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Robust routes for the synthesis of *N*-acylated-L-homoserine lactone (AHL) quorum sensing molecules with high levels of enantiomeric purity

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ABSTRACT

The ready availability of native quorum sensing molecules and related structural analogues is of significant biological interest in the development of methods to manipulate bacterial quorum sensing systems in a useful fashion. In this Letter we report robust routes for the synthesis of a range of *N*-acylated-L-homoserine lactone (AHL) quorum sensing molecules. Crucially, we have analysed the enantiopurity of the final AHLs and in all cases, excellent levels were observed.

Quorum sensing is a mechanism of intercellular communication employed by numerous species of bacteria. This signalling process, mediated by small diffusible molecules termed autoinducers, allows the cells comprising a bacterial colony to coordinate their genome expression in a cell-density dependent manner.[1] Quorum sensing has been shown to play a critical role in both pathogenic and symbiotic bacteria-host interactions.[2] For example the bacterium *Pseudomonas aeruginosa*, one of the most prevalent pathogens in a range of life-threatening nosocomial infections and the primary cause of mortality in cystic fibrosis sufferers, uses quorum sensing to regulate a variety of processes associated with virulence.[3] These include the formation of biofilms and the expression of virulence factors.[4]

Though quorum sensing systems are used by many bacterial pathogens to regulate virulence they are not essential for survival.[3a] Thus, disruption of quorum sensing (so-called 'quorum-quenching') should attenuate pathogenicity without imposing the level of selective pressure associated with antibacterial treatments.[4c,5,6] Consequently, the targeting of bacterial quorum sensing systems represents an attractive alternative therapeutic approach for the treatment of human bacterial infections.[4c,7] Indeed, there is proof-of-concept from animal studies that the virulence of the Gram-negative bacterium *P. aeruginosa* can be partially attenuated in vivo by the inhibition of quorum sensing.[4c,8]

The synthesis of small molecules which are capable of modulating bacterial quorum sensing systems has attracted significant attention in recent years.[9] Many of these molecules are based around the structures of known, native bacterial auto-inducers. *N*-Acylated-L-homoserine lactones (AHLs) are among the most common signal molecules produced and used by Gram-negative bacteria for intercellular communication.[5] For example, *P. aeruginosa* uses (at least) three types of quorum sensing signals. Two of these are AHL based (Fig. 1), employing *N*-(3-oxododecanoyl)-L-homoserine lactone (OdDHL) and *N*-butanoyl-L-homoserine lactone (BHL).[9]

Given the integral role of AHLs in quorum sensing systems it is unsurprising that there is a large body of work pertaining to their synthesis and that of other analogous structures.[9,10] However, detailed experimental protocols and full analytical data, particularly enantiomeric purity, has not always been provided. Indeed, accessing such compounds with reliably high levels of enantiomeric

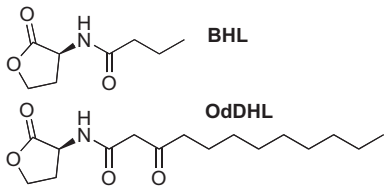

Figure 1. Molecules employed by *P. aeruginosa* in quorum sensing.

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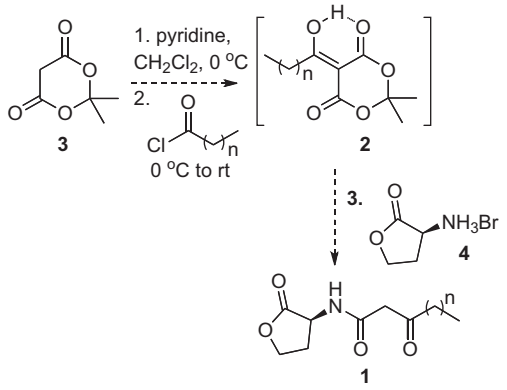


Scheme 1. Proposed one-pot synthesis of β-ketoamide AHLs.

purity remains a largely unmet challenge. Herein we report upon our research towards the development of general and efficient methods for the synthesis of a variety of AHLs. These studies have led to robust synthetic routes towards a variety of native AHLs from readily available starting materials, allowing access to these biologically important molecules in good yields, and crucially, with reliably excellent levels of enantiomeric purity. Full experimental details and analytical data for all the AHLs synthesized are given (Supplementary data) and we hope that this collection of information will be of significant assistance to the practising chemist in this field.

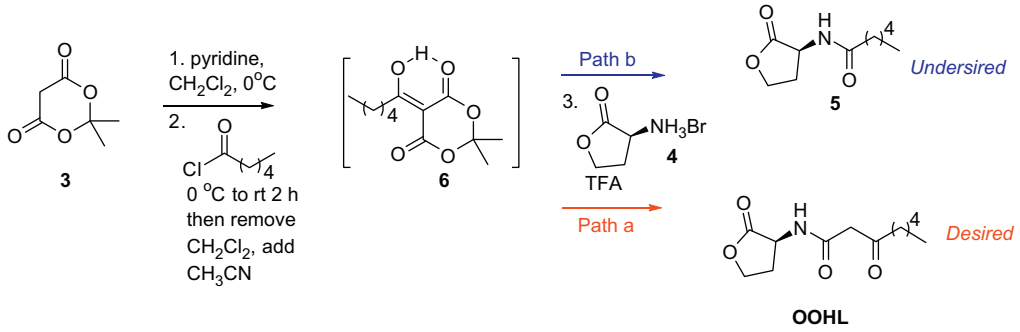
Our research was directed towards accessing the two major structural classes of AHLs; those with unfunctionalized acyl chains (e.g., BHL) and those containing an *N*-acyl β-diketone moiety (henceforth known as β-ketoamide AHLs, e.g., OdDHL). Initial studies focused upon the development of a one-pot method for the synthesis of β-ketoamide AHLs (**1**). Towards this end, we were inspired by a report from Chhabra et al. which detailed the synthesis of a variety of OdDHL analogues using adducts of the form **2**, produced from the DCC-mediated coupling of carboxylic acid derivatives with Meldrum's acid (**3**), as key intermediates (Scheme 1).[11] In their report, these adducts were isolated from the reaction mixture by a standard work-up procedure and treated in a separate vessel with the hydrochloride salt of (*S*)-(−)-α-amino-γ-butyrolactone (**4**) in the presence of a base to afford the corresponding AHL analogue. We envisaged a modification of this method whereby adduct **2**, formed by the reaction of Meldrum's acid with an acid chloride, could be isolated in situ by solvent removal upon completion of the reaction, rather than through a work-up process. Addition of **4** to the same reaction vessel would lead directly to the corresponding AHL derivative **1** (Scheme 1). Overall, this sequence would thus comprise a novel one-pot synthesis of AHLs carrying an *N*-acyl β-diketone moiety.

Xu et al. reported a related one-pot procedure for the formation of β-ketoamides via the reaction of amines with Meldrum's acid adducts **2**, with their data suggesting that the reaction requires a slightly acidic medium for optimum results.[12] Thus we initially decided to attempt one-pot AHL synthesis under acidic conditions. Proof-of-principle studies were directed towards *N*-3-oxoacyl-L-homserine lactone (OOHL) (Scheme 2). Pyridine and hexanoyl chloride were added to a solution of Meldrum's acid (**3**) in dichloromethane. Upon completion of the reaction the solvent was removed in vacuo and the residue was re-suspended in acetonitrile.[13]

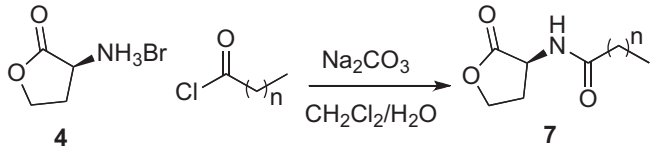
(*S*)-(−)-α-amino-γ-butyrolactone hydrobromide **(4)** was added followed by trifluoroacetic acid and the resulting solution was stirred at 45 °C until the reaction was observed to have proceeded to completion. The solvent was removed in vacuo and the crude product was purified by column chromatography to yield OOHL in a good overall yield and with an excellent enantiomeric purity (enantiomeric excess ~95% as determined by chiral HPLC). Our initial excitement at this result was quickly tempered by the realization that the reaction was extremely capricious. Indeed, the results of our initial experiment could not be obtained on a consistent basis. A significant problem was the formation of the undesired AHL side-product **5** at some point during the course of the reaction sequence. Unfortunately, **5** was found to co-elute with the desired product on silica and consequently it proved extremely difficult to isolate analytically pure product by column chromatography. It was eventually determined that this side-product arose in the second stage of the reaction sequence, presumably though amidation of the exocyclic carbonyl group of intermediate adduct **6** (Scheme 2, path b).[14] We speculated that the acidic reaction conditions may have influenced the regioselectivity of the nucleophilic attack on adduct **6** through some unknown mechanism.[15] To investigate this further we repeated the process outlined in Scheme 2 using triethylamine rather than TFA in the third step.[16] Under these basic reaction conditions, no formation of the side-product **5** was observed. Analytically pure OOHL could be isolated by column chromatography and the reaction proved to be fairly reproducible with a range of acid chlorides to furnish several AHL derivatives. However, isolated yields of products were relatively poor (<30%) as were enantiomeric purities (<70% enantiomeric excess).

Given the problems associated with the one-pot method described above, we were interested in exploring alternative routes towards β-ketoamide AHLs, as well as developing an efficient synthesis of AHLs with unfunctionalized acyl chains. The ability to access both of these structural classes of AHLs with reliably excellent levels of enantiomeric purity was a key goal.

AHLs with unfunctionalized acyl chains (**7**) could be readily obtained by adaptation of previously reported conditions involving the reaction of commercially available (*S*)-(−)-α-amino-γ-butyro-



Scheme 2. One-pot synthesis of OOHL.



Scheme 3. Synthesis of AHLs using Schotten–Baumann conditions.

Table 1
AHLs synthesized using the Schotten–Baumann coupling procedure

Compound	*n*	Yield (%)	ee[a] (%)
N-butanoyl-L-homoserine lactone (BHL)	2	86	>99
N-hexanoyl-L-homoserine lactone (HHL)	4	97	>99
N-octanoyl-L-homoserine lactone (OHL)	6	96	>99
N-decanoyl-L-homoserine lactone (DHL)	8	98	>99
N-dodecanoyl-L-homoserine lactone (dDHL)	10	96	>99

[a] ee = enantiomeric excess as determined by chiral HPLC by comparison with independently synthesized racemic samples.

lactone hydrobromide (**4**) with a variety of acid chlorides under Schotten–Baumann conditions (Scheme 3).[11,17] Using this methodology, a range of AHLs (Table 1) were prepared in good-to-excellent yields. The enantiomeric excesses of the AHL products were determined by chiral HPLC and found to be consistently excellent indicating the absence of any racemization during the coupling process. Full experimental details and comprehensive analytical data are provided for all final compounds synthesized by this route (Supplementary data).

A robust linear route towards the β-ketoamide AHLs, *N*-(3-oxooctanoyl-L-homoserine lactone (OOHL, *n* = 4), *N*-(3-oxodecanoyl)-L-homoserine lactone (ODHL, *n* = 6), *N*-(3-oxododecanoyl)-L-homoserine lactone (OdDHL, *n* = 8) and *N*-(3-oxotetradecanoyl)-L-homoserine lactone (OtDHL, *n* = 10) was developed by adaptation of previously reported procedures (Scheme 4).[17a,b,18] Reaction of Meldrum's acid (**3**) with the appropriate acid chloride generated adducts **2**. Subsequent treatment of this crude material with methanol yielded β-ketoesters **8**–**11**. Acetal protection generated compounds **12**–**15** and ester hydrolysis furnished acid derivatives **16**–**19**. EDC-mediated coupling with (*S*)-(−)-α-amino-γ-butyrolactone hydrobromide (**4**) proceeded to generate the protected amide products **20**–**23** in good yields. Finally, acid-catalyzed acetal deprotection furnished the final products.

All steps outlined in Scheme 4 generally proceeded in good-to-excellent yields, and were found to be amenable to scale-up, having been performed successfully on multi-gram scale. In all cases the spectroscopic data of the final compounds were consistent with those previously reported in the literature (where available), or those obtained from authentic samples. In addition, the enantiomeric excesses of the AHL products were excellent indicating that little racemization occurred during the reaction sequence. Thus this route provides a reliable, scalable method for synthesizing several β-ketoamide AHLs with excellent levels of enantiomeric purity.

In this Letter we have reported the development of robust and reliable synthetic routes to a variety of native quorum sensing molecules. Significantly, we have used chiral HPLC to analyse the enantiopurity of the final AHLs, and in all cases, excellent levels were observed. These results are timely and address a significant shortcoming of previously reported routes towards such compounds. Thus the protocols that we have developed serve as arguably the most reliable means to access a range of AHLs with excellent levels of enantiomeric purity. Full experimental details and analytical data for all final compounds reported in this manuscript are given, and we hope that this collection of information will be of assistance to the practising chemist in this field. Further analogue syntheses and structure–activity studies are ongoing and the results of this work will be reported in due course.

Acknowledgements

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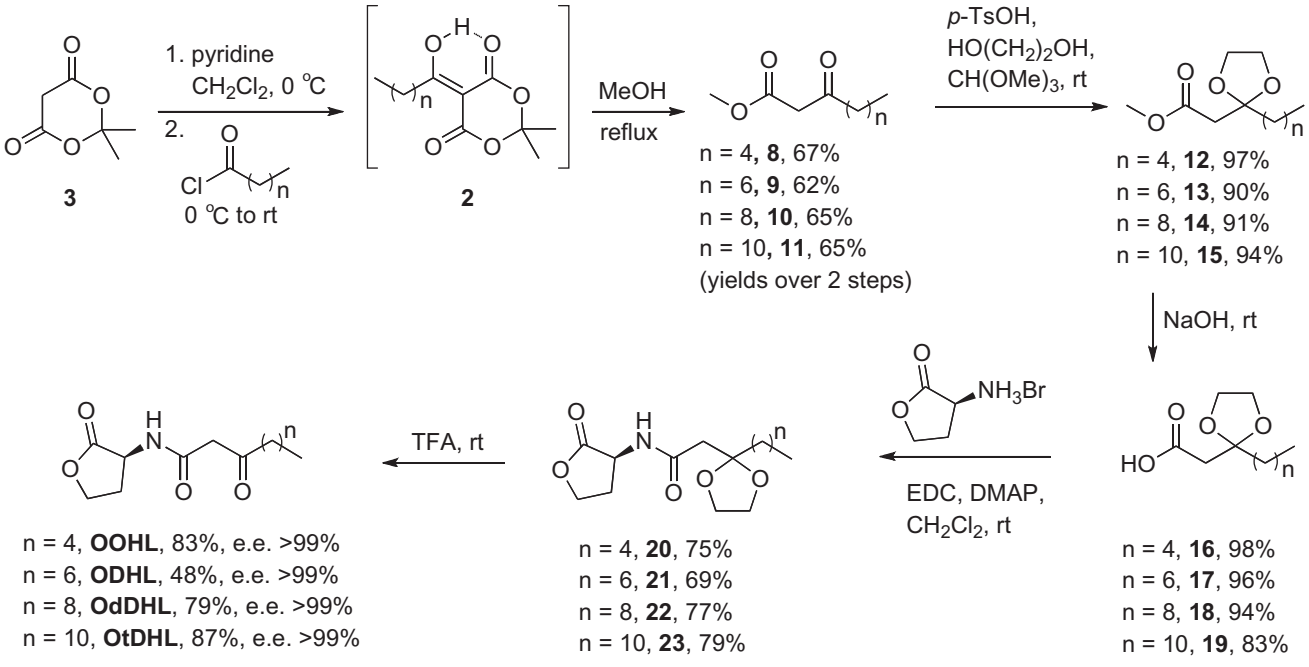

Scheme 4. Linear route towards β-ketoamide AHLs. rt = room temperature. ee = enantiomeric excess as determined by chiral HPLC by comparison with independently synthesized racemic samples.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.04.059.

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13. (*S*)-(−)-α-amino-γ-butyrolactone hydrobromide (**4**) was only sparingly soluble in dichloromethane but dissolved readily in acetonitrile.
14. The possibility that **5** may have arisen via water-mediated acid chloride hydrolysis to the corresponding carboxylic acid followed by coupling to the amine was discounted by the use of strictly anhydrous reaction conditions. Increasing the amount of Meldrum's acid relative to the chloride (in order to ensure complete consumption of the chloride before addition of the lactone) and attempts to hydrolyze any acid chloride remaining after the first stage of the reaction by the use of an aqueous work-up before addition of the lactone did not prevent formation of significant quantities of **5**, thus indicating that by-product formation occurred *via* adduct **6** rather than by direct coupling of the lactone to the acid chloride. Presumably, **5** therefore results via amidation of the exocylic carbonyl group of the tautomeric form of **6**. Chhabra et al. (see ref. 11) reported similar problems when reacting isolated adducts of the general form **2** with L-homoserine lactone hydrochloride under basic conditions in their syntheses of OdDHL analogues. However, the authors reported that these impurities were easily removed by column chromatography on silica.
15. In addition, Meldrum's acid adducts of the form **6** have been reported to be unstable in acid, which may also affect the efficiency of the one-pot process. See ref. 12 and references therein.
16. Chhabra et al. employed triethylamine in the second step of their 'two-pot' synthesis of OdDHL analogues via 5-acyl Meldrum's acid derivatives isolated by a standard work-up procedure. See Ref. 11
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