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# Influence of the D/L configuration of *N*-acyl-homoserine lactones (AHLs) and analogues on their Lux-R dependent quorum sensing activity

Si-Zhe Li [a], Rui Xu [a], Mohammed Ahmar [a], Catherine Goux-Henry [b], Yves Queneau [a,*], Laurent Soulère [a,*]

[a] Univ Lyon, INSA Lyon, CNRS, Université Lyon 1, CPE Lyon, UMR 5246, ICBMS, Institut de Chimie et de Biochimie Moléculaires et Supramoléculaires, Laboratoire de Chimie Organique et Bioorganique Bât. J. Verne, 20 avenue A. Einstein, F-69621 Villeurbanne, France

[b] Univ Lyon, Université Lyon 1, CNRS, INSA Lyon, CPE Lyon, UMR 5246, ICBMS, Institut de Chimie et de Biochimie Moléculaires et Supramoléculaires, CASYEN Bât Curien (CPE) 43, Bd du 11 Novembre 1918, 69622 Villeurbanne cedex, France

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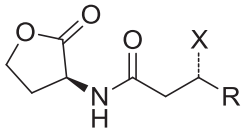
ABSTRACT

Whereas L-3-oxo-hexanoyl homoserine lactone (OHHL) is the active enantiomer of the of LuxR-regulated quorum sensing (QS) autoinducer, its D isomer is implicitly considered as inactive. The present work aims to clarify this L-specificity and investigate whether it extends to some analogues in the acyl homoserine lactone (AHL) family. For this purpose, OHHL and a series of AHL analogs were synthesized in racemic and enantiomerically pure D and L forms and their ability to induce or attenuate bioluminescence in the LuxR-dependent QS system was evaluated. In this study, L-isomers are confirmed as either the only, or as the most active, enantiomers. However, in several cases, especially for the natural ligand of LuxR (OHHL) and the very similar AHL agonist analogue **2**, the D-isomer cannot be considered as totally inactive on QS. Molecular modelling suggests that when the lactone moiety of the D-isomer is able to twist, enabling the lactone carbonyl group and the amide function to interact with the key residues in the binding site, then the D-isomer can exhibit some activity.



## 1. Introduction

Bacterial Quorum Sensing (QS) is an intercellular communication system employed by numerous species of bacteria [1–3]. This process involves a coordination or repression of the genome based on chemical messengers, called autoinducers, which are synthesized by the LuxI protein family and interact with the LuxR protein family [3–5]. Many proteobacteria have been found to have a LuxR-AHL based QS response, with each having at least the same key components [3]. In Gram negative bacteria, autoinducers are related to acylated L-homoserine lactones (AHL) with diverse structural variations, such as different acyl chain lengths or the eventual presence of a 3-oxo or 3-hydroxy function. As a potential antibacterial strategy, the investigation of QS mimics represents an attractive alternative therapeutic approach for the treatment of human and plant bacterial infections. This is why the synthesis of small molecules which are capable of modulating bacterial QS systems has been widely studied in recent years [2], notably with experiments designed around native bacterial auto-inducers [2,3,6].




Stereochemistry is a key aspect of molecular recognition for biological systems. As such, receptors and enzymes are often highly stereospecific, only recognizing one stereoisomer of a ligand. The first discovery of the AHL signal was in the L-configuration, from a comparison of the c.d. spectra of the natural product and two synthetic isomers [7]. In 2004, D.R. Spring et al. reported that the L-isomer is probably reponsible for the QS autoinducing activity in *P. aeruginosa* and *Serratia* 39,006 when both enantiomers of its autoinducer were assayed [8,9]. However, until now, there has been no systematic demonstration showing the stereochemical significance of QS autoinducers in various species of bacteria except for the few studies, cited above, pertaining to the investigation of the enantiomeric character of AHLs. In most cases, it was thought that probably only the L-isomers of AHL type autoinducers were responsible for the QS modulation and, consequently, only L-AHL analogues were considered for research without considering their D-isomers. For example, several families of

* Corresponding authors.
*E-mail addresses:* yves.queneau@insa-lyon.fr (Y. Queneau), laurent.soulere@insa-lyon.fr (L. Soulère).

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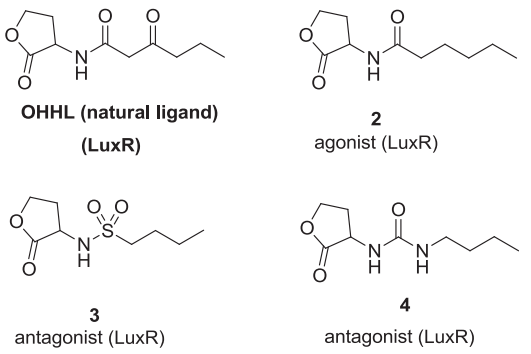
racemic active analogues have been reported by our group, over several years, with variations in the side chain or in the amide mimics [10–16]. However, the ability of each pure enantiomer of these analogues, such as sulfonamides [12], ureas [11], sulfonylureas [14] and β-ketoamide AHL derivatives [17], to attenuate or induce QS modulation has never been studied in a systematic manner. With the aim of determining to what extent the D/L configuration of AHLs is a key parameter in the design of QS modulators, we report below our investigation into the influence of the D/L configuration of OHHL and some analogues, chosen from various agonists or antagonists, on their ability to modulate QS.




In this study, we report our results on the synthesis and biological evaluation of a variety of optically pure AHLs and analogues, chosen from the QS-active compounds in our library and representative of several structural variations, namely OHHL, the natural ligand of *Vibrio fischeri* as L and D isomers, together with compounds **2**–**4**. Compound **2** was described as an agonist and compounds **3**–**4** as antagonists when tested as a racemic mixture on the LuxR-regulated QS system.[10–12] All the compounds were prepared from L- and D-homoserine lactone hydrobromide *via* amidation with reagents. The enantiomeric purity was assessed by polarimetry, with complementary measurements from two methods, namely NMR, using an NMR shift reagent, and chiral HPLC. The bioassay was conducted in *E. coli* by measuring the level of inducing or inhibiting bioluminescence. In addition, the molecular modelling study of these enantiomers has also been investigated.

## 2. Results and discussion

### 2.1. Synthesis

For the synthesis of AHLs, as previously described [18], L and D-methionine were alkylated with 2-bromoacetic acid, in acidic conditions, to give L- and D-homoserine lactone hydrobromide. These compunds were then acylated with the corresponding acylated Meldrum's acid (D-isomers of OHHL, the L form was purchased) to yield optically pure β-ketoamide AHLs (Scheme 1). This method was based on an adapted DCC/DMAP-mediated coupling of carboxylic acid derivatives with Meldrum's acid as the key intermediates [17]. This proved to be a concise and efficient synthetic route for preparing enantiomerical β-ketoamide AHLs with a C6 or longer side chain [19,20], compared with the reported, and widely used, method for the synthesis of β-ketoamide AHL using Meldrum's acid as the starting material in six steps [21]. With the process described here, the products could be easily isolated by silica column chromatography (Scheme 1).

D- and L-isomers of compounds **2**–**4** were prepared, in the presence of triethylamine, using L and D-homoserine lactone hydrobromide reacting with hexanoyl chloride, sulfonyl chloride or butyl isocyanate, respectively. Racemic compounds were synthesized from commercially available racemic homoserine lactone hydrobromide using the same process (Scheme 2). It should be noted that other methods were used with different bases, leading to consistent results regarding optical rotation.

To clearly demonstrate the enantiomeric purity of all compounds, and in particular OHHL and compound **2** for which the activity of the D-isomer was found to be not negligible (see Section 2.2), we used two different methods: one based on $^1$H NMR using chiral europium complex salts [22], and one based on chiral HPLC with a DAICEL Chiralpak AD coloumn. The NMR method provided a first clue regarding the efficiency of the enantiomeric selectivity of the synthetic sequence. For example, the spectra showed that the triplet of $CH_3$ in compound **2** was split into a double triplet which was consistent with L-2 and D-2. The HPLC method consistently confirmed the enantiomeric purity of the D and L-isomers of OHHL and compound **2** (see supplementary data).

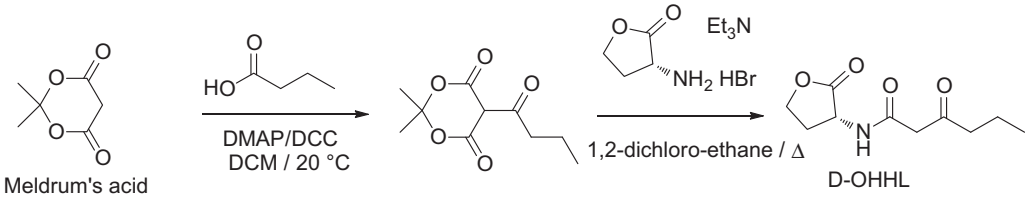
### 2.2. Biological evaluation

All compounds, either enantiomerically pure D or L form or as a racemic mixture, were then systematically tested with respect to their possible agonistic and antagonistic activity, regardless of their previous identification as agonists or antagonists.

#### 2.2.1. Agonistic activity

For OHHL, no significant differences were observed between the racemic mixture and the corresponding isomers at high concentrations (50–200 μM). However, at lower concentrations (0.4–1 μM), the activity of the D-isomers of OHHL was not negligible, with a relative bioluminescence ranging from 10 to 20, compared with the L-isomer, leading to a maximum of relative bioluminescence with values ranging from 50 to 60 (Fig. 1). It should be noted that the racemic OHHL, which is the natural, very active ligand, induces more than half of the bioluminescence measured for the l-isomer, probably because the maximum level of bioluminescence that could be induced has already been reached.

For the hexanoyl-AHL analog **2** (known agonist), the D-**2** isomer proved to be inactive, compared with the L-**2,** at low concentrations (0.4–1 μM) but it showed some activity at higher concentrations of between 10 μM and 200 μM (Fig. 2). For the sulfonamide and urea analogues **3** and **4** (known antagonists), neither the D nor the L-isomer exhibited any agonistic activity at low concentrations (Fig. 2), as expected Only high concentrations of the L-isomers of




**Scheme 1.** Synthesis of optically pure β-ketoamide AHLs.