encoding virulence factors and other functions.[1] This phenomenon has been termed quorum sensing (QS). The QS-system in most Gram-negative bacteria is composed basically of two components: a LuxI homologue which synthesizes an acyl homoserine lactone (AHL) autoinducer, and an autoinducer-dependent transcriptional activator, a LuxR homologue.

LuxI/LuxR from the bioluminescent marine bacteria *Vibrio fischeri* was the first AHL-based QS-system to be described.[2] LuxI constitutively synthesizes the autoinducer *N*-(3-oxohexanoyl)-L-homoserine lactone **1** (Fig. 1), which binds to the LuxR protein when a threshold concentration has been reached. AHL binding activates LuxR, which initiates transcription of the *lux* operon and, in turn, confers the bioluminescent phenotype.

Mutants with defects in their QS-system are significantly less virulent[3] and the QS-systems have therefore been envisaged as a potential novel target for development of anti-infective drugs.[4] A well established example is *Pseudomonas aeruginosa*, a prevalent opportunistic human pathogen that causes chronic pulmonary infections in patients with cystic fibrosis. *P. aeruginosa* produces a battery of extracellular virulence factors. Expression of many of these are controlled by QS regulation.[5] Evidence also suggests that QS is involved in formation of bacterial biofilms, which renders the sessile population less susceptible to anti-infectives.[6]

One strategy to modulate QS is by an autoinducer analogue, which inhibits activation by the natural autoinducer. Several reports describe the synthesis and screening of autoinducer analogues to investigate the specificity of autoinducer interaction with LuxR homologues and to identify small-molecule inhibitors.[7] These reports mainly focus on the effect of varying the fatty acid tail of the AHL molecules. Using this approach, a few inhibitors specific to certain bacterial QS-systems have been identified.[7] We envisioned that modification of the invariable homoserine lactone part of the autoinducer molecule could potentially generate a new, more general class of QS inhibitors.

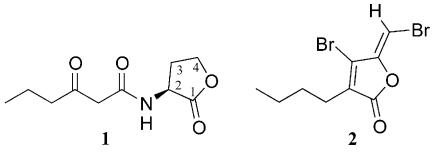


**Figure 1.** LuxR natural autoinducer **1** and furanone QS inhibitor **2**.

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Halogenated furanone type QS inhibitors **2** (Fig. 1),[8] isolated from the red marine alga *Delisea pulchra*, have been shown to inhibit several different QS-systems utilizing different autoinducer molecules. The furanone type inhibitors have substituents in the 3- and 4-positions of the furanone ring **2**. The structural resemblance between the furanone inhibitors and AHL suggests that it would be interesting to explore AHL substituted in the 3- and 4-positions (Fig. 1).

Only a few examples exist where the homoserine lactone part of the AHL molecules has been substituted. To the best of our knowledge there are no reports on analogues with substituents in the 3- and 4-positions of the homoserine lactone ring. Introduction of substituents should also provide interesting information of the interaction between the LuxR homologue and the autoinducer homoserine lactone moiety.

Herein, we describe a new strategy which enables the introduction of a variety of different substituents into the 3- and 4-positions of the homoserine lactone ring in a stereoselective way. These analogues have been screened for their ability to activate and inhibit a LuxR-based quorum sensing system.

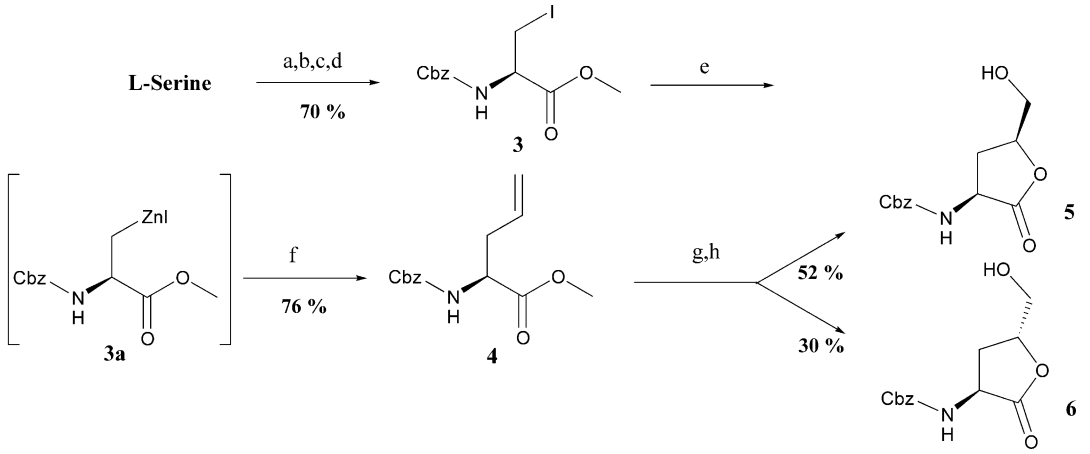
## Chemistry

We envisioned that a range of 3- and 4-substituted autoinducers could be synthesized in a parallel manner by using enantiopure *cis*- and *trans*-3-hydroxy and 4-hydroxymethyl substituted L-homoserine lactones as central scaffolds. Acylation, carbamoylation, and alkylation would then allow the introduction of sustituents with different size and character.

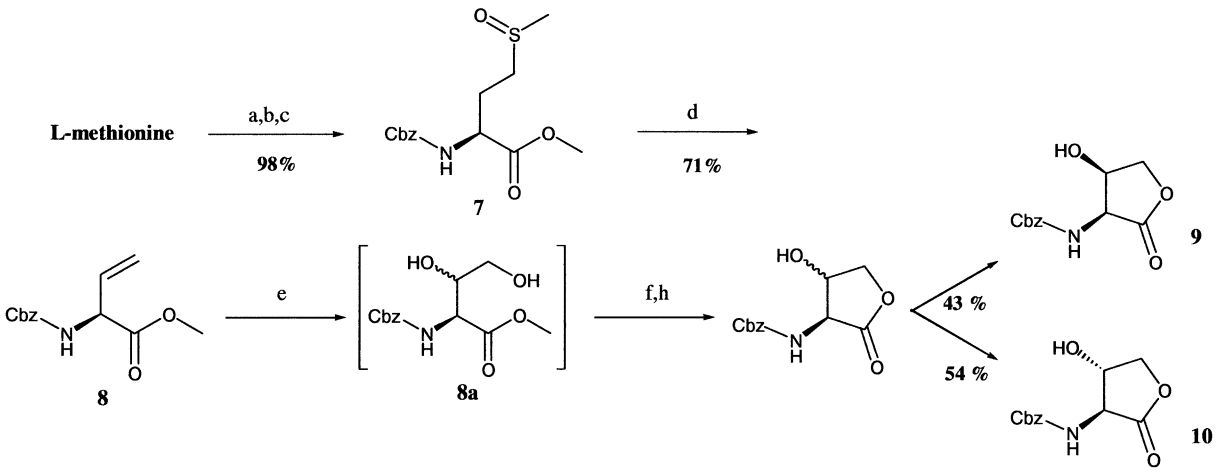
The *cis*-(**5**) and *trans*-(**6**) 4-hydroxymethyl substituted homoserine lactones[9] were synthesized (Scheme 1) in only six linear steps starting from L-serine via a new synthesis of protected L-allylglycine. First, L-serine was protected as the appropriate Cbz amino protected methyl ester. Tosylation of the hydroxy group followed by substitution with NaI afforded the iodide **3**. Similarly to methods developed for the equivalent Boc derivative, treatment with Zn afforded the insertion product **3a**.[10] Palladium catalyzed Negishi cross coupling reaction with vinyl bromide in presence of 5% $Pd_2(dba)_3$ and P(*o*-Tolyl)$_3$ afforded protected L-allylglycine **4**. Dihydroxylation by the Upjohn procedure[11] was followed by spontaneous ring closure to afford a diastereomeric mixture of the most stable γ-lactones **5** and **6**. The mixture was separated by flash chromatography to give enantiopure lactones **5** and **6**.[12]

The *cis*-(**9**) and *trans*-(**10**) 3-hydroxy substituted homoserine lactones[13] were synthesized (Scheme 2) from L-methionine in only six steps by taking advantage of a modified literature procedure for protected vinylglycine **8**.[14] Protection of L-methionine followed by oxidation with metaperiodate gave the sulfoxide **7**. Thermal elimination generated protected vinylglycine **8**. Dihydroxylation under the conditions as described for the allylglycine derivative afforded a mixture of **8a**, **9** and **10**. Full conversion to the γ-lactone was accomplished by heating in the presence of 5% PTSA in toluene. The diastereomeric mixture was purified by column chromatography to afford enantiopure **9** and **10.**[12]

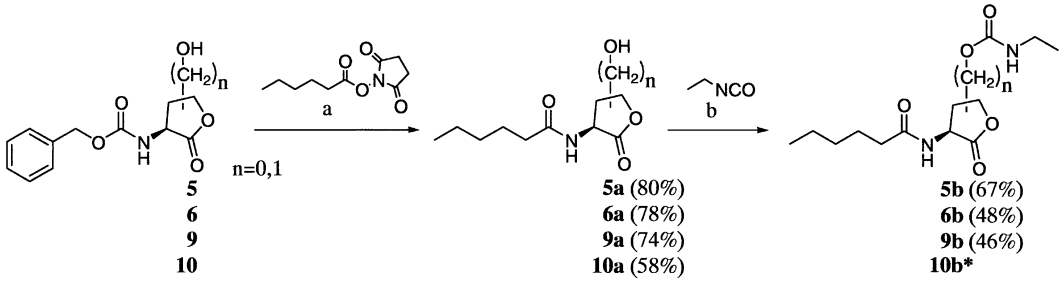
A new simple one-pot procedure for substituting the Cbz group with an appropriate fatty acid chain, similar to the natural autoinducers, was developed.[15] Hydrogenolysis of the Cbz group over Pd/C in MeOH was accomplished in the presence of hexanoic acid hydroxysuccinimide ester, which acylates the liberated amino group (Scheme 3). All four scaffolds (**5**, **6**, **9** and **10**) were subjected to this mild one-pot procedure to give the corresponding hexanoylated derivatives (**5a**, **6a**, **9a** and **10a**). Analogues with more bulky substituents derived from ethylisocyanate were synthesized on a small scale. The hydroxy group of the hexanoylated lactones (**5a**, **6a** and **9a**) underwent reaction with ethylisocyanate in the presence of CuCl in THF (Scheme 3) to give the corresponding carbamate lactones (**5a**, **6a** and **9a**).[16] Lactone (**10a**), however, decomposed under these reaction conditions as well as the Cbz-protected lactone **10**.[17]




**Scheme 1.** Reagents and conditions: (a) AcCl, MeOH, reflux; (b) CbzCl, $K_2CO_3$, EtOAc/$H_2O$; (c) TsCl, pyridine; (d) NaI, acetone, reflux; (e) Zn, DMA; (f) 1.0 M vinylbromide in THF, $Pd_2(dba)_3$ (5%), P(*o*-tolyl)$_3$; (g) $K_2OsO_4 \cdot 2H_2O$ (1%), NMO, acetone; $H_2O$; (h) separation of diastereomers by flash chromatography.




**Scheme 2.** Reagents and conditions: (a) AcCl, MeOH, reflux; (b) CbzCl, $K_2CO_3$, EtOAc/$H_2O$; (c) $NaIO_4$, MeOH/$H_2O$; (d) xylene, reflux; (e) $K_2OsO_4 \cdot 2H_2O$ (1%), NMO, AcOH, acetone, $H_2O$; (f) PTSA, toluene, 100 °C; (h) separation of diastereomers by column chromatography.




**Scheme 3.** Reagents and conditions: (a) $H_2$ (1 atm), 5% Pd/C ( 2.7 mol%), MeOH; (b) CuCl, THF. *not observed; decomposition of starting material **10a**.

## Biological Results and Discusion

The Cbz protected compounds (**5**, **6**, **9** and **10**) and the hexanoylated compounds (**5a**, **5b**, **6a**, **6b**, **9a**, **9b** and **10a**) were screened for their ability to activate and inhibit a LuxR-based QS reporter system (Fig. 2). The QS reporter system contains elements of *V. fischeri* quorum sensing circuit encoded by *luxR-$P_{luxI}$* fused to a reporter gene encoding an unstable form of the green fluorescent protein (Gfp).[18] The genetic construct, termed pJBA89, is harbored by a pUC18 vector maintained in *Escherichia coli* JM105. The green fluorescent QS reporter system is activated by exogenous autoinducers. Quantification of the Gfp generated fluorescence gives a direct measure of the degree of activation. *N*-Hexanoyl-L-homoserine lactone **11** was able to activate the QS reporter system and used as positive control. In correspondence with previous studies,[7] this indicates that the fatty acids' 3-oxo group is not an absolute requirement for activity.

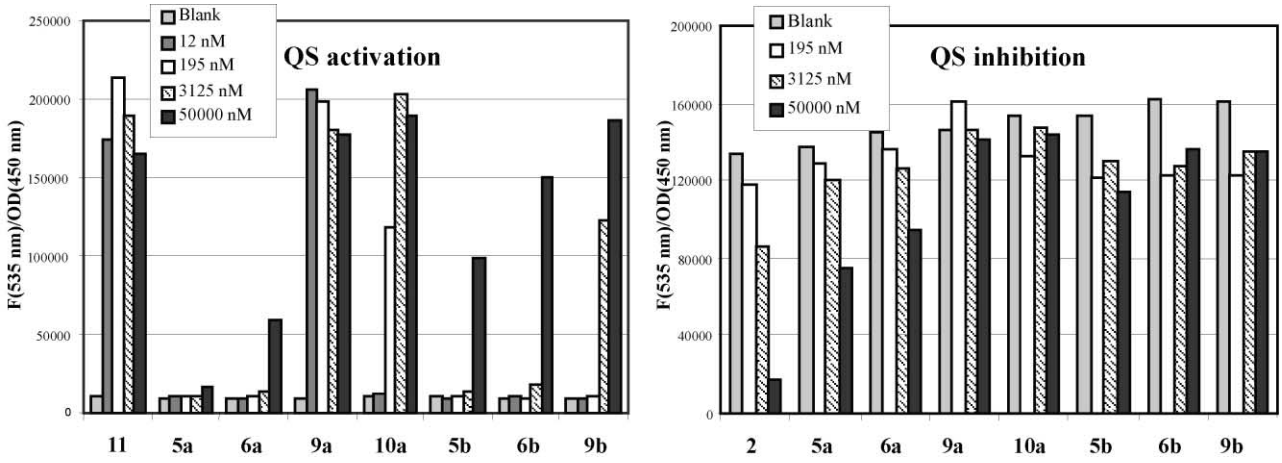



**Figure 2.** Ability of 3- and 4-substituted homoserine lactones to activate and inhibit a LuxR-based QS screening system. *N*-Hexanoyl-L-homoserine lactone **11** was included as positive control in screening for activation. Inhibition was measured by adding the natural autoinducer **1** (concentration 2 nM) together with the compound. A furanone-based inhibitor **2** was included in the experiment as a positive control. After 3 h, green fluorescence was measured at 535 nm and bacterial density (OD) at 450 nm.

The Cbz protected compounds (**5**, **6**, **9** and **10**) were neither able to activate nor inhibit the QS-system. This underlines the importance of the fatty acid chain moiety of these autoinducer molecules, as also demonstrated in other studies.[7] The 4-substituted analogues (**5a**, **5b**, **6a** and **6b**) were only very weak activators, implying that this part of the AHL molecule is crucial for recognition of the autoinducers by LuxR. One can speculate that this part of the molecules is embedded in a narrow binding pocket of the LuxR protein. In contrast, the 3-substituted analogues (**9a**, **9b** and **10a**) were significantly more potent as activators. Interestingly, **9a** was able to activate LuxR as efficiently as **11**, while inverting the hydroxy group as in **10a** reduced the potency by an order of magnitude. Derivatization of **9a** hydroxy group as in the case of **9b** reduced the potency dramatically.

Compounds **5a** and **6a** were able to inhibit QS at maximum concentration tested, though less efficiently than the furanone inhibitor **2**. Even though the other synthesized analogues did not show significant antagonistic activity, our results suggests that further exploration of 3- and 4-substituted analogues could generate a potent QS inhibitor. This is being pursued at the moment, together with 3- and 4-substituted analogues having a 3-oxo-dodecanoyl fatty acid side chain for targeting the therapeutically important QS-system of *P. aeruginosa*.

## Acknowledgements

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## References and Notes

1. (a) Dunny, G. M.; Winans, S. C. *Cell–Cell Signaling in Bacteria*; ASM: Washington, DC, 2000. (b) Parsek, M. R.; Greenberg, E. P. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 8789.
2. (a) Nealson, K. H.; Platt, T.; Hastings, J. W. *J. Bacteriol.* **1970**, *104*, 313. (b) Eberhard, A. *J. Bacteriol.* **1972**, *109*, 1101.
3. Tang, H. B.; DiMango, E.; Bryan, R.; Gambello, M.; Iglewski, B. H.; Goldberg, J. B.; Prince, A. *Infect. Immun.* **1996**, *64*, 37.
4. (a) Hartman, G.; Wise, R. *The Lancet* **1998**, *351*, 848. (b) Finch, R. G.; Pritchard, D. I.; Bycroft, B. W.; Williams, P.; Stewart, G. S. A. B. *J. Antimicrob. Chemother.* **1998**, *42*, 569.
5. Delden, C. V.; Iglewski, B. H. *Emerg. Infect. Dis.* **1998**, *4*, 551.
6. (a) Davies, D. G.; Parsek, M. R.; Pearson, J. P.; Iglewski, B. H.; Costerton, J. W.; Greenberg, E. P. *Science* **1998**, *280*, 295. (b) Costerton, J. W.; Stewart, P. S.; Greenberg, E. P. *Science* **1999**, *284*, 1318. (c) Lewis, K. *Antimicrob. Agents Chemother.* **2001**, *45*, 999.
7. (a) Eberhard, A.; Widrig, C. A.; McBath, P.; Schineller, J. B. *Arch. Microbiol.* **1986**, *146*, 35. (b) Chhabra, S. R.; Stead, P.; Bainton, N. J.; Salmond, G. P. C.; Stewart, G. S. A. B.; Williams, P.; Bycroft, B. W. *J. Antibiot.* **1993**, *46*, 441. (c) Schaefer, A. L.; Hanzelka, B. L.; Eberhard, A.; Greenberg, E. P. *J. Bacteriol.* **1996**, *178*, 2897. (d) Passador, L.; Tucker, K. D.; Guertin, K. R.; Journet, M. P.; Kende, A. S.; Iglewski, B. H. *J. Bacteriol.* **1996**, *178*, 5995. (e) Zhu, J.; Beaber, J. W. R.; More, M. I.; Fuqua, C.; Eberhard, A.; Winans, S. C. *J. Bacteriol.* **1998**, *180*, 5398. (f) Kline, T.; Bowman, J.; Iglewski, B. H.; Kievit, T. D.; Kakai, Y.; Passador, L. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3447.
8. (a) Givskov, M.; Nys, R. d.; Manefield, M.; Gram, L.; Maximilien, R.; Eberl, L.; Molin, S.; Steinberg, P. D.; Kjelleberg, S. *J. Bacteriol.* **1996**, *178*, 6618. (b) Manefield, M.; Nys, R. D.; Kumar, N.; Read, R.; Givskov, M.; Steinberg, P.; Kjelleberg, S. *Microbiology* **1999**, *145*, 283.
9. (a) Bernardo, S. D.; Tengi, J. P.; Sasso, G. J.; Weigele, M. *J. Org. Chem.* **1985**, *50*, 3457. (b) Girard, A.; Greck, C.; Genêt, J. P. *Tetrahedon Lett.* **1998**, *39*, 4259. (c) Liao, L.-X.; Zhou, W.-S. *Tetrahedron* **1998**, *54*, 12571.
10. Jackson, R. F. W.; Moore, R. J.; Dexter, C. S.; Elliott, J.; Mowbray, C. E. *J. Org. Chem.* **1998**, *63*, 7875.
11. VanRheenen, V.; Kelly, R. C.; Cha, D. Y. *Tetrahedron Lett.* **1976**, *23*, 1973.
12. The optical purity corresponded to the literature: **5** $[\alpha]_D^{22}$ $-2.0^\circ$ (*c* 0.57, MeOH) [lit.[10b] $[\alpha]_D^{22}$ $-1.0^\circ$ (*c* 0.6, MeOH]. **6** $[\alpha]_D^{22}$ $-48.2^\circ$ (*c* 0.48, MeOH) [lit.[10c] $[\alpha]_D^{22}$ $-46^\circ$ (*c* 1.3, MeOH)]. **9** $[\alpha]_D^{22}$ $+29.0^\circ$ (*c* 0.48, EtOAc) [lit.[14a] $[\alpha]_D^{22}$ $+36.9^\circ$ (*c* 0.5, EtOAc)]. **10** $[\alpha]_D^{22}$ $-54.2^\circ$ (*c* 0.98, MeOH) [lit.[14b] $[\alpha]_D^{22}$ $-59.2^\circ$ (*c* 1.01, MeOH)].
13. (a) Sendai, M.; Hashiguchi, S.; Tomimoto, M.; Kishimoto, S.; Matsuo, T.; Ochiai, M. *Chem. Pharm. Bull.* **1985**, *33*, 3798. (b) Herranz, R. J. *Chem. Soc., Perkin Trans. 1*, 649.
14. Afzali-Ardakani, A.; Rapoport, H. *J. Org. Chem.* **1980**, *45*, 4817.
15. Dzubeck, V.; Schneider, J. P. *Tetrahedron Lett.* **2000**, *41*, 9953.
16. Holten, R. A.; Zhang, Z.; Clarke, P. A.; Nadizadeh, H.; Procter, D. J. *Tetrahedron Lett.* **1997**, *39*, 2883.
17. The ethylisocyanate benzylalcohol adduct (*O*-benzyl-*N*-ethyl-carbamate) was isolated as major by-product for compound **10**. The reason has not yet been elucidated.
18. (a) Andersen, J. B.; Sternberg, C. B.; Poulsen, L. K.; Bjørn, S. P.; Givskov, M.; Molin, S. *Appl. Environ. Microbiol.* **1998**, *64*, 2240. (b) Andersen, J. B.; Heydorn, A.; Hentzer, M.; Eberl, L.; Geisenberger, O.; Christiansen, B. B.; Molin, S.; Givskov, M. *Appl. Environ. Microbiol.* **2001**, *67*, 575.