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Synthesis of Tritium Labelled and Photoactivatable *N*-Acyl-L-homoserine Lactones: Inter-Kingdom Signalling Molecules

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N-Acyl-L-homoserine lactones (AHLs) are signal molecules that are synthesized in nature by Gram-negative bacteria. They allow communication between bacteria (quorum sensing) and between microorganisms and their eukaryotic host cells (inter-kingdom signalling). However, very little is known about the mechanisms of the latter system. Therefore, tritium labelled photoactivatable AHLs were synthesized to identify specific receptors in immune cells (e.g., human polymorphonuclear neutrophils, PMN) that bind to AHL. A photoaffinity label – a diazirine group – was chosen as the smallest possible photoactivatable group that can be activated by light irradiation. The resulting highly active carbene will bind covalently to the closest chemical structure in the pocket of the receptor. The diazirine label was introduced into the AHL molecule according to a literature method. To isolate the labelled receptor from the cellular protein moiety, the AHL was additionally labelled with tritium. During the development of an effective isotopic labelling method, a simple route to hydrogen isotope incorporation into the AHLs was proposed and explored in detail. According to the new protocol, the photoactivatable diazirine-AHL was labelled with deuterium and tritium by a postsynthetic catalytic exchange of the hydrogen with its isotopes, using deuterium or tritium labelled water along with catalytic amounts of metal salts under mild basic conditions.

Introduction

N-Acyl-L-homoserine lactones (AHLs) are synthesized in nature by bacteria such as *Pseudomonas aeruginosa* and mediate bacterial cell-to-cell communication (quorum sensing).[1,2] Quorum-sensing molecules and their role in bacterial communication have been extensively studied[3–7] in the context of biofilm formation, which causes chronic, destructive infections. Recently, several studies have shown that signalling by these molecules is not restricted to bacteria, but that they also interact with eukaryotic cells in a phenomenon termed "inter-kingdom signalling".[8] *N*-(3-Oxododecanoyl)-L-homoserine lactone **1** (3OC12-HSL, Figure 1) is the most biologically active derivative in this class.[9] In contrast to the adverse effects reported in literature, 3OC12-HSL might also support the immune system defence of the host.[7,10] The host defence against bacteria or bacterial biofilms relies on phagocytic cells polymorphonuclear neutrophils (PMN) and their capacity to infiltrate into infected sites to recognize and eliminate pathogens.[11] However, very little is known about the detailed mechanism of the interaction between AHL and host cells.

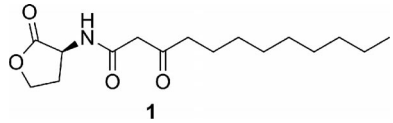

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Figure 1. Structure of the *N*-(3-oxododecanoyl)-L-homoserine lactone (3OC12-HSL).

Isotope-labelled compounds have several important applications. Compounds labelled with hydrogen isotopes have application in mechanistic studies in organic chemistry,[12] as well as in many other branches of science such as in the investigation of models of biological systems[13,14] or metabolism and in metabolism-mediated toxicity studies.[15] However, most labelling methods require the use of hydrogen gas, expensive metal-based catalysts, aggressive reagents, higher temperatures, or microwave irradiation.[16] Therefore, new, mild postsynthetic labelling strategies are still strongly desired.

Affinity labelling is a biochemical technique that is used for the investigation of structural and functional properties of biological systems. The method involves labelling of an active site of proteins (e.g., receptors) by a small molecule bearing a group that is capable of specifically binding to

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the active site, and a second group that is chemically reactive.[17–19] One of the most important variants of this method is photoaffinity labelling. In this approach, a functional group that is chemically unreactive in the dark is converted into a reactive intermediate upon photolysis that binds irreversibly to the biological receptor.[20] Photoactivatable reagents must possess a photoactive group and must be easily detectable by virtue of having either a radioactive isotope or a fluorophore moiety. However, the latter usually contain several combined aromatic groups, which change the structure of the ligand dramatically. Recent studies uncovered a drastic difference in activity between the alkynyl- and azido-tagged 3OC12-HSLs and their unmodified analogues.[2,21] Therefore, the most convenient label is the radioactive isotope, particularly tritium. Importantly, this radioactive isotope of hydrogen does not change the structure of the ligand, which preserves its specificity towards a target receptor. Moreover, the photoactivatable reagents must be bifunctional, i.e., must be linked reversibly to the biological ligand (for photoaffinity labelling) or to the biological macromolecule such as nucleic acids or lipids (for cross-linking), before photolysis. After activation by light, the photolytic intermediate reacts covalently within the site before it can dissociate.[22] Several photoactivatable groups are known, including azido, diazo, and azo groups, diazonium ions, benzophenone moieties or diazirines. The latter groups are found to be the most convenient because of their chemical stability, selective photolytic activity, and small size.[2,23–27]

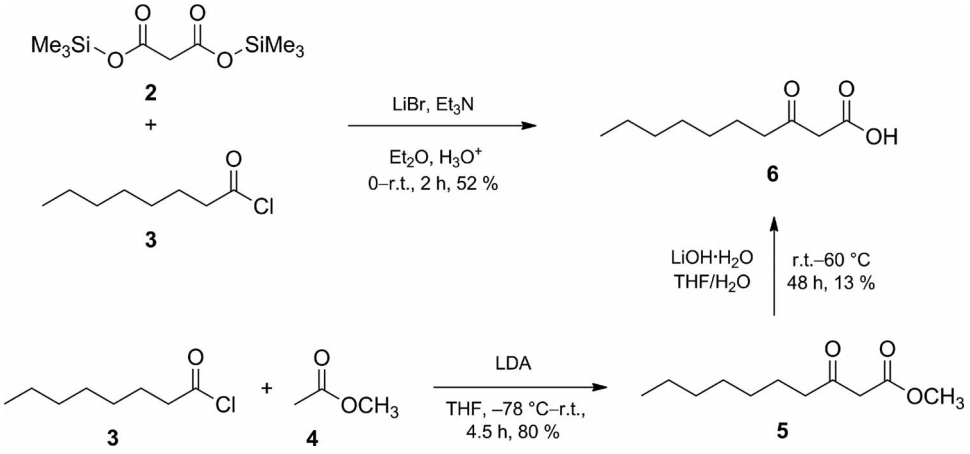
Our strategy entailed the design and synthesis of a 3OC12-HSL derivative that contained a photoactivatable group for affinity capture of the receptor and an additional radioactive label that can be used to isolate and identify the captured receptor from the cellular protein moiety. Our method demonstrates a straightforward and very target-oriented approach to the bi-labelling of a highly biologically active molecule, 3OC12-HSL.

Results and Discussion

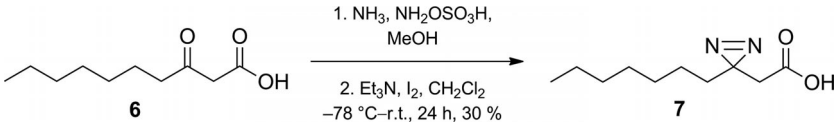
The first step of the synthesis of *N*-(3-oxo-5-diazirinedodecanoyl)-L-homoserine lactone (**9**) was based on the synthesis of β-ketodecanoic acid (**6**). For this purpose, two methods were tested (Scheme 1). In the first, bis(trimethylsilyl) malonate (BSM, **2**) was acylated with octanoyl chloride (**3**) by using a mild base such as triethylamine and lithium bromide, in good yield (52%).[28] Complexation of the lithium cation by BSM **2** increased the acidity of the α-carbon protons thus making them easier removed. As a consequence, the use of strong bases such as metal alkoxides, metal amides, or alkyllithium reagents was avoided.[29]

In the second method, a two-step synthesis was started from acylation of methyl acetate **4** by octanoyl chloride (**3**) in the presence of lithium diisopropylamide (LDA), with very good yield (Scheme 1).[30–32] The obtained methyl 3-oxodecanoate (**5**) was further hydrolysed under strong basic conditions. However, even the use of a strong base such as lithium hydroxide and heating under reflux for two days, yielded only 13% of the desired product **6**. Thus, the first method was chosen as a more efficient and convenient approach.

The diazirine group was chosen as the most convenient photoactivatable agent. Thus, 3-diazirine-decanoic acid (**7**) was prepared according to a procedure described by Husain et al.[1] β-Ketodecanoic acid (**6**) was treated with hydroxylamine-*O*-sulfonic acid in liquid ammonia to give an aziridine intermediate. Oxidation with iodine resulted in the for-



Scheme 1. The synthesis of β-ketodecanoic acid (**6**).



Scheme 2. Formation of 3-diazirinedecanoic acid (**7**).

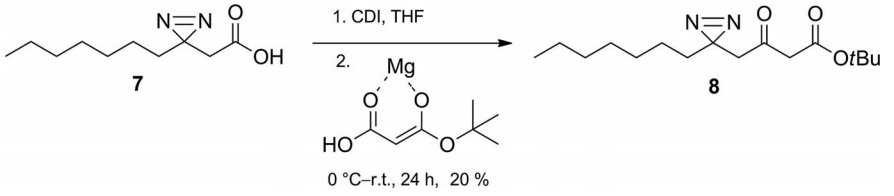
mation of diazirine **7** in 30% yield (Scheme 2).[1] 3-Diazirinedecanoic acid (**7**) was subsequently converted into *tert*-butyl ester **8** with 20% yield (Scheme 3).[1,33]

The ester group of compound **8** was deprotected with trifluoroacetic acid (TFA) and condensed with L-homoserine lactone in an EDC-mediated reaction. This gave the desired *N*-(3-oxo-5-diazirinedodecanoyl)-L-homoserine lactone (**9**) with 25% yield (Scheme 4).

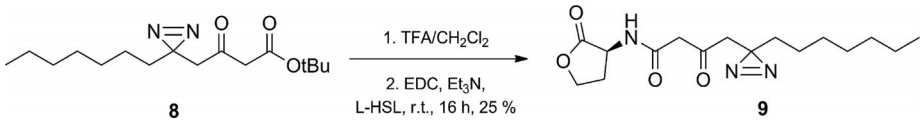
Deuterium and tritium labelling of **9** requires mild reaction conditions. A strong base, for instance, can cleave the lactone moiety, and elevated temperature can lead to premature decomposition of the diazirine group. Thus, the first method tested in this work was based on a postsynthetic hydrogen–deuterium(tritium) exchange by deuterated (tritiated) water under mild conditions (mild base, room temperature). Initial experimental exchange reactions were carried out with lactone **1** by using triethylamine and deuterium oxide. This reaction yielded only 31% of the monodeuterated isomer. The degree of deuteration was slightly increased by extension of the reaction time (See Scheme 5 and Table 1, entry 1).

The second approach relied on the application of the phase-transfer catalyst triethylbenzylammonium chloride (TEBA) in a solvent system consisting of a solution of 5% sodium deuterioxide and ethyl acetate (Table 1).[34] However, even after two days reaction time the deuterium content was not higher than 51%, thus, phase-transfer catalysis (PTC) conditions were not efficient in this system.

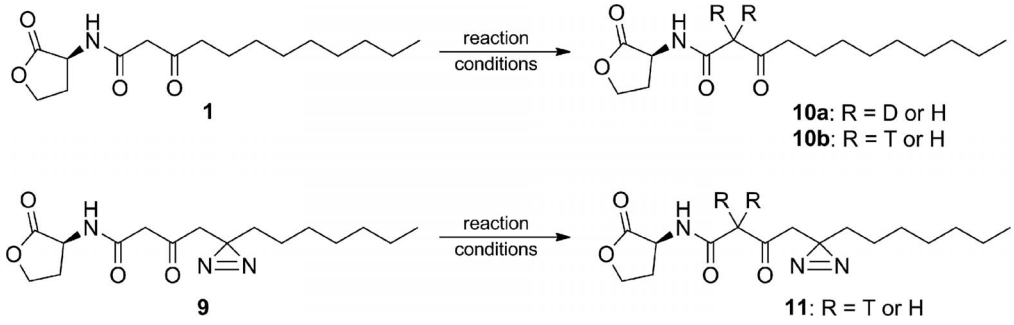
Another concept of deuterium labelling of these molecules was inspired by previously demonstrated activation of the acidic protons in malonic esters by metal salts.[28] Metal cations are chelated by the two carbonyl groups and the acidity of the α-protons is enhanced. Tritium labelling in this position is beneficial for the stability of the final product because the tritium–carbon bond is much stronger than a protium–carbon bond. Thus, application of lithium bromide to the labelling of substrate **1** increased the deuterium content up to 56%. The deuteration degree was still



Scheme 3. Synthesis of *tert*-butyl 3-oxo-5-diazirinedodecanoate (**8**); CDI = 1,1′ carbonyldiimidazole.



Scheme 4. Synthesis of *N*-(3-oxo-5-diazirinedodecanoyl)-L-homoserine lactone (**9**).



Scheme 5. Deuterium and tritium labelling of AHLs through exchange reactions.

Table 1. Deuterium and tritium labelling of **1** and **9**.

Entry	Substrate[a]	Reactions conditions	Time [h]	Yield [%]	Product[a]	Deuterium content[b] (radiochemical yield[c] [%])
1	**1**	Et_3N, D_2O, MeCN	12	93	**10a**	d_1 (41), d_0 (59)
2	**1**	5% NaOD in D_2O/EtOAc, TEBA, 30 °C	24	93	**10a**	d_1 (51), d_0 (49)
3	**1**	LiBr, Et_3N, D_2O, THF	48	94	**10a**	d_2 (29), d_1 (56), d_0 (15)
4	**1**	$Mg(OAc)_2$, Et_3N, D_2O, THF	48	98	**10a**	d_3 (6), d_2 (79), d_1 (6)
5	**1**	$Mg(OAc)_2$, Et_3N, HTO, THF	48	89	**10b**	22
6	**9**	$Mg(OAc)_2$, Et_3N, HTO, THF	48	99	**11**	14

[a] See Scheme 5. [b] Determined by ESI-TOF MS. [c] Determined with a liquid scintillation counter.

lower than expected, probably because of the low solubility of LiBr. Therefore, the inclusion of magnesium(II) acetate was tested, resulting a very high deuterium incorporation, up to 79% of double-deuterated isotopologue (Table 1, entry 4). This method was then applied in tritium labelling – first of substrate **1** and then of lactone **9**. As expected, very good yields and relatively good specific activities were obtained (Scheme 5, Table 1 and experimental section).

Conclusions

The *N*-(3-oxo-5-diazirinedodecanoyl-[2-$^3H_2$])-L-homoserine lactone (**11**) was successfully synthesized in five steps. It bears a photoactivatable diazirine group and is easily detectable because of its radioactive isotope label. The diazirine moiety is located close to the hydrophilic part of the molecule and affects the structure of the highly biologically active *N*-(3-oxododecanoyl)-L-homoserine lactone in a negligible way. An additional advantage of this approach is the fact that the tritium label is introduced in the last step of the synthesis, reducing the time required to handle radioactive material. The bifunctional molecule is being applied in ongoing photoaffinity labelling studies of a potential receptor for inter-kingdom signalling. In general, this mild and convenient method could be applied in labelling of other bioactive molecules for the investigation of their biological activities.

Experimental Section

General: Solvents and chemicals used for reactions were purchased from commercial suppliers. Solvents were dried under standard conditions; chemicals were used without further purification. All reactions were carried out under nitrogen in flame-dried glassware. Evaporation of solvents and concentration of reaction mixtures were performed in vacuo at 60 °C with a Heidolph Rotary Evaporator, Laborota 4000. Thin-layer chromatography (TLC) was carried out on silica gel plates (Kieselgel 60, Merck) with visualization by iodine vapour and Seebach solution.[35] Normal-phase silica gel (silica gel 60, 230–400 mesh, Merck) was used for flash chromatography. IR and Raman spectra were recorded with a Bruker Vertex 80 FTIR spectrometer (Bruker Optik GmbH, Ettlingen, Germany) with a single reflection "Golden Gate" diamond ATR sampling unit (Specac, UK). ^{1}H and ^{13}C NMR spectra were recorded with a Bruker-ACS-60, Ultrashield 500 Plus spectrometer. Chemical shifts are reported as δ parts per million (ppm) values relative to the $CHCl_3$ signal (^{1}H: δ = 7.26 ppm, ^{13}C: δ = 77.0 ppm). Coupling constants (*J*) are given in Hertz [Hz]; s = singlet, d = doublet, t = triplet, quint. = quintet, sept = septet, m = multiplet, dt = doublet of triplets, ddd = double doublet of doublets. ESI-TOF mass spectra were recorded with a ESI-TOF Mariner system (Applied Biosystems). UV/Vis spectra were recorded with an Agilent 8453 UV/Vis Photospectrometer. Absorption was measured in tetrahydrofuran solution from 200 to 800 nm with a resolution of 1 nm in a 8.5 mm 1 mL UV micro cuvette (Brand GmbH, Wertheim, Germany) at room temperature. Scintillation counting was carried out with a Wallac 1409 apparatus by using a "mélange scintillant III" cocktail from SDS company.

3-Oxodecanoic Acid (6): Anhydrous LiBr (powder; 95 mg, 1 mmol) was transferred to a 100 mL two-neck flask fitted with a septum and gas inlet tube with gas bubbler, and anhydrous Et_2O (30 mL) and bis(trimethylsilyl) malonate (**2**; 267.7 μL, 1.05 mmol) were added. Triethylamine (152.5 μL, 1.1 mmol) was added dropwise and a precipitate was formed almost instantly. After stirring for 10 min, the flask was cooled to 0 °C and octanoyl chloride (**3**; 170.7 μL, 1 mmol) was added (dropwise, slowly). After stirring at r.t. for 1 h, the reaction was quenched by the addition of cold satd. aq $NaHCO_3$ (15 mL) and the mixture was stirred for 10 min in an ice bath. The aqueous layer was separated and acidified to pH 2–3 by the dropwise addition of cold 1 M HCl. The resulting precipitate was extracted with ethyl acetate and the organic layer was washed several times with satd. aq $NaHCO_3$. After drying the combined organic solution over anhydrous sodium sulfate, the solvent was evaporated in vacuo. The desired product **6** (96 mg, 52%) was obtained as a colourless solid. R_f = 0.58 (hexane/EtOAc, 1:10). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.91 (*t*, ^3J = 6.7 Hz, 3 H, CH_3), 1.21–1.40 [m, 8 H, $CH_3(CH_2)_4$], 1.54–1.69 (m, 2 H, CH_2CH_2CO), 2.58 (t, ^3J = 7.4 Hz, 2 H, CH_2CO), 3.54 (s, 2 H, $COCH_2CO$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.1 (s, CH_3), 22.6 (s, C-9, CH_2), 23.4 (s, C-8, CH_2), 28.9 (m, C-7, CH_2), 29.6 (m, C-6, CH_2), 31.6 (s, C-5, CH_2), 43.3 (s, C-4, CH_2), 47.6 (s, C-2, CH_2), 170.8 (s, C-1, COOH), 209.7 (s, C-3, C=O) ppm. ESI-TOF MS: *m*/*z* (%) = 187 (100) $[M + H]^+$.

3-Diazirinedecanoic Acid (7): Anhydrous ammonia (100 mL) was condensed into a round-bottomed flask. 3-Oxodecanoic acid (**6**; 3.2 g, 17.2 mmol) was dissolved in a small amount of anhydrous MeOH (10 mL) and added to the flask, and the mixture was stirred at –35 to –40 °C for 5 h. The solution was cooled with dry-ice, and a solution of hydroxylamine-*O*-sulfonic acid (2.22 g, 19.6 mmol) in anhydrous MeOH (5 mL) was added over a period of 30 min. The dry-ice bath was removed, and the mixture was refluxed with stirring at –35 °C for 1 h. The mixture was warmed slowly to room temperature and stirred overnight, then the ammonia was allowed to evaporate. The resulting slurry was filtered and the filter cake was washed with several portions of methanol. The combined solution was concentrated in vacuo and the crude aziridine residue was dissolved in CH_2Cl_2 (10 mL) and treated with triethylamine (2.96 mL, 21.3 mmol). A solution of iodine (5.8 g, 23 mmol) in CH_2Cl_2 (20 mL) was slowly added with stirring until the appearance of a persistent orange-brown colour. The crude residue was purified by flash column chromatography (hexane/EtOAc, 1:1) to give the desired product (1.02 g, 30%) as an orange-brown oil. R_f = 0.39 (hexane/EtOAc, 1:10). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.89 (t, ^1J = 6.9 Hz, 3 H, CH_3), 0.90–1.14 [m, 2 H, $CH_2(CH_2)_4$], 1.15–1.26 [m, 8 H, $CH_3(CH_2)_4$], 1.55 (t, ^1J = 7.8 Hz, 2 H, CH_2CN_2), 2.33 (s, 2 H, CN_2CH_2CO), 10.10 (s, 1 H, COOH) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.1 (s, C-10, CH_3), 22.6 (s, C-9, CH_2), 23.7 (s, C-8, CH_2), 25.9 (s, C-7, CH_2), 28.9 (s, C-6–5, CH_2), 31.6 (s, C-4, CH_2), 32.4 (s, C-2, CH_2), 39.6 (s, C-3, CN_2), 175.8 (s, C-1, COOH) ppm. IR (ATR): $\tilde{\nu}$ = 2980, 1710, 1657, 1330, 1218, 1055 cm^{-1}. Raman: $\tilde{\nu}$ = 2899, 1693, 1592, 1258, 1045, 881 cm^{-1}. UV (THF): λ_{max} = 343 (ε = 69 M^{-1} cm^{-1}) nm. ESI-TOF MS: *m*/*z* (%) = 197 (100) $[M - H]^+$.

***tert*-Butyl 3-Oxo-5-diazirinedodecanoate (8):** *Solution 1:* To a solution of 3-diazirine-decanoic acid (**7**; 34 mg, 0.17 mmol) in anhydrous THF (2.5 mL) under a nitrogen atmosphere, 1,1′-carbonyldiimidazole (CDI; 33 mg, 0.2 mmol) was added at room temperature. The mixture was stirred at room temperature for 4 h. *Solution 2:* To a solution of mono *tert*-butyl malonate (32 μL, 0.2 mmol) in anhydrous THF (2.5 mL) at 0 °C under a nitrogen atmosphere, isopropylmagnesium chloride (2 M in THF, 187 μL, 0.37 mmol) was added dropwise. After 30 min at 0 °C the solution was heated at 50 °C for 30 min and cooled again to 0 °C. Solution 1 was added

by using a cannula and the mixture was warmed to room temperature. After stirring for 16 h, the reaction was quenched by the addition of 1 M HCl (6 mL) and the aqueous phase was extracted with ethyl acetate. The combined organic layers were washed with $NaHCO_3$, brine, dried with Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (hexane/EtOAc, 10:1) to give the desired product (10 mg, 20%) as an orange-brown oil. R_f = 0.47 (hexane/EtOAc, 1:10). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.89 (t, ^3J = 6.9 Hz, 3 H, CH_3), 0.91–1.12 (m, 2 H, CH_2CN_2), 1.14–1.32 (m, 10 H, 5 × CH_2), 1.48 (s, 9 H, 3 × CH_3), 2.47 (s, 2 H, CN_2CH_2CO), 3.38 (s, 2 H, $COCH_2CO$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.1 (s, C-12, CH_3), 22.6 (s, C-11, CH_2), 23.7 (s, C-10, CH_2), 27.9 (s, 3 × CH_3), 28.3 (s, C-9, CH_2), 29.2 (s, C-8, CH_2), 29.7 (s, C-7, CH_2), 31.6 (s, C-6, CH_2), 32.5 (s, C-4, CH_2), 47.8 (s, C-5, CN_2), 50.8 (s, C-2, CH_2), 82.4 [s, $C(CH_3)_3$], 165.8 (s, C-1, C=O), 199.3 (s, C-3, C=O) ppm. ESI-TOF MS: *m*/*z* (%) = 297 (100) $[M + H]^+$.

***N*-(3-Oxo-5-diazirinedodecanoyl)-l-homoserine Lactone (9):** *tert*-Butyl 3-oxo-5-diazirine-dodecanoate (**8**; 7.4 mg, 0.025 mmol) was stirred in TFA/CH_2Cl_2 (1:1, 540 μL) for 20 min. After solvent evaporation, the resulting β-keto acid (8 mg, 0.025 mmol) was dissolved in 1,4-dioxane (2 mL) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC; 6.7 mg, 0.035 mmol), hydroxybenzotriazole (HOBt; 3.4 mg, 0.025 mmol) and l-homoserine lactone hydrobromide (4.5 mg, 0.025 mmol) were added together with a few drops of water (1.6 mL) at room temperature. Triethylamine (7 μL, 0.05 mmol) was added and the solution was stirred at room temperature for 16 h. The mixture was evaporated and the residue was dissolved in ethyl acetate, washed with water, dried with Na_2SO_4 and the solvent was removed by rotary evaporation. The crude residue was purified by flash column chromatography (hexane/EtOAc, 1:3) to give the desired product (2 mg, 25%) as a yellow oil. R_f = 0.66 (hexane/EtOAc, 1:4). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.88 (t, ^1J = 7.1 Hz, 3 H, CH_3), 1.02–1.14 (m, 2 H, $CH_2CH_2CN_2$), 1.22–1.39 (m, 8 H, 4 × CH_2), 1.50 (t, ^1J = 8.1 Hz, 2 H, CH_2CN_2), 2.18–2.31 (m, 1 H, 3α-H), 2.49 (s, 2 H, $COCH_2CN_2$), 2.70–2.89 (m, 1 H, 3β-H), 3.51 (s, 2 H, $COCH_2CO$), 4.12–4.30 (m, 1 H, 4α-H), 4.51 (td, ^1J = 8.9, ^2J = 1.0 Hz, 1 H, 4β-H), 4.57–4.71 (m, 1 H, 2-H), 7.70–7.89 (m, 1 H, NH) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.1 (s, C′-12, CH_3), 22.6 (s, C′-11, CH_2), 23.7 (s, C′-10, CH_2), 26.6 (s, C′-9, CH_2), 28.9–29.8 (m, C′-8, CH_2), 29.9 (m, C′-7, CH_2), 30.3 (s, C-3, CH_2), 31.6 (s, C′-6, CH_2), 32.5 (s, C′-4, CH_2),47.7 (s, C′-5, CN_2), 48.4 (s, C′-2, CH_2), 49.2 (s, C-2, CH), 66.0 (s, C-4, CH_2), 166.0 (s, C′-1, C=O), 175.0 (s, C-1, C=O), 203.1 (s, C′-3, C=O) ppm. ESI-TOF MS: *m*/*z* (%) = 323 (100) $[M + H]^+$.

Preparation of Deuterium and Tritium Labelled *N*-(3-Oxoalkanoyl)-L-homoserine Lactones; General Procedure: To a stirred solution of *N*-(3-oxododecanoyl)-l-homoserine lactone **1** (1 equiv.) in THF (1 mL per 0.037 mmol) was added magnesium acetate tetrahydrate (1 equiv.), triethylamine (1.2 equiv.) and deuterium oxide or tritium oxide (1 mL per 0.037 mmol). The mixture was stirred at r.t. for 24–48 h then evaporated to dryness and the residue was redissolved in ethyl acetate. The ethyl acetate solution was sequentially washed with 1 M sodium hydrogen carbonate solution, 1 M potassium hydrogen sulfate solution, and saturated sodium chloride solution. After drying over anhydrous sodium sulfate, the solvent was evaporated in vacuo.

***N*-(3-Oxododecanoyl-[2-$^2H_2$])-L-homoserine Lactone (10a):** Obtained according to the general procedure from **1** (11 mg, 0.037 mmol), magnesium acetate tetrahydrate (8 mg, 0.037 mmol), deuterium oxide (41 mL, 56 mmol) and triethylamine (6 μL, 0.044 mmol) in THF (1 mL). The crude residue was purified by recrystallization (EtOAc with a few drops of hexane) to give the desired product (11 mg, 99%) as a white solid. R_f = 0.51 (hexane/EtOAc, 1:3). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.87 (t, ^3J = 6.5 Hz, 3 H, CH_3), 1.19–1.31 (m, 12 H, 6 × CH_2), 1.50–1.68 (m, 2 H, CH_2CH_2CO), 2.12–2.30 (m, 1 H, 3α-H), 2.52 (t, ^1J = 7.3 Hz, 2 H, CH_2CO), 2.60–2.80 (m, 1 H, 3β-H), 3.47 (s, 0.2 H, COCHDCO), 4.20–4.38 (m, 1 H, 4α-H), 4.40–4.56 (m, 1 H, 4β-H), 4.58–4.69 (m, 1 H, 2-H), 7.61–7.81 (m, 1 H, NH) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.1 (s, C′-12, CH_3), 22.5 (s, C′-11, CH_2), 23.4 (s, C′-10, CH_2), 28.9–29.8 (m, C′-6–9, CH_2), 29.9 (s, C-3, CH_2), 43.2 (quint., ^1J = 20.1 Hz, C′-2, CD_2), 43.9 (s, C′-4, CH_2), 49.0 (s, C-2, CH), 65.8 (s, C-4, CH_2), 166.3 (s, C′-1, C=O), 174.7 (s, C-1, C=O), 206.7 (s, C′-3, C=O) ppm. ESI-TOF MS: *m*/*z* (%) = 322 (100) $[M + Na]^+$, 323 (40), 321 (9); deuterium distribution: 79% d_2, 15% d_3, 6% d_1.

***N*-(3-Oxododecanoyl-[2-$^3H_2$])-L-homoserine Lactone (10b):** Obtained according to the general procedure from **1** (10 mg, 33.6 μmol), magnesium acetate tetrahydrate (7 mg, 33.6 μmol), tritium oxide (900 μL, 50 mmol, specific activity: 1 mCi/g) and triethylamine (5.5 μL, 40 μmol) in THF (1 mL). The desired product was obtained as a white solid. Chemical yield: 8 mg (89%); Radioactivity = 1.78 μCi (66 kBq); Specific activity = 0.22 mCi/g; Radiochemical yield: 22% R_f = 0.51 (hexane/EtOAc, 1:3).

***N*-(3-Oxo-5-diazirinedodecanoyl-[2-$^3H_2$])-L-homoserine Lactone (11):** Obtained according to the general procedure from **9** (1 mg, 3 μmol), magnesium acetate tetrahydrate (1 mg, 4 μmol), tritium oxide (100 μL, 5.5 mmol, specific activity: 1 mCi/g) and triethylamine (1 μL, 7 μmol) in THF (100 μL). The crude residue was purified by recrystallization (EtOAc with a few drops of hexane) to give the desired product as a white solid. Chemical yield (1 mg, 99%); Radioactivity = 0.14 μCi (5.2 kBq); Specific activity = 0.14 mCi/g; Radiochemical yield: 14% R_f = 0.65 (hexane/EtOAc, 1:3).

Supporting Information (see footnote on the first page of this article): Copies of ^{1}H and ^{13}C NMR spectra of key compounds.

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