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Rational design and synthesis of new quorum-sensing inhibitors derived from acylated homoserine lactones and natural products from garlic

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Received 12th October 2004, Accepted 29th October 2004
First published as an Advance Article on the web 1st December 2004

Parallel solution-phase synthesis of sulfide AHL analogues (**10a–s**) by one-pot or a sequential approach is reported. The corresponding sulfoxides **13a–e** and sulfones **14a–e** were prepared to expand the diversity of the 19-member array of sulfides **10a–s**. Likewise, dithianes **12a–c** were prepared with similarity both to sulfides **10a–s** and to bioactive structures from garlic. Design and biological screening of all compounds presented in this work targeted inhibition of quorum-sensing comprising competitive inhibition of transcriptional regulators LuxR and LasR. The design was based on critical interactions within the binding-site and structural motifs in molecular components isolated from garlic, **7** and **8**, shown to be quorum-sensing inhibitors but not antibiotics. A potent quorum-sensing inhibitor *N*-(heptylsulfanylacetyl)-L-homoserine lactone (**10c**) was identified. Together with data collected for the other analogues, the resulting structure–activity relationship led to a hypothesis in which competitive binding was assumed.

Introduction

N-Acyl-L-homoserine lactones (AHLs) play an important role in the pathogenicity of many gram-negative bacteria. These AHLs are signal molecules that accumulate during cell growth in a bacterial population. At a certain threshold level, a concentration-dependent burst of target gene expression is mediated in a process termed quorum-sensing (QS).[1] *N*-(3-Oxododecanoyl)-L-homoserine lactone (**1**, Fig. 1) forms a complex with LasR, a member of the LuxR (*Vibrio fischeri*) family of transcription factors. LasR controls multiple target genes in *Pseudomonas aeruginosa*, including biofilm formation and a second QS regulatory circuit denoted *rhl*. The corresponding LasR homologue, RhlR, responds to the concentration of another signalling molecule, *N*-butanoyl-L-homoserine lactone (**2**, Fig. 1).

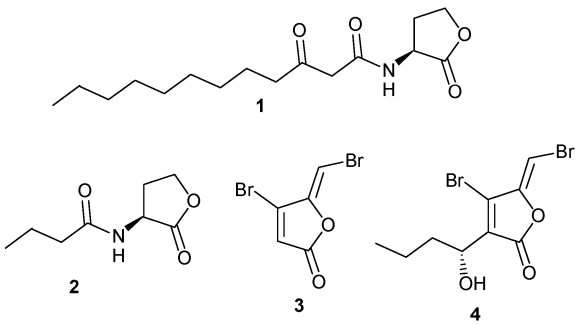


Fig. 1 Signal molecules *N*-(3-oxododecanoyl)-L-homoserine lactone (**1**) and *N*-butanoyl-L-homoserine lactone (**2**), and QS inhibitors (5*Z*)-4-bromo-5-(bromomethylene)-2(5*H*)-furanone (**3**) and (1′*R*, 5*Z*)-4-bromo-3-(1′-hydroxybutyl)-5-(bromomethylene)-2(5*H*)-furanone (**4**).

The involvement of QS in biofilm formation was originally reported for *P. aeruginosa*. A mutant unable to produce **1** formed flat and undifferentiated biofilms when compared to the wild-type or a mutant unable to produce **2**, which formed characteristic microcolonies.[2] In other studies, no differences between the biofilms of the wild-type and those formed by QS mutants were observed.[3] Biofilms produced by *P. aeruginosa* are much more tolerant to treatment with antibiotics than planktonic cells, hence, infections by bacteria in the biofilm mode are difficult to eradicate.[4] Blocking the QS receptors with **3** was recently shown to increase biofilm sensitivity to treatment with tobramycin.[5] Much work has been done on finding such antagonists (*V. fischeri*,[6,7] *Chromobacterium violaceum*,[8] and *Agrobacterium tumefaciens*[9]) but strikingly few, considering the clinical impact of *P. aeruginosa*, employed LasR as the target.[10] One possible explanation is that LasR is less susceptible to structural variations of the signal molecule, **1**. In fact, **1** has been shown to inhibit both RhlR[11] and LuxR,[6] whereas the signal molecules of RhlR and LuxR do not inhibit LasR. Likewise, experience from previous studies[12,13,14] on antagonist identification suggests lower discrimination towards structural modifications of the homoserine lactone (HSL) moiety in ligands of LuxR, as compared to LasR. However, there are excellent examples where the *las*/*rhl* cascade was blocked by non-HSL based molecules,[15] especially an *in vivo* experiment involving **3** showing attenuation of pathogenicity in a mouse model.[5] Brominated ylidenebutenolide (**3**) is a biomimetic of **1** and a synthetic analogue of naturally occurring fimbrolide, **4**, which is one out of many analogous secondary metabolites produced by red seaweed *Delisea pulchra* (Fig. 1). QS inhibitory activity has been documented for several of these.[16]

Due to lack of structural information of target receptors, most inhibitors have primarily been developed based on AHL-like structures. Even though pharmacophoric elements have been suggested, these do not deviate much from the structural elements revealed by **1**.[17] Only recently, a 3-D pharmacophore of the target receptor could be created from a crystal structure of TraR (*A. tumefaciens*, Fig. 2).[18] Even though the sequence alignment between TraR and LasR shows great dissimilarities, all amino acid residues forming hydrogen bonds to the ligand; Tyr53, Trp57, Asp70, Ala38 (glycine in LasR) and Thr129 (serine in LasR), are conserved between the two proteins. The two

DOI: 10.1039/b415761c

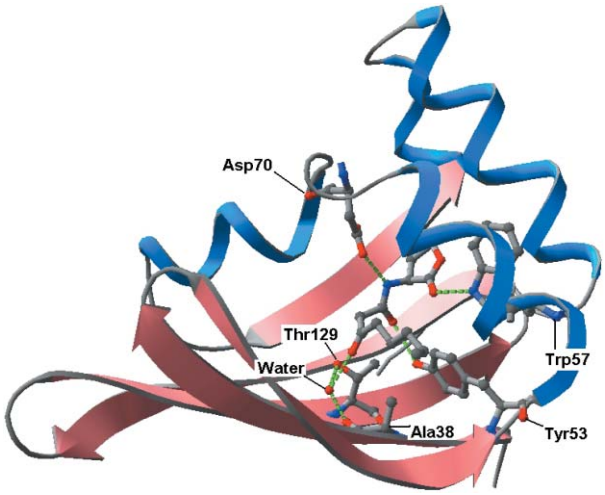


Fig. 2 Ribbon diagram of residues 34–139 of TraR (PDB entry [1L3L] reproduced with Swiss-Pdb viewer v37)[#346] in an active conformation showing hydrogen bonds (green) inside the binding-site between the ligand (*N*-[3-oxooctanoyl]-L-homoserine lactone) and five amino acid residues.[18]

latter interactions are mediated by a water molecule inside the binding pocket formed between a β-sheet (red) and 4 α-helices (blue). Ala38 and Thr129 are positioned on the β-sheet together with some hydrophobic residues responsible for van der Waals contacts on one side of the acyl chain. Likewise, on the opposite side of the acyl chain are lipophilic residues belonging to the α-helices. One striking deviation is Phe62 in TraR, which is not found in LasR. This residue is found near the end of the acyl chain, presumably deciding which AHLs to reject, since most of the AHL signal molecules differ only by length and functionality of the acyl chain and all embrace a lactone moiety. Several questions can be raised on how much this part of the cavity in the receptor changes when transforming into an active conformation: Are the oxygen atoms necessary for inhibition of the receptor or only for activation? Is the butyrolactone or butenolide a prerequisite? If not, can this be combined with other structural elements known to suppress the active conformation?

Antagonists of LasR, lacking the potential to interact with the receptor through a 3′-oxo-group (as in **1**) have been identified. These analogues had low inhibitory activity[10] but the reason is not obvious from the model used. Inhibition was measured as a degree of competition for the binding site, not the actual quenching of QS. The general perception of QS inhibition needs to be further unified to eliminate some of these uncertainties.

The body of literature on QS covers many ways to synthesize AHLs and other analogous structures, both by solution-phase[19,20] and solid-phase[21] techniques. Evidently, the L-homoserine lactone (HSL) moiety has a central role. Herein, we present an improved protocol for the synthesis of L-homoserine lactone hydrobromide (**5**). A number of procedures are also included, whereby substances of the general formulae **10** were obtained.

It is known from literature that garlic (genus *Allium sativum L.*) possess antibiotic activity.[22] The activity has mainly been attributed to 2-propenesulfenic acid, which in garlic gives allicin, a thiosulfinate with antibiotic properties.[23] During this study, we have investigated in more details if garlic extracts contained compounds, which solely inhibit QS, void of antibiotic activity, which, to the best of our knowledge, has not previously been investigated. Indeed, some individual molecular components inhibiting QS were identified after isolation from garlic extracts. The components differed structurally from **10** inferring affinity for another binding-site than for the AHLs, perhaps a nearby site. Grafting of two individual low-affinity binders to yield one ligand with high affinity, referred to as linked binding-elements by Maly *et al.*,[24] has been successfully applied before.[25] This possibility was also investigated here.

Results and discussion

In this study we wished to combine rational design with bioactive scaffolds from natural products. We anticipated that such natural compounds with quorum-sensing inhibitory activity could be isolated from garlic. In order to apply these structural motifs to the rationally derived compounds **10a–e**, design and synthesis of the subsequent analogues were performed side-by-side with the isolation and identification of non-toxic quorum-sensing inhibitors from garlic.

Identification of substances in garlic responsible for a QS inhibitory effect

Toluene extracts of garlic were shown to inhibit LuxR- and LasR-based QS systems.[26] Through bio-assay guided fractionation, six compounds (**6a–d**, **7** and **8**, Fig. 3) were identified by GC-MS analysis and NMR spectroscopy, that inhibited QS in a LuxR monitor system. This was also confirmed by screening synthetic **6a–d**, **7** and **8**, which are known components from garlic and obtained readily either from commercial sources (**6a** and **6b**) or by known synthetic procedures (**6c**, **6d**, **7** and **8**).[27,28] **6a–d** antagonized LuxR but they were also toxic to the bacteria. More significantly, compounds **7** and **8** possessed QS activity exclusively, albeit only in the LuxR monitor system.

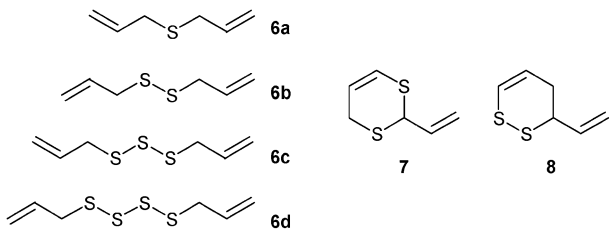
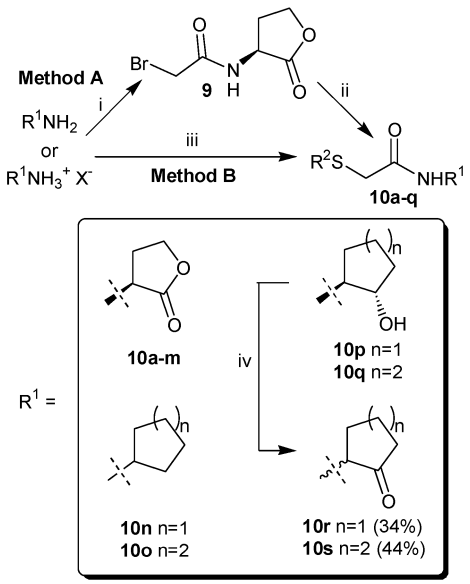


Fig. 3 Garlic components found to be antibiotics (**6a–d**) and QS antagonists (**6a–d**, **7** and **8**).

Notably, allicin, which is known to possess antibacterial activity,[29,30] did not show inhibitory activity against any of the QS systems tested (LuxR and LasR).

Synthesis and characterization of QS inhibitors

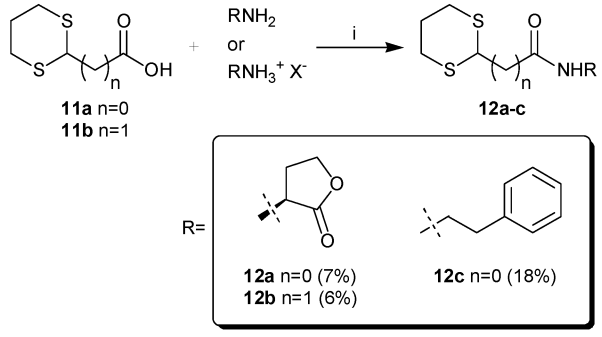
From the vast collection of QS attenuators reported in literature, those containing an HSL-moiety are in great majority. Therefore, most protocols published to date involve a carboxylic acid being coupled to HSL. The modest representation of compounds like **10** in literature offer no exception.[31,32] Therefore, to be able to vary R^1 and R^2 in a one-step procedure, a new strategy was developed. The chosen strategy provides the *N*-acyl part first and then diverts into **10a–q**. Since analogues **10a–m** were all derivatives of HSL, they could be produced from *N*-bromoacetyl homoserine lactone (**9**) by method A (Scheme 1). When **10c** and **10n–q** were synthesized, method B was applied. This one-pot approach allowed for high throughput synthesis of any sulfanyl-acetamides (**10**) from thiols and amines. All amines used were commercially available either as halide salts or their free amines. However, due to the large quantities needed of L-homoserine lactone hydrobromide (**5**), we developed an optimized synthetic protocol. S-Alkylation of L-methionine with bromoacetic acid and then immediate intramolecular nucleophilic substitution (lactonization) in acidic solution,[33] gave **5** by precipitation with hydrobromic acid. In our hands, the formerly described precipitation process, with hydrochloric acid,[34] was quite inefficient and it is likely that some product was lost at that stage. The optimized yield was 81% and the next step, addition of bromoacetyl bromide to L-homoserine lactone hydrobromide



Scheme 1 Reagents and conditions: (i) bromoacetyl bromide, TEA, CH_2Cl_2, −78 °C → RT, 70%; (ii) R^2-SH, TEA, EtOH, RT; (iii) bromoacetyl bromide, TEA, CH_2Cl_2, −20 °C → RT then R^2-SH, TEA; (iv) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C.

(**5**), gave **9** in 70%.[35] As a consequence of these high yields, products **10a**, **10b** and **10d–m** were furnished in a satisfying overall yield (20–56%) from L-methionine in three steps (Table 1 gives the yields of **10a–q** starting from the amines). Analogues **10e–m** were all synthesized by method A modified by omitting the washing procedure. Less satisfactory yields were obtained for analogues **10c** and **10n–q** using method B (3–46%). Method B also gave longer reaction times, but was more convenient during synthesis of analogues **10n–q**, due to variations in R^1 (starting materials for **10n**: cyclopentylamine, **10o**: cyclohexylamine, **10p**: *trans*-2-aminocyclopentanol hydrochloride, **10q**: *trans*-2-aminocyclohexanol hydrochloride). Two other analogues, **10r** and **10s**, were derived from **10p** and **10q** by Swern oxidation (Scheme 1). The presence of a sulfide resulted in complications reflected in the yields 34 and 44%, respectively.

It is a well-known tactic in medicinal chemistry to combine several discrete bioactive structural motifs into a larger, more potent pharmacophore.[24] Contemplating garlic component **7**, a second class of analogues, **12a** and **12b**, conformationally restricted isosteres of **10**, were synthesized from **11a** and **11b** (Scheme 2). These starting materials were accessible through hydrolysis of the corresponding esters,[36] commercially available or synthesized according to a published procedure.[37] Synthesis of **12c** served as a model system for optimization of the coupling reaction and only this product was stable enough for purification by silica gel flash chromatography, whereas both **12a** and **12b** had to be isolated by preparative RP-HPLC.



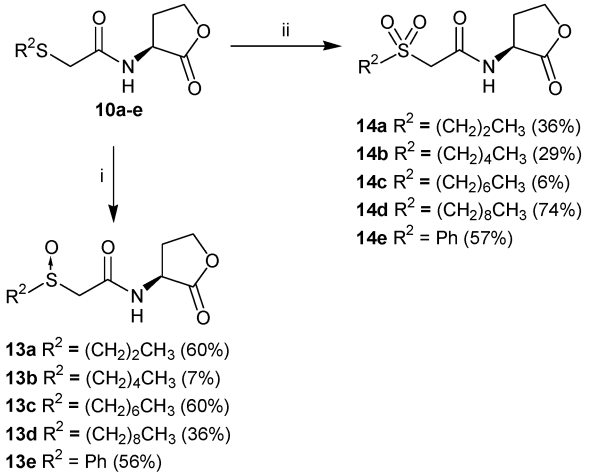
Scheme 2 Reagents and conditions: (i) isobutyl chloroformate, TEA, CH_2Cl_2, 0 °C.

Table 1 Yield of products **10a–q** using the sequential method (method A) or the one-pot method (method B)

R^1	R^2	Method	Product	Yield/%[a]
		A	**10a**	32
		A	**10b**	47
		B	**10c**	46
		A	**10d**	25
		A	**10e**	53
		A	**10f**	62
	HO	A	**10g**	25
	Cl	A	**10h**	64
	Cl	A	**10i**	64
	Cl	A	**10j**	64
		A	**10k**	69
		A	**10l**	69
		A	**10m**	64
		B	**10n**	27
		B	**10o**	37
OH		B	**10p**	3
OH		B	**10q**	6

[a] All yields are based on the amines or ammonium salts used as starting material and the products obtained after purification by flash chromatography.

Sulfinyl- and sulfonyl functionalities are isosteres of ketones and have analogous reactivity to sulfides and ketones (acidic α-proton). These inherent properties made analogues of **10** with such functionalities an interesting target. **13a–e** and **14a–e** were produced by oxidation of the corresponding sulfides (**10a–e**) with *m*-CPBA (Scheme 3). **13a–e** were obtained as mixtures of diastereomers and their ratio determined by ^{1}H NMR. The diastereomers could not be resolved by the standard RP-HPLC protocol.



Scheme 3 Reagents and conditions: (i) *m*-CPBA, CH_2Cl_2, −10 °C; (ii) *m*-CPBA, CH_2Cl_2, RT.

All substances applied in the micro-assay were confirmed as homogeneous by LC-MS (>99% pure at 215 nm). Exceptions were the products **10p** and **10q**, which appeared pure with NMR spectroscopy but contained a strongly UV-absorbing contaminant, tentatively assigned by HRMS as the corresponding sulfoxide impurity. NMR spectroscopy of all sulfonyls, **14a–e**, was also suggestive of pure products but for some of these, homogeneity could not be confirmed by LC-MS.

Biological activity and mechanistic considerations

All experiments made with the LasR system were preceded by tests in a LuxR screening system. The *V. fischeri* QS circuit is less specific, accepting a wider spectrum of ligands (*vide supra*). Therefore, qualitative tests were made to select candidates attractive enough for a more quantitative determination of QS inhibition in *P. aeruginosa* (Table 2). The initial screening employed the QS inhibitor selector system QSIS1, constructed by Rasmussen *et al.*[26] This system indicates if a compound is able to block LuxR mediated QS in the presence of *N*-(3-oxohexanoyl)-L-homoserine lactone, the natural agonist of LuxR. All the positive samples from the first screen were tested with the *las* system from *P. aeruginosa*. A fusion of the *lasB* promoter to *gfp* (ASV), harboured by *P. aeruginosa* PAO1, was used.[38] Grown without inhibitor, this monitor strain developed green fluorescence when the QS systems are activated. If an inhibitor is present in the growth medium, the recorded induction of green fluorescence is decreased or, in case of a strong inhibitor, abolished. The compounds were tested in this system at various concentrations to determine the strength of the inhibitor, in other words to which degree the induction could be prevented. The values given in Table 2 are the concentrations that gave half the induction of an untreated control. The maximum inhibition was also determined at the highest non-toxic concentration.

Screening of **10a–e** and **13a–e** in the LuxR monitor system was successful in all cases but for **13d**. Therefore, **13d** was not screened in the subsequent LasR monitor system. Neither were the sulfonyl analogues (**14a–e**), which were also incapable of antagonizing LuxR. Even though, as outlined above, the LuxR monitor system is more susceptible to structural variations of the signal molecule compared to the LasR monitor system, there is a risk of overlooking hits. However, at this stage all three functionalities; sulfides, sulfoxides and sulfones, were represented with the same acyl groups, usually critical for differentiating the receptor homologues.

The highest activities were observed for **10c** and **13c**, thus the optimal chain length was two carbon atoms shorter than for the natural ligand. This is not necessarily a consequence of pharmacodynamics since it was established a long time ago that the efficiency of diffusion of molecules with a long carbon chain through membranes is to a great extent determined by its length.[39] Inefficient diffusion has been observed also for the AHLs[40] and there is some evidence of an efflux system active in *P. aerugonosa*.[41] Another bias in this part of the screening might be that the metabolic stability towards reduction of the sulfoxide analogues is low[42] and what we are really observing is another less concentrated set of thioether analogues. However, this possibility is contradicted by the total absence of activity for **13d**. In that case, if it is the activity for the sulfoxide scaffold we observed, there is an even greater difficulty for the longer chain derivative **13d** than for **10d** to cross the cell membrane. It seems unlikely that the affinity would drop that much for **13d** and not the other sulfoxides (**13a–c** and **13e**) compared to **10a–e**.

Table 2 QS inhibition of LuxR and LasR controlled gene expression with **3** as reference

Analogue	LuxR[a]	LasR[b]	Maximum induction[e]
3	+	2	10%
6a	+	—[c]	NA[d]
6b	+	—[c]	NA[d]
6c	+	—[c]	NA[d]
6d	+	—[c]	NA[d]
7	+	—[c]	NA[d]
8	+	—[c]	NA[d]
10a	+	—[c]	67%
10b	+	300	33%
10c	+	6	13%
10d	+	50	10%
10e	+	300	33%
10f	+	50	50%
10g	+	100	50%
10h	+	—[c]	67%
10i	+	—[c]	67%
10j	+	—[c]	67%
10k	+	100	50%
10l	+	100	25%
10m	+	150	40%
10n	+	150	50%
10o	+	75	33%
10p	+	100	50%
10q	+	—[c]	NA[d]
10r	+	150	50%
10s	+	150	50%
12a	+	—[c]	NA[d]
12b	+	—[c]	NA[d]
12c	—	NA[d]	NA[d]
13a	+	—[c]	67%
13b	+	300	33%
13c	+	50	50%
13d	—	NA[d]	NA[d]
13e	+	—[c]	67%
14a	—	NA[d]	NA[d]
14b	—	NA[d]	NA[d]
14c	—	NA[d]	NA[d]
14d	—	NA[d]	NA[d]
14e	—	NA[d]	NA[d]

[a] ± indicates positive or negative response in the QSIS1 system, respectively.[26] [b] Concentration (μM) required to lower the activity of a *lasB-gfp* fusion to 50%.[38] [c] The compound was not able to lower activation to 50%. [d] NA = not available. [e] Maximum induction in relation to an untreated control of a *lasB-gfp* fusion in the presence of the highest, non-toxic, concentration of the test compound.

After the initial biological screening of compounds **10a–e**, **13a–e** and **14a–e** it was concluded that the most potent of the three types of functionalities was a thioether. Valuable structure–activity relationships were collected by a larger set of aromatic analogues. This was also an attempt to get around the detrimental effect that a highly lipophilic acyl chain could have on the pharmacokinetic properties but at the same time preserve the same approximate length of the acyl group. At this point, the low potency of **10e** was relevant only in light of more potent analogues. Indeed, both **10f** and **10g**, with a *para*-methyl and -hydroxyl group, respectively, were more potent, the former being the strongest of the aromatic QS inhibitors that were screened. A set of chloro-aryl analogues *ortho* (**10h**), *meta* (**10i**) and *para* (**10j**), were also screened to reveal any steric factors. However, **10h–j** were only active in the LuxR monitor system and therefore it was not possible to quantize the activity. The impact of the thioether functionality as a part of the scaffold could be evaluated by comparing all the 4-substituted phenyl derivatives. Since the activity of a 4-chloro derivative (**10j**) vanished compared to non-substituted **10e**, and both **10f** and **10g** were more potent, this was clearly a $-\sigma$ effect according to Topliss.[43] It is not as clear whether the higher potency of **10f** compared to **10g** can be assigned on the basis of a $+\pi$ effect or if the $-\sigma$ effect have an influence here as well.

Extending **10e** with a methyl group in the 4-position gave a more potent antagonist, but moving the phenyl group further away reduced their potency as inhibitors (**10k** and **10l**) and worsening as in **10m** with a naphthyl group. There are several aromatic residues in the binding-site likely to be responsible for favourable interactions and it should be stressed that the acyl chain of **10b**, an inactive analogue, extends to the same approximate distance as the acyl group of **10f**.

In **10n–s** the lactone moiety had been changed and all analogues were antagonists of LuxR. The design of **10p–s** was based on the acyl chain in potent **10c** but also on structures observed as inducers of QS by Smith *et al.*,[15] structures which had the same cyclic moiety as for **10p–s** but with the same acyl chain as **1**. Their corresponding analogues of **10p** and **10s** were antagonist of LasR, with the same relative potency as that for **10p** and **10s**. The thioether functionality and shorter acyl chain also made **10r** an antagonist of LasR. Likewise, the cyclopentyl and cyclohexyl analogues **10n** and **10o**, lacking the 1-oxo group, and thus their ability to form hydrogen bonds to residue Trp57 in LasR, were antagonists.

Even though these findings may provide aid in future architectures, it is tempting to return to the strongest antagonist **10c**. It was several times more potent than the previously reported **10d**[32] and **10l**[31], also included in this work. Whereas Reverchon *et al.* screened **10l** in a LuxR system and found it to be a potent QS inhibitor,[31] it had low potency compared to **10c** in our LasR assay. The key feature of this type of structure is a shorter acyl chain than in **1** and a 3′-oxo group substituted with a sulfur atom. Obviously, the water-mediated hydrogen bond involving receptor residues Gly38 (Ala38 in TraR) and Ser129 (Thr129 in TraR), which is important for signal binding, is extremely unlikely if the lactone/amide part of **10c** is positioned like **1** in the binding-pocket. According to our hypothesis, the β-sheet, to which residues Gly38 (Ala38 in TraR) and Ser129 (Thr129 in TraR) belong, will then be forced from the position where it is found in an active receptor–ligand complex (the activated complex is actually a dimer that binds to an operon[1]). If this is the reason why LasR is blocked in our biological assays, one may wonder if compounds like **1** lacking the 3′-oxo functionality, despite their less favourable physicochemical properties, also could be potent inhibitors. This type of compounds are known as inhibitors but they seem to be considerably weaker as such.[10] Even though a methylene and a sulfide group are bioisosteric, the acyl chain of **10c** is tilted because the C–C–C and C–S–C bond angles (109 and 105°, respectively) differ and so do the C–C and C–S bond lengths (1.54 and 1.82 Å, respectively).[44] Importantly, the ability of a sulfide group to stabilize α-anions is considered to be a significant difference. Electronic factors certainly do have influence on the inert binding caused by the thioethers. Otherwise the aromatic analogues **10e–n**, with different geometry than **10a–d**, would not contain the same approximate potencies.

The same requirements were not fulfilled by the conformationally restricted garlic-derived analogues **12a–c**, most strikingly **12a**, for which the effect should be stronger having two sulfur atoms in the β-position. Both **12a** and **12b** were antagonists of LuxR but not LasR and this was also the case for the QS inhibiting natural products found in garlic, **7** and **8**. Compound **12c** did not inhibit any of the two QS monitor systems it was screened against. The grafting strategy outlined at the end of the introduction was not successful since the two separate components, **7** and **10c**, each had the same or higher potency, respectively. The reason was perhaps the wrong linker between the two, or that the double bond in **7** was absent in **12a–c**.

Compound **3** was included as a reference in Table 2 since it is the most potent antagonist of LasR to date. However, the poor chemical stability observed for **3** under the conditions used makes it a less suitable candidate.

Conclusion

We have designed, synthesized and screened 32 compounds in two QS systems designed from the LuxR (*V. fischeri*) and LasR (*P. aeruginosa*) systems. A significant portion of these compounds proved to inhibit either one or both of the QS systems. When including the compounds lacking activity, the observed structure–activity relationships (SARs) gave rise to a preliminary hypothesis explaining the notable potency of the sulfide class of analogues. Thus, strong inhibition is obtained by competitive binding of 3′-sulfide analogues unable to interact with the β-sheet but with the ability to form other important contacts inside the binding-pocket. This includes van der Waals interactions between the α-helices and the lipophilic acyl group. Apparently, these contacts are critical for antagonizing receptors like LuxR or LasR. Furthermore, several natural products isolated from garlic were identified as inhibitors of QS without affecting microbial growth. Interestingly, these inhibitors were structurally different from the AHLs and the above-mentioned sulfide AHL analogues and a small series of conformationally restricted analogues, in which the sulfide AHL motif had been grafted together with a dithiane moiety (representing **7**), were synthesized. However, these garlic-derived structures were less potent than their more flexible sulfide analogues.

Experimental

Biology

The QSIS1 assay was performed as described elsewhere.[26] The concentration of *N*-(3-oxohexanoyl)-L-homoserine lactone was maintained constant at 100 nM during screening, whereas the concentrations for the analogues were increased in a gradient never exceeding 10 mM.

The *lasB-gfp* assay was performed in accordance with Hentzer *et al.*[38] This monitor system was prepared from a wild-type strain and therefore the concentration of **1** was maintained at a natural level.

Chemistry

Commercially available reagents (Aldrich) were used without further purification unless otherwise noted. Starting materials **11a** and **11b** were prepared as previously reported.[36,37] Solvents used for the synthesis were of analytical grade, dried over activated 4 Å molecular sieves when necessary (all solvents used under dry conditions had a water content <25 ppm). Triethylamine was distilled from P_2O_5 and stored over 4 Å molecular sieves. MilliQ water was used for RP-HPLC. Analytical

TLC was performed using pre-coated silica gel 60 F_{254} plates and visualized using either UV light, phosphomolybdic acid or potassium permanganate stain. Merck 60H silica gel was used for VLC. Flash chromatography was performed automatically on a Biotage Quad 3+ Parallel Flash Purification™ system with pre-packed columns (KP-SIL, 32–63 μm, 60 Å). Parallel reactions requiring cooling were performed in a Radley Greenhouse Parallel Synthesizer™ block. Corrected melting points were measured in open capillary tubes on a Gallenkamp electrothermal melting point apparatus. Optical rotation measurements were obtained on an Optical Activity Ltd. AA-1000 polarimeter using a 0.5 dm path length micro cell. Infrared spectrum was collected on a Perkin Elmer System 2000 FT-IR instrument. Preparative RP-HPLC was performed on a Waters 600 system equipped with a Waters 996 photodiode array (PDA) detector and three consecutive columns (40 × 100 mm prep. NOVA Pak HR C18 6 μm, 60 Å) with a flow of 35 ml min^{-1}. 80% → 50% A 0–29 min and then 50% → 0% A 30–65 min (A: water B: CH_3CN). ^1H and ^{13}C NMR spectral data was recorded on a Bruker Avance 300 using the deuterated solvent as lock. Chemical shifts are reported in ppm relative to the residual solvent peak (^1H NMR) or the solvent peak (^{13}C NMR) as the internal standard. GC-MS was carried out on a Trio 2 VG Masslab fitted with a 5809A Hewlett Packard gas chromatograph. Accurate mass and purity determinations were performed on a Waters 2795 system equipped with a Waters 996 PDA detector and a Waters Symmetry C18 Column (2.1 × 50 mm, 3.5 μm) with a flow of 0.2 ml min^{-1}. 100% → 0% A 0–10 min (A: 0.1% aq formic acid, B: 95% CH_3CN in 0.05% aq formic acid). The connected Micromass LCT apparatus was equipped with an AP-ESI probe calibrated with Leu-Enkephalin (556.2771 g mol^{-1}).

Separation and identification of garlic components. Garlic (1800 g) chopped in pieces was soaked in toluene (3750 ml) and stirred overnight. The toluene was decanted and concentrated to dryness. The remaining oil was partitioned between hexane and water–MeOH and the clear hexane-phase dried (Na_2SO_4) and concentrated *in vacuo* to afford 3.61 g of a viscous yellow oil. Flash chromatography (eluent: 0–10% EtOAc in hexane) yielded several bioactive fractions from which six known garlic compounds (**6a–d**, **7** and **8**) were identified by comparison of data (NMR spectroscopy and GC-MS analysis) with authentic samples. The authentic samples, either commercially available or accessible through known synthetic procedures,[27,28] served to verify that **6a–d**, **7** and **8**[45] were antagonists of LuxR and that **6a–d** were antimicrobials.

L-Homoserine lactone hydrobromide (5). A mixture of bromoacetic acid (1.54 g, 11.0 mmol) and L-methionine (1.52 g, 10.0 mmol) in 14.4 ml of an H_2O–2-propanol–AcOH mixture (5 : 5 : 2 v/v) was refluxed for 9 h. The solvent was then removed at reduced pressure. Further drying overnight by vacuum pump left a beige semi-solid. It was partly dissolved in 10 ml of a 4 : 1 mixture (v/v) of 2-propanol–HBr (30% in AcOH). The title compound was collected by filtration and the purification procedure repeated starting from evaporation of the orange filtrate to dryness. Compound **5** was collected as a white powder after drying of both portions *in vacuo* (m = 1.47 g, 81%), mp 226–228 °C (lit.ref. 34 mp 229–231 °C); $[\alpha]_D^{22} = -19.5$ ($c = 0.05$, H_2O); lit.ref. 34 $[\alpha]_D^{25} = -24.4$ ($c = 0.087$, H_2O). All other physical and analytical data were in agreement with those previously reported.[34]

***N*-Bromoacetyl-L-homoserine lactone (9).** Under dry conditions, triethylamine (290 μl, 2.10 mmol) was added to a suspension of **5** (182 mg, 1.00 mmol) in CH_2Cl_2 (50 ml) cooled at −78 °C. 2-Bromoacetyl bromide (96 μl, 1.10 mmol) was then added dropwise and the temperature allowed to rise to RT during a period of 1 h. Evaporation to dryness gave a white semi-solid. It was partly dissolved by EtOAc and the salt remaining separated off by filtration ($HNEt_3^+Br^-$ exclusively according to ^1H NMR). The product was again evaporated to dryness and purified by flash chromatography (eluent: 1% MeOH in CH_2Cl_2,). Yield: 155 mg (70%); mp 126–129 °C; $[\alpha]_D^{22} = 20.5$ ($c = 0.0074$, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.02 (br s, exch., 1 H, N*H*), 4.62–4.25 (m, 3 H, CH_2-5 and C*H*-3), 3.91 (ABq, $\Delta = 7$ Hz, $J_{AB} = 11$ Hz, 2 H, CH_2-2′), 2.87–2.77 (m, 1 H, CH_2-4), 2.30–2.14 (m, 1 H, CH_2-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.3 (1 C, C-2), 166.1 (1 C, C-1′), 65.8 (1 C, C-5), 49.5 (1 C, C-3), 29.7, 28.0 (2 C, C-4 and C-2′); LC-MS (ESI) $t_R = 1.18$ min (m/z 222 $[MH]^+$); HRMS $(M + H)^+$ calcd. for $C_6H_9BrNO_3$ 221.9766, found 221.9787.

General procedure for the preparation of 10a, 10b and 10d by method A. At ambient temperature, triethylamine (1.1 equiv.) was added to a 0.05 M solution of **9** (1.0 equiv.) in abs. EtOH turning it dark beige. Half an hour of stirring was followed by addition of thiol (1.1 equiv., R^2-SH, Table 1) and then another 3 h of stirring was ended by addition of 10% HCl. Extraction (3 × CH_2Cl_2) of the aqueous phase and drying of the combined organic phases ($MgSO_4$) gave the pure sulfides after removal of solvent *in vacuo* and purification by flash chromatography (eluent: CH_2Cl_2).

***N*-(Propylsulfanylacetyl)-L-homoserine lactone (10a).** 222 mg **9** gave 101 mg of a white solid (46% yield, >99% purity); mp 70–71 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.38 (br d, $^3J = 5$ Hz, 1 H, N*H*), 4.64–4.23 (m, 3 H, CH_2-5 and C*H*-3), 3.25 (ABq, $\Delta = 6$ Hz, $J_{AB} = 17$ Hz, 2 H, CH_2-2′), 2.81–2.72 (m, 1 H, CH_2-4), 2.54 (t, $^3J = 6$ Hz, 2 H, CH_2-4′), 2.27–2.15 (m, 1 H, CH_2-4), 1.61 (m, 2 H, CH_2-5′), 0.97 (t, $^3J = 7$ Hz, 3 H, CH_3′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.9 (1 C, C-2), 169.8 (1 C, C-1′), 65.8 (1 C, C-5), 49.2 (1 C, C-3), 35.7, 35.0, 29.9, 22.4 (4 C, C-4, C-2′ and 2 × CH_2′), 13.3 (1 C, CH_3′); LC-MS (ESI) $t_R = 1.35$ min (m/z 218 $[MH]^+$); HRMS $(M + H)^+$ calcd. for $C_9H_{16}NO_3S$ 218.0851, found 218.0845.

***N*-(Pentylsulfanylacetyl)-L-homoserine lactone (10b).** 1.60 g **9** gave 1.18 g of a white solid (67% yield, >99% purity); mp 73–75 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.35 (br d, $^3J = 5$ Hz, 1 H, N*H*), 4.64–4.25 (m, 3 H, CH_2-5 and C*H*-3), 3.27 (ABq, $\Delta = 6$ Hz, $J_{AB} = 17$ Hz, 2 H, CH_2-2′), 2.85–2.76 (m, 1 H, CH_2-4), 2.57 (t, $^3J = 6$ Hz, 2 H, CH_2-4′), 2.26–2.11 (m, 1 H, CH_2-4), 1.65–1.55 (m, 2 H, CH_2-5′), 1.41–1.25 (m, 4 H, 2 × CH_2′), 0.89 (t, $^3J = 7$ Hz, 3 H, CH_3′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.8 (1 C, C-2), 169.7 (1 C, C-1′), 65.9 (1 C, C-5), 49.2 (1 C, C-3), 35.9, 33.1, 30.9, 30.1, 28.8, 22.2 (6 C, C-4, C-2′ and 4 × CH_2′), 13.9 (1 C, CH_3′); LC-MS (ESI) $t_R = 6.00$ min (m/z 246 $[MH]^+$); HRMS $(M + H)^+$ calcd. for $C_{11}H_{20}NO_3S$ 246.1164, found 246.1145.

***N*-(Nonylsulfanylacetyl)-L-homoserine lactone (10d).** 312 mg **9** gave 149 mg of a white solid (35% yield, >99% purity); mp 94–95 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.34 (br d, $^3J = 5$ Hz, 1 H, N*H*), 4.64–4.25 (m, 3 H, CH_2-5 and C*H*-3), 3.27 (ABq, $\Delta = 6$ Hz, $J_{AB} = 17$ Hz, 2 H, CH_2-2′), 2.86–2.77 (m, 1 H, CH_2-4), 2.57 (t, $^3J = 6$ Hz, 2 H, CH_2-4′), 2.26–2.11 (m, 1 H, CH_2-4), 1.64–1.54 (m, 2 H, CH_2-5′), 1.39–1.26 (m, 12 H, 6 × CH_2′), 0.88 (t, $^3J = 7$ Hz, 3 H, CH_3′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.7 (1 C, C-2), 169.7 (1 C, C-1′), 65.9 (1 C, C-5), 49.3 (1 C, C-3), 35.9, 33.1, 31.8, 30.2, 29.4, 29.2, 29.2, 29.1, 28.7, 22.7 (10 C, C-4, C-2′ and 8 × CH_2′), 14.1 (1 C, CH_3′); LC-MS (ESI) $t_R = 7.65$ min (m/z 302 $[MH]^+$); HRMS $(M + H)^+$ calcd. for $C_{15}H_{28}NO_3S$ 302.1790, found 302.1770.

General procedure for the preparation of 10e–m (modified method A). Method A was also applied for the aromatic analogues **10e–m** but the work-up preceding flash chromatography was abandoned. Instead the crude was isolated by removal of solvent after completion of the reaction using a stream of air. Then it was dissolved in least amount of CH_2Cl_2 and absorbed onto silica gel for purification by flash chromatography.

***N*-(Phenylsulfanylacetyl)-L-homoserine lactone (10e).** 56 mg **9** gave 47 mg of a white solid (75% yield, >99% purity) after flash chromatography (eluent: 1% MeOH in CH_2Cl_2); mp 87–89 °C;

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36–7.22 (m, 5H, S–C_6H_5), 7.21 (br s, exch., 1 H, N*H*), 4.56–4.21 (m, 3 H, CH_2-5 and C*H*-3), 3.67 (s, 2 H, CH_2-2′), 2.80–2.70 (m, 1 H, CH_2-4), 2.09–1.95 (m, 1 H, CH_2-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.7 (1 C, C-2), 168.8 (1 C, C-1′), 134.1, 129.3, 128.9, 127.1 (6 C, S–C_6H_5), 65.9 (1 C, C-5), 49.3 (1 C, C-3), 37.6 (1 C, C-2′), 29.9 (1 C, C-4); LC-MS (ESI) t_R = 5.51 min (*m*/*z* 252 $[MH]^+$); HRMS (M + H)$^+$ calcd. for $C_{12}H_{14}NO_3S$ 252.0694, found 252. 0738.

***N*-(*p*-Tolylsulfanylacetyl)-L-homoserine lactone (10f).** 44 mg **9** gave 47 mg of a white solid (88% yield, >99% purity) after flash chromatography (eluent: 1% MeOH in CH_2Cl_2); mp 103–106 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (br s, exch., 1 H, N*H*), 7.26 (d, ^3J = 8 Hz, 2H, Ar-*H*), 7.21 (d, ^3J = 8 Hz, 2H, Ar-*H*), 4.57–4.19 (m, 3 H, CH_2-5 and C*H*-3), 3.60 (s, 2 H, CH_2-2′), 2.75–2.65 (m, 1 H, CH_2-4), 2.13–1.98 (m, 1 H, CH_2-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.8 (1 C, C-2), 169.0 (1 C, C-1′), 137.4, 130.4, 130.1, 129.7 (6 C, Ar), 65.9 (1 C, C-5), 49.3 (1 C, C-3), 38.3 (1 C, C-2′), 29.8 (1 C, C-4), 21.0 (1 C, $ArCH_3$); LC-MS (ESI) t_R = 5.83 min (*m*/*z* 266 $[MH]^+$); HRMS (M + H)$^+$ calcd. for $C_{13}H_{16}NO_3S$ 266.0851, found 266.0831.

***N*-(*p*-Hydroxyphenylsulfanylacetyl)-L-homoserine lactone (10g).** 44 mg **9** gave 19 mg of a colourless oil (36% yield, >99% purity) after flash chromatography (eluent: 1% MeOH in CH_2Cl_2). ^{1}H NMR (300 MHz, CD_3OD) δ 7.33 (d, ^3J = 9 Hz, 2H, Ar-*H*), 6.72 (d, ^3J = 9 Hz, 2H, Ar-*H*), 4.58–4.20 (m, 3 H, CH_2-5 and C*H*-3), 3.43 (s, 2 H, CH_2-2′), 2.49–2.39 (m, 1 H, CH_2-4), 2.21–2.07 (m, 1 H, CH_2-4); ^{13}C NMR (75 MHz, CD_3OD) δ 177.1 (1 C, C-2), 172.2 (1 C, C-1′), 159.1, 135.8, 135.5, 124.4 (6 C, Ar), 67.2 (1 C, C-5), 50.1 (1 C, C-3), 41.0 (1 C, C-2′), 29.5 (1 C, C-4); LC-MS (ESI) t_R = 4.92 min (*m*/*z* 268 $[MH]^+$); HRMS (M + H)$^+$ calcd. for $C_{12}H_{14}NO_4S$ 268.0644, found 268.0587.

***N*-(*o*-Chlorophenylsulfanylacetyl)-L-homoserine lactone (10h).** 44 mg **9** gave 53 mg of a white solid (92% yield, >99% purity) after flash chromatography (eluent: 1% MeOH in CH_2Cl_2); mp 140–141 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.14 (m, 5H, Ar-*H* and N*H*), 4.55–4.19 (m, 3 H, CH_2-5 and C*H*-3), 3.71 (s, 2 H, CH_2-2′), 2.74–2.65 (m, 1 H, CH_2-4), 2.14–1.99 (m, 1 H, CH_2-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.5 (1 C, C-2), 168.3 (1 C, C-1′), 133.6, 133.4, 129.9, 128.8, 127.8, 127.7 (6 C, Ar), 65.8 (1 C, C-5), 49.3 (1 C, C-3), 36.4 (1 C, C-2′), 29.7 (1 C, C-4); LC-MS (ESI) t_R = 5.85 min (*m*/*z* 286 $[MH]^+$); HRMS (M + H)$^+$ calcd. for $C_{12}H_{13}ClNO_3S$ 286.0305, found 286.0289.

***N*-(*m*-Chlorophenylsulfanylacetyl)-L-homoserine lactone (10i).** 44 mg **9** gave 53 mg of a white solid (92% yield, >99% purity) after flash chromatography (eluent: 1% MeOH in CH_2Cl_2); mp 98–99 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32–7.19 (m, 5H, Ar-*H* and N*H*), 4.60–4.20 (m, 3 H, CH_2-5 and C*H*-3), 3.66 (s, 2 H, CH_2-2′), 2.75–2.66 (m, 1 H, CH_2-4), 2.15–2.01 (m, 1 H, CH_2-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.8 (1 C, C-2), 168.3 (1 C, C-1′), 136.3, 134.9, 130.3, 128.5, 127.1, 126.7 (6 C, Ar), 65.9 (1 C, C-5), 49.3 (1 C, C-3), 37.2 (1 C, C-2′), 29.7 (1 C, C-4); LC-MS (ESI) t_R = 5.96 min (*m*/*z* 286 $[MH]^+$); HRMS (M + H)$^+$ calcd. for $C_{12}H_{13}ClNO_3S$ 286.0305, found 286.0296.

***N*-(*p*-Chlorophenylsulfanylacetyl)-L-homoserine lactone (10j).** 44 mg **9** gave 53 mg of a white solid (92% yield, >99% purity) after flash chromatography (eluent: 1% MeOH in CH_2Cl_2); mp 130–131 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31–7.25 (m, 4H, Ar-*H*), 7.22 (br s, exch., 1 H, N*H*), 4.58–4.20 (m, 3 H, CH_2-5 and C*H*-3), 3.63 (s, 2 H, CH_2-2′), 2.75–2.67 (m, 1 H, CH_2-4), 2.14–1.99 (m, 1 H, CH_2-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.8 (1 C, C-2), 168.5 (1 C, C-1′), 133.2, 132.6, 130.5, 129.4 (6 C, Ar), 65.9 (1 C, C-5), 49.3 (1 C, C-3), 37.8 (1 C, C-2′), 29.8 (1 C, C-4); LC-MS (ESI) t_R = 5.96 min (*m*/*z* 286 $[MH]^+$); HRMS (M + H)$^+$ calcd. for $C_{12}H_{13}ClNO_3S$ 286.0305, found 286.0290.

***N*-(Benzylsulfanylacetyl)-L-homoserine lactone (10k).** 44 mg **9** gave 52 mg of a white solid (98% yield, >99% purity) after flash chromatography (eluent: 1% MeOH in CH_2Cl_2); mp 77–79 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32–7.24 (m, 5H, Ar-*H*), 7.18 (br s, exch., 1 H, N*H*), 4.54–4.21 (m, 3 H, CH_2-5 and C*H*-3), 3.77 (s, 2 H, CH_2-2′), 3.16 (s, 2 H, CH_2-2′), 2.80–2.70 (m, 1 H, CH_2-4), 2.13–1.99 (m, 1 H, CH_2-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.9 (1 C, C-2), 169.3 (1 C, C-1′), 136.9, 129.1, 128.7, 127.4 (6 C, Ar), 65.9 (1 C, C-5), 49.1 (1 C, C-3), 37.0, 34.9 (2 C, C-2′ and C-4′), 29.8 (1 C, C-4); LC-MS (ESI) t_R = 5.68 min (*m*/*z* 266 $[MH]^+$); HRMS (M + H)$^+$ calcd. for $C_{13}H_{16}NO_3S$ 266.0851, found 266.0847.

***N*-(Phenethylsulfanylacetyl)-L-homoserine lactone (10l).** 44 mg **9** gave 55 mg of a white solid (99% yield, >99% purity) after flash chromatography (eluent: 1% MeOH in CH_2Cl_2); mp 67–70 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32–7.19 (m, 6H, Ar-*H* and N*H*), 4.61–4.22 (m, 3 H, CH_2-5 and C*H*-3), 3.26 (s, 2 H, CH_2-2′), 2.94–2.81 (m, 4 H, CH_2-4′ and CH_2-5′), 2.76–2.68 (m, 1 H, CH_2-4), 2.22–2.07 (m, 1 H, CH_2-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.8 (1 C, C-2), 169.5 (1 C, C-1′), 139.7, 128.5, 128.5, 126.5 (6 C, Ar), 65.8 (1 C, C-5), 49.1 (1 C, C-3), 35.8, 35.4, 34.3 (3 C, C-2′, C-4′ and C-5′), 29.7 (1 C, C-4); LC-MS (ESI) t_R = 5.92 min (*m*/*z* 280 $[MH]^+$); HRMS (M + H)$^+$ calcd. for $C_{14}H_{18}NO_3S$ 280.1007, found 280.0978.

***N*-(Naphthyl-2-sulfanylacetyl)-L-homoserine lactone (10m).** 44 mg **9** gave 55 mg of a white solid (91% yield, >99% purity) after flash chromatography (eluent: 1% MeOH in CH_2Cl_2); mp 133–134 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79–7.39 (m, 7H, Ar-*H*), 7.35 (br d, ^3J = 6 Hz, 1 H, N*H*), 4.55–4.33 (m, 3 H, CH_2-5 and C*H*-3), 3.74 (s, 2 H, CH_2-2′), 2.67–2.58 (m, 1 H, CH_2-4), 2.08–1.93 (m, 1 H, CH_2-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.7 (1 C, C-2), 168.7 (1 C, C-1′), 133.7, 132.0, 131.5, 128.9, 127.6, 127.3, 127.3, 126.8, 126.6, 126.2, (10 C, Ar), 65.8 (1 C, C-5), 49.3 (1 C, C-3), 37.5 (1 C, C-2′), 29.6 (1 C, C-4); LC-MS (ESI) t_R = 6.17 min (*m*/*z* 302 $[MH]^+$); HRMS (M + H)$^+$ calcd. for $C_{16}H_{16}NO_3S$ 302.0851, found 302.0832.

***N*-(Heptylsulfanylacetyl)-L-homoserine lactone (10c) by method B.** Under dry conditions triethylamine (3.07 ml, 22.0 mmol) was added to a suspension of **5** (1.86 g, 10.0 mmol) in CH_2Cl_2 (50 ml) at RT. While cooling at, −20 °C 2-bromoacetylbromide (11.0 mmol, 0.958 ml) was added dropwise and the mixture was then allowed to warm to ambient temperature during a period of 1.5 h whereupon a white solid appeared. After another 2.5 h of stirring, additional triethylamine (1.54 ml, 11.0 mmol) was added together with 1-heptanethiol (1.69 ml, 11.0 mmol), in that order. The reaction was ended after 17 h by washing the mixture with 10% HCl (25 ml) and the organic phase was dried ($MgSO_4$), filtrated and evaporated to dryness. **10c** was isolated as a white solid (1.30 g, 46% yield, >99% purity) after VLC (eluent: CH_2Cl_2) in); mp 86–89 °C; $[a]_D^{22}$ = −9.6 (*c* = 0.084, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 3307 (CONH), 2924 (CH), 1774 (ring CO), 1649 (amide CO), 1549, 1176; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (br d, ^3J = 5 Hz, 1 H, N*H*), 4.64–4.24 (m, 3 H, CH_2-5 and C*H*-3), 3.26 (ABq, Δ = 6 Hz, J_{AB} = 17 Hz, 2 H, CH_2-2′), 2.84–2.75 (m, 1 H, CH_2-4), 2.56 (t, ^3J = 6 Hz, 2 H, CH_2-4′), 2.26–2.14 (m, 1 H, CH_2-4), 1.63–1.54 (m, 2 H, CH_2-5′), 1.38–1.25 (m, 8 H, 4 × CH_2′), 0.87 (t, ^3J = 7 Hz, 3 H, CH_3′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.8 (1 C, C-2), 169.8 (1 C, C-1′), 65.9 (1 C, C-5), 49.2 (1 C, C-3), 35.8, 33.1, 31.6, 30.1, 29.1, 28.8, 28.7, 22.5 (8 C, C-4, C-2′ and 6 × CH_2′), 14.0 (1 C, CH_3′); LC-MS (ESI) t_R = 6.79 min (*m*/*z* 274 $[MH]^+$); HRMS (M + H)$^+$ calcd. for $C_{13}H_{24}NO_3S$ 274.1477, found 274.1481.

General procedure for the preparation of 10n–q by modified method B. While cooling a mixture of triethylamine (614 µl, 4.4 mmol. In the synthesis of **10p** and **10q** only 2.2 mmol triethylamine was added initially since these starting materials were not salts) and amine or ammonium salt (2.0 mmol) in CH_2Cl_2 (2 ml) under dry conditions at −20 °C, 2-bromoacetylbromide (2.2 mmol, 196 µl) was added dropwise. The mixture was then allowed to warm to ambient temperature during a period of

1 h. Additional triethylamine (307 μl, 2.2 mmol) was added together with 1-heptanethiol (355 μl, 2.2 mmol) in that order. After another 24 h of stirring the crude was isolated by removal of solvent using a stream of air. It was then absorbed onto silica gel by aid of the least amount of chromatography eluent possible (the triethylammonium halide salt was left behind by filtration) for purification by flash chromatography.

***N*-Cyclopentyl-2-heptylsulfanyl-acetamide (10n).** oil. 27% yield (>99% purity) after flash chromatography (eluent: 10% EtOAc in hexane). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95 (br s, exch., 1 H, N*H*), 4.28–4.16 (m, 1 H, C*H*-1), 3.22 (s, 2 H, CH_2-2′), 2.50 (t, $^3J = 8$ Hz, 2 H, CH_2-4′), 1.70–1.27 (m, 18 H, CH_2-2, CH_2-3, CH_2-4, CH_2-5 and 5 × CH_2′), 0.88 (t, $^3J = 6$ Hz, 3 H, CH_3′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.3 (1 C, C-1′), 51.3 (1 C, C-1), 36.1, 33.1, 32.8, 31.5, 29.1, 28.6, 28.5, 23.5, 22.4 (11 C, C-2, C-3, C-4, C-5 and 7 × CH_2′), 13.9 (1 C, CH_3′); LC-MS (ESI) $t_R = 8.04$ min (*m*/*z* 258 $[MH]^+$); HRMS $(M + H)^+$ calcd. for $C_{14}H_{28}NOS$ 258.1892, found 258.1911, $(M + CH_3CN + H)^+$ calcd. for $C_{16}H_{31}N_2OS$ 299.2157, found 299.2154.

***N*-Cyclohexyl-2-heptylsulfanyl-acetamide 10o.** White solid. 37% yield (>99% purity) after flash chromatography (eluent: 10% EtOAc in hexane); mp 55–56 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95 (br d, $^3J = 8$ Hz, 1 H, N*H*), 3.82–3.68 (m, 1 H, C*H*-1), 3.16 (s, 2 H, CH_2-2′), 2.47 (t, $^3J = 8$ Hz, 2 H, CH_2-4′), 1.89–1.10 (m, 20 H, CH_2-2, CH_2-3, CH_2-4, CH_2-5, CH_2-6 and 5 × CH_2′), 0.88 (t, $^3J = 7$ Hz, 3 H, CH_3′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.7 (1 C, C-1′), 48.2 (1 C, C-1), 36.3, 33.1, 32.9, 32.9, 31.6, 29.2, 28.7, 28.6, 25.4, 24.6, 24.6, 22.5 (12 C, C-2, C-3, C-4, C-5 C-6 and 7 × CH_2′), 13.9 (1 C, CH_3′); LC-MS (ESI) $t_R = 8.51$ min (*m*/*z* 272 $[MH]^+$); HRMS $(M + H)^+$ calcd. for $C_{15}H_{30}NOS$ 272.2048, found 272.2063, $(M + CH_3CN + H)^+$ calcd. for $C_{17}H_{33}N_2OS$ 313.2314, found 313.2301.

(±)-2-Heptylsulfanyl-*N*-(*trans*-2-hydroxy-cyclopentyl)-acetamide (10p). Yellow oil. 3% yield after flash chromatography (eluent: 2% MeOH in CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.02 (br s, exch., 1 H, N*H*), 4.02–3.80 (m, 2 H, C*H*OH and C*H*NH), 3.22 (s, 2 H, CH_2-2′), 2.53 (t, $^3J = 8$ Hz, 2 H, CH_2-4′), 2.22–1.26 (m, 16 H, CH_2-3, CH_2-4, CH_2-5 and 5 × CH_2′), 0.88 (t, $^3J = 6$ Hz, 3 H, CH_3′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1 (1 C, C-1′), 79.5 (1 C, C-1), 60.8 (1 C, C-2), 36.0, 33.3, 32.6, 31.7, 30.3, 29.2, 28.8, 28.7, 22.6, 21.3, (10 C, C-3, C-4, C-5 and 7 × CH_2′), 13.9 (1 C, CH_3′); LC-MS (ESI) $t_R = 6.85$ min (*m*/*z* 274 $[MH]^+$); HRMS $(M + H)^+$ calcd. for $C_{14}H_{28}NO_2S$ 274.1841, found 274.1856. The sulfoxide impurity of **10p** was also identified by LC-MS (ESI) $t_R = 5.70$ min (*m*/*z* 290 $[MH]^+$); HRMS $(M + H)^+$ calcd. for $C_{14}H_{28}NO_3S$ 290.1790, found 290.1914.

(±)-2-Heptylsulfanyl-*N*-(*trans*-2-hydroxy-cyclohexyl)-acetamide (10q). Yellow oil. 6% yield after flash chromatography (eluent: 2% MeOH in CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.02 (br d, $^3J = 7$ Hz, 1 H, N*H*), 3.69–3.28 (m, 2 H, C*H*OH and C*H*NH), 3.24 (ABq, $\Delta = 8$ Hz, $J_{AB} = 17$ Hz, 2 H, CH_2-2′), 2.53 (t, $^3J = 8$ Hz, 2 H, CH_2-4′), 2.08–1.17 (m, 18 H, CH_2-3, CH_2-4, CH_2-5, CH_2-6 and 5 × CH_2′), 0.86 (t, $^3J = 6$ Hz, 3 H, CH_3′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6 (1 C, C-1′), 75.1 (1 C, C-1), 55.9 (1 C, C-2), 36.2, 34.4, 33.1, 31.6, 31.3, 29.1, 28.8, 28.7, 24.5, 24.0, 22.5, (11 C, C-3, C-4, C-5, C-6 and 7 × CH_2′), 14.0 (1 C, CH_3′); LC-MS (ESI) $t_R = 7.07$ min (*m*/*z* 288 $[MH]^+$); HRMS $(M + H)^+$ calcd. for $C_{15}H_{30}NO_2S$ 288.1997, found 288.1989. The sulfoxide impurity of **10q** was also identified by LC-MS (ESI) $t_R = 5.91$ min (*m*/*z* 304 $[MH]^+$); HRMS $(M + H)^+$ calcd. for $C_{14}H_{28}NO_3S$ 304.1946, found 304.1859.

Oxidation of 10p and 10q. Swern reagent was prepared under dry conditions by adding DMSO (0.080 mmol, 5.7 μl) to a CH_2Cl_2 solution (0.2 ml) of oxalyl chloride (0.040 mmol, 3.6 μl) cooled at −78 °C. The mixture was transferred immediately and dropwise to another CH_2Cl_2 solution (0.2 ml) containing **10p** or **10q** (0.037 mmol) keeping the temperature constant at −78 °C. After 15 min triethylamine (0.16 mmol, 22 μl) was added and as the mixture warmed to RT it turned opaque. The 0.1 M HCl (10 ml), added to the reaction mixture after another 10 min of stirring, was extracted (3 × 10 ml EtOAc) and the combined organic phases washed (10 ml sat. $NaHCO_3$ and 10 ml brine in that order) and dried ($MgSO_4$). Concentration to dryness under reduced pressure gave a crude resolved by flash chromatography (1% MeOH in CH_2Cl_2).

(±)-2-Heptylsulfanyl-*N*-(2-oxo-cyclopentyl)-acetamide (10r). Yellow oil. 34% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20 (br s, exch., 1 H, N*H*), 4.22–4.13 (m, 1 H, C*H*NH), 3.25 (ABq, $\Delta =$ 6 Hz, $J_{AB} = 17$ Hz, 2 H, CH_2-2′), 2.66–1.26 (m, 18 H, CH_2-3, CH_2-4, CH_2-5 and 6 × CH_2′), 0.88 (t, $^3J = 7$ Hz, 3 H, CH_3′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 214.5 (1 C, C-1), 169.4 (1 C, C-1′), 58.0 (1 C, C-2), 36.0, 34.9, 33.1, 31.7, 29.8, 29.1, 28.8, 28.7, 22.6, 18.1 (10 C, C-3, C-4, C-5 and 7 × CH_2′), 13.9 (1 C, CH_3′); LC-MS (ESI) $t_R = 7.10$ min (*m*/*z* 272 $[MH]^+$); HRMS $(M + H)^+$ calcd. for $C_{14}H_{26}NO_2S$ 272.1684, found 272.1686.

(±)-2-Heptylsulfanyl-*N*-(2-oxo-cyclohexyl)-acetamide (10s). Yellow oil. 44% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 (br d, $^3J = 7$ Hz, 1 H, N*H*), 4.53–4.44 (m, 1 H, C*H*NH), 3.23 (s, 2 H, CH_2-2′), 2.66–1.25 (m, 20 H, CH_2-3, CH_2-4, CH_2-5, CH_2-6 and 6 × CH_2′), 0.87 (t, $^3J = 7$ Hz, 3 H, CH_3′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 207.2 (1 C, C-1), 168.7 (1 C, C-1′), 58.1 (1 C, C-2), 41.2, 36.2, 35.3, 33.1, 31.7, 29.1, 28.8, 28.7, 27.9, 24.1, 22.6, (11 C, C-3, C-4, C-5, C-6 and 7 × CH_2′), 14.1 (1 C, CH_3′); LC-MS (ESI) $t_R = 7.59$ min (*m*/*z* 286 $[MH]^+$); HRMS $(M + H)^+$ calcd. for $C_{15}H_{28}NO_2S$ 286.1841, found 286.1888.

General procedure for the synthesis of 12a–c. While cooling a slurry of acid (**11a** or **10b**, 1.0 equiv.) and amine or ammonium salt (1.0 equiv.) in CH_2Cl_2 (5 ml) under dry conditions at 0 °C, isobutyl chloroformate (1.0 equiv.) and triethylamine (2.2 equiv.). In the synthesis of **12c** only 1.1 equiv. triethylamine was added since this starting material was no salt) was added dropwise and simultaneously over a period of 1 h. The suspension was stirred for another 2 h while the temperature was allowed to rise to RT and then 5 ml of 4 M HCl was added. The resulting mixture was dissolved in 100 ml CH_2Cl_2 and subsequently washed with 30 ml brine, dried (Na_2SO_4) and concentrated *in vacuo* to give a crude purified by preparative RP-HPLC or flash chromatography.

***N*-[1,3]Dithiane-2-carboxyl-L-homoserine lactone (12a).** 530 mg **11a** gave 59 mg of a white fluffy solid (7% yield, >98% purity) after preparative RP-HPLC; mp 177–182 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.54 (br d, $^3J = 3$ Hz, 1 H, N*H*), 4.60–4.51 (m, 1 H, C*H*-3), 4.52 (s, 1 H, C*H*-2′), 4.49–4.17 (m, 2 H, CH_2-5), 3.25–3.16 (m, 2 H, CH_2-4′), 2.77–2.67 (m, 2 H, CH_2-4′), 2.48–2.38 (m, 1 H, CH_2-4), 2.22–2.08 (m, 1 H, CH_2-4), 2.00–1.89 (m, 2 H, CH_2-5′); ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.7 (1 C, C-2), 168.7 (1 C, C-1′), 65.2 (1 C, C-5), 48.0 (1 C, C-3), 43.0 (1 C, C-2′), 28.0, 26.8, 26.7, 24.9 (4 C, C-4, C-5′ and 2 × C-4′); LC-MS (ESI) $t_R = 4.84$ min (*m*/*z* $[MH]^+$); HRMS $(M + H)^+$ calcd. for $C_9H_{14}NO_3S_2$ 248.0415, found 248.0416.

***N*-[1,3]Dithiane-2-acetyl-L-homoserine lactone (12b).** 230 mg **11b** gave 21 mg of a white solid (6% yield, >99% purity) after preparative RP-HPLC (an additional 186 mg was isolated in fractions containing minor impurities); mp 185–187 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.31 (br s, exch., 1 H, N*H*), 4.58–4.24 (m, 4 H, CH_2-5, C*H*-3 and C*H*-3′), 2.99–1.81 (m, 10 H, CH_2-4, CH_2-2′, 2 × CH_2-5′ and CH_2-6′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.1 (1 C, C-2), 169.3 (1 C, C-1′), 66.2 (1 C, C-5), 49.6 (1 C, C-3), 42.6, 42.0 (2 C, C-2′ and C-3′), 30.4, 30.2, 30.2 (3 C, C-4 and 2 × C-5′), 25.2 (1 C, C-6′); LC-MS (ESI) $t_R = 4.96$ min (*m*/*z* $[MH]^+$); HRMS $(M + H)^+$ calcd. for $C_{10}H_{16}NO_3S_2$ 262.0572, found 262.0592.

***N*-[1,3]Dithiane-2-carboxylphenethyl-amide (12c).** 260 mg **11a** gave 82 mg of a white solid (18% yield, >95% purity) after flash chromatography (eluent: hexane then 10% EtOAc in hexane); mp 131–133 °C; ^{1}H NMR (300 MHz, DMSO-d$_6$) δ 7.27–7.14 (m, 5 H, Ar-*H*), 5.23 (br s, 1 H, N*H*), 4.25 (s, 1 H, C*H*-2′), 3.51 (q, J = 22, 2 H, CH_2-1), 2.95–2.87 (m, 2 H, CH_2-4′), 2.79 (t, J = 23, 2 H, CH_2-2), 2.65–2.57 (m, 2 H, CH_2-4′), 1.96–1.89 (m, 2 H, CH_2-5′); ^{13}C NMR (75 MHz, DMSO-d$_6$) δ 167.6 (1 C, C-1′), 138.5, 128.8, 128.6, 126.6 (6 C, Ar), 46.7, 41.3 (2 C, C-1 and C-2′), 35.4 (1 C, C-2), 28.1, (2 C, 2 × C-4′), 25.1 (1 C, C-5′); LC-MS (ESI) t_R = 6.39 min (m/z [MH]$^+$); HRMS (M + H)$^+$ calcd. for $C_{13}H_{18}NOS_2$ 268.0830, found 268.0831.

General procedure for the preparation of sulfoxides 13a–e. Over a period of 15 min, a 0.5 M solution of *m*-CPBA (1.0 equiv., based on the lowest purity reported on the *m*-CPBA label) in CH_2Cl_2 (*m*-CPBA is potentially explosive when highly purified and therefore the solution was just dried with $MgSO_4$ and filtered into the reaction mixture) was added to a clear 0.1 M solution of sulfide (1.0 equiv.) in CH_2Cl_2 cooled at −10 °C. It turned yellow–orange and then after a few min colourless again. After 3 h of stirring the reaction mixture was warmed to RT, washed with 5% $NaHCO_3$ (20 ml), dried ($MgSO_4$) and evaporated to dryness. The product was isolated as a diastereomeric mixture by flash chromatography (eluent: 1% MeOH in CH_2Cl_2).

***N*-(Propylsulfinylacetyl)-L-homoserine lactone (13a).** 98 mg **10a** gave 63 mg of a white solid. 60% yield (>99% purity); ^{1}H NMR (300 MHz, $CDCl_3$, 53:47 diastereomeric mixture) δ 7.98 (br d, ^3J = 7 Hz, 0.53 H, N*H*), 7.92 (br d, ^3J = 7 Hz, 0.47 H, N*H*), 4.77–4.20 (m, 3 H, CH_2-5 and C*H*-3), 3.75 (d, ^3J = 14 Hz, 0.53 H, CH_2-2′), 3.72 (d, ^3J = 14 Hz, 0.47 H, CH_2-2′), 3.45 (d, ^3J = 14 Hz, 0.47 H, CH_2-2′), 3.38 (d, ^3J = 14 Hz, 0.53 H, CH_2-2′), 3.07–2.23 (m, 4 H, CH_2-4 and CH_2-4′), 1.85–1.71 (m, 2 H, CH_2-5′), 1.07 (t, ^3J = 7 Hz, 3 H, CH_3′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.4, 174.4 (2 C, 2 × C-2), 164.9, 164.4 (2 C, 2 × C-1′), 65.8, 65.7 (2 C, 2 × C-5), 53.9, 53.6, 53.3, 53.0 (4 C, 2 × C-2′ and 2 × C-4′), 49.4, 48.8 (2 C, 2 × C-3), 29.0, 28.5, 16.5, 16.4 (4 C, 2 × C-4 and 2 × CH_2′), 13.2, 13.2 (2 C, 2 × CH_3′); LC-MS (ESI) t_R = 5.29 min (m/z 234 [MH]$^+$); HRMS (M + H)$^+$ calcd. for $C_9H_{16}NO_4S$ 234.0800; found 234.0795.

***N*-(Pentylsulfinylacetyl)-L-homoserine lactone (13b).** 491 mg **10b** gave 35 mg of a white solid. 7% yield (>99% purity); ^{1}H NMR (300 MHz, $CDCl_3$, 56:44 diastereomeric mixture) δ 7.85 (br d, ^3J = 9 Hz, 0.56 H, N*H*), 7.82 (br d, ^3J = 9 Hz, 0.44 H, N*H*), 4.83–4.22 (m, 3 H, CH_2-5 and C*H*-3), 3.77 (d, ^3J = 14 Hz, 0.56 H, CH_2-2′), 3.74 (d, ^3J = 14 Hz, 0.44 H, CH_2-2′), 3.38 (d, ^3J = 14 Hz, 0.44 H, CH_2-2′), 3.32 (d, ^3J = 14 Hz, 0.56 H, CH_2-2′), 3.14–2.23 (m, 4 H, CH_2-4 and CH_2-4′), 1.82–1.69 (m, 2 H, CH_2-5′), 1.51–1.30 (m, 4 H, 2 × CH_2′), 0.91 (t, ^3J = 7 Hz, 3 H, CH_3′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.4, 174.4 (2 C, 2 × C-2), 165.0, 164.5 (2 C, 2 × C-1′), 65.8, 65.7 (2 C, 2 × C-5), 52.8, 52.7, 51.2, 51.0 (4 C, 2 × C-2′ and 2 × C-4′), 49.5, 48.7 (2 C, 2 × C-3), 30.7, 30.7, 29.2, 28.6, 22.6, 22.6, 22.2, 22.2 (8 C, 2 × C-4 and 6 × CH_2′), 13.7, 13.7 (2 C, 2 × CH_3′); LC-MS (ESI) t_R = 5.03 min (m/z 262 [MH]$^+$); HRMS (M + H)$^+$ calcd. for $C_{11}H_{20}NO_4S$ 262.1113; found 262.1126.

***N*-(Heptylsulfinylacetyl)-L-homoserine lactone (13c).** 123 mg **10c** gave 78 mg of a white solid. 60% yield (>99% purity); ^{1}H NMR (300 MHz, $CDCl_3$, 54 : 46 diastereomeric mixture) δ 7.96 (br d, ^3J = 6 Hz, 0.54 H, N*H*), 7.90 (br d, ^3J = 7 Hz, 0.46 H, N*H*), 4.76–4.20 (m, 3 H, CH_2-5 and C*H*-3), 3.75 (d, ^3J = 14 Hz, 0.54 H, CH_2-2′), 3.71 (d, ^3J = 14 Hz, 0.46 H, CH_2-2′), 3.44 (d, ^3J = 14 Hz, 0.46 H, CH_2-2′), 3.37 (d, ^3J = 14 Hz, 0.54 H, CH_2-2′), 3.07–2.23 (m, 4 H, CH_2-4 and CH_2-4′), 1.78–1.67 (m, 2 H, CH_2-5′), 1.49–1.26 (m, 8 H, 4 × CH_2′), 0.85 (t, ^3J = 6 Hz, 3 H, CH_3′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.6, 174.6 (2 C, 2 × C-2), 164.9, 164.4 (2 C, 2 × C-1′), 65.8, 65.7 (2 C, 2 × C-5), 53.8, 53.5, 51.5, 51.2 (4 C, 2 × C-2′ and 2 × C-4′), 49.4, 48.8 (2 C, 2 × C-3), 31.4, 31.4, 29.0, 28.7, 28.7, 28.6, 28.6, 28.6, 22.8, 22.7, 22.4, 22.4 (12 C, 2 × C-4, and 6 × CH_2′), 13.9, 13.9 (2 C, 2 × CH_3′); LC-MS (ESI) t_R = 8.06 min (m/z 290 [MH]$^+$); HRMS (M + H)$^+$ calcd. for $C_{13}H_{24}NO_4S$ 290.1426; found 290.1439.

***N*-(Nonylsulfinylacetyl)-L-homoserine lactone (13d).** 207 mg **10d** gave 65 mg of a white solid. 36% yield (>99% purity); ^{1}H NMR (300 MHz, $CDCl_3$, 57 : 43 diastereomeric mixture) δ 7.85 (br d, ^3J = 9 Hz, 0.57 H, N*H*), 7.82 (br d, ^3J = 9 Hz, 0.43 H, N*H*), 4.83–4.22 (m, 3 H, CH_2-5 and C*H*-3), 3.77 (d, ^3J = 14 Hz, 0.57 H, CH_2-2′), 3.74 (d, ^3J = 14 Hz, 0.43 H, CH_2-2′), 3.38 (d, ^3J = 14 Hz, 0.43 H, CH_2-2′), 3.32 (d, ^3J = 14 Hz, 0.57 H, CH_2-2′), 3.14–2.23 (m, 4 H, CH_2-4 and CH_2-4′), 1.81–1.68 (m, 2 H, CH_2-5′), 1.49–1.26 (m, 12 H, 6 × CH_2′), 0.87 (t, ^3J = 7 Hz, 6 H, 2 × CH_3′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.4, 174.4 (2 C, 2 × C-2), 165.0, 164.5 (2 C, 2 × C-1′), 65.8, 65.6 (2 C, 2 × C-5), 52.8, 52.6, 51.3, 51.0 (4 C, 2 × C-2′ and 2 × C-4′), 49.5, 48.7 (2 C, 2 × C-3), 31.8, 31.8, 29.2, 29.2, 29.2, 29.1, 29.1, 29.1, 29.1, 29.1, 28.7 28.6, 23.0, 22.9, 22.6, 22.6 (16 C, 2 × C-4, and 14 × CH_2′), 14.1, 14.1 (2 C, 2 × CH_3′); LC-MS (ESI) t_R = 6.40 min (m/z 318 [MH]$^+$); HRMS (M + H)$^+$ calcd. for $C_{15}H_{28}NO_4S$ 318.1739; found 318.1716.

***N*-(Phenylsulfinylacetyl)-L-homoserine lactone (13e).** 25 mg **10e** gave 15 mg of a white solid. 56% yield (>99% purity); ^{1}H NMR (300 MHz, $CDCl_3$, 57:43 diastereomeric mixture) δ 7.70–7.53 (m, 5H, Ar-*H*), 7.45 (br s, 1 H, N*H*), 4.59–4.17 (m, 3 H, CH_2-5 and C*H*-3), 3.83 (d, ^3J = 14 Hz, 0.57 H, CH_2-2′), 3.82 (d, ^3J = 14 Hz, 0.43 H, CH_2-2′), 3.55 (d, ^3J = 14 Hz, 0.43 H, CH_2-2′), 3.49 (d, ^3J = 14 Hz, 0.57 H, CH_2-2′), 2.71–2.54 (m, 1 H, CH_2-4), 2.23–2.07 (m, 1 H, CH_2-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.3, 174.2 (2 C, 2 × C-2), 164.2, 163.9 (2 C, 2 × C-1′), 141.2, 140.9, 131.8, 131.7, 129.5, 129.4, 124.1, 124.1 (12 C, Ar), 65.8, 65.7 (2 C, 2 × C-5), 58.1, 58.1 (2 C, 2 × C-2′), 49.1, 49.0 (2 C, 2 × C-3), 29.6, 29.6 (2 C, 2 × C-4); LC-MS (ESI) t_R = 6.04 min (m/z 268 [MH]$^+$); HRMS (M + H)$^+$ calcd. for $C_{12}H_{14}NO_4S$ 268.0643; found 268.0621.

General procedure for the preparation of 14a–e. At ambient temperature *m*-CPBA (2.0 equiv., based on the lowest purity reported on the *m*-CPBA label) was added to a 0.1 M solution of sulfide (1.0 equiv.) in CH_2Cl_2. The mixture was stirred for 2 h and then washed with 5% $NaHCO_3$. The organic phase was dried ($MgSO_4$) and evaporated to dryness. Purification by flash chromatography (eluent: 1% MeOH in CH_2Cl_2) afforded the product.

***N*-(Propylsulfonylacetyl)-L-homoserine lactone (14a).** 51 mg **10a** gave 21 mg of a white solid. 36% yield (73% purity); mp 90–94 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (br d, ^3J = 5 Hz, 1 H, N*H*), 4.65–4.24 (m, 3 H, CH_2-5 and C*H*-3), 3.96 (s, 2 H, CH_2-2′), 3.24–3.18 (m, 2 H, CH_2-4′), 2.73–2.64 (m, 1 H, CH_2-4), 2.44–2.29 (m, 1 H, CH_2-4), 1.97–1.84 (m, 2 H, CH_2-5′), 1.09 (t, ^3J = 7 Hz, 3 H, CH_3′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.5 (1 C, C-2), 162.2 (1 C, C-1′), 66.0 (1 C, C-5), 58.5, 54.9 (2 C, C-2′ and C-4′), 49.6 (1 C, C-3), 28.7 (1 C, C-4), 15.7, 13.0 (2 C, C-5′ and CH$_3$′); LC-MS (ESI) t_R = 4.51 min (m/z 250 [MH]$^+$); HRMS (M + H)$^+$ calcd. for $C_9H_{16}NO_5S$ 250.0749, found 250.0786.

***N*-(Pentylsulfonylacetyl)-L-homoserine lactone (14b).** 123 mg **10b** gave 72 mg of a white solid. 29% yield (>99% purity); mp 97–100 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.08 (br d, ^3J = 5 Hz, 1 H, N*H*), 4.62–4.25 (m, 3 H, CH_2-5 and C*H*-3), 3.92 (s, 2 H, CH_2-2′), 3.24–3.18 (m, 2 H, CH_2-4′), 2.78–2.69 (m, 1 H, CH_2-4), 2.41–2.27 (m, 1 H, CH_2-4), 1.93–1.82 (m, 2 H, CH_2-5′), 1.50–1.33 (m, 4 H, 2 × CH_2′), 0.92 (t, ^3J = 7 Hz, 3 H, CH_3′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.1 (1 C, C-2), 162.0 (1 C, C-1′), 65.9 (1 C, C-5), 58.5, 53.3 (2 C, C-2′ and C-4′), 49.6 (1 C, C-3), 30.3, 28.9, 22.0, 21.6 (4 C, C-4 and CH$_2$′), 13.7 (1 C, CH$_3$′); LC-MS (ESI) t_R = 5.44 min (m/z 278 [MH]$^+$); HRMS (M + H)$^+$ calcd. for $C_{11}H_{20}NO_5S$ 278.1062, found 278.1085.

***N*-(Heptylsulfonylacetyl)-L-homoserine lactone (14c).** 700 mg **10c** gave 60 mg of a white solid. 6% yield (80% purity);

mp 103–106 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.13 (br d, ^3J = 5 Hz, 1 H, N*H*), 4.64–4.25 (m, 3 H, CH_2-5 and C*H*-3), 3.92 (s, 2 H, CH_2-2′), 3.24–3.18 (m, 2 H, CH_2-4′), 2.68–2.77 (m, 1 H, CH_2-4), 2.42–2.27 (m, 1 H, CH_2-4), 1.92–1.81 (m, 2 H, CH_2-5′), 1.50–1.25 (m, 8 H, 4 × CH_2′), 0.88 (t, ^3J = 7 Hz, 3 H, CH_3′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.1 (1 C, C-2), 162.0 (1 C, C-1′), 65.9 (1 C, C-5), 58.5, 53.3 (2 C, C-2′ and C-4′), 49.6 (1 C, C-3), 31.4, 28.9, 28.6, 28.2, 22.5, 21.9 (6 C, C-4 and 5 × CH_2′), 14.0 (1 C, CH_3′); LC-MS (ESI) t_R = 6.23 min (*m*/*z* 306 [MH]$^+$); HRMS (M + H)$^+$ calcd. for $C_{13}H_{24}NO_5S$ 306.1375, found 306.1357.

***N*-(Nonylsulfonylacetyl)-L-homoserine lactone (14d).** 98 mg **10d** gave 81 mg of a white solid. 74% yield (69% purity); mp 113–115 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (br d, ^3J = 5 Hz, 1 H, N*H*), 4.63–4.25 (m, 3 H, CH_2-5 and C*H*-3), 3.93 (s, 2 H, CH_2-2′), 3.24–3.19 (m, 2 H, CH_2-4′), 2.77–2.67 (m, 1 H, CH_2-4), 2.42–2.28 (m, 1 H, CH_2-4), 1.91–1.81 (m, 2 H, CH_2-5′), 1.50–1.26 (m, 12 H, 6 × CH_2′), 0.88 (t, ^3J = 7 Hz, 3 H, CH_3′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.1 (1 C, C-2), 162.0 (1 C, C-1′), 65.9 (1 C, C-5), 58.5, 53.4 (2 C, C-2′ and C-4′), 49.6 (1 C, C-3), 31.8, 29.2, 29.1, 29.0, 29.0, 28.3, 22.6, 21.9 (8 C, C-4 and 7 × CH_2′), 14.1 (1 C, CH_3′); LC-MS (ESI) t_R = 6.93 min (*m*/*z* 334 [MH]$^+$); HRMS (M + H)$^+$ calcd. for $C_{15}H_{28}NO_5S$ 334.1688, found 334.1679.

***N*-(Phenylsulfonylacetyl)-L-homoserine lactone (14e).** 20 mg **10e** gave 13 mg of a white solid. 57% yield (>99% purity); mp 163–164 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99 (d, ^3J = 8 Hz, 2H, *o*-SO_2–C_6H_5), 7.71 (d, ^3J = 8 Hz, 1H, *p*-SO_2–C_6H_5), 7.60 (d, ^3J = 8 Hz, 2H, *m*-SO_2–C_6H_5), 7.30 (br d, ^3J = 5 Hz, 1 H, N*H*), 4.63–4.23 (m, 3 H, CH_2-5 and C*H*-3), 4.09 (s, 2 H, CH_2-2′), 2.77–2.67 (m, 1 H, CH_2-4), 2.33–2.19 (m, 1 H, CH_2-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.1 (1 C, C-2), 161.1 (1 C, C-1′), 137.8, 134.6, 129.5, 128.3 (6 C, S–C_5H_6), 65.8 (1 C, C-5), 49.4 (1 C, C-3), 49.4 (1 C, C-2′), 29.3 (1 C, C-4); LC-MS (ESI) t_R = 4.95 min (*m*/*z* 222 [MH]$^+$); HRMS (M + H)$^+$ calcd. for $C_{12}H_{14}NO_5S$ 284.0593, found 284.0603.

Acknowledgements

Financial support was in part obtained from the Royal Veterinary and Agricultural University, the Technical University of Denmark, Hede Nielsen Foundation, the Danish Technical Research Council (Grant no. 56-00-0134) and the Villum Kann Rasmussen Foundation (Grant 1002–12). Dr. J. Øgaard Madsen is thanked for assistance with GC-MS and Søren Ryborg for technical assistance. QSI Pharma A/S is acknowledged for their generous donation of *trans*-2-aminocyclopentanol hydrochloride.

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