Synthetic Analogues of the Bacterial Signal (Quorum Sensing) Molecule *N*-(3-Oxododecanoyl)-L-homoserine Lactone as Immune Modulators

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Comparative immune modulatory activity for a range of synthetic analogues of a *Pseudomonas aeruginosa* signal molecule, *N*-(3-oxododecanoyl)-L-homoserine lactone (3O, C_{12}-HSL), is described. Twenty-four single or combination systematic alterations of the structural components of 3O, C_{12}-HSL were introduced as described. Given the already defined immunological profile of the parent compound, 3O, C_{12}-HSL, these compounds were assayed for their ability to inhibit murine and human leucocyte proliferation and TNF-α secretion by lipopolysaccharide (LPS) stimulated human leucocytes in order to provide an initial structure−activity profile. From IC_{50} values obtained with a murine splenocyte proliferation assay, it is apparent that acylated L-homoserine lactones with an 11−13 C side chain containing either a 3-oxo or a 3-hydroxy group are optimal structures for immune suppressive activity. These derivatives of 3O, C_{12}-HSL with monounsaturation and/or a terminal nonpolar substituent on the side chain were also potent immune suppressive agents. However, structures lacking the homoserine lactone ring, structures lacking the L-configuration at the chiral center, and those with polar substituents were essentially devoid of activity. The ability of compounds selected from the optimal activity range to modulate mitogen-driven human peripheral blood mononuclear cell proliferation and LPS-induced TNF-α secretion indicates the suitability of these compounds for further investigation in relation to their molecular mechanisms of action in TNF-α driven immunological diseases, particularly autoimmune diseases such as psoriasis, rheumatoid arthritis, and type 1 (autoimmune) diabetes.

Introduction

Immunological diseases, including common disorders such as rheumatoid arthritis, psoriasis, type 1 diabetes mellitus, and autoimmune thyroid disease, as well as less common conditions such as multiple sclerosis and myasthenia gravis, cause chronic morbidity and disability. In a recent study of 24 well-characterized autoimmune diseases in the U.S., it was estimated that approximately 8 500 000, or 1 in 31 individuals in the U.S., suffer from one of these disorders,[1] which are not successfully treated with existing therapies. The currently employed immunosuppressive drugs and their mechanism of action have recently been reviewed.[2] Steroid treatment is in the main nonselective, cyclosporin A (CsA) and FK506 can be nephrotoxic, and antibody therapy to Th1 cytokines is expensive and can cause immune-mediated toxicity. Nevertheless, use of the potent immunosuppressive agents such as CsA, FK506, and rapamycin, which have a degree of selective action on cells of the immune system and are derived from fungal or bacterial sources, has led to a dramatic increase in the success rate of transplant surgery, indicating not only the value of immunosuppressive natural products to clinical medicine but also an understanding of molecular mechanisms involved.[2] The recent observation[3] that *N*-(3-oxododecanoyl)-L-homoserine lactone (3O, C_{12}-HSL),[4] one of the cell-density-dependent (quorum sensing) signal molecules derived from *Pseudomonas aeruginosa*, also possesses immune modulatory activity in animals suggested that a more detailed evaluation of synthetic analogues of this molecule might ultimately provide a better understanding of their mechanism of action and lead to the development of novel therapeutic agents in due course.

Although *N*-acylhomoserine lactones (AHLs) have so far largely been considered as effectors of prokaryotic gene expression, they are also capable of influencing eukaryotic cell behavior and potentially mediating disease processes.[3,5] In particular, 3O, C_{12}-HSL (but not the shorter-chain 3O, C_6-HSL homologue) suppresses human and murine leucocyte proliferation and inhibits LPS-induced secretion of TNF-α and IL-12, an immunological response modification associated with many autoimmune diseases. AHLs also promote other immunological responses associated with recovery from some autoimmune diseases, indicating immunological selectivity of action,[3] although recent papers have presented contradictory data at high dosage.[6,7] What is not in doubt is the confirmed biological activity of this class of molecule in immunological assay.

The present study compares immune-mediated responses in a number of standard cellular assays, produced by a range of synthetic analogues of 3O, C_{12}-HSL, where single or combination systematic alterations have been made to the structural components of

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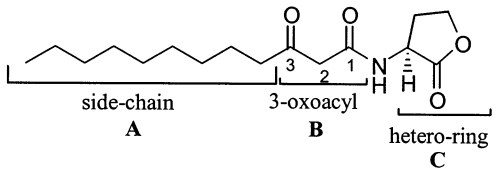


Figure 1. Structure of *N*-(3-oxododecanoyl)-L-homoserine lactone. A, B, and C are the structural segments that were modified for SAR studies.

3O, C_{12}-HSL in order to establish the basic inherent structural requirements for activity.

Chemistry

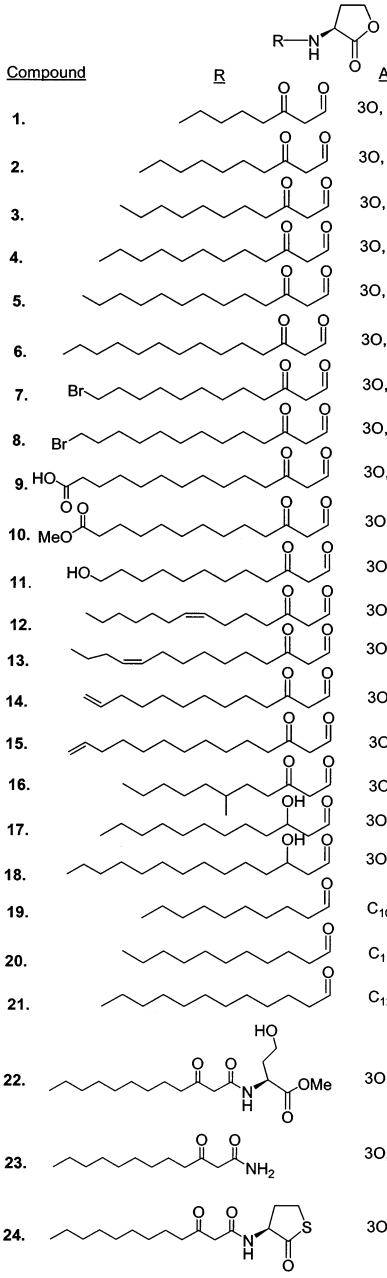
To evaluate the molecular basis for the immune modulatory activity of the native autoinducer 3O, C_{12}-HSL,[3] single or combination systematic structural variations were introduced within the structural components A, B, and C of 3O, C_{12}-HSL (Figure 1).

Chemical syntheses of some of these analogues have been previously reported by us[8−11] and other groups.[12−14] *N*-(3-Oxoalkanoyl) derivatives were prepared by the acylation of L-homoserine lactone (L-HSL) with activated 3-oxoalkanoic acids. The acids were either prepared from the commercially available 3-oxo esters or chemically synthesized from appropriate acid chlorides and malonates.[10] These procedures involved the protection and deprotection of the 3-oxo substituent, which could be shortened by employing 5-acyl Meldrum's acid (2,2-dimethyl-1,3-dioxane-4,6-dione) intermediates **I** (Scheme 1) as a combined means of 3-oxo protection and alkanoic acid activation.[15]

The 5-acyl Meldrum's acid derivatives **I** were prepared in nearly quantitative yields by the DCCI/DMAP catalyzed acylation of Meldrum's acid with an appropriate carboxylic acid in CH_2Cl_2.[16] The isolated products were used without further purification. Their characterization by ^{1}H NMR established not only their identity but also the presence of H-bonded enolic structures **II** with the enol proton resonating at δ 14−15. Amidation of **II** with L-HSL HCl in the presence of triethylamine in CH_3CN afforded the desired *N*-(3-oxoalkanoyl) analogues **III** in varying yields. Often, small quantities of side products **IV**, arising from the amidation of the exocyclic carbonyl, were present but could be easily removed by chromatographic separation on a silica column. Final purification was achieved by RP-HPLC. Compounds **1**−**16** (Table 1) were obtained by this procedure.

The corresponding fatty acids required for the preparation of **II** for the analogues **1**−**6**, **8**, **9**, **11**, **12**, **14**, and **16** were commercially available. Preparation of **11**, required prior protection of the terminal hydroxyl group as an acetate (Ac_2O and pyridine)[17,18] to avoid self-condensation. Acid hydrolysis of the final protected product with 1 M HCl afforded the free alcohol. Undecandioic acid monomethyl ester required for the preparation of **10** was obtained by first converting undecandioic acid to its dimethyl ester followed by saponification with 1 M equiv of NaOH. For compounds **7** and **13**, the corresponding carboxylic acids were produced by Jones' oxidation[19] of the commercially available 10-bromo-1-decanol and *cis*-8-dodecen-1-ol, respectively, and to prepare **15**, 11-dodecen-1-yl acetate was purchased and hydrolyzed to the alcohol and then oxidized to the desired acid using Jones' reagent.

Table 1. Chemical Structures, Abbreviations, and Mouse Splenocyte Proliferation Data for All Compounds Synthesized for SAR Studies

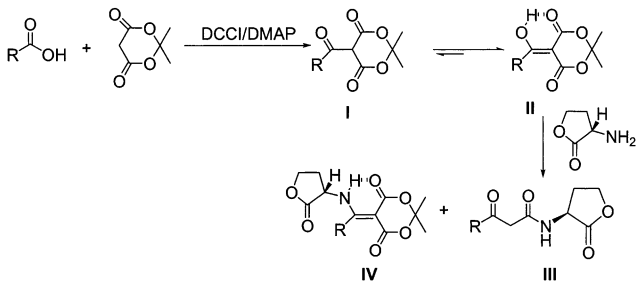


Compound	Abbreviation	IC50 (μM)
1.	3O, C_8-HSL	~ 400
2.	3O, C_{10}-HSL	21
3.	3O, C_{11}-HSL	8.9
4.	3O, C_{12}-HSL	4
5.	3O, C_{13}-HSL	4.3
6.	3O, C_{14}-HSL	6.3
7.	3O, 12Br, C_{12}-HSL	6
8.	3O, 13Br, C_{13}-HSL	3
9.	3O, 13CO_2H, C_{13}-HSL	not active
10.	3O, 12CO_2Me, C_{12}-HSL	60
11.	3O, 12OH, C_{12}-HSL	8
12.	3O,Δ^{7Z}, C_{14}-HSL	17
13.	3O, Δ^{10Z}, C_{14}-HSL	15
14.	3O, Δ^{12}, C_{13}-HSL	7
15.	3O, Δ^{13}, C_{14}-HSL	10
16.	3O, 6Me, C_{11}-HSL	5
17.	3OH, C_{12}-HSL	12.5
18.	3OH, C_{14}-HSL	7
19.	C_{10}-HSL	33
20.	C_{11}-HSL	90
21.	C_{12}-HSL	52
22.	3O, C_{12}-Hse.OMe	53
23.	3O, C_{12}-NH_2	not active
24.	3O, C_{12}-HTL	150

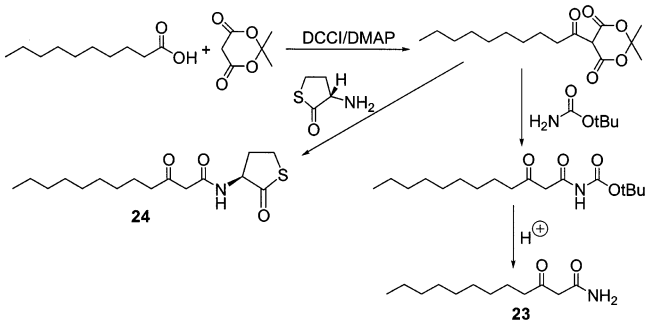
The amidation of the 5-decanoyl Meldrum's acid with *tert*-butyl carbazate and L-homocysteine thiolactone respectively furnished analogues **23** and **24** (Scheme 2). The open-ring analogue **22** was prepared by methanolysis of compound **4** with NaOMe at room temperature.

3-Hydroxy derivatives **16** and **17** were obtained as mixtures of diastereoisomers in good yields by NaC-NBH_3 reduction of the corresponding 3-oxo derivatives **4** and **6**[10] at pH 3−4 in MeOH. Finally analogues **19**−**21** were synthesized by the acylation of L-HSL either with the corresponding carboxylic acid activated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride in a water/1,4-dioxane (1:1) solvent system (ref 10) or with the corresponding acid chlorides in DCM in the presence of triethylamine.

Scheme 1



Scheme 2



Results and Discussion

The present study was undertaken to determine the broad structural requirements for the immune modulatory activity of 3O, C_{12}-HSL, following our initial observation that this molecule possessed immunological activity whereas the shorter-chain analogue 3O, C_6-HSL lacked such activity.[3]

A total of 24 compounds were synthesized and characterized. Their purity was established by elemental analyses. Their homogeneity was confirmed by reverse-phase HPLC using a linear gradient of acetonitrile in water (70−100%, v/v) over a 20 min period. The parent compound *N*-(3-oxododecanoyl)-L-homoserine lactone (3O, C_{12}-HSL) (Table 1, compound **4**) isolated from the spent culture of the Gram-negative bacterium *Pseudomonas aeruginosa* suppresses murine and human leucocyte proliferation. To determine the molecular basis of this activity, structure alterations have been performed initially on three different components of this molecule, namely, the alkyl chain, the 3-oxo substituent, and the heterocyclic ring. These compounds were assayed for their ability to modulate murine and human leucocyte proliferation stimulated with concanavalin A (ConA) and TNF-α secretion by LPS stimulated human leucocytes.

Compound activity in the murine splenocyte proliferation assay demonstrated that analogues with an alkyl chain length shorter than 8 C (data not shown) had no activity (Table 1, Figure 2A). The upper limit of the alkyl chain was dictated by analogue solubility. Thus, our subsequent studies were confined to analogues with a chain length of 8−14 C. Table 1 illustrates that activity in a murine splenocyte assay gradually increased when the alkyl chain was lengthened and peaked at 12 C. However, higher homologues with 13 and 14 C, compounds **5** and **6**, had similar activities. Branched-chain analogue (compound **16**) was slightly less potent than the parent structure (**4**). Data are presented as the IC_{50} of the compound in μM, generated from the type of data plot illustrated in Figure 2A, where the 12 C and 6 C analogues are compared against the vehicle DMSO. Splenocytes stimulated with concanavalin A are stimulated to incorporate [^{3}H]-thymidine into newly synthesized DNA, indicated as counts per minute. The IC_{50} for 3O, C12-HSL in this figure would be calculated to be in the region of 4−6 μM.

Unsaturation in the 12−14 C alkyl chain had little effect on activity. Interestingly, the terminally unsaturated compounds **14** and **15** were more active than compounds **12** and **13** with unsaturation within the chain. Activity increased, albeit slightly, as the double bond is moved away from the 3-oxoacyl moiety, suggesting that flexibility of the chain is of importance for eliciting the decrease in mouse splenocyte response. These studies were conducted only with the (*Z*)-alkenes because of the availability of appropriate precursors from commercial sources.

The same range of activity was observed when the alkyl chain was terminally substituted with bromine (**7** and **8**), hydroxyl (**11**), and CO_2Me (**10**) groups, although the last, more polar compound was much less active. However, the analogue with a free CO_2H functionality (**9**) had markedly less activity. It thus appears that lipophilicity is important for activity.

The presence of either a 3-oxo or a 3-hydroxyl substituent (compare compounds **6** and **18** and **3** and **17**) appears to retain activity, since their absence in **19**, **20**, and **21** caused a distinct drop in activity compared to **2**, **3**, and **4**. The 3-hydroxy derivatives were assayed as mixtures of the diastereoisomers because they could not be resolved easily by standard HPLC protocols.

Opening of the lactone ring structure demonstrates that the homoserine lactone is critical for the immunosuppressive activity based on the observed IC_{50} values for **4** and **22**. The ring-opened analogue methyl ester **22** also had poor activity. Furthermore, the thio analogue **24** was only weakly active compared to **4**. Complete removal of the HSL as represented by **23** rendered the molecule totally inactive. Finally the L-configuration of the homoserine lactone moiety also appears to be critical, since the D-isomer had considerably reduced activity (IC_{50} = 100 μM).

To confirm and extend our earlier findings[3] that the parent molecule also inhibited mitogen-driven proliferation of human lymphocytes, the 3-oxo compounds with chain lengths ranging from 8 to 14 C (**1**−**6** listed in Table 1) were selected for assay against Con A stimulated peripheral blood monocyte (PBMC), using three separate donors. These data are presented in Table 2, and a representative data plot is given in Figure 2B. Analysis of this table and figure illustrates once again that bioactivity in immunological assay appears to increase with increasing chain length (8−12 C); the 3O, C_6-HSL compound remains inactive (Figure 2B).

Two analogues were then taken forward for testing in a human PBMC assay in which TNF-α secretion is driven by bacterial lipopolysaccharide. From Table 2 it can be seen that each compound was bioactive in this assay; a representative data plot is given in Figure 3. Our previous publication indicated that 3O, C_6-HSL was totally inactive in preventing LPS-driven TNF-α secretion, supporting our assumption that bioactivity in all proliferative assays translates to bioactivity in TNF-α

Table 2. Mouse Cell Proliferation, Human PBMC Proliferation, and Human TNF-α Releases Data for Selected Compounds

compound	mouse cell proliferation, IC_{50} (μM)	human PBMC proliferation, IC_{50} (μM)			human TNF-α release, IC_{50} (μM)
		donor A	donor B	donor C	
1, 3O, C_8-HSL	~400	250	*a*	230	*a*
2, 3O, C_{10}-HSL	21	130	*a*	65	*a*
3, 3O, C_{11}-HSL	8.9	60	30	45	*a*
4, 3O, C_{12}-HSL	4	60	10	35	15
5, 3O, C_{13}-HSL	4.3	35	30	25	*a*
6, 3O, C_{14}-HSL	6.3	30	15	30	35

[a] Not determined.

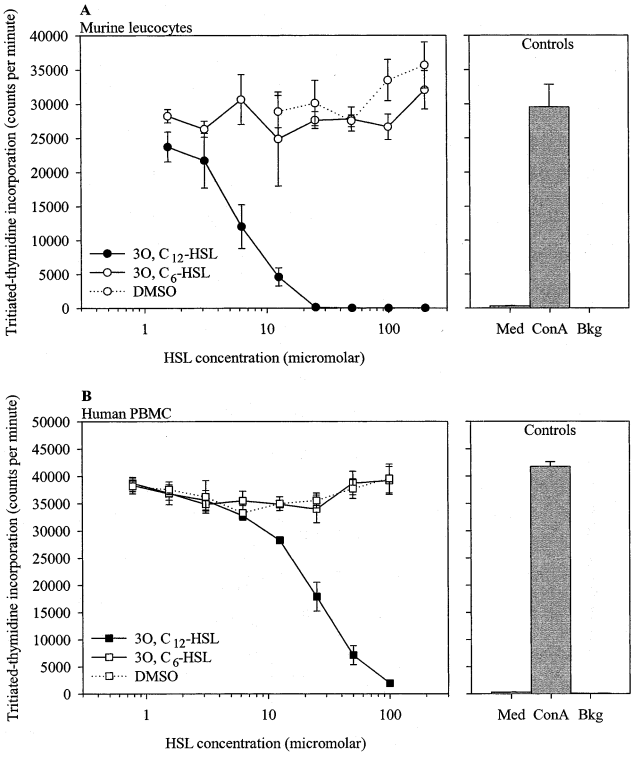


Figure 2. Dose−response curves of 3O, C_{12}-HSL and 3O, C_6-HSL in the proliferation of (A) murine and (B) human leukocytes, peripheral blood monocytes, in ConA stimulated assays. DMSO was used as a diluent control. The results are presented to illustrate stimulations to ConA compared with medium (Med) and background (Bkg).

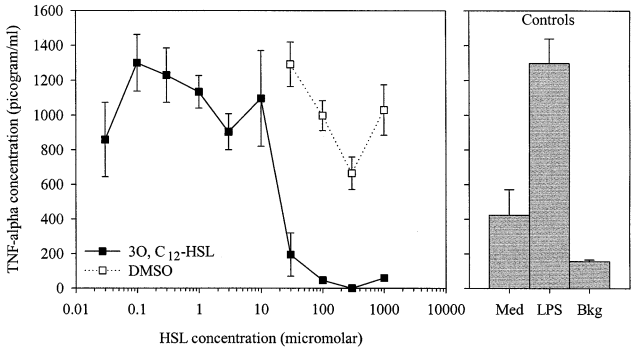


Figure 3. Dose−response curves of 3O, C_{12}-HSL in the secretion of TNF-α by *E. coli* LPS-stimulated human leukocytes. DMSO was used as a diluent control. The results are presented to illustrate stimulations to ConA compared with medium (Med) and background (Bkg).

secretion assays. Nevertheless, after a basic structure−activity profile for 3O, C_{12}-HSL was secured in immune assay, it is now possible to explore further chemical modifications to improve on the rather narrow dose−

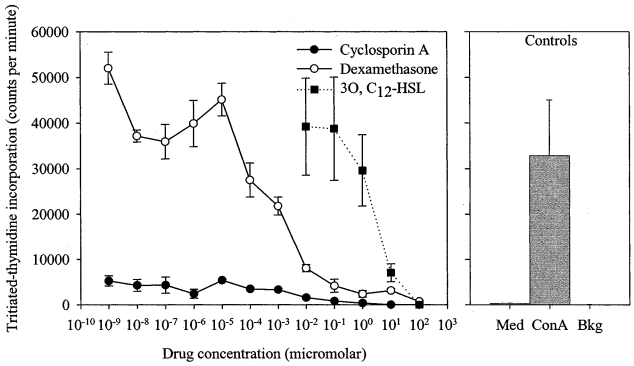


Figure 4. The comparative potency of 3O, C_{12}-HSL compared with two standard immune suppressive agents dexmethasone and cyclosporin A was assessed in a concanavalin A driven murine splenocyte proliferation assay. It is clear from this experiment that cell proliferation was completely inhibited by cyclosporin along the dose range used, with dexmethasone exhibiting an IC_{50} in the region of 10^{-3}−10^{-4} μM. 3O, C_{12}-HSL was the least potent compound in this assay.

response range illustrated in this publication. Figure 4 clearly illustrates the superior potency of cyclosporin A and dexamethasone in cell proliferative assays. However, potency does not always equate with efficacy in vivo, and the effective dose range for 3O, C_{12}-HSL illustrated in the present study may in fact be sufficient for application to some immunological diseases.

Conclusions

From these initial structure−activity-relationship studies, it is apparent that the L-homoserine lactone ring acylated with a 3-oxo or 3-hydroxy 12−14 carbon acyl side chain is optimal for immune suppressive activity. The overall lipophilic character of the compound also appears to be important for activity. Terminal substitution of the acyl chain with a bromine or hydroxyl functionality retains activity, while polar carboxylic acid or methyl ester substitution decreases activity. Unsaturation within the side chain also retains activity. 3-Hydroxy derivatives, even as a mixture of diastereoisomers, were equipotent with the corresponding 3-oxo derivatives. These preliminary observations using cellular assays have established a general picture of structure−activity-relationships and allowed the selection of a number of active compounds for more detailed evaluation in well-established animal models of autoimmune disease.

On the basis of the studies described here, methods are now available for the synthesis of radioisotope and fluorescence labeled compounds for further molecular mechanistic studies to identify molecular targets for these compounds in eukaryotic cells. To support this

work, sites for attachment to solid supports within the acyl side chain of active molecules have been identified and currently affinity matrixes are being constructed for the isolation and identification of the target proteins.[20]

Experimental Section

Chemistry. L-Homoserine was obtained from NovaBiochem (Nottingham, U.K.). Carboxylic acids and other starting materials were purchased from Aldrich Chemical Co. The solvents used were HPLC grade. Dichloromethane was dried by storing it over anhydrous calcium chloride and distillation.

Melting points were determined with a Kofler hot stage and are uncorrected. Infrared spectra through the range 4000−600 cm^{-1} were obtained with KBr pellets or as thin films using an Avatar 360 Nicolet FT-IR spectrophotometer. ^1H NMR spectra were recorded on Varian EM 390 and Bruker AMX-250 spectrophotometers operating at 90 and 250 MHz, respectively, and were recorded with tetramethylsilane as an internal standard. ^{13}C NMR data were obtained in deuteriochloroform with a Bruker AMX-250 instrument operating at 62.9 MHz. Chemical shifts are reported relative to an internal $CDCl_3$ standard on a broad band decoupled mode, and assignments were done using a DEPT pulse sequence. Electrospray mass spectra (ES-MS) were recorded using Micromass VG platform and Micromass LCT spectrometers for low- and high-resolution spectra, respectively. Elemental analyses were obtained with a Perkin-Elmer 240 D elemental analyzer.

Thin-layer chromatography (TLC) was performed using Merck silica gel 60 GF_{254} precoated (0.2 mm) alumina plates. Preparative thin-layer chromatography (PLC) was performed using Merck silica gel 60 GF_{254} coated (1.0 mm) glass plates (20 cm × 20 cm). Flash chromatography was performed using Merck Kieselgel 60 (230−400 mesh) silica.

Analytical reverse-phase high-performance liquid chromatography (RP-HPLC) was performed using an analytical column (HiChrom Kromasil KR100-5 C 8; 250 mm × 4.6 mm) with a Waters 625 LC system attached to a Waters 996 photodiode array system operating with a Millenium 2010 Chromatograph Manager. Fractions were eluted with a linear gradient of acetonitrile in water (70−100%, v/v) over a 20 min period at a flow rate of 0.7 mL min^{-1} and monitored at 210 nm. Semipreparative HPLC was performed with a C_8 reverse-phase preparative column (HiChrom Kromasil KR100-5 C 8; 250 mm × 8.0 mm) using a Gilson system. Fractions were eluted with an isocratic system, and determined from the analytical HPLC data, at a flow rate of 2.0 mL min^{-1}.

Synthesis of *N*-(3-Oxoalkanoyl)-L-homoserine Lactones (Table 1, Compounds 1−16). General Method A. To a dry dichloromethane solution containing 2 mmol of the appropriate carboxylic acid (20 mL) was added 4-(dimethylamino)pyridine (2.1 mmol), *N*,*N*′-dicyclohexylcarbodiimide (2.2 mmol), and Meldrum's acid (2 mmol). This solution was stirred at room temperature overnight and then filtered to remove the precipitated *N*,*N*′-dicyclohexylurea. The filtrate was evaporated to dryness, and the residue was redissolved in ethyl acetate. The ethyl acetate solution was washed with 2 M hydrochloric acid (20 mL), dried over anhydrous magnesium sulfate, and concentrated to afford the acylated Meldrum's acids, which were used without further purification in the next step.

To a stirred solution of an acylated Meldrum's acid (1 mmol) in acetonitrile (30 mL) was added L-homoserine lactone hydrochloride (1 mmol) and triethylamine (1.2 mmol). The mixture was stirred at room temperature for 2 h and then heated under reflux for 3 h. The solvent was removed by rotary evaporation to give a residue that was redissolved in ethyl acetate. The organic solution was sequentially washed with saturated sodium hydrogen carbonate solution, 1 M potassium hydrogen sulfate solution, and saturated sodium chloride solution. After drying over anhydrous magnesium sulfate, the organic solution was evaporated and the residue was purified by preparative layer chromatography on silica plates. Further purification was achieved by RP-HPLC.

***N*-(3-Oxooctanoyl)-L-homoserine Lactone (1).** Mp: 78−80 °C. RP-HPLC: t_R = 5.23 min. IR (KBr): 3290 (NH), 1790 (ring C=O), 1715 (ketone C=O), 1650 (amide C=O), 1545, 1175 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.89 (3H, t, J = 7.0 Hz, CH_3), 1.29 (4H, m, $CH_3CH_2CH_2$), 1.59 (2H, m, CH_2CH_2CO), 2.22 (1H, m, 4α-H), 2.52 (2H, t, J = 7.3 Hz, CH_2CO), 2.76 (1H, m, 4β-H), 3.47 (2H, s, $COCH_2CO$), 4.27 (1H, m, 5α-H), 4.48 (1H, td, J = 1.3 and 9 Hz, 5β-H), 4.58 (1H, m, 3-H), 7.64 (1H, br s, NH). EI-MS *m*/*z* (%): 241.1312 (11, M^+, $C_{12}H_{19}NO_4$ requires *m*/*z* 241.1314), 185 (65), 143 (39), 113 (28), 102 (68), 99 (49), 71 (36), 57 (86). Anal. (C_{12} $H_{19}NO_4$) C, H, N.

***N*-(3-Oxodecanoyl)-L-homoserine Lactone (2).** Mp: 79−81 °C. RP-HPLC: t_R = 6.77 min. IR (KBr): 3294 (NH), 1785 (ring C=O), 1716 (ketone C=O), 1648 (amide C=O), 1545, 1176 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.88 (3H, t, CH_3), 1.21 (8H, m, $CH_3(CH_2)_4$), 1.58 (2H, m, CH_2CH_2CO), 2.25 (1H, m, 4α-H), 2.55 (2H, t, CH_2CO), 2.74 (1H, m, 4β-H), 3.48 (2H, s, $COCH_2CO$), 4.29 (1H, m, 5α-H), 4.48 (1H, td, 5β-H), 4.58 (1H, m, 3-H), 7.73 (1H, d, NH). ES-MS *m*/*z*: 270.1 (MH^+, $C_{14}H_{24}NO_4$ requires *m*/*z* 270.17). Anal. ($C_{14}H_{23}NO_4$) C, H, N.

***N*-(3-Oxoundecanoyl)-L-homoserine Lactone (3).** Mp: 80−82 °C. RP-HPLC: t_R = 7.62 min. IR (KBr): 3295 (NH), 1782 (ring C=O), 1717 (ketone C=O), 1646 (amide C=O), 1545, 1177 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.9 (3H, t, CH_3), 1.27 (10H, m, $CH_3(CH_2)_5$), 1.59 (2H, m, CH_2CH_2CO), 2.22 (1H, m, 4α-H), 2.52 (2H, t, CH_2CO), 2.76 (1H, m, 4β-H), 3.47 (2H, s, $COCH_2CO$), 4.27 (1H, m, 5α-H), 4.48 (1H, td, 5β-H), 4.58 (1H, m, 3-H), 7.64 (1H, d, NH). ES-MS *m*/*z*: 284.4 (MH^+, $C_{15}H_{26}NO_4$ requires *m*/*z* 284.18). Anal. ($C_{15}H_{25}NO_4$) C, H, N.

***N*-(3-Oxododecanoyl)-L-homoserine Lactone (4).** Mp: 84−85 °C. RP-HPLC: t_R = 8.81 min. IR (KBr): 3297 (NH), 1778 (ring C=O), 1718 (ketone C=O), 1643 (amide C=O), 1547, 1178, 1015 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.9 (3H, t, CH_3), 1.27 (12H, m, $CH_3(CH_2)_6$), 1.59 (2H, m, CH_2CH_2CO), 2.22 (1H, m, 4α-H), 2.52 (2H, t, CH_2CO), 2.76 (1H, m, 4β-H), 3.47 (2H, s, $COCH_2CO$), 4.27 (1H, m, 5α-H), 4.48 (1H, td, 5β-H), 4.58 (1H, m, 3-H), 7.64 (1H, d, NH). ^{13}C NMR ($CDCl_3$): δ 14.1, 22.7, 23.4, 26.4, 29.0, 29.3, 29.4, 29.9, 31.9, 43.7, 49.0, 66.0, 166.8, 175.3 and 206.3. ES-MS *m*/*z*: 298.0 (MH^+, $C_{16}H_{28}NO_4$ requires *m*/*z* 298.19). Anal. ($C_{16}H_{27}NO_4$) C, H, N.

***N*-(3-Oxotridecanoyl)-L-homoserine Lactone (5).** Mp: 84−86 °C. RP-HPLC: t_R = 10.28 min. IR (KBr): 3292 (NH), 1787 (ring C=O), 1724 (ketone C=O), 1643 (amide C=O), 1541, 1187, 1015 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.9 (3H, t, CH_3), 1.27 (14H, m, $CH_3(CH_2)_7$), 1.59 (2H, m, CH_2CH_2CO), 2.22 (1H, m, 4α-H), 2.52 (2H, t, CH_2CO), 2.76 (1H, m, 4β-H), 3.47 (2H, s, $COCH_2CO$), 4.27 (1H, m, 5α-H), 4.48 (1H, td, 5β-H), 4.58 (1H, m, 3-H), 7.64 (1H, d, NH). ES-MS *m*/*z*: 312.2 (MH^+, $C_{17}H_{30}NO_4$ requires *m*/*z* 312.21). Anal. ($C_{17}H_{29}NO_4$) C, H, N.

***N*-(3-Oxotetradecanoyl)-L-homoserine Lactone (6).** Mp: 90−91 °C. RP-HPLC: t_R = 11.79 min. IR (KBr): 3296 (NH), 1777 (ring C=O), 1718 (ketone C=O), 1643 (amide C=O), 1547, 1178, 1015 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.9 (3H, t, CH_3), 1.27 (16H, m, $CH_3(CH_2)_8$), 1.59 (2H, m, CH_2CH_2CO), 2.22 (1H, m, 4α-H), 2.52 (2H, t, CH_2CO), 2.76 (1H, m, 4β-H), 3.47 (2H, s, $COCH_2CO$), 4.27 (1H, m, 5α-H), 4.48 (1H, td, 5β-H), 4.58 (1H, m, 3-H), 7.64 (1H, d, NH). ES-MS *m*/*z*: 326.3 (MH^+, $C_{18}H_{32}NO_4$ requires *m*/*z* 326.23). Anal. ($C_{18}H_{31}NO_4$) C, H, N.

***N*-(12-Bromo-3-oxododecanoyl)-L-homoserine Lactone (7).** Mp: 92−94 °C. RP-HPLC: t_R = 8.01 min. IR (KBr): 3288 (NH), 1786 (ring C=O), 1716 (ketone C=O), 1641 (amide C=O), 1539, 1187 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.28 (10H, m, $BrCH_2CH_2(CH_2)_5$), 1.42 (2H, m, $BrCH_2CH_2$), 1.58 (2H, m, CH_2CH_2CO), 2.27 (1H, m, 4α-H), 2.54 (2H, t, CH_2CO), 2.75 (1H, m, 4β-H), 3.48 (2H, s, $COCH_2CO$), 3.53 (2H, t, CH_2Br), 4.27 (1H, m, 5α-H), 4.44 (1H, td, 5β-H), 4.61 (1H, m, 3-H), 7.74 (1H, d, NH). ES-MS *m*/*z*: 376.3 and 378.3 (MH^+, $C_{16}H_{27}NO_4Br$ requires *m*/*z* 376.1 and 378.1). Anal. ($C_{16}H_{26}NO_4Br$) C, H, N.

***N*-(13-Bromo-3-oxotridecanoyl)-L-homoserine Lactone (8).** Mp: 100−102 °C. RP-HPLC: t_R = 7.96 min. IR (KBr): 3288 (NH), 1786 (ring C=O), 1716 (ketone C=O), 1641 (amide C=O), 1539, 1187 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.28 (12H, m,

$BrCH_2CH_2(CH_2)_6$), 1.42 (2H, m, $BrCH_2CH_2$), 1.58 (2H, m, CH_2CH_2CO), 2.27 (1H, m, 4α-H), 2.54 (2H, t, CH_2CO), 2.75 (1H, m, 4β-H), 3.48 (2H, s, $COCH_2CO$), 3.53 (2H, t, CH_2Br), 4.27 (1H, m, 5α-H), 4.44 (1H, td, 5β-H), 4.61 (1H, m, 3-H), 7.74 (1H, d, NH). ES-MS *m/z*: 390.6 and 392.6 (MH^+, $C_{17}H_{29}NO_4Br$ requires *m/z* 390.3 and 392.3). Anal. ($C_{17}H_{28}NO_4Br$) C, H, N.

***N*-(13-Carboxy-3-oxotridecanoyl)-L-homoserine Lactone (9).** Mp: 91−94 °C. RP-HPLC: t_R = 3.44 min. IR (KBr): 3300 (NH), 2930, 2854, 1777 (ring C=O), 1717 (ketone C=O), 1647 (amide C=O), 1544, 1178 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.31 (12H, m, $HO_2C(CH_2)_2(CH_2)_6$), 1.59 (4H, m, $HO_2CCH_2CH_2$ and CH_2CH_2CO), 2.33 (2H, t, HO_2CCH_2), 2.40 (1H, m, 4α-H), 2.55 (2H, t, CH_2CO), 2.70 (1H, m, 4β-H), 3.51 (2H, s, $COCH_2CO$), 4.28 (1H, m, 5α-H), 4.47 (1H, td, 5β-H), 4.66 (1H, m, 3-H), 7.88 (1H, d, NH), 8.90 (1H, br s, CO_2H). ES-MS *m/z*: 356.2 (MH^+, $C_{18}H_{30}NO_6$ requires *m/z* 356.2). Anal. ($C_{18}H_{29}NO_6$) C, H, N.

***N*-(12-Methoxycarbonyl-3-oxododecanoyl)-L-homoserine Lactone (10).** Mp: 66−68 °C. RP-HPLC: t_R = 5.60 min. IR (KBr): 3295 (NH), 1778 (ring C=O), 1731 (ester C=O), 1717 (ketone C=O), 1643 (amide C=O), 1547, 1177 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.27 (10H, m, $CH_3OCOCH_2CH_2(CH_2)_5$), 1.59 (4H, m, CH_2CH_2CO and $CH_3OCOCH_2CH_2$), 2.22 (1H, m, 4α-H), 2.25 (2H, t, CH_3OCOCH_2), 2.52 (2H, t, CH_2CO), 2.76 (1H, m, 4β-H), 3.47 (2H, s, $COCH_2CO$), 3.58 (3H, s, CH_3O), 4.27 (1H, m, 5α-H), 4.48 (1H, td, 5β-H), 4.58 (1H, m, 3-H), 7.64 (1H, d, NH). ES-MS *m/z*: 356.0 (MH^+, $C_{18}H_{30}NO_6$ requires *m/z* 356.2). Anal. ($C_{18}H_{29}NO_6$) C, H, N.

***N*-(12-Hydroxy-3-oxododecanoyl)-L-homoserine Lactone (11).** Mp: 64−66 °C. RP-HPLC: t_R = 4.30 min. IR (KBr): 3293 (NH), 1781 (ring C=O), 1715 (ketone C=O), 1649 (amide C=O), 1547, 1174 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.29 (10H, m, $HOCH_2CH_2(CH_2)_5$), 1.62 (4H, m, $HOCH_2CH_2$ and CH_2CH_2CO), 1.88 (1H, m, OH), 2.27 (1H, m, 4α-H), 2.54 (2H, t, CH_2CO), 2.75 (1H, m, 4β-H), 3.48 (2H, s, $COCH_2CO$), 3.63 (2H, t, CH_2OH), 4.27 (1H, m, 5α-H), 4.44 (1H, td, 5β-H), 4.61 (1H, m, 3-H), 7.74 (1H, d, NH). ES-MS *m/z*: 314.1 (MH^+, $C_{16}H_{28}NO_5$ requires *m/z* 314.2). ES-HRMS *m/z*: 336.1808 (M + Na, $C_{16}H_{27}NO_5Na$ requires *m/z* 336.1787).

***N*-(3-Oxo-7*Z*-tetradecenoyl)-L-homoserine Lactone (12).** Mp: 59−60 °C. RP-HPLC: t_R = 10.14 min. IR (KBr): 3293 (NH), 1780 (ring C=O), 1717 (ketone C=O), 1645 (amide C=O), 1547, 1175 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.84 (3H, t, CH_3), 1.27 (8H, m, $CH_3(CH_2)_4$), 1.57 (2H, m, CH_2CH_2CO), 1.96 (4H, m, $CH_2CHCHCH_2$), 2.26 (1H, m, 4α-H), 2.50 (2H, t, CH_2CO), 2.68 (1H, m, 4β-H), 3.43 (2H, s, $COCH_2CO$), 4.24 (1H, m, 5α-H), 4.43 (1H, m, 5β-H), 4.58 (1H, m, 3-H), 5.30 (2H, m, CHCH), 7.72 (1H, br d, NH). ES-MS *m/z*: 323.8 (MH^+, $C_{18}H_{30}NO_4$ requires *m/z* 324.2). Anal. ($C_{18}H_{29}NO_4$) C, H, N.

***N*-(3-Oxo-10*Z*-tetradecenoyl)-L-homoserine Lactone (13).** Mp: 55−57 °C. RP-HPLC: t_R = 10.09 min. IR (KBr): 3293 (NH), 1777 (ring C=O), 1718 (ketone C=O), 1648 (amide C=O), 1548, 1175 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.90 (3H, t, CH_3), 1.30 (8H, m, CH_3CH_2 and $(CH_2)_3(CH_2)_2CO$), 1.58 (2H, m, CH_2CH_2CO), 1.99 (4H, m, $CH_2CHCHCH_2$), 2.30 (1H, m, 4α-H), 2.54 (2H, t, CH_2CO), 2.71 (1H, m, 4β-H), 3.48 (2H, s, $COCH_2CO$), 4.26 (1H, m, 5α-H), 4.45 (1H, m, 5β-H), 4.60 (1H, m, 3-H), 5.35 (2H, m, CHCH), 7.76 (1H, br d, NH). ES-MS *m/z*: 323.8 (MH^+, $C_{18}H_{30}NO_4$ requires *m/z* 324.2). Anal. ($C_{18}H_{29}NO_4$) C, H, N.

***N*-(3-Oxo-12-tridecenoyl)-L-homoserine Lactone (14).** Mp: 83−85 °C. RP-HPLC: t_R = 8.74 min. IR (KBr): 3297 (NH), 1778 (ring C=O), 1718 (ketone C=O), 1643 (amide C=O), 1547, 1177, 1015 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.25 (12H, m, $(CH_2)_5(CH_2)_2CO$), 1.57 (2H, m, CH_2CH_2CO), 2.04 (2H, m, CH_2CHCH_2), 2.27 (1H, m, 4α-H), 2.54 (2H, t, CH_2CO), 2.69 (1H, m, 4β-H), 3.47 (2H, s, $COCH_2CO$), 4.29 (1H, m, 5α-H), 4.47 (1H, m, 5β-H), 4.61 (1H, m, 3-H), 4.94 (2H, m, CH_2CHCH_2), 5.79 (1H, m, CH_2CHCH_2), 7.77 (1H, br d, NH). ES-MS *m/z*: 310.2 (MH^+, $C_{17}H_{28}NO_4$ requires *m/z* 310.2). Anal. ($C_{17}H_{27}NO_4$) C, H, N.

***N*-(3-Oxo-13-tetradecenoyl)-L-homoserine Lactone (15).** Mp: 76−78 °C. RP-HPLC: t_R = 10.14 min. IR (KBr): 3297 (NH), 1778 (ring C=O), 1718 (ketone C=O), 1643 (amide C=O), 1547, 1177, 1015 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.25 (12H, m, $(CH_2)_6(CH_2)_2CO$), 1.57 (2H, m, CH_2CH_2CO), 2.04 (2H, m, CH_2CHCH_2), 2.27 (1H, m, 4α-H), 2.54 (2H, t, CH_2CO), 2.69 (1H, m, 4β-H), 3.47 (2H, s, $COCH_2CO$), 4.29 (1H, m, 5α-H), 4.47 (1H, m, 5β-H), 4.61 (1H, m, 3-H), 4.94 (2H, m, CH_2CHCH_2), 5.79 (1H, m, CH_2CHCH_2), 7.77 (1H, br d, NH). ES-MS *m/z*: 323.6 (MH^+, $C_{18}H_{30}NO_4$ requires *m/z* 324.2). ES-HRMS *m/z*: 346.1991 (M + Na, $C_{18}H_{29}NO_4Na$ requires *m/z* 346.1994).

***N*-(6-Methyl-3-oxoundecanoyl)-L-homoserine Lactone (16).** Mp: 72−74 °C. RP-HPLC: t_R = 8.75 min. IR (KBr): 3289 (NH), 1786 (ring C=O), 1716 (ketone C=O), 1649 (amide C=O), 1543, 1176 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.87 (6H, 2 × t, $CHCH_3$ and CH_2CH_3), 1.26 (8H, m, $CH_3(CH_2)_4$), 1.38 (2H, m, $COCH_2CH_2$), 1.63 (1H, m, CH_3CH), 2.28 (1H, m, 4α-H), 2.53 (2H, t, $COCH_2$), 2.57 (1H, m, 4β-H), 3.50 (2H, s, $COCH_2CO$), 4.27 (1H, m, 5α-H), 4.44 (1H, dt, 5β-H), 4.63 (1H, m, 3-H), 7.77 (1H, d, NH). ES-MS *m/z*: 298.2 (MH^+, $C_{16}H_{28}NO_4$ requires *m/z* 298.2). Anal. ($C_{16}H_{27}NO_4$) C, H, N.

.Synthesis of *N*-(3-Hydroxyacyl)-L-homoserine Lactones (Table 1, Compounds 17−18). General Method B. To a solution of the *N*-(3-oxoacyl)-L-homoserine lactone (1 mmol) in methanol (5 mL) was added sodium cyanoborohydride (1.5 mmol). The reaction mixture was maintained at pH 3−4 by addition of 3% HCl in methanol. Three further additions of sodium cyanoborohydride (3 × 1.5 mmol) were made at 4 h intervals while the pH was kept at 3−4. The solvent was removed by rotary evaporation, and the residue was extracted with hot ethyl acetate (4 × 20 mL). The ethyl acetate extracts were combined and concentrated to yield the crude product, which was purified by silica column chromatography (10% MeOH/DCM). Further purification was achieved by RP-HPLC.

***N*-(3-Hydroxydodecanoyl)-L-homoserine Lactone (17).** Mp: 94−96 °C. RP-HPLC: t_R = 7.75 min. IR (KBr): 3297 (NH), 1778 (ring C=O), 1643 (amide C=O), 1547, 1178, 1015 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.85 (3H, t, CH_3), 1.26 (12H, m, $CH_3(CH_2)_6$), 1.42−1.52 (2H, m, CH_2CH_2CHOH), 1.67 (2H, m, CH_2CHOH), 2.19 (1H, m, 4α-H), 2.40 (2H, m, CH_2CO), 2.81 (1H, m, 4β-H), 3.18 (1H, br d, OH), 4.01 (1H, m, CHOH), 4.31 (1H, m, 5α-H), 4.49 (1H, td, 5β-H), 4.57 (1H, m, 3-H), 6.62 (1H, d, NH). ES-MS *m/z*: 300.4 (MH^+,$C_{16}H_{30}NO_4$ requires *m/z* 300.2). Anal. ($C_{16}H_{29}NO_4$) C, H, N.

***N*-(3-Hydroxytetradecanoyl)-L-homoserine Lactone (18).** Mp: 110−112 °C. RP-HPLC: 10.41 min. IR (KBr): 3360 (OH), 3297 (NH), 1775 (ring C=O), 1645 (amide C=O), 1547, 1190, 1176 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.85 (3H, t, CH_3), 1.26 (16H, m, $CH_3(CH_2)_8$), 1.42−1.52 (2H, m, CH_2CH_2CHOH), 1.67 (2H, m, CH_2CHOH), 2.19 (1H, m, 4α-H), 2.40 (2H, m, CH_2CO), 2.81 (1H, m, 4β-H), 3.18 (1H, br d, OH), 4.01 (1H, m, CHOH), 4.31 (1H, m, 5α-H), 4.49 (1H, td, 5β-H), 4.57 (1H, m, 3-H), 6.62 (1H, d, NH). ^{13}C NMR: δ 14.1, 22.7, 25.5, 29.4, 29.5, 29.6, 29.6, 29.7, 30.4, 31.9, 37.0, 37.0, 42.6, 49.2, 66.1, 68.7, 173.0, 175.4. ES-MS *m/z*: 328.3 (MH^+, $C_{18}H_{34}NO_4$ requires *m/z* 328.2). Anal. ($C_{18}H_{33}NO_4$) C, H, N.

Synthesis of *N*-Acyl-L-homoserine Lactones (Table 1, Compounds 19−21). General Method C. To a suspension of L-homoserine lactone hydrochloride (1 mmol) in dry DCM (20 mL) at 0 °C was added triethylamine (2.4 mmol). The solution was stirred at 0 °C for 30 min when the alkanoyl chloride (1 mmol) was added dropwise over 5 min. The reaction mixture was allowed to come to room temperature and was stirred for 4 h. The solvent was removed by rotary evaporation. The residue was redissolved in ethyl acetate and washed sequentially with saturated sodium hydrogen carbonate solution (2 × 20 mL), 1 M potassium hydrogen sulfate solution (2 × 20 mL), and brine (1 × 10 mL). After the mixture was dried over $MgSO_4$, the ethyl acetate was removed by rotary evaporation to leave the crude product that was purified by column chromatography on silica in ethyl acetate. Further purification was achieved by RP-HPLC.

***N*-Decanoyl-L-homoserine Lactone (19).** Mp: 132−134 °C. RP-HPLC: t_R = 8.48 min. IR (KBr): 3318 (NH), 1777 (ring C=O), 1646 (amide C=O), 1549, 1172, 1013 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.88 (3H, t, CH_3), 1.26 (12H, m, $CH_3(CH_2)_6$), 1.67 (2H, m, $COCH_2CH_2$), 2.13 (1H, m, 4α-H), 2.25 (2H, t, $COCH_2$), 2.86 (1H, m, 4β-H), 4.28 (1H, m, 5α-H), 4.44 (1H, td, 5β-H),

4.56 (1H, m, 3-H), 6.12 (1H, d, NH). ES-MS *m/z*: 256.2 (MH^+, $C_{14}H_{26}NO_3$ requires *m/z* 256.2). Anal. ($C_{14}H_{25}NO_3$) C, H, N.

***N*-Undecanoyl-L-homoserine Lactone (20).** Mp: 129−130 °C. RP-HPLC: t_R = 9.85 min. IR (KBr): 3319 (NH), 1778 (ring C═O), 1646 (amide C═O), 1549, 1173, 1013 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.88 (3H, t, CH_3), 1.28 (14H, m, $CH_3(CH_2)_7$), 1.64 (2H, m, CH_2CH_2CO), 2.12 (1H, m, 4α-H), 2.25 (2H, t, CH_2-CO), 2.84 (1H, m, 4β-H), 4.28 (1H, m, 5α-H), 4.43 (1H, td, 5β-H), 4.52 (1H, m, 3-H), 6.04 (1H, d, NH). ES-MS *m/z*: 270.4 (MH^+, $C_{15}H_{28}NO_3$ requires *m/z* 270.2). Anal. ($C_{15}H_{27}NO_3$) C, H, N.

***N*-Dodecanoyl-L-homoserine Lactone (21).** Mp: 128−129 °C. RP-HPLC: t_R = 11.36 min. IR (KBr): 3318 (NH), 1778 (ring C═O), 1647 (amide C═O), 1549, 1173, 1013 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.88 (3H, t, CH_3), 1.26 (16H, m, $CH_3(CH_2)_8$), 1.62 (2H, m, CH_2CH_2CO), 2.12 (1H, m, 4α-H), 2.25 (2H, t, CH_2-CO), 2.88 (1H, m, 4β-H), 4.28 (1H, m, 5α-H), 4.48 (1H, td, 5β-H), 4.55 (1H, m, 3-H), 6.02 (1H, d, NH). ^{13}C NMR: δ 14.11, 22.67, 25.42, 29.20, 29.31, 29.31, 29.44, 29.44, 29.59, 30.75, 36.21, 49.30, 66.11, 173.75, 175.49. ES-MS *m/z*: 284.4 (MH^+, $C_{16}H_{30}NO_3$ requires *m/z* 284.2). Anal. ($C_{16}H_{29}NO_3$) C, H, N.

***N*-(3-Oxododecanoyl)-L-homoserine Methyl Ester (22).** A solution of compound **4** (1 mmol) in MeOH (5 mL) was stirred with a solution of sodium methoxide (1 mmol) in methanol (5 mL) for 6 h at room temperature. The methanol was removed, and the residue was redissolved in ethyl acetate. The solution was washed with water and brine, dried ($MgSO_4$), and rotary-evaporated to give *N*-(3-oxododecanoyl)-L-homoserine methyl ester as an amorphous solid. RP-HPLC: t_R = 7.95 min. ^1H NMR ($CDCl_3$): δ 0.88 (3H, t, CH_3), 1.32 (12H, m, $CH_3(CH_2)_6$), 1.59 (2H, m, CH_2CH_2CO), 1.71 (1H, m, β-H), 2.18 (1H, m, β-H), 2.54 (2H, t, CH_2CO), 2.76 (1H, m, OH), 3.47 (2H, s, $COCH_2CO$), 3.60 (1H, m, γ-H), 3.70 (1H, m, γ-H), 3.78 (3H, s, CO_2CH_3), 4.78 (1H, m, α-H), 7.74 (1H, br d, NH). ES-MS *m/z*: 330.2 (MH^+, $C_{17}H_{32}NO_5$ requires *m/z* 330.2).

3-Oxododecanamide (23). A solution of 5-decanoyl Meldrum's acid (1 mmol) (prepared by general method A) and *tert*-butyl carbazate (1 mmol) in acetonitrile (20 mL) was stirred at room temperature overnight and then heated under reflux for 2 h. The solvent was removed, and the residue was chromatographed on a silica column in ethyl acetate. The product, *N*-*tert*-butoxycarbonyl-3-oxododecanamide, was taken up in 3 M HCl/ethyl acetate and left at room temperature for 3 h. Evaporation of the solvent afforded 3-oxododecanamide as crystalline material after crystallization from ethyl acetate. Mp: 105−106 °C. RP-HPLC: t_R = 8.00 min. IR (KBr): 3382, 3186 (NH), 1706 (C═O), 1657 (amide C═O) cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.88 (3H, t, CH_3), 1.26 (12H, m, $CH_3(CH_2)_6$), 1.59 (2H, m, $COCH_2CH_2$), 2.53 (2H, t, $COCH_2$), 3.35 (2H, s, $COCH_2$-CO), 5.55 (1H, br d, NH), 7.07 (1H, br d, NH). ES-MS *m/z*: 214.2 (MH^+, $C_{12}H_{24}NO_2$ requires *m/z* 214.2). Anal. ($C_{12}H_{23}NO_2$) C, H, N.

***N*-(3-Oxododecanoyl)-L-homocysteine Thiolactone (24).** Compound **24** was synthesized from decanoic acid using general method A replacing L-homoserine lactone with L-homocysteine thiolactone. Mp: 83−85 °C. RP-HPLC: t_R = 10.54 min. IR (KBr): 3267 (NH), 1702 (C═O), 1648 (amide C═O), 1561, 1178 cm^{-1}. ^1H NMR ($CDCl_3$): δ 0.81 (3H, t, CH_3), 1.22 (12H, m, $CH_3(CH_2)_6$), 1.55 (2H, m, CH_2CH_2CO), 1.94 (1H, m, 4α-H), 2.16 (2H, t, CH_2CO), 2.70 (1H, m, 4β-H), 3.18 (1H, m, 5α-H), 3.24 (1H, m, 5β-H), 3.45 (2H, s, $COCH_2CO$), 4.52 (1H, m, 3-H), 6.63 (1H, d, NH). ^{13}C NMR ($CDCl_3$): δ 14.49, 23.04, 23.75, 27.87, 29.38, 29.63, 29.74, 29.77, 31.82, 32.24, 44.22, 48.89, 59.61, 166.76, 205.10, 206.87. ES-MS *m/z*: 314.1 (MH^+, $C_{16}H_{28}NO_3S$ requires *m/z* 314.2). Anal. ($C_{16}H_{27}NO_3S$) C, H, N.

Immunological Assays. Concanavalin A (ConA) Mitogen-Stimulated Proliferation of Murine Splenocytes. A ConA stimulated cell proliferation assay was used to assess the effect of *N*-acylhomoserine lactones (AHLs) on T-cell stimulated proliferation. Proliferation was assessed by the incorporation of [^{3}H]-thymidine into DNA. Eight-week-old female BALB/c mice were obtained from Harlan (Bicester, Oxon, U.K.) and given food and water ad libitum. Splenocyte suspensions were prepared by removing the spleen and placing them into RPMI 1640 medium (Sigma, Poole, U.K.). Spleens were forced through 70 μm pore size wire gauzes using the plunger from a 5 mL syringe to produce a single cell suspension. Cells were pelleted by centrifugation, and erythrocytes were lysed with 0.017 M Tris, 0.144 M ammonium chloride buffer, pH 7.2. Leukocyte pellets were washed twice with RPMI 1640 medium with 2% (v/v) fetal calf serum (FCS) and resuspended in complete T-cell culture medium (CTCM) consisting of RPMI 1640 medium with 5% FCS, 2 mM L-glutamine, and 5×10^{-5} M 2-mercaptoethanol (Sigma, Poole, U.K.). Test compounds were studied using doubling-down dilutions ranging from 1 mM to 0.1 μM in a final volume of 200 μL of CTCM containing ConA (Sigma, Poole, U.K.) at 1 μg/mL and 100 000 spleen cells. Following incubation for 48 h at 37 °C in 5% CO_2/air, 0.25 μCi [^{3}H]-thymidine (Amersham Pharmacia Biotech, U.K.) in 10 μL of RPMI 1640 medium was added and cells were incubated for a further 24 h. Cells were collected onto fiberglass filters with a filtermate harvester (Packard Bioscience, Pangbourne, U.K.). After the addition of 25 μL of MicroScint-O (Packard Bioscience, Pangbourne, U.K.) to each well, these filters were counted with a Packard TopCount scintillation counter.

ConA Mitogen-Stimulated Proliferation of Human PBMC. With ethical clearance and full donor consent, blood specimens were obtained from healthy human volunteers. Human peripheral blood mononuclear cells were isolated from heparinized whole blood by buoyant density centrifugation over Histopaque 1077 (Sigma, Poole, U.K.) at 600 g for 20 min. Cells harvested from "buffy" layers were washed twice with RPMI 1640 medium and resuspended in CTCM. Test compounds were tested using similar dilutions as used for murine splenocyte studies in 200 μL of CTCM containing 1 μg/mL ConA and 100 000 PBMC. Human PBMC were incubated for 48 h at 37 °C in 5% CO_2/air, followed by pulsing with 0.25 μCi [^{3}H]-thymidine (see above). After a further incubation of 24 h, cells were collected onto fiberglass filters and then counted in MicroScint-O with a Packard TopCount scintillation counter.

TNF-α Secretion from LPS-Stimulated Human PBMC. Bacterial lipopolysaccharide (LPS) stimulates the production of a variety of cytokines, including TNF-α, from human PBMC; these cytokines in turn influence the development of T cells, supporting a T helper 1 conducive milieu. Human PBMC prepared from whole blood by buoyant density centrifugation was resuspended in CTCM. Test compounds were again studied using similar dilutions as used for murine splenocytes in 200 μL of CTCM containing 1×10^{-5} μg/mL LPS *Escherichia coli* strain 055:B5 (Sigma, Poole, U.K.) and 100 000 PBMC. Following incubation for 24 h at 37 °C in 5% CO_2/air, cell culture supernatants were collected and tested for TNF-α levels by "sandwich" ELISA. Briefly, 96-well Nunc MaxiSorp (Life Technologies, Paisley, U.K.) plates were coated with 50 μL of a 2 μg/mL solution of mouse antihuman TNF-α monoclonal antibody (BD Pharmingen, U.K.) in 0.05 M carbonate/bicarbonate buffer, pH 9.6, overnight at 4 °C. After these plates were washed three times with PBS-Tween, which contained phosphate-buffered saline (PBS) with 0.5% (v/v) Tween 20 (Sigma, Poole, U.K.), plates were blocked with 1% (w/v) bovine serum albumin (BSA) (Sigma, Poole, U.K.) at room temperature for 2 h. Following three washes with PBS-Tween, 50 μL of cell culture supernatant was added and the mixture was incubated overnight at 4 °C; standard human TNF-α (BD Pharmingen, U.K.) ranging from 2000 to 31.25 pg/mL was added to each plate. After four washes with PBS-Tween, 50 μL of a second antibody, biotinylated mouse antihuman TNF-α monoclonal antibody (BD Pharmingen, U.K.), was added at 0.5 μg/mL dilution in 1% BSA in PBS-Tween and incubated at room temperature for 1 h. Following four washes, the bonded biotinylated antibody was measured with 50 μL of a 1:1000 dilution of streptavidin/peroxidase (BD Pharmingen, U.K.). At the end of an hour of incubation at room temperature, these plates were thoroughly washed six times with PBS-Tween and the assay developed by the addition of 100 μL of 0.1 mg/mL of 3,3′,5,5′-tetramethylbenzidine substrate (Sigma,

Poole, U.K.) in 0.1 M sodium acetate buffer, pH 6, containing 0.03% H_2O_2. The enzyme reaction was stopped with 50 μL of 2.5 M H_2SO_4 after an incubation of 10 min at room temperature, and the absorbance was measured at 450 nm with a spectrophotometric 96-well plate reader (Dynex Technologies).

Optimization of Cell Culture Conditions. In the cell culture assays, the number of mouse splenocytes and human PBMC cells used was initially optimized to 100 000 cells per well. The optimal dose of ConA, 1 μg/mL, used in the cell proliferation assays was determined from ConA titration curves. A similar titration curve was established for LPS stimulation to obtain an LPS concentration that stimulated a suboptimal level of TNF-α release from human PBMC.

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