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Immunosuppressive but Non-LasR-Inducing Analogues of the *Pseudomonas aeruginosa* Quorum-Sensing Molecule *N*-(3-Oxododecanoyl)-L-homoserine Lactone

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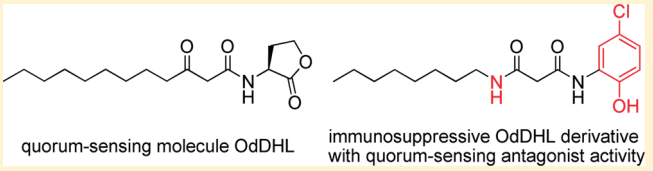
Supporting Information

ABSTRACT: The *Pseudomonas aeruginosa* quorum-sensing molecule *N*-(3-oxododecanoyl)-L-homoserine lactone (**1**) is involved not only in bacterial activation but also in subversion of the host immune system, and this compound might thus be used as a template to design immunosuppressive agents, provided derivatives devoid of quorum-sensing activity could be discovered. By use of a leukocyte proliferation assay and a newly developed bioluminescent *P. aeruginosa* reporter assay, systematic modification of **1** allowed us to delineate the bacterial LasR-induction and host immunosuppressive activities. The main determinant is replacement of the methylene group proximal to the β-ketoamide in the acyl chain of **1** with functions containing heteroatoms, especially an NH group. This modification can be combined with replacement of the homoserine lactone system in **1** with stable cyclic groups. For example, we found the simple compound N^1-(5-chloro-2-hydroxyphenyl)-N^3-octylmalonamide (**25d**) to be over twice as potent as **1** as an immune suppressor while displaying LasR-induction antagonist activity.



INTRODUCTION

Chronic respiratory infection by *Pseudomonas aeruginosa* is the major source of morbidity and mortality in cystic fibrosis patients, and infections by this Gram-negative opportunistic pathogen are also particularly prevalent in immune-compromised patients, e.g., those suffering from burn wounds and leg ulcers or those undergoing cytotoxic chemotherapy.[1] *P. aeruginosa* produces extracellular virulence factors that promote host invasion, biofilm formation, and tissue damage. These factors are regulated by quorum-sensing (QS), a regulatory mechanism that enables bacteria to coordinate gene expression as a function of cell population density through the production of one or more quorum sensing signal molecules (QSSMs).[2] These low molecular weight compounds or "autoinducers" activate or repress QS-dependent target genes once a critical threshold concentration of QSSMs has been reached. *P. aeruginosa* produces at least two chemically distinct classes of QSSMs, the *N*-acylhomoserine lactones and the 2-alkyl-4-quinolones.[2] Among these is *N*-(3-oxododecanoyl)-L-homoserine lactone (OdDHL, **1**, Figure 1),[3] which is produced by the LasI synthase and sensed via the transcriptional activator protein LasR, which in turn controls the expression of a number of virulence factors, including the secreted metalloprotease LasB, and also promotes biofilm maturation.[3]

In addition, **1** promotes persistent infection by subverting the host immune system, as we have shown previously.[1,4] Lipopolysaccharide (LPS) from invading Gram-negative bacteria activates the innate host immune system through Toll-like receptors (TLR4 and TLR2), which leads to the induction of the nuclear transcription factor NF-κB and the production of proinflammatory cytokines.[5] However, despite activation of the innate immune system, highly virulent opportunistic bacteria such as *P. aeruginosa* are able to cause acute severe disease and also to establish persistent infection. The QSSM **1** modulates the functions of many host immune cell types, including lymphocytes,[4,6] macrophages and monocytes,[7] neutrophils,[7] and mast cells,[8] as well as other cells such as respiratory epithelial cells, fibroblasts, and vascular smooth muscle cells.[9−11] Immune cell functions are suppressed or redirected in response to the presence of **1** through antiproliferative effects, as well as altered cytokine expression profiles.[12] It is now clear that **1** can modulate the function of host transcriptional regulators involved in inflammation and immunity, including peroxisome proliferator activated receptors (PPARs) and NF-κB.[13,14] While there is evidence that **1** may interact

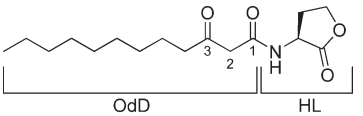


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Figure 1. Chemical structure of OdDHL and numbering convention for its analogues.

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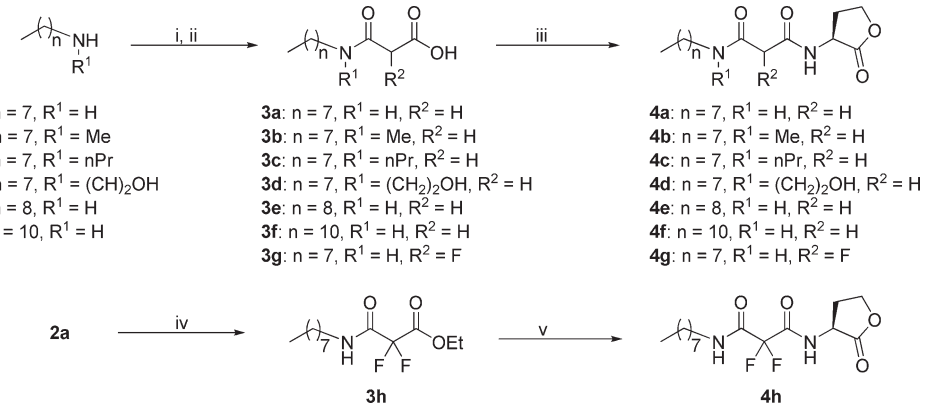
Scheme 1. Synthesis of 4-aza-OdDHL Derivatives 4[a]



2a: n = 7, R^1 = H
2b: n = 7, R^1 = Me
2c: n = 7, R^1 = nPr
2d: n = 7, R^1 = $(CH)_2OH$
2e: n = 8, R^1 = H
2f: n = 10, R^1 = H

3a: n = 7, R^1 = H, R^2 = H
3b: n = 7, R^1 = Me, R^2 = H
3c: n = 7, R^1 = nPr, R^2 = H
3d: n = 7, R^1 = $(CH_2)_2OH$, R^2 = H
3e: n = 8, R^1 = H, R^2 = H
3f: n = 10, R^1 = H, R^2 = H
3g: n = 7, R^1 = H, R^2 = F

4a: n = 7, R^1 = H, R^2 = H
4b: n = 7, R^1 = Me, R^2 = H
4c: n = 7, R^1 = nPr, R^2 = H
4d: n = 7, R^1 = $(CH_2)_2OH$, R^2 = H
4e: n = 8, R^1 = H, R^2 = H
4f: n = 10, R^1 = H, R^2 = H
4g: n = 7, R^1 = H, R^2 = F

[a] Reagents and conditions: (i) $ClCOCHR^2COOEt$, dry CH_2Cl_2, 0 °C to room temp, 2 h; (ii) aq NaOH, EtOH, room temp, 4 h; (iii) L-HSL·HCl, EDC, Et_3N, H_2O—dioxane, room temp, 16 h or L-HSL·HCl, CDI, Et_3N, dry CH_2Cl_2, room temp, 16 h; (iv) $EtCOOCF_2COOEt$, EtOH, room temp, 2 h; (v) L-HSL·HCl, Et_3N, MeCN, reflux, 16 h, then aq NaOH, MeOH, room temp, 3 h.

directly with PPARγ,[15] the molecular targets responsible for disruption of NF-κB-dependent gene expression by **1** remain unknown.[16]

Given the intrinsic immunosuppressive activity of **1** and the potential for immunosuppressive QSSMs to act as chemical templates for the development of a new generation of therapeutics to treat immunological diseases, it became of interest to us to investigate the possibility of divorcing the QS capacity of **1** and its analogues from their immunosuppressive activity. Clearly it would be highly undesirable for immunosuppressive treatment regimens based on QSSMs to promote bacterial infection in immune compromised patients. In a previous communication[17] we reported on the immune suppression SARs of modifications in the acyl chain and at C^2 of **1** but we observed that the active compounds also retained QS activity. We have now carried out further SAR studies based on **1**, which have allowed us to identify the structural requirements for immunosuppression while minimizing bacterial LasR-induction activity.

CHEMISTRY

The 4-aza-OdDHL derivatives **4** were prepared (Scheme 1) by acylation of amines **2** with appropriate malonic acid esters or chlorides, followed by saponification of the resulting monoamides to the intermediates **3**, which were condensed using carbodiimide- or carbonyldiimidazole-mediated coupling with L-HSL to afford products **4**. In the case of the difluoro derivative **4h**, the ester **3h** was substituted with L-HSL directly. The 8-oxa (**7a**) and 7,10-dioxa (**7b**) analogues (Scheme 2) of 4-aza-OdDHL were obtained similarly from amines **5** via intermediates **6**.

Amine **5b** (Scheme 2) was prepared from di(ethylene glycol) ethyl ether by Mitsunobu reaction with phthalimide and hydrazinolysis.[18] Initial attempts to synthesize 3-aza-OdDHL analogues **10** were based on reductive condensation of 1-nonanal with *O*-benzylhydroxylamine, but reduction of the major oxime product to the *O*-alkylhydroxylamine **8a** under a variety of conditions[19,20] was inefficient and removal of the benzyl group from **10a** difficult.[21] Reductive amination of 1-nonanal with *O*-*tert*-butylhydroxylamine, on the other hand, furnished **8b** in high yield. The corresponding carbazate **8c** was obtained by alkylation of *tert*-butyl carbazate with 1-bromononane. Intermediates **8** were then alkylated with benzyl bromoacetate and the benzyl ester groups removed hydrogenolytically to provide the acids **9**, which were converted to the products **10** by L-HSL coupling and acidolytic removal of the *tert*-butyl protecting groups. For the preparation of the 4-oxa-OdDHL analogue **13**, monobenzyl malonate was esterified with 1-octanol **11** and the product **12** was elaborated in the usual manner. For the synthesis of the 3-thia-OdDHL derivatives **16**, the starting materials were the thiols **14**, which were alkylated with *tert*-butyl bromoacetate under conditions that led to in situ deprotection of the *tert*-butyl ester groups to provide the thioethers **15a** and **15b** directly.[22] These were then oxidized to the sulfoxides **15c** and **15d** and sulfones **15e** and **15f** using acidic bromate—bromide[22] and permanganate[23] conditions, respectively. The intermediates **15** were coupled with L-HSL as usual; the diastereomeric sulfoxide products **16b** and **16c** were not resolved.

The sulfonamide 4-aza-OdDHL derivatives **19** (Scheme 3) were prepared from the common precursor methyl 2-(chlorosulfonyl)-acetate[24] **17** by amidation with 1-octylamine and L-HSL, respectively, to provide intermediates **18** following methyl ester hydrolysis. Further condensation afforded the sulfonamides **19**. The racemic 3-acetoxy-OdDHL analogue **20** was prepared from **1** by successive cyanoborohydride reduction and acetylation (Scheme 4). The tetramic acid derivative **21**, a nonenzymatic degradation product of **1**, was prepared by intramolecular Claisen condensation as described (Scheme 4).[25]

A number of different ring-modified OdDHL and 4-aza-OdDHL analogues were prepared (Scheme 5); these included compounds in which the γ-lactone ring of L-HSL was replaced with pyrrolidine-2,5-dione-3-yl (**23a**), piperidine-2,6-dione-3-yl (**23b**), thiazol-2-yl (**23c** and **25a**), cyclopentanol-2-yl (**24**), cyclohexanol-2-yl (**25b**), hydroxyphen-2-yl (**25c**), 4-chloro-(hydroxyphen-2-yl) (**25d**), carbamoylphen-2-yl (**25e**), (dimethylcarbamoyl)phen-2-yl (**25f**), carbamoylphen-3-yl (**25g**), 1*H*-indazol-5-yl (**25h**), pyrid-2-yl (**25i**), and cycloheptanyl (**25j**) systems. With the exception of **23**, these were prepared from cyclic amine precursors analogously to 4-aza-ODdHL **4a** and derivatives **7**. Compounds **23a**, **23b**, and **23c** were synthesized by acylation of 5-decanoyl Meldrum's acid **22** with L-aspartimide, L-glutarimide, and 2-aminothiazole, respectively, under similar conditions as for acylation with L-HSL.[17]

RESULTS AND DISCUSSION

Design. The SARs of OdDHL derivatives with respect to bacterial *P. aeruginosa* autoinduction have been studied in some detail (reviewed in ref 26), and it is clear that only very conservative structural changes are tolerated in order to retain LasR agonist activity: a

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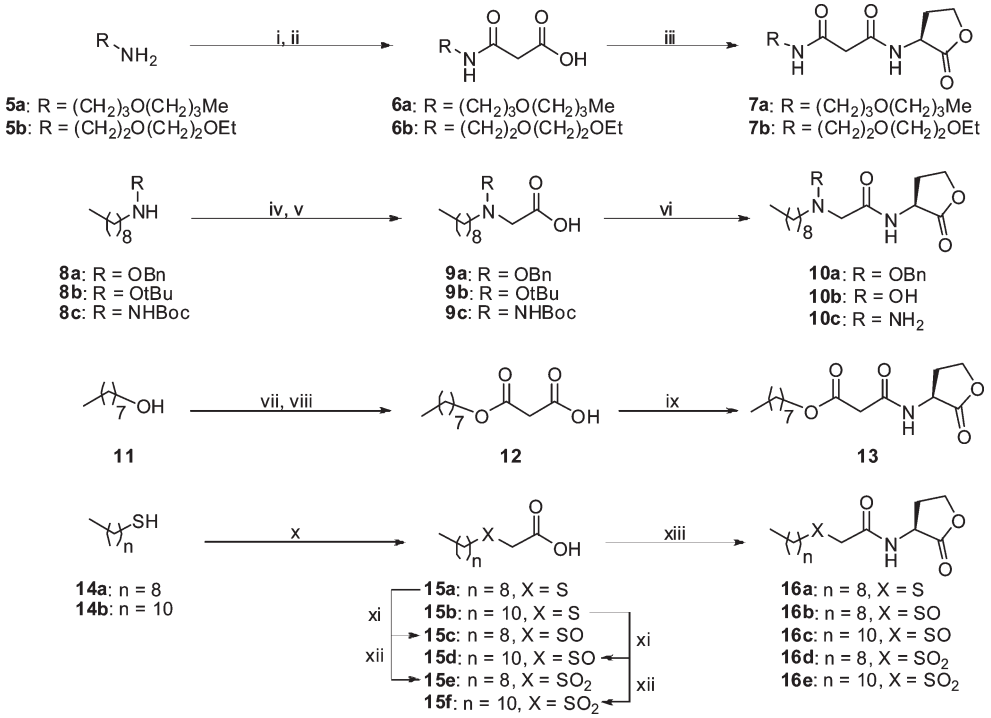
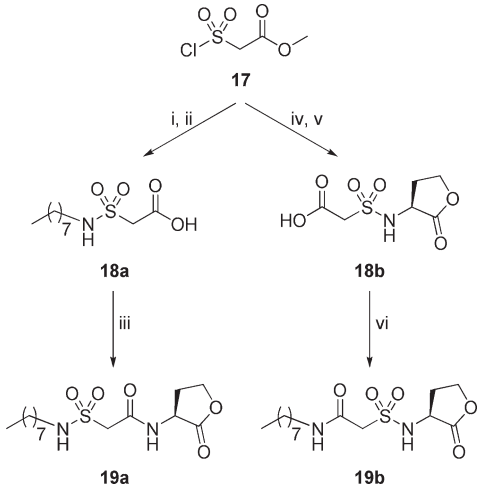
Scheme 2. Synthesis of Modified 4-aza-OdDHL Derivatives[a]



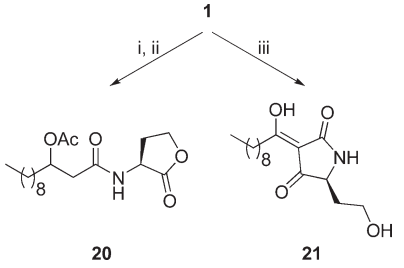
[a] Reagents and conditions: (i) $ClCOOCH_2COOEt$, CH_2Cl_2, 0 °C to room temp, 16 h; (ii) aq NaOH, EtOH, room temp, 6 h; (iii) L-HSL·HCl, EDC·HCl, Et_3N, H_2O−dioxane, room temp, 16 h; (iv) $BrCH_2COOBn$, *i*-Pr_2NEt, THF, reflux, 16 h, then H_2, Pd/C, EtOH, room temp, 8 h; or (v) $BrCH_2COOtBu$, *i*-Pr_2NEt, THF, reflux, 18 h, then CF_3COOH, EtOAc, room temp, 3 h; (vi) L-HSL·HCl, EDC·HCl, Et_3N, H_2O−dioxane, room temp, 16 h, then 50% v/v CF_3COOH/CH_2Cl_2, room temp, 6 h; (vii) $BnOCOCH_2COOH$, DCI, DMAP, dry CH_2Cl_2, room temp, 16 h; (viii) H_2, Pd/C, EtOH, room temp, 8 h; (ix) L-HSL·HCl, CDI, Et_3N, dry CH_2Cl_2, room temp, 16 h; (x) $BrCH_2COO$-*t*-Bu, 18% w/v aq NaOH, 90 °C, 14 h; (xi) 0.5 M bromate−bromide soln, HCl, AcOH, 0 °C, 45 min; (xii) 4% w/v $KMnO_4$, AcOH, 50 °C to room temp, 40 min; (xiii) L-HSL·HCl, EDC·HCl, Et_3N, H_2O−dioxane, room temp, 16 h (for sulfoxides) or L-HSL·HCl, CDI, Et_3N, dry CH_2Cl_2, room temp, 16 h.

Scheme 3. Synthesis of Sulfonamide Derivatives of OdDHL[a]



[a] Reagents and conditions: (i) $CH_3(CH_2)_7NH_2$, DBU, K_2CO_3, dry CH_2Cl_2, reflux, 3 h; (ii) aq NaOH, MeOH, room temp, 4 h; (iii) L-HSL·HCl, EDC·HCl, Et_3N, H_2O−dioxane, room temp, 16 h; (iv) L-HSL·HCl, DMAP, DBU, dry CH_2Cl_2, 0 °C to room temp, 16 h; (v) 1.0 M aq HCl, reflux, 4 h; (vi) $CH_3(CH_2)_7NH_2$, DCI, DMAP, dry CH_2Cl_2, room temp, 16 h.

Scheme 4. Synthesis of 3-Acetoxy- and Tetramic Acid Derivatives of OdDHL[a]



[a] Reagents and conditions: (i) $NaBH_3CN$, MeOH−HCl, room temp, pH 4.0, 16 h; (ii) Ac_2O, reflux, 2 h; (iii) NaOMe, MeOH, 55 °C, 6 h.

minimum acyl chain length of six carbon atoms, the presence of the 3-oxo function, and the γ-lactone ring of **1** (or certain other similar five- or six-membered alicyclic systems containing a hydrogen bond-acceptor function) are all required for agonism. On the other hand, more drastic structural changes are tolerated in terms of LasR binding, but these generally result in compounds with antagonistic activity. Some of the modifications in this category include sterically bulky and structurally diverse acyl chains, as well as a variety of substituted and unsubstituted alicyclic (and especially aromatic) systems in place of the γ-lactone ring. Although the structural basis of the recognition of **1** by the *P. aeruginosa* autoinduction target LasR was elucidated recently through a complex X-ray crystal structure,[27] it remains difficult to rationalize the different structural requirements for agonist versus antagonist activity.

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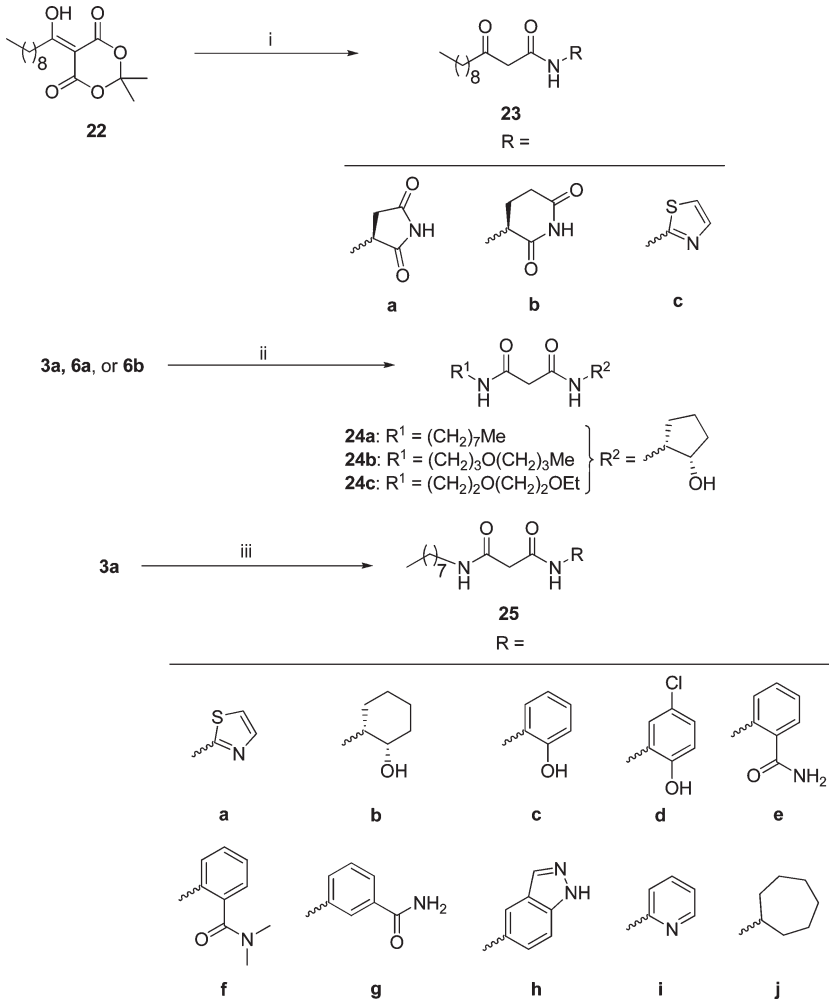
Scheme 5. Synthesis of Heterocyclic 4-aza-OdDHL Analogues[a]



[a] Reagents and conditions: (i) RNH_2, Et_3N, MeCN, room temp, 16 h; (ii) (1*S*,2*R*)-2-(benzyloxy)cyclopentylamine, EDC·HCl, Et_3N, dry CH_2Cl_2, room temp, 16 h, then H_2, Pd(C), EtOH, room temp, 8 h; (iii) EDC, Et_3N, H_2O−dioxane, room temp, 16 h.

Preliminary SAR studies on analogues of **1** showed that L-HSL acylated with a 3-oxo or 3-hydroxyl C_{12-14} acyl chain was optimal for immunosuppressive activity.[17] The fact that **1** was able to modulate immune functions in vivo suggested that novel therapeutic immunosuppressive agents based on **1** might be designed.[6,28] However, our early close analogues of **1** retained quorum sensing activity.[17] Because properties of an immunosuppressive agent that might promote bacterial virulence are highly undesirable, we sought to examine if it were possible to delineate the LasR-induction and immune modulation activities. Since the molecular targets responsible for these activities are different, it was not unreasonable to expect that this should be feasible. Our initial strategy for structural changes of **1** included introduction of heteroatoms to probe SARs and to increase aqueous solubility, as well as replacement of functional groups likely to be metabolically labile, including the β-ketoamide and the γ-lactone. We therefore embarked on a program of research in which we introduced structural changes in the L-HSL ring system, the β-ketoamide portion, and the alkyl chain of **1** and assessed the resulting derivatives for their ability to suppress host immune cell proliferation and to activate the bacterial LasR system.

Pharmacological Assays. We used the ex vivo concanavalin A stimulated murine peripheral blood leukocyte proliferation assay, in which cell proliferation is measured as a function of cellular ^{3}H-thymidine incorporation, as a surrogate measure for mammalian host immune suppression.[17] Immune suppression was determined as the half-maximal effective dose (EC_{50}) of test compounds required to inhibit cell proliferation compared to mock-treated cells (Table 1). In order to assess compound cytotoxicity, the effect of test compounds on the viability of Jurkat E6.1 cells was also measured using a trypan blue exclusion assay. Jurkat E6.1 is an immortalized human T-lymphocyte cell line that replicates constitutively in a manner not dependent upon activation of the T-cell receptor or the presence of interleukin-2.[29,30] The use of this cell line thus allowed us to define toxicity in a relevant cell while excluding the immunosuppressive effects associated with T-cell activation.

To evaluate the effects of the test compounds on the activation of LasR or the antagonism of OdDHL-dependent LasR activation, a simple rapid assay was developed in which the promoter of the *lasB* gene (a direct target for LasR/OdDHL activation) was coupled to the *luxCDABE* genes. When introduced into a *P. aeruginosa lasI* mutant, the resulting strain (termed PAKR02) is dark and emits light only on the exogenous provision of **1**. The EC_{50} for **1** in this assay was ~40 nM (Figure 2). For QS agonist

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Table 1. Immune Suppression and Quorum Sensing Activity of OdDHL Analogues

	immune suppression activity		QS agonist and antagonist activity	
compd	inhibition of murine peripheral blood leukocyte proliferation[a] EC_{50} (μM)	Jurkat cytotoxicity[b] EC_{50} (μM)	LasR-induction ratio at 10^{-4} M test compd[c]	remaining LasR-induction in presence of 5 μM **1** at 10^{-4} M test compd (%)
1	21 ± 13 (n = 8)	250	12.3	100
4a	35 ± 25 (n = 6)	200	1.06	20 ± 2
4b	22 ± 12 (n = 6)	>1000	0.91	60 ± 2
4c	28 ± 15 (n = 5)	200	0.99	101 ± 1
4d	41 ± 16 (n = 4)	300	1.01	16 ± 2
4e	23 ± 13 (n = 4)	530	1.01	3.4 ± 0.4
4f	12 ± 9 (n = 7)	140	0.72	14 ± 2
4g	26 ± 10 (n = 4)	800	0.91	57 ± 2
4h	97 ± 20 (n = 9)	>1000	*d*	6.0 ± 1
7a	101 ± 17 (n = 3)	>1000	0.97	108 ± 2
7b	>1000 (n = 3)	>1000	1.02	61 ± 3
10a	17 ± 11 (n = 7)	63	*d*	*d*
10b	13 ± 11 (n = 4)	400	0.99	69 ± 1
10c	8.9 ± 9.4 (n = 4)	410	1.03	55 ± 2
13	37 ± 24 (n = 7)	500	1.18	46 ± 4
16a	>1000 (n = 3)	>1000	0.90	51 ± 1
16b	27 ± 31 (n = 7)	720	*d*	*d*
16c	8.2 ± 6.6 (n = 7)	45	0.77	63 ± 2
16d	31 ± 26 (n = 7)	200	0.92	72 ± 2
16e	8.3 ± 5.4 (n = 7)	170	0.75	76 ± 1
19a	13 ± 10 (n = 3)	900	1.02	101 ± 2
19b	9.1 ± 2.6 (n = 3)	220	1.04	86 ± 2
20	5.2 ± 5.2 (n = 4)	120	1.13	70 ± 2
21	29 ± 32 (n = 3)	52	0.96	21 ± 1
23a	>1000 (n = 3)	N/A	1.04	82 ± 1
23b	77 ± 5 (n = 4)	N/A	1.03	71 ± 2
23c	25 ± 9 (n = 4)	800	0.87	51 ± 2
24a	43 ± 16 (n = 4)	400	0.96	55 ± 2
24b	160 ± 25 (n = 3)	>1000	0.94	111 ± 1
24c	192 ± 80 (n = 3)	700	1.03	89 ± 1
25a	27 ± 13 (n = 6)	600	0.90	64 ± 1
25b	32 ± 6 (n = 3)	400	0.92	45 ± 3
25c	22 ± 4 (n = 4)	>1000	0.79	29 ± 2
25d	9.3 ± 5.7 (n = 4)	>1000	0.62	66 ± 1
25e	25 ± 9 (n = 4)	120	0.80	23 ± 2
25f	22 ± 15 (n = 5)	160	0.91	37 ± 4
25g	15 ± 5 (n = 3)	700	0.81	59 ± 1
25h	55 ± 33 (n = 4)	>1000	1.10	62 ± 2
25i	18 ± 11 (n = 4)	>1000	0.91	41 ± 2
25j	14 ± 6 (n = 6)	78	0.90	19 ± 4

[a] Measured using the ex vivo concanavalin A stimulated murine peripheral blood leukocyte proliferation assay. [b] From at least two independent observations. [c] Ratio of relative luminescence (as a function of bacterial cell density) of cell culture with test compound to cell culture alone in bacterial reporter assay. [d] Measurement not performed.

activity, the induction of bioluminescence (recorded as a function of bacterial cell population) following addition of test compounds was measured, and in Table 1 this activity is expressed as a ratio of bacterial bioluminescence between test compound and negative control (diluent only). The results shown are for a test compound concentration of 100 μM (compounds were also assayed at 1 mM with similar results). In order to determine antagonist activity, competition assays were performed with test compounds at three concentrations (1 mM, 100 μM, 10 μM) in the presence of the cognate agonist **1** (5 μM). Dose-response effects were observed for antagonistic compounds (not shown), and the results in Table 1 (at 100 μM) were calculated as percentage inhibition of relative bioluminescence by a test compound compared to the positive control **1**.

Immunosuppression and Bacterial LasR-Induction SARs. The β-ketoamide group in **1** can exist in different tautomeric forms, and it has been reported that constrained enolic derivatives of **1** are devoid of LasR activity, whereas the nonenolizable *gem*-difluoro derivative of **1** apparently retains agonist activity.[31] We therefore asked if a similar situation pertained with immunosuppression activity by introducing heteroatoms at C^1 or C^3, reduction at C^3, and fluorine substitution at C^2 (refer to Figure 1 for numbering convention).

It is expected that at physiological pH enolic tautomers of C^4-aza derivatives of **1** (e.g., **4a**), in which the ketone function of the β-ketoamide group present in **1** has been replaced with another amide, would be far less significant. Surprisingly, we found that LasR agonist activity was strongly reduced with all of the C^4-aza analogues (**4a**–**f**). Instead, these compounds, with the exception of the *N*-alkyl derivatives (NMe, **4b**; N-*i*-Pr, **4c**) acted as strong LasR antagonists. On the other hand, replacement of the acyl chain C^4 methylene with an NH group (**4a**, **4e**, **4f**) led to full retention of immunosuppressive activity of the parent compound **1**, with the C_{15} acyl chain length analogue **4f** displaying somewhat enhanced activity

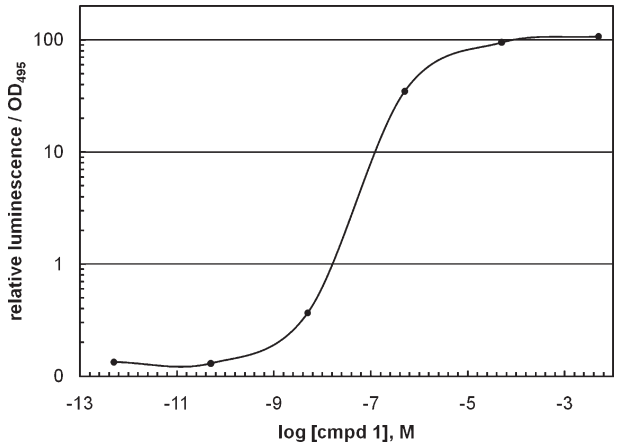

Figure 2. Dose-response curve for **1** in *P. aeruginosa* reporter strain PAKR02 (*lasI*::Gm^R mini-CTX::*lasB-luxCDABE*).

(Table 1). Similar replacements with N-substituted groups (NMe, **4b**; N-*i*-Pr, **4c**) were also tolerated, although with some loss of activity in the polar *N*-hydroxyethyl analogue **4d**.

Despite the fact that preventing enolization under physiological conditions for C^4-aza derivatives of **1** is probably superfluous, we investigated the effects of C^2 fluorination, since this may stabilize against predicted metabolism at this position. We observed that immunosuppresive activity was maintained when one of the C^2-H atoms in the alkyl chain of the 4-aza lead compound **4a** was replaced with fluorine (**4g**), but replacement of both C^2-H atoms with fluorine (**4h**) significantly reduced such activity. Both C^2-fluoro-4-aza derivatives (**4g** and **4h**) displayed much reduced LasR agonist activity, but both acted as antagonists.

The replacement of the C^3═O function with N-OBn (**10a**) or N-OH (**10b**) also maintained immunosuppression activity relative to **1**, whereas replacement with N-NH$_2$ (**10c**) led to increased activity. Replacement of the C^3 carbonyl group with an O-acetylated carbinol in **20** afforded the most potent compound in our series in terms of immune suppression, with somewhat enhanced potency compared to the corresponding free carbinol compound reported earlier (compound **17** in ref 17). Like the C^4-modified compound discussed above, these C^3-modified derivatives **10a**−**c** and **20** also exhibited very little LasR agonist activity but some antagonist activity.

Of the compounds in which the C^1 or C^3 carbonyls of **1** were replaced with sulfur-containing functional groups, the C^3-modified thioether derivative **16a** showed no immunosuppressive activity whereas the corresponding sulfones (**16b** and especially **16c**) were active. A similar situation was observed with the sulfoxides **16d** and **16e**; again, the extended acyl chain derivative (**16e**) was more potent. Replacement of the C^3 carbonyl group with a sulfonyl group in the context of the 4-aza derivative **4a** afforded the sulfonamide analogue **19a**, which also retained full immunosuppressive activity. Similarly, the C^1-modified sulfonamide analogue **19b** was also active. These derivatives, too, were practically devoid of LasR agonist activity but retained some antagonist activity.

A number of ring-modified analogues of **1** have been reported as agonists and antagonists of bacterial autoinduction,[32,33] and we therefore decided to investigate such compounds in terms of their immune suppression activity. It is known, for example, that replacement of the ring O in **1** with S is tolerated and the resulting thiolactone compound has full LasR-inducing activity, whereas the corresponding lactam arising from replacement of the ring O with NH is poorly tolerated in this respect.[10,34] In our hands replacement of the L-HSL system with L-aspartimide (**23a**) resulted in complete loss of immunosuppressive activity and QS agonism and antagonism activity. The corresponding L-glutarimide derivative **23b**, on the other hand, exhibited markedly reduced but measurable immunosuppressive activity while showing insignificant LasR-induction activity and some QS antagonism.

Introduction of the nonchiral 2-aminothiazole ring system in **23c** surprisingly resulted in retention of immunosuppressive activity while abolishing LasR-induction ability. Again, the same modification was similarly successful in the context of the 4-aza derivative **4a** (analogue **25a**). These results motivated us to investigate a series of simple (hetero)aromatic and alicyclic replacements for the lactone ring in the 4-aza derivative **4a**. The 2-cyclopentanol (**24a**), 2-cyclohexanol (**25b**), and 2-phenol (**25c**) analogues, all known QS antagonists in the context of **1**,[35,36] retained activity but did not show any improvement over **1** or **4a**. The substituted phenol derivative **25d** and the 2-cycloheptane analogue **25j**, on the other hand, showed potent immunosuppressive activity, although in the latter compound this was accompanied by appreciable cellular toxicity. All three benzamide compounds (**25e**−**g**) and the indazole (**25h**) and pyridine (**25i**) derivatives displayed similar immunosuppressive activity while lacking QS activity. These results suggest that while the LasR/OdDHL-driven activation of *lasB* requires the lactone ring of **1**, immune suppression activity is not influenced strongly by the nature of the cyclic system. Furthermore, these results provide evidence that the hydrolytically and metabolically labile homoserine lactone unit can be replaced with more druglike systems in prospective immunosuppressive lead compounds based upon **4a**.

The tetramic acid derivative **21**, a known nonenzymatic degradation product of **1**, has antimicrobial properties toward Gram-positive bacteria[25] but in contrast to **1** was reported to have no cytotoxicity toward human bone marrow-derived macrophages at concentrations up to 100 μM.[37] However, we observed that **21** has immunosuppressive activity, accompanied by cytotoxicity against Jurkat cells at concentrations above ~50 μM.

One of the drawbacks of derivatives of **1** as leads for drug discovery is their high lipophilicity and comparatively low aqueous solubility. We attempted to alleviate this situation by insertion of oxygen atoms into the acyl chain. It can be seen from the list of RP-HPLC retention times (Supporting Information Table 1) that the relevant compounds (**7a**, **7b**, **24b**) are comparatively more polar than other derivatives of **1**. Replacement of the C^4 methylene group in **1** with O (derivative **13**) resulted in biological activity similar to that from replacement with NH (**4a**), whereas oxygen insertion into the alkyl chain further from the β-ketoamide group was less well tolerated. Thus, the derivative of **1** with an oxygen replacement at C^8 (**7a**) had reduced immunosuppressive activity, and the corresponding compound with oxygen replacements at both C^7 and C^{10} (**7b**) was completely inactive. Similarly, both corresponding derivatives **24b** and **24c** based on the cyclopentanol compound **24a** had much reduced immunosuppressive activity. It would therefore appear that the lipophilic nature of the acyl chain is important not only for QS but also for immunosuppressive activity.

The separation of inhibition of murine peripheral blood leukocyte proliferation and Jurkat cytotoxicity for the natural compound **1**, at approximately 12-fold (Table 1), was somewhat narrow, and better separation would be desirable in an immunosuppressive lead compound. The majority of the analogues investigated here did indeed show similar or enhanced separation of these activities, although some compounds displayed a narrower window between

immune suppression and cytotoxicity, e.g., **4a**, **4c**, **4d**, **16d**, **25e**, **25f**, and especially **10a**, **16c**, **21**, and **25j**. It is not clear at this stage what the structural features responsible for the separation of these activities are.

CONCLUSIONS

The key *P. aeruginosa* QSSM **1**, due to its contribution to virulence gene regulation and host immune suppression activities, has been studied intensively since its discovery in 1994.[3] Much of this research has been based on the expectation that **1** might serve as a starting point for the design of new antibiotic and immunosuppressive therapeutic agents. We have been particularly interested in the latter topic. We have examined the SARs of a series of analogues of **1**, and here we have shown for the first time that a key determinant for the separation of bacterial autoinduction and host immune suppression is the C^4 methylene group in the acyl chain of **1**. When this is replaced with certain functions based on heteroatoms, especially an NH group, derivatives of **1** are no longer able to act as agonists at LasR, although in some cases LasR antagonist activity is observed. However, this replacement was not detrimental to immune suppression activity. Furthermore, we have discovered that the structural requirements in the cyclic portion of **1** are less restrictive for immune suppression activity than for QS in *P. aeruginosa*. It is thus possible to combine the C^4-aza modification with replacement of the lactone ring in the L-HSL system with simple nonchiral cyclic groups while retaining or even improving the immunosuppressive potency of **1** and abolishing LasR-induction activity. Such compounds are expected to be much more druglike than **1** in terms of metabolic stability. Unfortunately our efforts to increase the polarity of the somewhat lipophilic compound **1** by introducing heteroatoms into the acyl chain were not tolerated in terms of biological activity, and this aspect, as well the optimization of immune suppression potency, remains to be studied in more detail. The most promising lead compounds from the present study for further optimization and development are those that are devoid of bacterial LasR-induction and that are either equipotent with **1** (e.g., compounds **4b**, **4e**, and **23c**) or more potent than **1** (compounds **4f**, **10b**, **10c**, **16e**, **19a**, **19b**, **20**, **25c**, **25d**, **25g**, and **25i**) in terms of immune suppression, provided that they display a similar or better window between immunosuppressive and cytotoxic activity and especially if they also possess QS antagonist properties (compounds **4f**, **4e**, **25c**, and **25i**), which may be desirable in a future immunosuppressive agents. On the basis of our preliminary exploration, we envisage that such QS-derived agents, provided the current lead molecules can be optimized against appropriate parameters such as immunosuppressive potency, bioavailability, and safety pharmacology, may have potential to be developed as novel therapeutics for immunological diseases such allergy, type I diabetes, rheumatoid arthritis, inflammatory bowel disease, psoriasis, etc.[38]

EXPERIMENTAL METHODS

L-HSL, obtained from NovaBiochem (Nottingham, U.K.), was used to prepare L-HSL·HCl. Carboxylic acids and other starting materials were purchased from Aldrich Chemical Co. and Lancaster Chemicals. The solvents used were of HPLC grade. Dichloromethane was dried by storage over anhydrous $CaCl_2$ before distillation. Melting points were measured using a Kofler Hot Stage melting point apparatus and are reported uncorrected. FT-IR spectra were recorded on an Avatar 360 Nicolet FT-IR spectrophotometer using the range 4000−600 cm^{-1}, with samples either in the form of KBr pellets or as thin films. ^1H NMR spectra were recorded as $CDCl_3$ or DMSO-d_6 sample solutions on Bruker AMX-250 or Bruker Avance-400 instruments operating at 250 and 400 MHz, respectively. ^{13}C NMR spectra were recorded similarly using the Bruker Avance-400 instrument operating at 100 MHz. Chemical shifts were referenced to an internal standard $SiMe_4$ or residual protic solvents on a broad band decoupled mode, and assignments were made using a DEPT pulse sequence. ES-MS spectra were recorded using Micromass VG Platform and Micromass LCT spectrometers for low- and high-resolution spectra, respectively. TLC was performed using Merck silica gel 60 GF_{254} precoated (0.2 mm) aluminum plates. Preparative TLC was performed using Merck silica gel 60 GF_{254} coated (1.0 mm) glass plates (20 cm × 20 cm). Flash chromatography[39] was performed using Merck Kieselgel 60 (230−400 mesh). All products during chromatography were visualized using UV light (λ = 254 nm) or by staining with dilute potassium permanganate or phosphomolybdic acid solutions. Analytical RP-HPLC was performed using a Shimadzu UFLCXR system coupled to an Applied Biosystems API2000 UV−MS instrument. Two columns, thermostated at 40 °C, were used: (1) Gemini-NX 3 μm to 110 Å, 50 mm × 2 mm (column A) and (2) Luna 3 μm (PFP2) to 110 Å, 50 mm × 2 mm (column B). The flow rate was 0.5 mL/min. Gradient elution was from 10% to 98% solvent B (0.1% HCOOH in MeCN) in solvent A (0.1% HCOOH in H_2O) over 2 min, followed by isocratic elution at 98% solvent B for 2 min. UV detection was at 220 and 254 nm. Compound purities by RP-HPLC were ≥95% for all the final products. For general procedures 1 and 8−11, refer to Supporting Information.

General Procedure 2 (GP2). Ethyl 3-chloro-3-oxopropanoate (5 mmol) was added dropwise over 20 min to a stirred solution of an appropriate amine **2** (10 mmol) in dry CH_2Cl_2 (25 mL) at 0 °C. Stirring was continued overnight. The mixture was diluted with CH_2Cl_2 (15 mL) and washed with saturated aqueous $NaHCO_3$ (3 × 20 mL), 2 M aqueous HCl (3 × 15 mL), and brine (25 mL). The organic layer was dried over $MgSO_4$ and evaporated. The product was crystallized from Et_2O.

General Procedure 3 (GP3). A solution of NaOH (6 mmol) in H_2O (15 mL) was added dropwise to a stirred solution of the appropriate ester (5 mmol) in EtOH or MeOH (25 mL). Stirring was continued for 4 h. The mixture was evaporated, and the residue was dissolved in H_2O (20 mL). The solution was washed with EtOAc (2 × 10 mL). The aqueous layer was acidified with 2 M aqueous HCl to pH 2 and extracted with EtOAc or CH_2Cl_2 (3 × 15 mL). The combined extracts were dried over $MgSO_4$, filtered, and evaporated.

3-(Methyl(octyl)amino)-3-oxopropanoic Acid (3b). The ethyl ester precursor of **3b** was prepared from **2b** (97%) using GP2. ^1H NMR ($CDCl_3$): δ 0.87 (3H, t, *J* 6.7), 1.26−1.32 (13H, m), 1.55 (2H, bs), 2.96 (3H, dd, *J* 2.9, 8.6), 3.24 (1H, *J* 7.6), 3.39 (1H, quint, *J* 2.9, 7.6), 3.44 (2H, d, *J* 2.9), 4.2 (2H, 2 × q). The title compound was obtained as white crystals (93%) from this material using GP3. FT-IR (KBr): 3296, 1718, 1649 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.89 (3H, t, *J* 6.0 Hz), 1.10−1.45 (10H, m), 1.58 (2H, bs), 3.03 (3H, s), 3.24 and 3.44, (2H, t, *J* 7.6), 3.37 and 3.39 (2H, 2 × s), 10.15 (1H, s, COO*H*).

3-(Nonylamino)-3-oxopropanoic Acid (3e). The ethyl ester precursor of **3e** was prepared from **2e** as pink crystals (66%) using GP2. Mp: 185−187 °C. FT-IR (KBr): 3294, 1744, 1654, 1560 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.89 (3H, t, *J* 6.8 Hz), 1.21−1.32 (15H, m), 1.50−1.55 (2H, bm, *J* 7.2), 3.29 (2H, t, *J* 7.2), 3.31 (2H, s), 4.21 (2H, q, *J* 7.2), 7.13 (1H, s). The title compound was obtained as white crystals (94%) from this material using using GP3. Mp: 82−84 °C. FT-IR (KBr): 3300, 1708, 1638, 1561 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 0.90 (3H, t, *J* 7.0), 1.24−1.38 (12H, m), 1.53−1.58 (2H, bm), 3.32 (2H, s), 3.35 (2H, t, *J* 7.2), 6.30 (1H, s), 11.21 (1H, s). ^{13}C NMR ($CDCl_3$): δ 168.66, 168.18, 40.14, 38.22, 31.82, 29.43, 29.20, 29.17, 29.14, 26.80, 22.64, 14.08.

3-Oxo-3-(undecylamino)propanoic Acid (3f). The ethyl ester precursor of **3f** was prepared from **2f** as pale yellow crystals (93%) using GP2. Mp: 196−198 °C. FT-IR (KBr): 3292, 1745, 1636, 1548 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.90 (3H, t, *J* 6.8), 1.25−1.37 (19H, m), 1.50−1.56 (2H, bm, *J* 7.2), 3.28 (2H, t, *J* 7.2), 3.31 (2H, s), 4.21 (2H, q, *J* 7.2), 7.12

(1H, s). ^{13}C NMR ($CDCl_3$): δ 168.13, 166.87, 61.52, 41.05, 39.60, 31.90, 29.57, 29.51(2), 29.36, 29.32, 29.27, 26.90, 22.68, 14.10, 14.05. The title compound was obtained from this material as white crystals (87%) using GP3. Mp: 92−94 °C. FT-IR (KBr): 3317, 1708, 1638, 1553 cm^{-1}. ^{1}H NMR ($CDCl_3$) δ: 0.90 (3H, t, *J* 7.0), 1.25−1.38 (16H, m), 1.53−1.59 (2H, bm), 3.31 (2H, s), 3.34 (2H, t, *J* 7.2), 6.22 (1H, s), 12.6 (1H, bs). ^{13}C NMR ($CDCl_3$) δ: 171.68, 166.67, 40.17, 38.10, 31.89, 29.57, 29.54 (2), 29.46, 29.30, 29.15, 26.80, 22.67, 14.10.

General Procedure 4 (GP4). A solution of L-HSL·HCl (1 mmol) in a minimum amount of H_2O (∼2 mL) was added to a stirred solution of an appropriate carboxylic acid **3** (1 mmol) in a minimum volume of dioxane (4−6 mL). To this was added Et_3N (3 mmol), followed by *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC·HCl, 1.5 mmol), and stirring was continued for 16 h. Solvents were removed under reduced pressure and the residue was dissolved in EtOAc (20 mL). The solution was sequentially washed with H_2O (3 × 10 mL), saturated aqueous $NaHCO_3$ (3 × 10 mL), and brine (15 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated.

(*S*)-*N*^{1}-Methyl-*N*^{1}-octyl-*N*^{3}-(2-oxotetrahydrofuran-3-yl)malonamide (4b). White crystalline solid in (20%) from **3b** using GP4 and purification by preparative TLC (EtOAc−hexane, 1:1). Mp: 71−73 °C. FT-IR (KBr): 3300, 1777, 1684, 1655, 1640, 1557 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.88 (3H, t, *J* 5.6), 1.10−1.31 (10H, m), 1.53 (2H, bs), 2.29 (1H, m), 2.66 (1H, m), 2.96 and 3.03 (3H, 2 × s), 3.30 (2H, t, *J* 7.5), 3.38 (2H, dd, *J* 4.9), 4.28 (1H, m), 4.48 (1H, t, *J* 9.1), 4.6 (1H, m), 8.84 (1H, d, *J* 7.4). ^{13}C NMR ($CDCl_3$): δ 174.2, 166.62 (2), 65.88, 53.82, 42.97, 39.76, 31.92, 30.16, 29.72(2), 29.42(2), 26.81, 22.82, 14.02. ES-MS: *m*/*z* 313.2134 $[M + H]^+$, $C_{16}H_{29}N_2O_4$ requires 313.2127.

(*S*)-*N*^{1}-Nonyl-*N*^{3}-(2-oxotetrahydrofuran-3-yl)malonamide (4e). Pinkish white crystals (31%) from **3e** using GP4. Mp: 122−124 °C. FT-IR (KBr): 3301, 1778, 1679, 1642, 1550 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.90 (3H, t, *J* 6.8), 1.27−1.38 (12H, m), 1.51−1.56 (2H, bm) 2.26−2.31 (1H, m), 2.72−2.79 (1H, m), 3.24 (2H, s), 3.28 (2H, t, *J* 7.0), 4.26−4.33 (1H, m), 4.48−4.53 (1H, m), 4.55−4.61 (1H, m), 6.49 (1H bs), 7.78 (1H, d, *J* 6.0). ^{13}C NMR ($CDCl_3$): δ 174.67, 167.67, 166.73, 65.81, 49.05, 42.46, 39.86, 31.85, 29.56, 29.48, 29.31, 29.25(2), 26.89, 22.67, 14.12. ES-MS: *m*/*z* 313.2138 $[M + H]^+$, $C_{16}H_{29}N_2O_4$ requires 313.2127.

(*S*)-*N*^{1}-(2-Oxotetrahydrofuran-3-yl)-*N*^{3}-undecylmalonamide (4f). Pinkish white crystals (55%) from **3f** using GP4. Mp: 123−125 °C. FT-IR (KBr): 3293, 1771, 1686, 1644, 1554 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.90 (3H, t, *J* 7.0), 1.27−1.33 (16H, m), 1.50−1.58 (2H, bm), 2.25−2.30 (1H, m), 2.72−2.78 (1H, m), 3.25 (2H, s), 3.27 (2H, t, *J* 7.0), 4.26−4.33 (1H, m), 4.48−4.53 (1H, m), 4.55−4.62 (1H, m), 6.48 (1H s), 7.75 (1H, d, *J* 6.0). ^{13}C NMR ($CDCl_3$): δ 175.17, 167.67, 166.13, 65.78, 54.12, 43.05, 41.46, 32.26, 30.25(2), 29.38, 29.19, 26.82, 22.74, 14.12. ES-MS: *m*/*z* 341.2434 $[M + H]^+$, $C_{18}H_{33}N_2O_4$ requires 341.2440.

General Procedure 5 (GP5). The pH of a solution of *O*-benzylhydroxylamine (0.25 g, 2 mmol) in MeOH (20 mL) at 0 °C or of *O*-*tert*-butylhydroxylamine (released from its hydrochloride salt (0.25 g, 2 mmol) in MeOH (20 mL) at 0 °C by adding a solution of sodium methoxide (from Na (0.08 g, 2 mmol) in MeOH)) was adjusted to 4 with AcOH. 1-Nonanal (344 μL, 2 mmol) was added, and the mixture was allowed to warm to room temperature. The pH was maintained by further addition of AcOH. After 1 h of being stirred, the reaction mixture was cooled to 0 °C, and a first portion of $NaCNBH_3$ (0.14 g, 2.2 mmol) was added. The pH was maintained, and the reaction mixture was warmed to room temperature. After intervals of 1 h at pH 4 two further portions of $NaCNBH_3$ (0.06 g, 1.1 mmol, each) were added. The reaction mixture was stirred for 4 h and was evaporated. The residue was dissolved in EtOAc (40 mL), and the solution was washed with water (3 × 10 mL), saturated aqueous $NaHCO_3$ (3 × 15 mL), and brine (20 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated.

***O*-Benzyl-*N*-nonylhydroxylamine (8a).** Colorless oil (58%) using GP5 and purification by flash chromatography (EtOAc−hexane, 1:9). ^{1}H NMR ($CDCl_3$): δ 0.90 (3H, t, *J* 6.8), 1.25−1.38 (12H, m), 1.49−1.58 (2H, quint, *J* 7.2), 2.95 (2H, t, *J* 7.2), 4.73 (2H, s), 7.30−7.35 (5H, bm). ^{13}C NMR ($CDCl_3$): δ 138.02, 128.38(2), 128.36(2), 127.78, 76.20, 52.24, 31.89, 29.54(2), 29.28, 27.33, 27.20, 22.69, 14.13. ES-MS: *m*/*z* 250.2159 $[M + H]^+$, $C_{16}H_{28}NO$ requires 250.2171.

***O*-*tert*-Butyl-*N*-nonylhydroxylamine (8b).** Colorless oil (72%) using GP5 and purification by flash chromatography (EtOAc−hexane, 1:9). ^{1}H NMR ($CDCl_3$): δ 0.89 (3H, t, *J* 6.8), 1.19 (9H, s), 1.92−1.34 (12H, m), 1.45−1.48 (2H, m), 2.86 (2H, t, *J* 7.2), 4.88 (1H, bs). ^{13}C NMR ($CDCl_3$): δ 79.10, 42.83, 32.14, 29.52, 28.87(2), 27.38(3), 27.10, 26.92, 22.58, 14.13. ES-MS: *m*/*z* 216.2341 $[M + H]^+$, $C_{13}H_{30}NO$ requires 216.2327.

***tert*-Butyl *N*^{2}-Nonylcarbazate (8c).** A solution of 1-bromononane (571 μL, 3 mmol) and *tert*-butyl carbazate (0.264 g 2 mmol,) in THF (25 mL) was refluxed for 18 h. The solution was cooled and evaporated. The residue was purified by flash chromatography (EtOAc−hexane, 1:3) to afford the title compound as a yellow oil (25%). FT-IR (KBr): 3315, 1713, 1458, 1367 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.89 (3H, t, *J* 6.8), 1.20−1.34 (12H, m), 1.42−1.45 (2H, m), 1.47 (9H, s), 2.83 (2H, t, *J* 7.2), 3.92 (1H, bs), 6.09 (1H, bs). ^{13}C NMR ($CDCl_3$): δ 158.68, 80.37, 52.18, 31.88, 29.57, 29.52, 29.27, 28.37(3), 27.86, 27.09, 22.68, 14.12. ES-MS: *m*/*z* 259.2392 $[M + H]^+$, $C_{14}H_{31}N_2O_2$ requires 259.2386.

General Procedure 6 (GP6). Benzyl 2-bromoacetate or *tert*-butyl bromoacetate (8 mmol) was added dropwise to a stirred solution of **8** (4 mmol) in THF (30 mL) containing *i*-Pr_2NEt (8 mmol), and the mixture was refluxed overnight. The solution was cooled and evaporated. The residue was dissolved in EtOAc (40 mL), and the solution was washed with water (3 × 10 mL), saturated aqueous $NaHCO_3$ (3 × 15 mL), 1 M aqueous $KHSO_4$ (2 × 15 mL), and brine (20 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated.

2-(Benzyloxy(nonyl)amino)acetic Acid (9a). *tert*-Butyl 2--(benzyloxy(nonyl)amino)acetate was prepared from **8a** using GP6 as a viscous oil (89%): ^{1}H NMR ($CDCl_3$): δ 0.90 (3H, t, *J* 6.8), 1.25−1.36 (12H, m), 1.51 (9H, s), 1.61−1.64 (2H, m), 2.80 (2H, t, *J* 7.6), 3.47 (2H, s), 4.88 (2H, s), 7.34−7.35 (5H, bm). ^{13}C NMR ($CDCl_3$): δ 168.57, 137.37, 128.79(2), 128.31(2), 127.86, 81.07, 75.34, 61.25, 59.48, 31.91, 29.54(2), 29.30(2), 28.13(3), 27.23, 22.70, 14.15. ES-MS: *m*/*z* 364.2867 $[M - H]^-$, $C_{22}H_{38}NO_3$ requires 364.2852. This *tert*-butyl ester was deprotected by acidolysis (CF_3COOH) to afford the title compound (73%). ^{1}H NMR ($CDCl_3$): δ 0.91 (3H, t, *J* 6.8), 1.23−1.39 (12H, m), 1.57−1.64 (2H, quint, *J* 7.2), 2.88 (2H, t, *J* 7.6), 3.61 (2H, s), 4.83 (2H, s), 7.31−7.36 (5H, bm), 10.38 (1H, bs). ES-MS: *m*/*z* 306.2078 $[M - H]^-$, $C_{18}H_{28}NO_3$ requires 306.2069.

2-(*tert*-Butoxy(nonyl)amino)acetic Acid (9b). Benzyl 2-(*tert*-butoxy(nonyl)amino)acetate was prepared from **8b** using GP6 as an oil (85%). ^{1}H NMR ($CDCl_3$): δ 0.90 (3H, t, *J* 6.8), 1.19 (9H, s), 1.27−1.35 (12H, m), 1.38−1.43 (2H, m), 2.86 (1H, t, *J* 7.2), 2.94 (1H, bs), 3.62 (2H, d, *J* 12.8), 5.17 (2H, s), 7.32−7.39 (5H, bm). ES-MS: *m*/*z* 364.2878 $[M - H]^-$, $C_{22}H_{38}NO_3$ requires 364.2852. This material was debenzylated by hydrogenolysis (H_2/Pd−C in EtOH) to afford the title compound (85%). ^{1}H NMR ($CDCl_3$): δ 0.89 (3H, t, *J* 7.0), 1.19−1.28 (21H, m), 1.35−1.46 (2H, m), 2.86 (2H, bt), 3.68 (2H, s), 10.23 (1H, s). ^{13}C NMR ($CDCl_3$) δ: 171.18, 75.17, 61.21, 59.60, 31.84, 29.49, 29.46, 29.24, 27.23(3), 26.99, 26.81, 22.68, 14.10. ES-MS: *m*/*z* 272.2238 $[M - H]^-$, $C_{15}H_{30}NO_3$ requires 272.2226.

2-(2-(*tert*-Butoxycarbonyl)-1-nonylhydrazinyl)acetic Acid (9c). *tert*-Butyl 2-(2-benzyloxy-2-oxoethyl)-2-nonylhydrazinecarboxylate was prepared from **8c** using GP6 as a yellow oil (82%). FT-IR (KBr): 3364, 1739, 1456, 1366 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.89 (3H, t, *J* 7.0), 1.24−1.31 (12H, m), 1.46 (9H, s), 1.51 (2H, bs), 2.88 (2H, t, *J* 7.4), 3.77 (2H, s), 5.18 (2H, s), 6.56 (1H, bs), 7.34−7.40 (5H, bm).

^{13}C NMR ($CDCl_3$): δ 168.15, 158.71, 135.21, 128.67 (2), 128.50, 128.35 (2), 81.73, 75.32, 66.40, 62.52, 31.89, 29.52(2), 29.29, 28.34(3), 27.60, 26.99, 22.68, 14.13. ES-MS: m/z 407.2889 $[M + H]^+$, $C_{23}H_{39}N_2O_4$ requires 407.2910. This material was debenzylated by hydrogenolysis (H_2/Pd−C in EtOH) to afford the title compound as a white solid (74%). Mp: 76−78 °C. FT-IR (KBr): 3342, 2578, 1724, 1503, 1368 cm^{-1}). ^{1}H NMR ($CDCl_3$): δ 0.91 (3H, t, J 7.2), 1.24−1.35 (12H, m), 1.47 (9H, s), 1.50 (2H, bs), 2.86 (2H, t, J 7.2), 3.58 (2H, s), 5.92 (1H, bs), 10.5 (1H, bs). ES-MS: m/z 315.2272 $[M - H]^-$, $C_{16f}H_{31}N_2O_4$ requires 315.2284.

(*S*)-2-(Benzyloxy(nonyl)amino)-*N*-(2-oxotetrahydrofuran-3-yl)acetamide (10a). Solid (27%) from **9a** using GP4 and purification by flash chromatography (EtOAc). Mp: 82−84 °C. FT-IR (KBr): 3403, 3378, 1778, 1678, 1664, 1519 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.91 (3H, t, J 6.8), 1.23−1.37 (12H, m), 1.54−1.59 (2H, m), 1.94−2.05 (1H, m), 2.75−2.81 (1H, m), 2.82−2.87 (2H, dt, J 7.2, 2.0), 3.49 (2H, s), 4.24−4.31 (1H, m), 4.43−4.47 (1H, m), 4.48−4.55 (1H, m), 4.75 (2H, dd, J 28.4, 11.2), 7.17 (1H, d, J 6.4), 7.31−7.36 (5H,bm).

General Procedure 7 (GP7). The compound to be deprotected (0.1 mmol) was treated with a solution of CF_3COOH in CH_2Cl_2 (50% v/v, 3 mL) for 2 h. The solution was evaporated, re-evaporated with MeCN (3 × 10 mL), and dried under a stream of N_2. The residue was dissolved in EtOAc (15 mL), and the solution was filtered. The filtrate was evaporated.

(*S*)-2-(Hydroxy(nonyl)amino)-*N*-(2-oxotetrahydrofuran-3-yl)-acetamide (10b). The *tert*-butyl-protected precursor of the title compound was obtained (55%) from **9b** using GP4 and purification by flash chromatography (Me_2CO). FT-IR (KBr): 3372, 1775, 1761, 1519, 1368 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.89 (3H, t, J 6.8), 1.20 (9H, s), 1.23−1.27 (12H, s), 1.38−1.51 (2H, bs), 2.10−2.21 (1H, m), 2.79 (2H, bs), 2.84−2.91 (1H, m), 3.59(2H, bs), 4.28−4.34 (1H, m), 4.47−4.52 (1H, m), 4.48−4.55 (1H, m), 7.54 (1H, d, J 5.6). ES-MS: m/z 357.2762 $[M + H]^+$, $C_{19}H_{37}N_2O_4$ requires m/z 357.2753. This material was deprotected using GP7 to afford the title compound as white crystals (97%). FT-IR (KBr): 3480, 1769, 1678, 1520 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.90 (3H, t, J 6.8), 1.21−1.37 (12H, m), 1.74 (2H, bs), 1.94 (2H, t, J 7.2), 2.33−2.38 (1H, m), 2.76−2.81 (1H, m), 3.89 (2H, t, J 7.2), 4.28−4.34 (1H, m), 4.50−4.54 (1H, m), 4.65−4.71 (1H, m), 7.13 (1H, s), 10.37 (1H, d, J 6.8). ^{13}C NMR ($CDCl_3$): δ 175.63, 171.53, 74.23, 59.17, 57.31, 49.03, 31.80 (2), 29.34, 29.15, 29.11, 26.51, 22.64 (2), 14.08. ES-MS m/z 301.2140 $[M + H]^+$, $C_{15}H_{29}N_2O_4$ requires 301.2127.

(*S*)-2-(1-Nonylhydrazinyl)-*N*-(2-oxotetrahydrofuran-3-yl)-acetamide (10c). The Boc-protected precursor of the title compound was obtained (60%) from **9c** using GP4 and purification by flash chromatography (Me_2CO) as a yellow solid. Mp: 60−62 °C. FT-IR (KBr): 3339, 3288, 1779, 1705, 1692, 1661, 1529 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.90 (3H, t, J 6.8), 1.35−1.43 (12H, m), 1.45 (9H, s), 1.47−1.52 (2H, bs), 2.34−2.39 (1H, m), 2.52−2.61 (1H, m), 2.75−2.80 (2H, dt, J 6.8), 3.46 (2H, dd, J 37.6, 8.8), 4.25−4.28 (1H, m), 4.47−4.52 (1H, m), 4.61−4.74 (1H, m), 5.51 (1H, s), 8.75 (1H, d, J 6.8). ES-MS: m/z 400.2798 $[M + H]^+$, $C_{20}H_{38}N_3O_5$ requires 400.2811. This material was deprotected using GP7 to afford the title compound as light yellow crystals (99%). Mp: 99−101 °C. FT-IR (KBr): 3396, 3287, 1778, 1704, 1530 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.89 (3H, t, J 6.8), 1.26−1.34 (12H, m), 1.69 (2H, bs), 2.33−2.44 (1H, m), 2.61−2.67 (1H, m), 3.16 (2H, t, J 7.8), 3.90 (2H, dd, J 23.6, 16.0), 4.28−4.35 (1H, m), 4.49−4.53 (1H, m), 4.62−4.67 (1H, m), 5.50 (2H, bs), 8.66 (1H, d, J 6.8). ^{13}C NMR ($CDCl_3$): δ 175.63, 171.53, 72.89, 62.37, 57.31, 49.03, 31.80 (2), 29.34, 29.15, 29.11, 26.51, 22.64 (2), 14.08. ES-MS: m/z 300.2279 $[M + H]^+$, $C_{15}H_{30}N_3O_3$ requires 300.2287.

***N*-((*S*)-2-Oxotetrahydrofuran-3-yl)-2-(undecylsulfinyl)acetamide (16c).** Off-white crystals (18%, 1:1 diastereomer ratio) from **15d** using GP4. Mp: 114−116 °C. FT-IR (KBr): 3316, 1773, 1648, 1543, 1037 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.88 (3H, t, J 6.8), 1.26−1.37 (14H, m), 1.45−1.51 (2H, m), 1.73−1.78 (2H, m), 2.22−2.34 (0.5H, m), 2.41−2.50 (0.5H, m), 2.64−2.73 (1H, m), 2.79−2.90 (1H, m), 3.01−3.11 (0.5H, m), 3.12−3.19 (0.5H, m), 3.27 (1H, dd, J 16.4, 14.4), 3.77 (1H, dd, J 14.4, 2.4), 4.28−4.33 (1H, m), 4.39−4.48 (0.5H, m), 4.49−4.54 (1H, m), 4.81−4.89 (0.5H, m), 7.63 (0.5H, d, J 6.4), 7.70 (0.5H, d, J 6.4). ES-MS: m/z 346.2037 $[M + H]^+$, $C_{17}H_{32}NO_4S$ requires 346.2052.

(*S*)-*N*-(2-Oxotetrahydrofuran-3-yl)-2-(undecylsulfonyl)acetamide (16e). Off-white crystals (86%) from **15f** using GP4. Mp: 110−112 °C. FT-IR (KBr): 3306, 1777, 1662, 1556, 1328, 1131 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 0.91 (3H, t, J 7.0), 1.21−1.37 (14H, m), 1.41−1.52 (2H, m), 1.82−1.91 (2H, m), 2.34−2.41 (1H, m), 2.70−2.74 (1H, m, 3.23 (2H, t, J 8.8), 3.96 (2H, s), 4.28−4.35 (1H, m), 4.50−4.55 (1H, m), 4.57−4.63 (1H, m), 7.23 (1H, d, J 6.8). ^{13}C NMR ($CDCl_3$): δ 175.47, 165.72, 65.66, 55.74, 53.24, 51.02, 31.92, 30.31, 29.72 (3), 29.38 (2), 28.83, 22.79, 21.08, 14.11. ES-MS: m/z 362.2012 $[M + H]^+$, $C_{17}H_{32}NO_5S$ requires 362.2001.

2-(*N*-Octylsulfamoyl)acetic Acid (18a). Compound **17**[40] (0.176 g, 1 mmol) was added to a solution of DBU (150 μL, 1 mmol), octan-1-amine (165 μL, 1 mmol), and K_2CO_3 (0.138 g, 1 mmol) in dry CH_2Cl_2 (15 mL) at 0 °C. The mixture was refluxed for 3 h, cooled, and evaporated. The residue was dissolved in EtOAc (20 mL). The solution was washed with 1 M aqueous HCl (3 × 10 mL) and brine (15 mL), dried over $MgSO_4$, and evaporated. The dark brown oily residue containing methyl 2-(*N*-octylsulfamoyl)acetate was used in the next step without purification. ^{1}H NMR ($CDCl_3$): δ 0.90 (3H, t, J 7.0), 1.34−1.39 (10H, m), 1.59−1.62 (2H, m), 3.16 (2H, dd, J 13.6, 7.2), 3.83 (3H, s), 4.02 (2H, s), 4.71 (1H, t). ES-MS: m/z 266.1382 $[M + H]^+$, $C_{11}H_{24}NO_4S$ requires m/z 266.1426. A cold solution of NaOH (0.1 g, 2.5 mmol) in H_2O (5 mL) was added dropwise to a stirred solution of this material (0.530 g, 2 mmol) in MeOH (10 mL). Stirring was continued for 4 h, and the solution was evaporated. The residue was dissolved in H_2O (20 mL) and washed with EtOAc (2 × 10 mL). The aqueous layer was acidified with 2 M aqueous HCl to pH 2 and extracted with EtOAc (3 × 10 mL). The combined extracts were dried over $MgSO_4$, filtered, and evaporated to afford the title compound (72%). ^{1}H NMR ($CDCl_3$): δ: 0.89 (3H, t, J 6.8), 1.27−1.35 (10H, m), 1.54−1.62 (2H, m), 3.13 (2H, dd, J 12.8, 6.8), 3.96 (2H, s), 5.02 (1H, bs), 9.87 (1H,bs). ^{13}C NMR ($CDCl_3$): δ 172.15, 54.67, 43.77, 31.73, 29.19, 29.12, 29.07, 26.54, 22.60, 14.06. ES-MS: m/z 250.1098 $[M - H]^-$, $C_{10}H_{20}NO_4S$ requires 250.1113.

(*S*)-2-(*N*-(2-Oxotetrahydrofuran-3-yl)sulfamoyl)acetic Acid (18b). A solution of L-HSL·HCl (0.344 g, 2.5 mmol), DBU (374 μL, 2.5 mmol), and DMAP (0.306 g, 2.5 mmol) in dry CH_2Cl_2 (15 mL) was stirred at 0 °C for 10 min. To this **17**[40] (0.618 g, 3.5 mmol) was added, and stirring was continued for 16 h. The solution was evaporated, and the residue was dissolved in EtOAc (30 mL). The solution was washed with 1 M aqueous HCl (2 × 10 mL) and brine (1 × 15 mL), dried over $MgSO_4$, and evaporated. The residue was purified by flash chromatography (EtOAc−MeCN, 1:1) to afford methyl (*S*)-methyl 2-(*N*-(2-oxotetrahydrofuran-3-yl)sulfamoyl)acetate (47%). FT-IR (KBr): 3309, 1782, 1756, 1457, 1348, 1143 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 2.29−2.40 (1H, m), 2.71−2.84 (1H, m), 3.84 (3H, s), 4.23 (1H, d, J 16), 4.27−4.34 (1H, m), 4.80 (1H, d, J 16), 4.46−4.52 (1H, m), 4.57−4.63 (1H, m), 5.65 (1H, d, J 7.2). ^{13}C NMR ($CDCl_3$): δ 174.71, 164.99, 65.83, 57.23, 53.24, 52.82, 30.46. ES-MS: m/z 236.0209 $[M - H]^-$, $C_7H_{10}NO_6S$ requires m/z 236.0229. This material (0.237 g, 1 mmol) was refluxed in 1 M aqueous HCl (15 mL) for 4 h. The solution was evaporated and the residue dissolved in EtOAc (20 mL). The solution was washed with brine (15 mL), dried over $MgSO_4$, and evaporated to afford the title compound as white crystals (77%). FT-IR (KBr): 3395, 2658, 1771, 1636, 1456, 1340, 1143 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 2.29−2.40 (1H, m), 2.71−2.84 (1H, m), 4.16 (1H, d, J 15.6), 4.19−4.26 (1H, m), 4.32 (1H, d, J 15.6), 4.38−4.43 (1H, m), 4.45−4.48 (1H, m), 6.86 (1H, bs), 10.56 (1H, s). ^{13}C NMR ($CDCl_3$): δ

174.87, 166.813, 65.69, 52.51, 49.36, 30.24. ES-MS: m/z 222.0038 $[M - H]^-$, $C_6H_8NO_6S$ requires 222.0072.

(*S*)-2-(*N*-Octylsulfamoyl)-*N*-(2-oxotetrahydrofuran-3-yl)-acetamide (19a). White crystals (64%) yield from **18a** using GP4 and purification by preparative TLC (EtOAc). Mp: 132−134 °C. FT-IR (KBr): 3325, 3252, 1773, 1671, 1542, 1320, 1147 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.89 (3H, t, J 7.2), 1.28−1.37 (10H, m_s), 1.54−1.61 (2H, m), 2.39−2.49 (1H, m), 2.65−2.74 (1H, m), 3.16 (2H, dd, J 13.2, 7.2), 4.01 (2H, s), 4.30−4.36 (1H, m), 4.56−4.61 (1H, m), 4.62−4.66 (1H, m), 5.26 (1H, t, J 6.0), 7.01 (1H, d, J 7.6). ES-MS: m/z 335.1628 $[M + H]^+$, $C_{14}H_{27}N_2O_5S$, requires 335.1641.

(*S*)-*N*-Octyl-2-(*N*-(2-oxotetrahydrofuran-3-yl)sulfamoyl)acetamide (19b). *n*-Octylamine (2 mmol), DMAP (3 mmol), and DCI (2.2 mmol) were added sequentially to a stirred solution of **18b** (2 mmol) in dry CH_2Cl_2 (15 mL). Stirring was continued for 16 h. After evaporation, the residue was dissolved in EtOAc (25 mL) and the solution was washed with saturated aqueous $NaHCO_3$ solution (3 × 10 mL), 1 M aqueous HCl (3 × 10 mL), and brine (15 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated. The residue was dissolved in cold Me_2CO (2−4 mL) and left to stand. When remaining DCU had crystallized, this was filtered off. The filtrate was evaporated and the residue purified by preparative TLC (EtOAc) to afford the title compound as white crystals (22%). Mp: 94−96 °C. FT-IR (KBr): 3386, 3323, 1761, 1683, 1560, 1327, 1168 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.89 (3H, t, J 6.8), 1.27−1.33 (10H, m), 1.52−1.57 (2H, m), 2.33−2.46 (1H, m), 2.71−2.79 (1H, m), 3.28 (2H, m), 4.07 (1H, s, J 14.2), 4.26 (1H, s, J 14.2), 4.27−4.34 (1H, m), 4.45−4.52 (1H, m), 4.46−4.51 (1H, m), 6.21 (1H, d, J 7.6), 6.69 (1H, t). ES-MS: m/z 335.1638 $[M + H]^+$, $C_{14}H_{27}N_2O_5S$ requires 335.1641.

General Procedure 12 (GP12). The appropriate amine (1 mmol) and Et_3N (155 μL, 1.1 mmol) were added to a stirred solution of **22**[17,34] (300 mg, 1 mmol) in dry MeCN (30 mL). The mixture was stirred for 16 h and then refluxed for 3 h. The solution was cooled, evaporated, and the residue was dissolved in EtOAc. The solution was washed with 1 M aqueous $KHSO_4$ (2 × 20 mL), dried over $MgSO_4$, and evaporated.

3-Oxo-*N*-(thiazol-2-yl)dodecanamide (23c). White solid (30%) from thiazol-2-amine using GP12 and purification by preparative TLC (EtOAc−hexane, 1:1). Mp: 137−139 °C. FT-IR (KBr): 3427, 1719, 1679, 1587 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.9 (3H, t, J 7.0), 1.22−1.29 (12H, m), 1.62 (2H, bm), 2.57 (2H, quart, J 7.2, 4.2), 3.34 (2H, s), 7.12 (1H, d, J 3.6), 7.56 (1H, d, J 3.6), 8.21 (1H, s). ^{13}C NMR ($CDCl_3$): δ 207.14, 164.4, 162.92, 138.6, 107.95, 47.93, 42.6, 32.17, 29.72, 29.38(2), 29.18, 23.2, 22.79, 14.12. ES-MS: m/z 297.1624 $[M + H]^+$, $C_{15}H_{25}N_2O_2S$ requires 297.1637.

General Procedure 13 (GP13). Et_3N (210 μL, 1.5 mmol), EDC·HCl (0.23 g, 1.2 mmol), and the appropriate amine (1.2 mmol) were added to a stirred solution of the appropriate carboxylic acid (1 mmol, 0.215 g) in CH_2Cl_2 or H_2O−dioxane (15 mL). Stirring was continued for 16 h. After evaporation, the residue was dissolved in EtOAc−CH_2Cl_2 (20 mL). The solution was washed with saturated aqueous $NaHCO_3$ solution (3 × 10 mL), 1 M aqueous HCl (3 × 10 mL), and brine (15 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated.

***N*^{1}-(2-Hydroxyphenyl)-*N*^{3}-octylmalonamide (25c).** Lustrous white crystals (82%) from **3a** and 2-aminophenol using GP13 (H_2O−dioxane). Mp: 84−86 °C. FT-IR (KBr): 3327, 3261, 3095, 1676, 1643, 1614, 1601, 1455, 1546, 744 cm^{-1}. ^1H NMR ($CDCl_3$ with a drop of DMSO): δ 0.82 (3H, t, J 6.4), 1.18−1.31 (10H, m), 1.47 (2H, bs), 3.20 (2H, t, J 6.4), 3.39 (1H, s), 6.78 (1H, t, J 7.6), 6.90 (1H, d, J 8.0), 6.98 (1H, t, J 7.6), 7.47 (1H, d, J 8.0), 7.65 (1H, s), 8.0 (1H, bs), 9.97 (1H, s). ^{13}C NMR (DMSO): δ 166.64, 165.07, 150.1, 128.1. 126.46, 123.16, 121.98, 116.12, 42.31, 40.1, 32.18, 29.99, 29.51, 29.27, 27.21, 23.22, 14.13. ES-MS: m/z 307.2034 $[M + H]^+$, $C_{17}H_{27}N_2O_3$ requires 307.2022.

***N*^{1}-(5-Chloro-2-hydroxyphenyl)-*N*^{3}-octylmalonamide (25d).** White solid (36%) from **3a** and 2-amino-4-chlorophenol using GP13 (H_2O−dioxane). Mp 80−82 °C. FT-IR (KBr): 3373, 3253, 3100, 1677, 1644, 1608, 1598, 1422, 1544, 809 cm^{-1}. ^1H NMR (DMSO-d_6): δ 0.85 (3H, t, J 6.8), 1.21−1.26 (10H, m), 1.39−1.42 (2H, m), 3.08 (2H, dd, J 12.8, 6.8), 3.35 (1H, s), 6.86 (1H, d, J 8.4), 6.93 (1H, dd, J 8.4, 2.4), 8.15 (1H, d, J 2.8), 8.21 (1H, t, J 5.6), 9.97 (1H, bs), 10.27 (1H, s). ^{13}C NMR (DMSO): δ 167.64, 166.07, 145.99, 128.18. 123.56, 122.56, 119.96, 116.25, 43.93, 39.15, 31.68, 29.35, 29.15, 29.12, 26.83, 22.55, 14.41. ES-MS: m/z 341.1625 $[M + H]^+$, $C_{17}H_{26}N_2O_3Cl$ requires 341.1632.

***N*^{1}-(2-Carbamoylphenyl)-*N*^{3}-octylmalonamide (25e).** White solid (52%) from 2-aminobenzamide and **3a** using GP13 (CH_2Cl_2). Mp: 120−121 °C. FT-IR (KBr): 3402, 3306, 3201, 1685, 1634, 1592, 1449, 1521, 752 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.89 (3H, t, J 7.2), 1.26−1.31 (10H, m), 1.52−1.59 (2H, m), 3.34 (2H, dd, J 12.8, 6.8), 3.46 (1H, s), 6.04 (1H, d), 6.26 (3H, s), 7.14−7.18 (1H, dt, J 8.0, 1.2), 7.50 (1H, bs), 7.52−7.60 (2H, m), 8.57 (1H, d, J 8.0), 11.59 (1H, s). ^{13}C NMR ($CDCl_3$): δ 167.61, 165.52, 165.01, 138.38. 133.25, 127.37, 124.67, 123.53, 121.81, 44.36, 39.75, 31.80, 29.35, 29.25, 29.19, 26.95, 22.65, 14.11. ES-MS: m/z 334.2123 $[M + H]^+$, $C_{18}H_{28}N_3O_3$ requires 334.2131, 317.1853 $[M - NH_2]^+$.

***N*^{1}-(2-(Dimethylcarbamoyl)phenyl)-*N*^{3}-octylmalonamide (25f).** Pink solid (66%) from 2-(dimethylamino)benzamide and **3a** using GP13 (CH_2Cl_2). Mp: 59−60 °C. FT-IR (KBr): 3304, 3160, 1685, 1660, 1605, 1484, 1530, 722 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.90 (3H, t, J 6.8), 1.24−1.34 (10H, m), 1.49−1.55 (2H, m), 3.03 (3H, s), 3.15 (3H, s), 3.28 (2H, dd, J 13.2, 7.2), 3.36 (1H, s), 7.14−7.18 (1H, dt, J 8.0, 1.2), 7.21 (1H, bs), 7.27−7.29 (1H, dd, J 8.0, 1.2), 7.38−7.43 (1H, dt, J 8.2, 1.2), 9.71 (1H, s). ^{13}C NMR ($CDCl_3$): δ 168.76, 166.55, 164.51, 139.98. 130.35, 127.71, 124.31(2), 121.43, 42.43, 39.59, 37.71(2), 31.98, 30.09, 29.45(2), 26.82, 22.86, 14.11. ES-MS: m/z 362.2459 $[M + H]^+$, $C_{20}H_{32}N_3O_3$ requires 362.2444; 317.1866 $[M - NMe_2]^+$.

***N*^{1}-(3-Carbamoylphenyl)-*N*^{3}-octylmalonamide (25g).** Pink solid (33%) from **3a** and 3-aminobenzamide using GP13 (H_2O−dioxane). Mp: 185−187 °C. FT-IR (KBr): 3374, 3307, 3171, 1659, 1637, 1586, 1452, 1541, 791 cm^{-1}. ^1H NMR (DMSO-d_6): δ 0.87 (3H, t, J 6.8), 1.24−1.27 (10H, m), 1.35−1.41 (2H, m), 3.06 (2H, dd, J 12.8, 6.8), 3.23 (1H, s), 7.34 (1H, s), 7.37 (1H, t, J 8.0), 7.53−7.55 (1H, dt, J 8.0, 1.2), 7.74−7.76 (1H, dd, J 8.0, 1.2), 7.94 (1H, bs), 8.02 (1H, t, J 2.0), 8.05 (1H, d, J 8.0), 10.21 (1H, s). ^{13}C NMR ($CDCl_3$): δ 168.16, 166.62, 164.15, 138.63. 134.45, 129.17, 125.17, 123.63, 117.28, 42.46, 39.71, 31.90, 30.15, 29.42(2), 26.81, 22.83, 14.10. ES-MS: m/z 334.2146 $[M + H]^+$, $C_{18}H_{28}N_3O_3$ requires 334.2131.

***N*^{1}-Octyl-*N*^{3}-(pyridin-2-yl)malonamide (25i).** Purple crystals (17%) from pyridine-2-amine and **3a** using GP13 (CH_2Cl_2, washing of EtOAc solution with aqueous HCl was omitted). Mp: 134−136 °C. FT-IR (KBr): 3306, 3117, 1645, 1679, 1578, 1438, 1555, 778 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.88 (3H, t, J 6.8 Hz), 1.27−1.30 (10H, m), 1.52−1.58 (2H, m), 2.86 (1H, s), 3.28 (2H, dd, J 9.6, 6.8), 3.57 (1H, s), 7.16 (1H, s), 7.23 (1H, t, J 6.4), 7.93 (1H, t, J 8.0), 8.19 (1H, t, J 8.8), 8.44 (1H, d, J 4.8). ^{13}C NMR ($CDCl_3$): δ 167.1, 164.10, 150.16, 146.15, 139.1, 120.21, 116.21, 42.58, 39.76, 31.89, 30.1, 29.37, 29.32, 26.81, 22.76, 14.1. ES-MS: m/z: 292.2016 $[M + H]^+$, $C_{16}H_{26}N_3O_3$ requires 292.2025.

***N*^{1}-Cycloheptyl-*N*^{3}-octylmalonamide (25j).** Off-white crystals (39%) from cycloheptylamine and **3a** using GP13 (CH_2Cl_2). Mp: 53−55 °C. FT-IR (KBr): 3299, 1662, 1552 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.90 (3H, t, J 5.2), 1.21−1.39 (10H, m), 1.43−1.61 (8H, m), 1.63−1.67 (4H, m), 1.89−1.95 (2H, m), 3.13 (2H, s), 3.24−3.29 (2H, m), 3.95 (1H, m), 6.72 (1H, bs), 6.88 (1H, d). ^{13}C NMR ($CDCl_3$): δ 167.18, 166.10, 50.69, 43.37, 39.68, 34.82(2), 31.79, 29.36, 29.23, 29.19, 27.97(2), 26.91, 24.00(2), 22.64, 14.09. ES-MS: m/z 311.2687 $[M + H]^+$, $C_{18}H_{35}N_2O_2$ requires 311.2699.

Ex Vivo Concanavalin A Stimulated Murine Peripheral Blood Leukocyte Proliferation Assay. This was carried out as previously described.[17] Test compounds were dissolved at 10 mM in DMSO and then diluted with tissue culture medium and DMSO to a final constant DMSO concentration of 1% (v/v) for the entire test concentration range.

Cytotoxicity Assay. Jurkat E6.1 cells were exposed to serially diluted test compounds in physiological saline (control) at 37 °C in 5%

CO_2/air for 24 h. Cells were washed thoroughly with physiological saline and then stained with 0.4% trypan blue dye. The number of viable cells and stained damaged cells were counted in a hemocytometer (counting chamber) in three microscopic fields. Cell viability was determined as the ratio of viable cells to all cells counted in the fields. Experiment was performed in triplicate.

QS Agonist and Antagonist Assays. To evaluate the activities of the OdDHL analogues synthesized, the *P. aeruginosa* strain PAKR02 (*lasI*::Gm^R mini-CTX::*lasB-luxCDABE*) was constructed. The *lasB* gene promoter region was amplified from *P. aeruginosa* PAO1 genomic DNA by PCR using oligonucleotide primers PlasBFW (TGGCCCAAGCTTACCGTGCG) and PlasBRV (ACCCAGGATCCCAACGAACAAC). The amplicon obtained was digested with the restriction enzymes *Hind*III and *Bam*HI, cloned into pHKBS1,[41] and digested with the same enzymes to create plasmid pKR6. After confirmation of the presence of the *lasB::luxCDABE* fusion in pKR6 (by sequencing with primer LUXCRV (TCGGGAAAGATTTCAACCTGGC)), the plasmid was mobilized by conjugation from *E. coli* S17-1λpir into *P. aeruginosa* strain PAKR94. This strain is a *lasI* mutant which was obtained by transformation with the suicide plasmid, pSB219.8.[42] Transfer of pKR6 into *P. aeruginosa* PAKR94 results in the integration of the *lasB::luxCDABE* fusion into the *attB* site of the *P. aeruginosa* chromosome giving strain PAKR02. The functionality of the *lasB::luxCDABE* fusion in PAKR02 was confirmed by the induction of bioluminescence in response to exogenously provided OdDHL.

For OdDHL agonist and antagonist assays, PAKR02 was cultured overnight at 37 °C in Luria−Bertani (LB) broth, centrifuged and the resulting cell pellet washed with LB before resuspending in LB to an optical density at 600 nm (OD_{600}) of 0.001. To evaluate the induction of bioluminescence as a function of growth, light levels were monitored over 24 h at 37 °C using an Anthos LUCY1 combined microplate photometer/luminometer. The EC_{50} was determined for OdDHL by quantifying the maximal bioluminescence output over a range of concentrations from 1 pM to 1 mM. For agonist assays, test compounds were added to microtiter wells containing PAKR02 at a range of concentrations up to 100 μM; OdDHL (5 μM) and solvent (5% v/v MeOH) were included as positive and negative controls, respectively. Values were expressed as a percentage of the maximal bioluminescence measured after exposure of the bacterial reporter cells to the analogues (approximately 8 h after inoculation at 37 °C) relative to the positive control (100%). For QS-inhibition (antagonist) assays mixtures of 5 μM **1** and test compounds (at 1 mM and 100 μM) were assayed. Assays were repeated up to five times.

■ ASSOCIATED CONTENT

Supporting Information. General procedures 1 and 8−11; synthesis and characterization of compounds **2c**, **2d**, **3a**, **3c**, **3d**, **3g**, **3h**, **4a**, **4c**, **4d**, **4g**, **4h**, **5b**, **6a**, **6b**, **7a**, **7b**, **12**, **13**, **15a**, **15c**−**15f**, **16a**, **16b**, **16d**, **20**, **21**, **23a**, **23b**, **24a**−**24c**, **25a**, **25b**, and **25h**; RP-HPLC analysis of OdDHL analogues; supplementary references. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

CDI, carbonyldiimidazole; DBU, 1,8-diazabicycloundec-7-ene; DCI, *N,N′*-dicyclohexylcarbodiimide; DCU, *N,N′*-dicyclohexylurea; DMAP, 4-dimethylaminopyridine; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; L-HSL, L-homoserine lactone; OdDHL, *N*-(3-oxododecanoyl)-L-homoserine lactone; QS, quorum sensing; QSSM, quorum sensing signal molecule; SAR, structure−activity relationship

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