# AUTOREGULATION OF CARBAPENEM BIOSYNTHESIS IN *Erwinia carotovora* BY ANALOGUES OF *N*-(3-OXOHEXANOYL)-L-HOMOSERINE LACTONE

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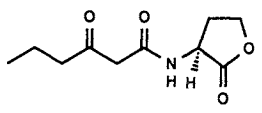
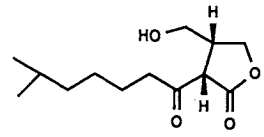
*N*-(3-Oxohexanoyl)-L-homoserine lactone (HSL) (**I**) is the autoregulator controlling carbapenem antibiotic biosynthesis in *Erwinia carotovora* ATCC 39048. The chemical synthesis and biological evaluation of analogues of HSL are described. These include alterations of chirality, side-chain modifications, ring size and ring hetero atom. A number of compounds are reported which are capable of restoring the phenotype to a HSL negative mutant but at higher concentrations than HSL.

A-factor, the autoregulator of streptomycin biosynthesis in *Streptomyces griseus*, was not active as an inducer of carbapenem biosynthesis in *E. carotovora*.

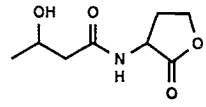
Autoregulators possessing a $\gamma$-butyrolactone ring constitute a diverse and expanding family of signalling molecules implicated in the control of many important aspects of bacterial secondary metabolism. A-factor, (2*S*,3*R*)-2-(6-methylheptanoyl)-3-hydroxymethyl-4-butanolide (**II**), the most studied of the actinomycete autoregulators, was isolated by KHOKHLOV *et al.*[1] during investigations of streptomycin biosynthesis. Mutagenesis of the streptomycin producer *Streptomyces griseus* yielded mutants that fell into two phenotypically distinct cross-feeding groups. One group were simply deficient in streptomycin biosynthesis. The other group were not only streptomycin deficient but also morphologically impaired in both aerial mycelium development and sporulation. A-factor, secreted by group 1 mutants was effective at nanomolar concentration in restoring the parental phenotype with respect to both streptomycin biosynthesis and morphology to the group 2 mutants[2,3].



I



II



III

A-factor is one of a number of related butyrolactones produced by the actinomycetes whose structure and functions have been elucidated; *e.g.* virginiamycin butanolides A to E from *Streptomyces virginiae* which regulate virginiamycin production[4,5], anthracycline inducing factors from *Streptomyces cyaneofuscatus* and *Streptomyces bikiniensis*[6] and IM-2, the regulator of blue pigment production in *Streptomyces* sp. FRI-5[7].

The production of light by certain marine bacteria of the genus *Vibrio* has also been demonstrated to be under autoregulatory control. *N*-(3-Oxohexanoyl)-L-homoserine lactone (HSL) has been shown to regulate bioluminescence in *Vibrio fischeri*[8] whilst a closely related molecule, *N*-(3-hydroxybutyryl)homoserine lactone (**III**) regulates bioluminescence in *Vibrio harveyi*[9]. We have recently shown that HSL also regulates the biosynthesis of a carbapenem antibiotic, 1-carbapen-2-em-3-carboxylic acid in *Erwinia carotovora*[10] and have furthermore demonstrated its presence in a wide range of terrestrial genera.[11]

In order to further investigate the role of HSL in the induction of carbapenem biosynthesis and other regulatory processes, a number of analogues were chemically synthesised and evaluated. A HSL-negative mutant of *E. carotovora* ATCC 39048 (GS101) served as the test organism. This paper deals with both the chemical synthesis and biological evaluation of these compounds as inducers of carbapenem biosynthesis.

## Chemistry

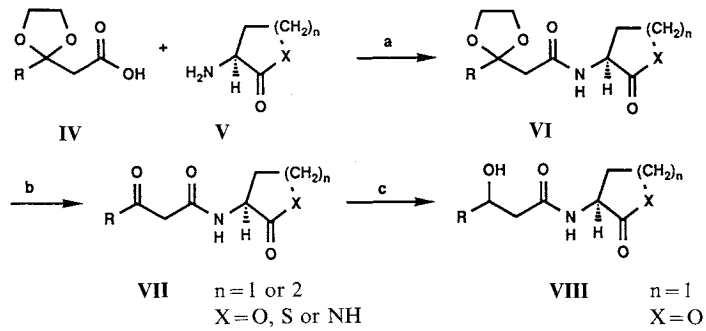
The autoregulator, HSL and some of its racemic analogues have been previously synthesised by EBERHARD *et al.*[12] to study the mechanism of induction of luminescence in *V. fischeri*. Our method of synthesis was a modified procedure of the one reported and is much simpler and consistently gives pure compounds in better yields.

The 3-hydroxy derivatives prepared by EBERHARD[12] required lengthy procedures requiring protection and deprotection of the hydroxy group. Straightforward reduction of the corresponding 3-oxo derivatives by sodium cyanoborohydride afforded these derivatives in good yields (60~65%)[13].

Synthetic steps for the preparation of autoregulator, its 3-oxo analogues (Table 1, compounds **1**~**9**, **15**~**17** and **22**) and 3-hydroxy analogues (**10**, **11**, **13** and **21**) are summarized in the Scheme 1.

Coupling of the acid (**IV**) and amine (**V**) was accomplished in aqueous solution using water soluble carbodiimide, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride. The reactions were clean and high yielding. The products (**VI**) were almost pure and did not require chromatographic separation. The

Scheme 1. Synthesis of the analogues of *N*-(3-oxohexanoyl)-L-homoserine lactone.




Reagents and conditions: (a) $EtN{=}C{=}N(CH_2)_3NMe_2 \cdot HCl$; (b) $HClO_4$ in $CH_2Cl_2$; (c) $NaCNBH_3$, pH 3~4.

use of dicyclohexylcarbodiimide gave lower yields and removal of dicyclohexylurea invariably required chromatographic separation. Acid catalysed removal of the ketal group in (**VI**) was achieved by perchloric acid in methylene chloride to afford the 3-oxo analogues (**VII**) having 90~95% purity. Further purification was obtained by HPLC on a reverse phase column.

Sodium cyanoborohydride reduction of the 3-oxo analogues (**VII**) gave 3-hydroxy derivatives (**VIII**) as a mixture of two diastereoisomers. In the case of 3-hydroxyhexanoyl ($R=C_3H_7$), the two diastereoisomers could be successfully separated by HPLC. The absolute stereochemistry at 3-hydroxy centre in either of the isomer has not been established.

The preparation of the unsubstituted acylated analogues (**12**, **14** and **18~20**) was achieved by a reaction of the amine (**V**) either with an acid anhydride or with an acid in the presence of water soluble carbodiimide.

D-Homoserine lactone hydrochloride was prepared by the regioselective reduction of D-aspartic acid with $BH_3$-THF by a procedure described by GONG and LYNN[14].

L-3-Amino-2-pyrrolidinone employed in the preparation of analogue **15** (Table 1) was prepared by refluxing a mixture of L-2,4-diaminobutyric acid dihydrochloride with hexamethyldisilazane in acetonitrile by a reported method[15].

*N*-(3-Oxo-4-hexenoyl)-L-homoserine lactone (analogue **17**) was synthesised by the method of KAPLAN *et al.*[16].

L-α-Amino-δ-valerolactone, required for the preparation of analogue **22** (Table 1) was prepared by the acid hydrolysis and lactonisation of *tert*-butyl 2-*tert*-butoxycarbonylamino-5-hydroxypentanoate[17].

Ethyl 3-oxoheptanoate was not commercially available and was synthesised by acylation of *tert*-butyl ethyl malonate with valeryl chloride by way of the ethoxymagnesium derivative. Hydrolysis and decarboxylation to the desired 3-oxo derivative was accomplished by heating in acid solution[18].

## Results and Discussion

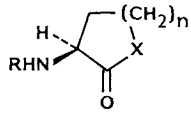
The structure of the natural autoregulator, HSL (**I**), the analogues synthesised together with their relative biological activities determined in the cross-feeding bio-assay are shown in Table 1.

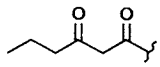
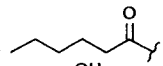
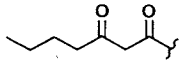
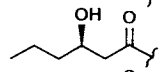
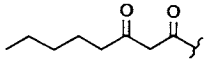
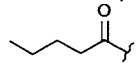
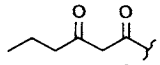
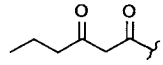
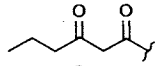
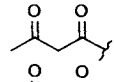
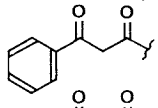
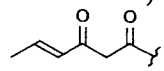
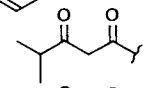
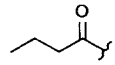
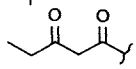
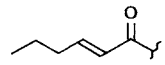
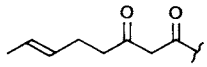
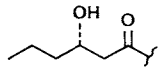
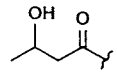
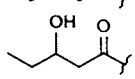
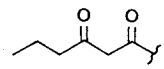
In order to confirm that the antibiotic induced in *E. carotovora* mutant PNP22 by HSL was indeed 1-carbapen-2-em-3-carboxylic acid, cultures were grown in ECP medium in the presence of exogenous HSL, supernatant concentrated and the carbapenem assayed by HPLC. The carbapenem elutes with a retention time of about 9.0 minutes and can be abolished by incubating the sample with 10 μg/ml β-lactamase at 30°C for 15 minutes. HPLC analysis of extracts of supernatants from mutant PNP22 revealed no peak with a similar retention time to that of the carbapenem or with β-lactamase sensitivity. However, when incubated with 10 μg/ml of exogenous autoinducer, carbapenem production was observed. As in the case of strain GS101, this peak could be selectively abolished by incubation with β-lactamase.

Fig. 1 shows some dose-response curves generated using the cross-feeding bio-assay, for analogues differing in the length of the side chain terminus. Increasing the length of the hydrocarbon terminus by one methylene unit results in a drop in activity of some 50%. Decreasing chain length by one methylene unit has a more profound effect on inducing activity, resulting in a ten-fold decrease.

Fig. 2 illustrates the effect of reduction or removal of the β-keto (3-oxo) oxygen on inducing activity. Activity falls as the keto group is firstly reduced to hydroxy, then oxygen removed altogether. The

Table 1. Structures of analogues of *N*-(3-oxohexanoyl)-L-homoserine lactone.



| Compound | R | n | X | Relative[a] activity | Compound | R | n | X | Relative activity |
|---|---|---|---|---|---|---|---|---|---|
| **1** |  | 1 | O | 100 | **12** |  | 1 | O | 0.3 |
| **2** |  | 1 | O | 50 | **13**[b] |  | 1 | O | 0.3 |
| **3** |  | 1 | O | 10 | **14** |  | 1 | O | 0.25 |
| **4** |  | 1 | S | 10 | **15** |  | 1 | N | 0.15 |
| **5** (D isomer) |  | 1 | O | 10 | **16** |  | 1 | O | 0.15 |
| **6** |  | 1 | O | 2 | **17** |  | 1 | O | 0.1 |
| **7** |  | 1 | O | 2 | **18** |  | 1 | O | 0.05 |
| **8** |  | 1 | O | 2 | **19** |  | 1 | O | 0 |
| **9** |  | 1 | O | 1 | **20** |  | 1 | O | 0 |
| **10**[b] |  | 1 | O | 1 | **21** |  | 1 | O | 0 |
| **11** |  | 1 | O | 0.5 | **22** |  | 2 | O | 0 |

[a] This refers to the threshold concentration of the analogues which triggers the carbapenem production expressed relative to HSL.

[b] Chirality at 3-hydroxy may be reversed.

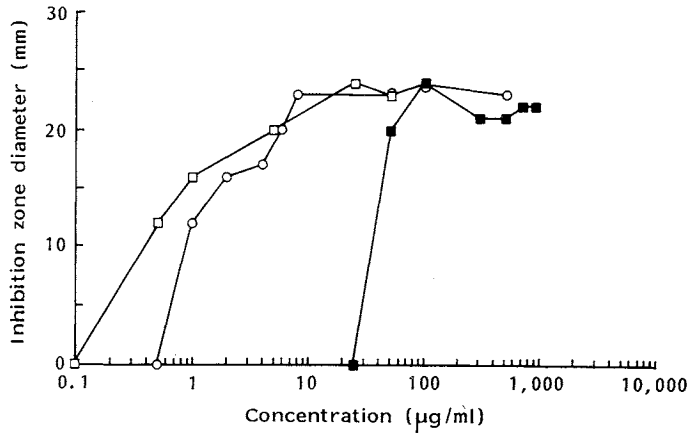
diastereoisomeric pair of hydroxy compounds (**10** and **13** in Table 1) possess markedly different activities. The less active of the pair is no more active than the *N*-hexanoyl homoserine lactone which lacks a $\beta$-keto oxygen.

Fig. 3 demonstrates the importance of the oxygen ring hetero atom in inducing activity. Replacement with sulphur or nitrogen atom resulted in marked decrease in activity.

Given that HSL, the regulatory molecule isolated from *E. carotovora* is identical to the autoregulator of bioluminescence in *V. fischeri*, it would seem highly probable that this molecule functions in an analogous manner in both organisms. In *V. fischeri* the phenomenon of bioluminescence involves both structural and regulatory genes collectively termed the *lux* operon. The bioluminescent phenotype is regulated by two genes upstream from the *lux* structural genes named *luxI* and *luxR*. *luxI* is believed to code for a protein directing synthesis of the *lux* autoregulator, HSL[19]. A positive regulatory element coded by *luxR* is required along with the autoregulator to induce transcription of the *lux* structural genes. The *luxR* gene

Fig. 1. The effect of side chain length on carbapenem inducing ability of analogues.

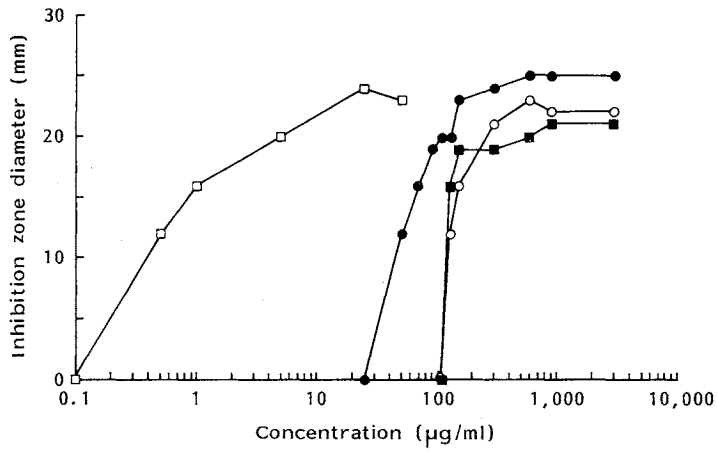
□ Natural product, ○ compound **2**, ■ compound **8**.




The ability of different analogues at various concentrations to induce antibiotic production in *E. carotovora* strain PNP22 was assessed by measuring the diameter of inhibition zones produced on DST agar plates seeded with *E. coli* ESS following incubation at 26°C for 24 hours.

Fig. 2. The significance of the 3-oxo group in the induction of carbapenem production in *E. carotovora* strain PNP22 by various analogues.

□ Natural product, ● compound **10**, ■ compound **12**, ○ compound **13**.



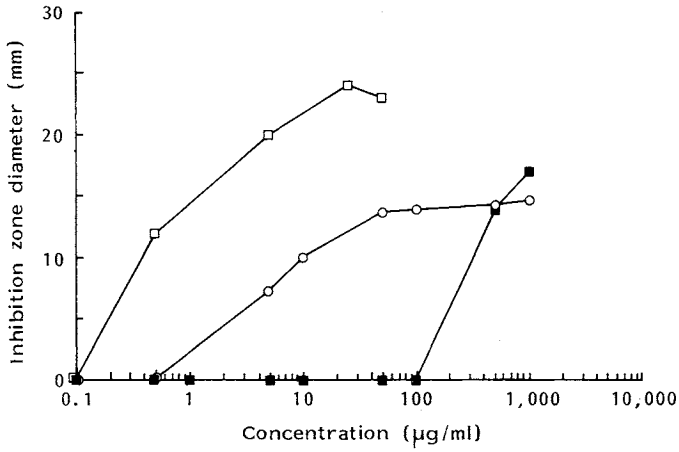

The importance of the 3-oxo function in the induction of antibiotic biosynthesis in strain PNP22 was assessed by measuring the inhibition of *E. coli* ESS growth in DST agar plates.

codes for a 28 kDa protein believed to be a receptor for the autoregulator[20]. The control of bioluminescence is thought to be exerted at the transcriptional level *via* a two component regulatory coupling which in essence relies upon binding of the autoregulator to a binding protein (LuxR) then binding of the complex to *lux* DNA facilitating transcription of the *lux* structural genes and hence light production[21].

A directly analogous system is thought to operate amongst actinomycetes which produce γ-butyrolactone autoregulators. Experiments with tritiated A-factor revealed a binding protein in the cytoplasmic fraction of *S. griseus* which bound A-factor in a 1:1 molar ratio. A molecular weight of

Fig. 3. The significance of ring hetero atom in the induction of carbapenem biosynthesis in *E. carotovora* strain PNP22 by various analogues.

□ Natural product, ○ compound **4**, ■ compound **15**.




The antibiotic-inducing potency of compounds containing oxygen, sulphur and nitrogen atoms in the hetero ring was measured from inhibition zones observed in *E. coli* ESS-seeded DST agar plates.

26 kDa was determined by gel filtration[22]. Similarly KIM *et al.*[23] identified an analogous protein in *S. virginiae* that bound tritiated virginiae butanolide C, possessing a molecular weight of 20 kDa. The low number of binding sites per genome (30 to 40) suggested that such proteins may act as receptors, mediating the pleiotropic effects of autoregulatory molecules in an as yet undefined manner.

Substrate specificity for the autoregulator binding protein (LuxR) in *V. fischeri* appears to be quite high. EBERHARD *et al.*[12] synthesised several analogues of HSL in order to ascertain which structural features were important for bioluminescent activity. A broad range of activities emerged amongst those compounds synthesised. In general any structural modification resulted in a significant decrease in activity, though alterations to the 3-oxohexanoyl side chain were better tolerated than alterations to the lactone ring.

Similarly NIHIRA *et al.*[24] synthesised a wide range of analogues of the actinomycete autoregulator virginiae butanolide C— over 40 were synthesised. A structure-activity profile emerged where optimal inducing activity required the presence of two free hydroxyl groups in common with the parent, a *cis* configuration for butyrolactone ring substituents, and a hydrophobic side chain 7 or 8 carbon atoms long. Deviation from this rigid specification rapidly led to loss of inducing activity.

A similar tight substrate specificity is seen amongst the analogues synthesised and evaluated in this work. The homoserine lactone nucleus itself showed no activity, indicating that the 3-oxohexanoyl side chain was required for induction of carbapenem biosynthesis. Similarly chirality of the homoserine lactone nucleus would appear to be critical since *N*-(3-oxohexanoyl)-D-homoserine lactone possessed a relative activity of 10% compared with the natural product. The requirements for the terminal region of the side chain were investigated by synthesizing analogues which varied both in the length and branching of the hydrocarbon chain.

An increase by one methylene unit decreased activity by 50%, and a two methylene unit extension reduced activity to 10%. Shortening the chain length by one methylene unit decreased activity ten-fold and an even greater drop in activity is observed with the branched isopropyl unit. Introduction of a double

bond into the side chain of (**1**), *i.e.* (**9**), (**17**) and (**19**) results in almost complete loss of activity possibly due to the rigidity introduced in the molecule. However, replacement of the *n*-propyl group with phenyl (**6**) results in activity equivalent to that of the isopropyl (**7**) and the chain-shortened analogue (**8**).

The $\beta$-carbonyl functionality also appears to be essential for induction of carbapenem production activity since removal of the 3-keto group (*cf.* **12**) results in a relative activity of only 0.3%. Reduction of this group to hydroxy gave a mixture of two diastereoisomers (compounds **10** and **13**) which could be resolved by HPLC and assayed separately. Both isomers were at least two orders of magnitude less active than the natural product, however their activities show significant differences from each other.

Replacement of the lactone ring oxygen with sulfur (**4**) results in a compound with 10% of the activity of the natural product. When oxygen was replaced by nitrogen (**15**), the activity dropped even further. Enlargement of the lactone ring by one methylene unit to give the $\delta$-valerolactone (**22**) abolished activity altogether.

Finally A-factor, the autoregulator of streptomycin biosynthesis in *S. griseus*, was inactive up to a level of 500 $\mu$g/ml as an inducer of carbapenem biosynthesis in *E. carotovora*, and neither was the autoregulator of bioluminescence in *V. harveyi*, *N*-(3-hydroxybutyryl)homoserine lactone (**21**).

Given that our experiments were carried out using intact cells, the structure-activity relationship will be influenced by both interaction at a putative protein binding site and by the mechanism of transport of HSL. Experiments using tritiated HSL in *V. fischeri* have indicated that autoinducer enters the bacterial cell by diffusion[23]. Assuming all the compounds described in Table 1 are taken-up by the organism in a similar way, none of the analogues synthesized are as active as HSL. Nevertheless a number of side chain modifications (Figs. 1 and 2) and hetero atom replacements within the ring (Fig. 3) are capable of restoring the phenotype at remarkably low concentrations. These observations offer a number of opportunities, not least the construction of affinity ligands for the isolation of the putative HSL protein receptor.

## Experimental

### Bacterial Strains

*Escherichia coli* ESS, a strain supersensitive to $\beta$-lactam antibiotics was provided by SmithKline Beecham Pharmaceuticals, Worthing, W. Sussex, U.K.

*E. carotovora* ATCC 39048 GS101, a restriction-less Tn5 mutant which carries the pTroy9 plasmid was derived from strain ATCC 39048 as previously described[10]. This strain has a carbapenem (car) and autoregulator producing phenotype ($car^+$; $HSL^+$).

*E. carotovora* mutant PNP22 ($car^-$; $HSL^-$) was derived from strain GS101 by chemical mutagenesis as previously described[10].

### Assays for Carbapenem Inducing Activity

#### HPLC Assay

The response of *E. carotovora* mutant PNP22 to exogenous HSL was analysed by HPLC. The organism was grown to stationary phase in LB broth at 26°C with rotary shaking. 2% v/v inocula were transferred to production media (ECP medium[10]; 50 ml in 250-ml Erlenmeyer flasks) which had been supplemented with varying concentrations of synthetic HSL. Incubation was continued for approximately 6 hours, or until an $OD_{600}$ 2.0 had been reached.

Cells were removed by centrifugation (10,000 rpm for 10 minutes). Supernatant was taken and extracted with 20% by volume of a solution of trioctylmethylammonium chloride (Aliquat 336, 4% w/v) in dichloromethane. The emulsion was broken by centrifugation (10,000 rpm for 10 minutes), dichloromethane

layer taken and shaken vigorously with a solution of sodium iodide (3% w/v) in water. The sodium iodide layer was taken and centrifuged at 13,000 rpm for 30 seconds to remove emulsified dichloromethane. The aqueous concentrate thus obtained was analysed for the presence of the carbapenem by HPLC under the following conditions: Mobile phase; 100 mM potassium dihydrogen orthophosphate in water, adjusted to pH 7 with 10 N NaOH, column type; $C_{18}$ reverse phase (Spherisorb S5ODS2) 25 cm × 2.5 cm, detection; UV at 254 nm.

Where β-lactamase was added to samples, 2 μl of a 0.1 mg/ml solution of β-lactamase [E. C. 3.5.2.6] (Calbiochem Novabiochem Ltd., UK) was added to 100 μl of sample and incubated at 30°C for 30 minutes.

Cross-feeding Bio-assay for the Carbapenem

Bio-assay plates were prepared by growing *E. coli* ESS to stationary phase in brain-heart infusion broth at 37°C, DST agar (500 ml) was prepared by autoclaving and cooling to 40°C. 1.5 ml of the *E. coli* culture was pipetted into the molten agar, mixed, and plates poured. Solidified plates were overdried for 1 hour at 37°C, then stored at 4°C until required. Plates prepared in this manner could be stored for up to two weeks without appreciable loss of viability of the seeded organism.

Circular wells were cut into the agar using the reverse ends of sterile Pasteur pipettes. Around the rim of each well was inoculated a thin smear of *E. carotovora* mutant PNP22. Solutions of HSL or its analogues were prepared in distilled water and filter sterilised. 50 μl aliquots were pipetted into wells, then plates incubated at 2°C for 24 hours. Induction of carbapenem biosynthesis in mutant PNP22 resulted in an inhibition zone around a well.

Relative potencies between analogues were determined by comparing threshold concentrations required to elicit a measurable response (an inhibition zone) in this assay. Dose-response curves were determined for each analogue by measuring inhibition zone diameters over a wide range of concentrations for each analogue.

Analytical Methods

Melting points were determined on a Kofler Hot Stage and are uncorrected. IR spectra were determined in KBr pellets on a Perkin-Elmer 257 spectrophotometer. $^1$H NMR spectra were measured on a Varian EM390 or Bruker AM-400 spectrometer operating at 90 and 400 MHz respectively and were recorded using TMS as an internal standard except for the compounds dissolved in $D_2O$ when sodium 3-(trimethylsilyl)propane sulfonate was used as an external standard. Electron impact and fast atom bombardment mass spectra were determined on VG-Micromass 70E and VG updated AEI MS902 spectrometers, respectively. Accurate mass measurements of molecular ions were carried out on compounds shown to be homogeneous by TLC and HPLC.

HPLC was carried out using PC-controlled Gilson model 306 pumps, manometric module model 805, dynamic mixer model 811B and Applied Biosystems UV detector model 759A. Analytical and semi-preparative reverse-phase chromatographic separations were carried out using Hichrom Kromasil KR100 columns with a mobile phase of 20% to 30% methanol-water, with a monitoring wavelength of 210 nm. Progress of most reactions was followed by TLC using Merck Kieselgel 60 $PF_{254}$ (0.25 mm) coated on glass plates.

Reagents

L-Homoserine, γ-benzyl L-glutamate, cysteine thiolactone hydrochloride and various 3-oxo esters, except ethyl 3-oxoheptanoate were obtained from Aldrich Chemical Company.

L-Homoserine Lactone Hydrochloride (**V**, n=1, X=O)

A solution of L-homoserine lactone (1.0 g) in 3 M HCl (40 ml) was heated under reflux for 2 hours and then evaporated to dryness. The residue was triturated with acetone and the solid hydrochloride (0.98 g, 85%) collected by suction and dried. mp. 218~220°C (lit.[20] mp. 219~220°C); $[\alpha]_D^{22}$ −26.1° ($c$=1, $H_2O$) (lit.[20] $[\alpha]_D^{20}$ −26.8° ($c$=1, $H_2O$)).

D-Homoserine Lactone Hydrochloride

D-Homoserine lactone hydrochloride was prepared in 44% yield from D-aspartic acid by reduction

with $BH_3$-THF as previously described[20]. The product was recrystallised from $H_2O$-acetone, mp. 213~215°C, $[\alpha]_D^{22}$ +25.2° ($c=1$, $H_2O$) (lit.[20] $[\alpha]_D^{22}$ +27.1° ($c=1$, $H_2O$)).

L-α-Amino-δ-valerolactone Hydrochloride

A solution of *tert*-butyl L-2-*tert*-butoxycarbonylamino-5-hydroxypentanoate (prepared from γ-benzyl L-glutamate by the method of OLSEN *et al.*[17]) (0.5 g) in 3 M HCl (20 ml) was heated under reflux for 3 hours. Evaporation and trituration of the residue with acetone gave the title L-α-amino-δ-valerolactone hydrochloride as an oil (0.24 g, 92%). $^1$H NMR ($D_2O$) δ 1.3~2.2 (4H, m, 4-$H_2$ and 5-$H_2$), 3.9~4.55 (3H, m, 3-H and 6-$H_2$).

Ethyl 3-Oxoheptanoate

Dry ethanol (2 ml) and dry carbon tetrachloride (0.2 ml) were added to magnesium turnings (0.6 g, 25 mmol). The reaction which commenced almost immediately was allowed to proceed for about 5 minutes. Dry diethyl ether (15 ml) was added and the flask was placed in a warm water bath and allowed the reaction mixture to reflux gently while a solution of *tert*-butyl ethyl malonate (4.7 g, 25 mmol) in dry ether (5 ml) was added with stirring. After 2.5 hours heating under reflux when all the magnesium had reacted, valeryl chloride (3.01 g, 25 mmol) in dry ether (10 ml) was added with vigorous stirring and the heating continued for a further period of 2 hours. Dilute sulfuric acid (5 ml) was added to the cooled solution and the ether layer separated. The aqueous layer was extracted with ether (2 × 10 ml) and the ether extracts pooled, washed with water (1 × 10 ml) and brine (1 × 10 ml). Drying ($MgSO_4$) and concentration *in vacuo* gave an oil. The oil was taken up in $CF_3COOH$ (10 ml) and left at room temperature for 2 hours. The solution was rotary evaporated and re-evaporated from toluene (2 × 25 ml) to remove $CF_3COOH$ to furnish the desired ethyl 3-oxoheptanoate as an oil (4.0 g, 93%). $^1$H NMR (90 MHz, $CDCl_3$) δ 0.90 (3H, t, $J=7.0$ Hz, $CH_3$), 1.30 (3H, t, $J=7.0$ Hz, $CO_2CH_2CH_3$), 1.0~1.8 (4H, m, $CH_3CH_2CH_2$), 2.56 (2H, t, $J=7.0$ Hz, $CH_2CO$), 3.46 (2H, s, $COCH_2$), 4.20 (2H, q, $J=7.0$ Hz, $CO_2CH_2$).

3,3-(Ethylenedioxy)alkanoic Acid (**IV**)

General Procedure

A mixture of ethyl or methyl alkanoate (0.04 mol), dry ethylene glycol (2.73 g, 0.44 mol) and toluene-*p*-sulfonic acid (5 mg) in dry benzene (60 ml) was heated under reflux for 16 hours using Dean-Stark apparatus for the azeotropic removal of water. The solution was cooled to room temperature, washed with 10% NaOH solution (30 ml), $H_2O$ (5 × 20 ml) and finally with brine (1 × 20 ml). After drying ($MgSO_4$), the solution was concentrated in vacuum to afford the title products as oils in yields of 75~80%. $^1$H NMR included characteristic absorptions at δ 2.6 ($CH_2CO_2R$), ~4.0 ($OCH_2CH_2O$).

A mixture of a solution of 3,3-(ethylenedioxy)alkanoate (0.02 mol) in MeOH (10 ml) and 1 M NaOH (22 ml, 0.22 mol) was stirred at room temperature for 6 hours. Methanol was removed under reduced pressure and the aqueous solution washed once with ethyl acetate to remove any unreacted ester. After acidification (pH 2~3) with 1 M HCl, the solution was extracted with ethyl acetate (3 × 5 ml). Drying ($MgSO_4$) and evaporation gave the desired 3,3-(ethylenedioxy)alkanoic acids as oils in 80~85% yield.

Methyl 3,3-(ethylenedioxy)octanoate required for the preparation of analogue **7** (Table 1) was prepared as follows:

Methyl 3-oxo-6-octenoate (Aldrich) was converted into the 3,3-(ethylenedioxy) derivative in 86% yield by the standard procedure given above.

A solution of methyl 3,3-(ethylenedioxy)-6-octenoate (0.5 g) in MeOH (5 ml) was hydrogenated (1.5 hours) in the presence of 10% Pd-C catalyst (50 mg). The catalyst was removed by filtration through a Celite pad and the filtrate rotary evaporated to yield methyl 3,3-(ethylenedioxy)octanoate as an oil (0.4 g, 80%). $^1$H NMR (90 MHz, $CDCl_3$) δ 0.90 (3H, t, $J=7.0$ Hz, $CH_3$), 1.0~1.6 (6H, m, $CH_3(CH_2)_3$), 1.6~1.9 (2H, m, $CH_2COO$), 2.66 (2H, s, $CH_2CO$), 3.70 (3H, s, $CO_2CH_3$), 4.0 (4H, s, $OCH_2CH_2O$).

Synthesis of *N*-(3-Oxohexanoyl)-L-homoserine Lactone and its Analogues (Table 1)

Synthesis of *N*-(3-Oxoalkanoyl) Analogues (**VII**)

General Method (Table 1, Compounds **1~9**, **15~17** and **22**)

Triethylamine (1 mmol) was added to a stirred solution of homoserine lactone hydrochloride (the

L- or D-isomer), L-homocysteine thiolactone hydrochloride, L-α-amino-δ-valerolactone hydrochloride or L-3-amino-2-pyrrolidinone hydrochloride (1 mmol) in water (2 ml) followed by the addition of 3,3-(ethylenedioxy)alkanoic acid (1 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1 mmol). The mixture was stirred for 20 hours and then rotary evaporated to dryness at 35°C. The light orange residue was extracted with warm ethyl acetate (5 × 5 ml) and the extracts pooled and washed successively with water (1 × 3 ml), 5% sodium bicarbonate solution (1 × 3 ml) and finally brine (1 × 5 ml). Drying ($MgSO_4$) and evaporation of solvent *in vacuo* gave the 3,3-(ethylenedioxy)alkanoylated derivatives (**VI**) (yield 40~50%).

Perchloric acid (60%, 0.25 ml) was added to an ice-cooled solution of the 3,3-(ethylenedioxy)alkanoylated derivatives (**VI**) (0.5 mmol) in dichloromethane (15 ml). The mixture was stirred at 0°C for 0.5 hours and then at room temperature for 1.5 hours. The solvent was removed *in vacuo* and the residue redissolved in ethyl acetate (20 ml). The solution was washed with cold water (2 × 5 ml) and brine (1 × 5 ml), dried ($MgSO_4$) and rotary evaporated to obtain the desired *N*-(3-oxoalkanoyl) analogues (**VII**) (yield 55~60%).

Synthesis of *N*-Acylated-L-homoserine Lactones (Table 1, Compounds **12**, **14**, **18**~**20**)

General Method

Triethylamine (1 mmol) was added to a stirred solution of L-homoserine lactone hydrochloride (1 mmol) in water (2 ml) followed either by the addition of acid anhydride (3 mmol) (compounds **18** and **20**) or acid (1.5 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.5 mmol) (compounds **12**, **14** and **19**). The mixture was stirred at room temperature overnight and then evaporated *in vacuo* to dryness. The residue was partitioned between water (5 ml) and ethyl acetate (20 ml) and the organic layer successively washed with 5% $NaHCO_3$ solution (2 × 5 ml), 1 M $KHSO_4$ solution (1 × 5 ml) and brine (1 × 5 ml). Drying ($MgSO_4$) and removal of solvent gave the title acylated lactones (20~60% yield).

Synthesis of *N*-(3-Hydroxyalkanoyl)-L-homoserine Lactones (Table 1, Compounds **10**, **11**, **13** and **21**)

General Method

*N*-(3-Oxoalkanoyl)-L-homoserine lactone (0.2 mmol) was dissolved in methanol (5 ml) and the solution made acidic (pH 3~4) with 2 M HCl-methanol. Sodium cyanoborohydride (0.5 mmol) was added in one lot with stirring and the reaction mixture maintained at pH 3~4 by the occasional addition of 2 M HCl-methanol. After 2 hours, solvent was removed *in vacuo* and ethyl acetate extracts (3 × 5 ml) of the residue were combined, dried ($MgSO_4$) and evaporated by yield the title hydroxy derivatives. The products were purified by preparative layer chromatography on silica plates in $CHCl_3$-MeOH (9:1) and repurified by HPLC. The latter also resolved and separated the diastereoisomers in the case of compounds **10** and **13**.

The analogues prepared by these methods were more than 90% pure and were further purified by isocratic reverse-phase HPLC using a 1 × 25 cm Hichrom Kromasil KR100 semi-prep column eluted with 20~30% MeOH-$H_2O$.

Spectroscopic Data for the Compounds Listed in Table 1

Compound **1** (*N*-(3-Oxohexanoyl)-L-homoserine Lactone): IR (KBr) $cm^{-1}$ 3295 (NH), 1780 (ring C=O), 1710 (ketone C=O), 1650 (amide C=O), 1550, 1170; EI-MS *m/z* (%) 213.0989 (47, $M^+$, $C_{10}H_{15}NO_4$ requires *m/z* 213.1001), 185 (13), 170 (8), 155 (7), 143 (33), 128 (8), 113 (19), 102 (56), 101 (35), 71 (82), 57 (100); $^1H$ NMR (400 MHz, $CDCl_3$) δ 0.9 (3H, t, $J$=7.4 Hz, $CH_3$), 1.64 (2H, sextet, $J$=7.4 Hz, $CH_3CH_2$), 2.22 (1H, m, 4α-H), 2.51 (2H, t, $J$=7.25 Hz, $CH_2CO$), 2.77 (1H, m, 4β-H), 3.47 (2H, s, $COCH_2CO$), 4.28 (1H, m, 5α-H), 4.48 (1H, td, $J$=1.3 and 9 Hz, 5β-H), 4.59 (1H, m, 3-H), 7.65 (1H, brs, NH); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ 13.58 ($CH_3$), 16.91 ($CH_2CH_3$), 29.94 (4-$CH_2$), 45.85 ($CH_2CO$), 48.13 ($COCH_2CO$), 49.12 (3-CH), 65.94 (5-$CH_2$), 166.38 (CONH), 174.83 (ring C=O), 206.54 (C=O).

Compound **2** (*N*-(3-Oxoheptanoyl)-L-homoserine Lactone): IR (KBr) $cm^{-1}$ 3920 (NH), 1785 (ring C=O), 1725 (ketone C=O), 1645 (amide C=O), 1540, 1190, 1175; EI-MS *m/z* (%) 227.1139 (15, $M^+$, $C_{11}H_{17}NO_4$ requires *m/z* 227.1157), 198 (9), 185 (96), 143 (49), 127 (20), 102 (83), 101 (43), 85 (88), 57 (100); $^1H$ NMR (400 MHz, $CDCl_3$) δ 0.91 (3H, t, $J$=7.4 Hz, $CH_3$), 1.32 (2H, sextet, $J$=7.5 Hz, $CH_3CH_2$), 1.58 (2H, t, $J$=7.5 Hz, $CH_3CH_2CH_2$), 2.23 (1H, m, 4α-H), 2.53 (2H, t, $J$=7.25 Hz, $CH_2CO$), 2.76 (1H, m, 4β-H), 3.47 (2H, s, $COCH_2CO$), 4.28 (1H, m, 5α-H), 4.48 (1H, td, $J$=1.3 and 9 Hz, 5β-H), 4.59 (1H,

m, 3-H), 7.66 (1H, br s, NH).

Compound **3** (*N*-(3-Oxooctanoyl)-L-homoserine Lactone): IR (KBr) $cm^{-1}$ 3290 (NH), 1790 (ring C=O), 1715 (ketone C=O), 1650 (amide C=O), 1545, 1175; 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); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 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, 4$\alpha$-H), 2.52 (2H, t, $J=7.3$ Hz, $CH_2CO$), 2.76 (1H, m, 4$\beta$-H), 3.47 (2H, s, $COCH_2CO$), 4.27 (1H, m, 5$\alpha$-H), 4.48 (1H, td, $J=1.3$ and 9 Hz, 5$\beta$-H), 4.58 (1H, m, 3-H), 7.64 (1H, br s, NH).

Compound **4** (*N*-(3-Oxohexanoyl)-L-homocysteine Thiolactone): IR (KBr) $cm^{-1}$ 3250 (NH), 1715 (ketone C=O), 1690 (ring C=O), 1645 (amide C=O), 1555, 1185, 1130, 1060; EI-MS *m/z* (%) 229.0745 (6, $M^+$, $C_{10}H_{15}NO_3S$ requires *m/z* 229.0773), 201 (100), 173 (21), 113 (28), 89 (60), 71 (62), 56 (49); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 0.93 (3H, t, $J=7.4$ Hz, $CH_3$), 1.64 (2H, sextet, $J=7.3$ Hz, $CH_3CH_2$), 2.02 (1H, m, 4$\alpha$-H), 2.52 (2H, t, $J=7.2$ Hz, $CH_2CO$), 2.85 (1H, m, 4$\beta$-H), 3.27 (1H, m, 5$\alpha$-H), 3.36 (1H, m, 5$\beta$-H), 3.46 (2H, s, $COCH_2CO$), 4.59 (1H, m, 3-H), 7.46 (1H, br s, NH).

Compound **6** (*N*-Benzoylacetyl-L-homoserine Lactone): IR (KBr) $cm^{-1}$ 3295 (NH), 1765 (ring C=O), 1690 (ketone C=O), 1650 (amide C=O), 1555, 1192; EI-MS *m/z* (%) 247.0824 (24, $M^+$, $C_{13}H_{13}NO_4$ requires *m/z* 247.0844), 147 (12), 105 (100), 77 (37); $^1H$ NMR (90 MHz, $CDCl_3$ - DMSO-$d_6$) $\delta$ 2.20~2.80 (2H, m, 4-$H_2$), 3.94 (2H, s, $COCH_2CO$), 4.10~4.80 (3H, m, 5-$H_2$ and 3-H), 7.30~7.70 (3H, m, ArH), 7.95 (2H, m, ArH), 8.56 (1H, br s, NH).

Compound **7** (*N*-(4-Methyl-3-oxopentanoyl)-L-homoserine Lactone): IR (KBr) $cm^{-1}$ 3295 (NH), 1770 (ring C=O), 1710 (ketone C=O), 1645 (amide C=O), 1555, 1190; EI-MS *m/z* (%) 213.1007 (100, $M^+$, $C_{10}H_{15}NO_4$ requires *m/z* 213.1001), 170 (34), 155 (7), 128 (14), 112 (26), 102 (53), 71 (20), 57 (22); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 1.14 (6H, d, $J=6.9$ Hz, $(CH_3)_2$), 2.22 (1H, m, 4$\alpha$-H), 2.69 (1H, septet, $J=6.9$ Hz, CHCO), 2.75 (1H, m, 4$\beta$-H), 3.52 (2H, s, $COCH_2CO$), 4.28 (1H, m, 5$\alpha$-H), 4.48 (1H, td, $J=1.3$ and 9.1 Hz, 5$\beta$-H), 4.59 (1H, m, 3-H), 7.71 (1H, br s, NH).

Compound **8** (*N*-(3-Oxopentanoyl)-L-homoserine Lactone): IR (KBr) $cm^{-1}$ 3280 (NH), 1780 (ring C=O), 1710 (ketone C=O), 1645 (amide C=O), 1550, 1170; EI-MS *m/z* (%) 199.0845 (25, $M^+$, $C_9H_{13}NO_4$ requires *m/z* 199.0845), 170 (9), 154 (7), 141 (6), 125 (7), 102 (34), 101 (34), 57 (100), 43 (36); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 1.09 (3H, t, $J=7.3$ Hz, $CH_3$), 2.23 (1H, m, 4$\alpha$-H), 2.57 (2H, q, $J=7.2$ Hz, $CH_2CO$), 2.77 (1H, m, 4$\beta$-H), 3.48 (2H, s, $COCH_2CO$), 4.28 (1H, m, 5$\alpha$-H), 4.48 (1H, td, $J=1.3$ and 9.1 Hz, 5$\beta$-H), 4.58 (1H, m, 3-H), 7.60 (1H, br s, NH).

Compound **9** (*N*-[(*E*)-3-Oxo-6-octenoyl]-L-homoserine Lactone): IR (KBr) $cm^{-1}$ 3290 (NH), 1785 (ring C=O), 1715 (ketone C=O), 1650 (amide C=O), 1550, 1185; EI-MS *m/z* (%) 239.1184 (29, $M^+$, $C_{12}H_{17}NO_4$ requires *m/z* 239.1157), 185 (23), 170 (20), 151 (31), 139 (20), 138 (32), 113 (27), 102 (100), 101 (27), 96 (41), 69 (54), 68 (82), 57 (54); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 1.64 (3H, dd, $J=6.1$ and 1.3 Hz, $CH_3$), 2.23 (1H, m, 4$\alpha$-H), 2.28 (2H, m, $CH=CHCH_2$), 2.59 (2H, t, $J=7.2$ Hz, $CH_2CO$), 2.76 (1H, m, 4$\beta$-H), 3.46 (2H, s, $COCH_2CO$), 4.28 (1H, m, 5$\alpha$-H), 4.48 (1H, td, $J=1.3$ and 9.1 Hz, 5$\beta$-H), 4.58 (1H, m, 3-H), 5.34~5.50 (2H, m, CH=CH), 7.65 (1H, br s, NH).

Compound **10** (*N*-[(*S*)-3-Hydroxyhexanoyl]-L-homoserine Lactone): IR (KBr) $cm^{-1}$ 3360 (OH), 3300 (NH), 1775 (ring C=O), 1645 (amide C=O), 1545, 1190, 1177; EI-MS *m/z* (%) 197.0995 (9, $M^+-H_2O$, $C_{10}H_{15}NO_3$ requires *m/z* 197.1052), 172.0629 (52, $M^+-C_3H_7$, $C_7H_{10}NO_4$ requires *m/z* 172.0610), 154 (4), 143 (31), 102 (100); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 0.94 (3H, t, $J=7.4$ Hz, $CH_3$), 1.43 (2H, m, $CH_3CH_2$), 1.55 (2H, m $CH_2CHOH$), 2.19 (1H, m, 4$\alpha$-H), 2.33 (1H, dd, $J=15.4$ and 8.9 Hz, $CH_\alpha H_\beta CO$), 2.46 (1H, dd, $J=15.4$ and 2.7 Hz, $CH_\alpha H_\beta CO$), 2.83 (1H, m, 4$\beta$-H), 4.05 (1H, m, CHOH), 4.30 (1H, m, 5$\alpha$-H), 4.48 (1H, m, 5$\beta$-H), 4.55 (1H, m, 3-H), 6.50 (1H, br s, NH).

Compound **11** (*N*-[(*RS*)-3-Hydroxypentanoyl]-L-homoserine Lactone): EI-MS *m/z* (%) 201.0939 (2, $M^+$, $C_9H_{15}NO_4$ requires *m/z* 201.1001), 183.0931 (8, $M^+-H_2O$, $C_9H_{13}NO_3$ requires *m/z* 183.0895), 172.0677 (24, $M^+-C_2H_5$, $C_7H_{10}NO_4$ requires *m/z* 172.0610), 143 (28), 102 (44), 101 (32), 57 (100); $^1H$ NMR (90 MHz, $CDCl_3$) $\delta$ 0.93 (3H, t, $J=7.0$ Hz, $CH_3$), 1.50 (2H, quintet, $J=7.0$ Hz, $CH_3CH_2$), 2.15 (1H, m, 4$\alpha$-H), 2.33 (2H, d, $J=6.5$ Hz, $CH_2CO$), 2.60 (1H, m, 4$\beta$-H), 2.70 (1H, br s, $D_2O$ exchangeable, OH), 4.0~4.80 (3H, m, 5-$H_2$ and 3-H), 6.90 (1H, br s, $D_2O$ exchangeable, NH).

Compound **12** (*N*-Hexanoyl-L-homoserine Lactone): IR (KBr) $cm^{-1}$ 3315 (NH), 1775 (ring C=O), 1645 (amide C=O), 1550, 1175; EI-MS *m/z* (%) 199.1256 (5, $M^+$, $C_{10}H_{17}NO_3$ requires *m/z* 199.1208), 170 (5), 156 (8), 143 (100), 85 (10); $^1H$ NMR (90 MHz, $CDCl_3$) $\delta$ 0.87 (3H, t, $J=7.0$ Hz, $CH_3$), 1.0~1.40

(4H, m, $CH_3CH_2CH_2$), 1.40~1.85 (2H, m, $CH_2CH_2CO$), 1.85~2.45 (3H, m, $CH_2CO$ and 4α-H), 2.45~2.90 (1H, m, 4β-H), 4.0~4.75 (3H, m, 5-$H_2$ and 3-H), 6.43 (1H, d, $J$=6.0 Hz, NH).

Compound **13** (*N*-[(*R*)-3-Hydroxyhexanoyl]-L-homoserine Lactone): IR (KBr) $cm^{-1}$ 3360 (OH), 3300 (NH), 1775 (ring C=O), 1645 (amide C=O), 1545, 1190, 1177; EI-MS *m/z* (%) 197.1066 (11, $M^+-H_2O$, $C_{10}H_{15}NO_3$ requires *m/z* 197.1052), 172.0612 (53, $M^+-C_3H_7$, $C_7H_{10}NO_4$ requires *m/z* 172.0610), 143 (27), 102 (100); $^1H$ NMR (400 MHz, $CDCl_3$) δ 0.94 (3H, t, $J$=7.4 Hz, $CH_3$), 1.43 (2H, m, $CH_3CH_2$), 1.55 (2H, m, $CH_2CHOH$), 2.19 (1H, m, 4α-H), 2.33 (1H, dd, $J$=15.4 and 8.9 Hz, $CH_\alpha H_\beta CO$), 2.46 (1H, dd, $J$=15.4 and 2.7 Hz, $CH_\alpha H_\beta CO$), 2.83 (1H, m, 4β-H), 4.05 (1H, m, CHOH), 4.30 (1H, m, 5α-H), 4.48 (1H, m, 5β-H), 4.55 (1H, m, 3-H), 6.50 (1H, br s, NH).

Compound **14** (*N*-Valeryl-L-homoserine Lactone): IR (KBr) $cm^{-1}$ 3310 (NH), 1775 (ring C=O), 1645 (amide C=O), 1550, 1175; EI-MS *m/z* (%) 143.0602 (58, $M^+-C_3H_6$, $C_6H_9NO_3$ requires *m/z* 143.0582), 125 (8), 102 (7), 101 (10), 85 (33), 57 (100); FAB-MS *m/z* (%) 186 (100, $M^++H$), 208 (21, $M^++Na$), 371 (12, $2M^++H$), 393 (6, $2M^++Na$); $^1H$ NMR (400 MHz, $CDCl_3$) δ 0.93 (3H, t, $J$=7.4 Hz, $CH_3$), 1.36 (2H, sextet, $J$=7.5 Hz, $CH_3CH_2$), 1.64 (2H, quintet, $J$=7.7 Hz, $CH_3CH_2CH_2$), 2.12 (1H, m, 4α-H), 2.26 (2H, t, $J$=7.0 Hz, $CH_2CO$), 2.88 (1H, m, 4β-H), 4.28 (1H, m, 5α-H), 4.47 (1H, td, $J$=1.3 and 9 Hz, 5β-H), 4.54 (1H, m, 3-H), 5.96 (1H, br s, NH).

Compound **15** (L-3-(3-Oxohexanoyl)amino-2-pyrrolidinone): EI-MS *m/z* (%) 212.1102 (15, $M^+$, $C_{10}H_{16}N_2O_3$ requires *m/z* 212.1160), 184 (8), 156 (5), 142 (18), 130 (33), 113 (34), 101 (78), 100 (92), 99 (100), 83 (33), 71 (88), 56 (22); $^1H$ NMR (400 MHz, $CDCl_3$) δ 0.93 (3H, t, $J$=7.4 Hz, $CH_3$), 1.60 (2H, sextet, $J$=7.4 Hz, $CH_3CH_2$), 2.00 (1H, m, 4α-H), 2.52 (2H, t, $J$=7.2 Hz, $CH_2CO$), 2.74 (1H, m, 4β-H), 3.39 (2H, m, 5-$H_2$), 3.44 (2H, s, $COCH_2CO$), 4.43 (1H, m, 3-H), 5.80 (1H, br s, NH), 7.43 (1H, br s, NH).

Compound **16** (*N*-(3-Oxobutanoyl)-L-homoserine Lactone): IR (KBr) $cm^{-1}$ 3280 (NH), 1780 (ring C=O), 1710 (ketone C=O), 1640 (amide C=O), 1550, 1170; EI-MS *m/z* (%) 185.0701 (3, $M^+$, $C_8H_{11}NO_4$ requires *m/z* 185.0687), 140 (4), 127 (7), 102 (4), 101 (21), 57 (72), 43 (100); $^1H$ NMR (400 MHz, $CDCl_3$) δ 2.23 (1H, m, 4α-H), 2.28 (3H, s, $CH_3$), 2.77 (1H, m, 4β-H), 3.50 (2H, s, $COCH_2CO$), 4.28 (1H, m, 5α-H), 4.48 (1H, td, $J$=1.3 and 9 Hz, 5β-H), 4.59 (1H, m, 3-H), 7.60 (1H, br s, NH).

Compound **17** (*N*-(3-Oxo-4-hexenoyl)-L-homoserine Lactone): FAB-MS *m/z* (%) 212 (94, $M^++H$), 234 (18, $M^++Na$), 423 (6, $2M^++H$); $^1H$ NMR (400 MHz, $CDCl_3$) δ 1.96 (3H, dd, $J$=6.8 and 1.6 Hz, $CH_3$), 2.23 (1H, m, 4α-H), 2.76 (1H, m, 4β-H), 3.59 (2H, s, $COCH_2CO$), 4.28 (1H, m, 5α-H), 4.48 (1H, m, 5β-H), 4.58 (1H, m, 3-H), 6.18 (1H, dq, $J$=15.75 and 1.6 Hz, $CH_3CH=CH$), 7.00 (1H, dq, $J$=15.7 and 6.8 Hz, $CH_3CH=CH$), 7.52 (1H, br s, NH).

Compound **18** (*N*-Butyryl-L-homoserine Lactone): IR (KBr) $cm^{-1}$ 3310 (NH), 1775 (ring C=O), 1640 (amide C=O), 1545, 1175; EI-MS *m/z* (%) 171.0922 (12, $M^+$, $C_8H_{13}NO_3$ requires *m/z* 171.0896), 153 (4), 143 (65), 128 (5), 125 (5), 102 (10), 101 (10), 71 (57), 57 (65), 43 (100); $^1H$ NMR (400 MHz, $CDCl_3$) δ 0.96 (3H, t, $J$=7.4 Hz, $CH_3$), 1.68 (2H, sextet, $J$=7.46 Hz, $CH_3CH_2$), 2.19 (1H, m, 4α-H), 2.24 (2H, t, $J$=7.1 Hz, $CH_2CO$), 2.82 (1H, m, 4β-H), 4.29 (1H, m, 5α-H), 4.47 (1H, td, $J$=1.3 and 9 Hz, 5β-H), 4.59 (1H, m, 3-H), 6.26 (1H, br s, NH).

Compound **19** (*N*-[(*E*)-2-Hexenoyl]-L-homoserine Lactone): IR (KBr) $cm^{-1}$ 3310 (NH), 1780 (ring C=O), 1675 (amide C=O), 1635 (C=C), 1555, 1175; EI-MS *m/z* (%) 197.1088 (11, $M^+$, $C_{10}H_{15}NO_3$ requires *m/z* 197.1052), 154 (9), 97 (100), 85 (3); $^1H$ NMR (400 MHz, $CDCl_3$) δ 0.94 (3H, t, $J$=7.4 Hz, $CH_3$), 1.48 (2H, sextet, $J$=7.4 Hz, $CH_3CH_2$), 2.17 (3H, m, 4α-H and $CH_2CH=CH$), 2.86 (1H, m, 4β-H), 4.31 (1H, m, 5α-H), 4.48 (1H, td, $J$=1.3 and 9 Hz, 5β-H), 4.63 (1H, m, 3-H), 5.83 (1H, dt, $J$=15.35 and 1.5 Hz, $CH=CHCO$), 6.11 (1H, br s, NH), 6.90 (1H, dt, $J$=15.35 and 7.0 Hz, $CH=CHCO$).

Compound **20** (*N*-Acetyl-L-homoserine Lactone): IR (KBr) $cm^{-1}$ 3300 (NH), 1785 (ring C=O), 1640 (amide C=O), 1535, 1185; EI-MS *m/z* (%) 143.0546 (6, $M^+$, $C_6H_9NO_3$ requires *m/z* 143.0582), 125 (5), 116 (2), 101 (3), 98 (11), 57 (93), 43 (100); $^1H$ NMR (400 MHz, $CDCl_3$) δ 2.06 (3H, s, $CH_3$), 2.19 (1H, m, 4α-H), 2.78 (1H, m, 4β-H), 4.29 (1H, m, 5α-H), 4.47 (1H, td, $J$=1.3 and 9 Hz, 5β-H), 4.62 (1H, m, 3-H), 6.57 (1H, br s, NH).

Compound **21** (*N*-[(*RS*)-3-Hydroxybutyryl]-L-homoserine Lactone): EI-MS *m/z* (%) 187.0820 (2, $M^+$, $C_8H_{13}NO_4$ requires *m/z* 187.0844), 172.0614 (11, $M^+-CH_3$, $C_7H_{10}NO_4$ requires *m/z* 172.0610), 169.0761 (6, $M^+-H_2O$, $C_8H_{11}NO_3$ requires *m/z* 169.0739), 143 (34), 102 (46), 101 (18), 57 (100); $^1H$ NMR (90 MHz, $D_2O$) δ 1.20 (3H, d, $J$=6.5 Hz, $CH_3$), 2.20 (1H, m, 4α-H), 2.40 (2H, d, $J$=6.5 Hz, $CH_2CO$), 2.57 (1H, m, 4β-H), 4.0~4.70 (4H, m, 5-$H_2$, 3-H and $CHCH$).

Compound **22** (L-α-(3-Oxohexanoyl)amino-δ-valerolactone): EI-MS *m/z* (%) 227.1174 (4, $M^+$, $C_{11}H_{17}NO_4$ requires *m/z* 227.1157), 199 (3), 184 (3), 156 (39), 115 (34), 71 (80); $^1H$ NMR (400 MHz, $CDCl_3$) δ 0.93 (3H, t, $J=7.4$ Hz, $CH_3$), 1.63 (2H, sextet, $J=7.4$ Hz, $CH_3CH_2$), 1.84 (4H, m, 4-$H_2$ and 5-$H_2$), 2.52 (2H, t, $J=7.2$ Hz, $CH_2CO$), 3.44 (2H, s, $COCH_2CO$), 4.37 (2H, t, $J=6.0$ Hz, 6-$H_2$), 4.66 (1H, m, 3-H), 7.62 (1H, br s, NH).

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