Synthesis of isotope labelled and photoactivatable *N*-acyl-L-homoserine lactones – inter kingdom signalling molecules

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1 Introduction

N-Acyl-L-homoserine lactones (AHLs) are synthesized by Gram-negative bacteria. These quorum sensing molecules play an important role in the context of bacterial infection and biofilm formation. They also allow communication between microorganisms and their eukaryotic host cells (inter-kingdom signalling). Very little is known about the detailed mechanism of the interaction between AHL and host cells. This work focuses on a special bacteria-derived mediator, *N*-(3-oxododecanoyl)-L-homoserine lactone **3** (3OC12-HSL) and its derivatives. Because usually only one isomer of the given AHL is biologically active, we undertook the detailed structural analysis supported by the theoretical studies to characterise the obtained compounds.[1]

Since 3OC12-HSL retains intracellular activity,[2] an intracellular receptor seems most likely, but an extracellular membrane-bound receptor cannot be excluded. Nevertheless, in order to interact with intracellular components, 3OC12HSL must cross the cell membrane. A receptor mediated path and membrane crossing by diffusion are both feasible. Due to the similarity in amphiphilic character of the lipids and AHL, non-receptor mediated diffusion through the cell membrane is reasonable.[3] So far, investigation of interactions between AHLs and cell membranes were only mentioned.[4,32]

The aim of this work is the synthesis of isotope labelled and photoactivatable *N*-acyl-L-homoserine lactones in order to investigate inter kingdom signalling mechanism. The synthesis and structure of some unlabeled AHL analogues have been reported by various groups,[5-9] but the synthesis of the deuterated derivatives has − to the best of our knowledge − only been mentioned by Kai *et al.*[44] and Gould *et al.*[10] Such, deuterium labelled compounds have several important applications in many branches of natural science e.g. in the investigation of biological activity,[44] reaction mechanisms[43] or kinetic studies.[11] There are several ways to use isotopes in mechanistic investigations, e.g. by following the diffusion path of the molecule. In this context, deuterium labelled AHLs were synthesized and applied in the study of insertion of deuterated AHLs in supported lipid bilayers (SLBs), using vibrational sum-frequency-generation (SFG) spectroscopy.[1,12] The synthesis of the deuterium labelled AHLs was optimised and high yields of the final products were obtained.

Moreover, obtained deuterium as well as tritium labelled AHLs were used to study cellular up-take of AHLs and their distribution within the cell. Herein a, novel method of deuterium and tritium labelling of terminally unsaturated AHLs via catalytic reduction of the double bond was developed and successfully applied. This uncommon reduction of the double bond using sodium borohydride and its isotopologues was performed in the presence of palladium (II) acetate as a catalyst in the liquid nitrogen.

In the next step, isotope labelled photoactivatable AHLs were synthesised to identify specific receptors in immune cells (e.g. human polymorphonuclear neutrophils, PMN) that bind to AHL. Photoaffinity label – a diazirine group – was chosen as the smallest possible photoactive group that can be activated by light irradiation. The resulting highly active carbene will bind to the closest chemical structure in the cell. The diazirine label was introduced to the AHL molecule according to the methodology of Dubinsky *et al*.[13] On our way to a simple and effective isotopic labelling method, the third route of the hydrogen isotopes incorporation into the AHLs was proposed and explored in details. According to a new protocol, the photoactive diazirine-AHL was labelled with deuterium and tritium by a post-synthetic catalytic exchange of the hydrogen with its isotopes, using deuterium or tritium labelled water along with catalytic amounts of metal salts in mild basic conditions.

2 Theoretical background

2.1 Biological role of *N*-Acyl-L-homoserine lactones (AHLs)

N-Acyl-L-homoserine lactones (AHLs) are produced in nature by gram-negative bacteria such as *Pseudomonas aeruginosa* and mediate bacterial cell-to-cell communication (quorum sensing, QS). Quorum-sensing molecules and their role in bacterial communication have been extensively studied,[14-19] particularly in the context of the production of virulence factors and biofilm formation, which is increasingly recognized as "a common cause of persistent infections",[20] in chronic and destructive inflammatory processes, such as chronic sinusitis, implant-associated osteomyelitis, and wound infection. Biofilm formation is common in *P. aeruginosa* infection and is the leading cause of morbidity and mortality in patients with cystic fibrosis, with open, non-healing wounds or those requiring indwelling catheters or ventilation tubes.[21]

Recently, several studies have shown that signalling by these molecules is not restricted to bacteria, but they also interact with eukaryotic cells, in a phenomenon termed "inter-kingdom signalling".[22] It was reported that AHL's alter gene expression in mammalian cells including up-regulation of proinflammatory cytokine expression, which often leads to the modulation of the host's immune response and finally to bacterial pathogenesis.[24]

N-(3-Oxododecanoyl)-L-homoserine lactone **3** (3OC12-HSL, Figure 2.1) is the most prominent molecule in this class.[23,24] However the other AHL derivatives – with shorter and longer acyl chains (Figure 2.1) – also exhibit a diverse biological activity.[16,18]

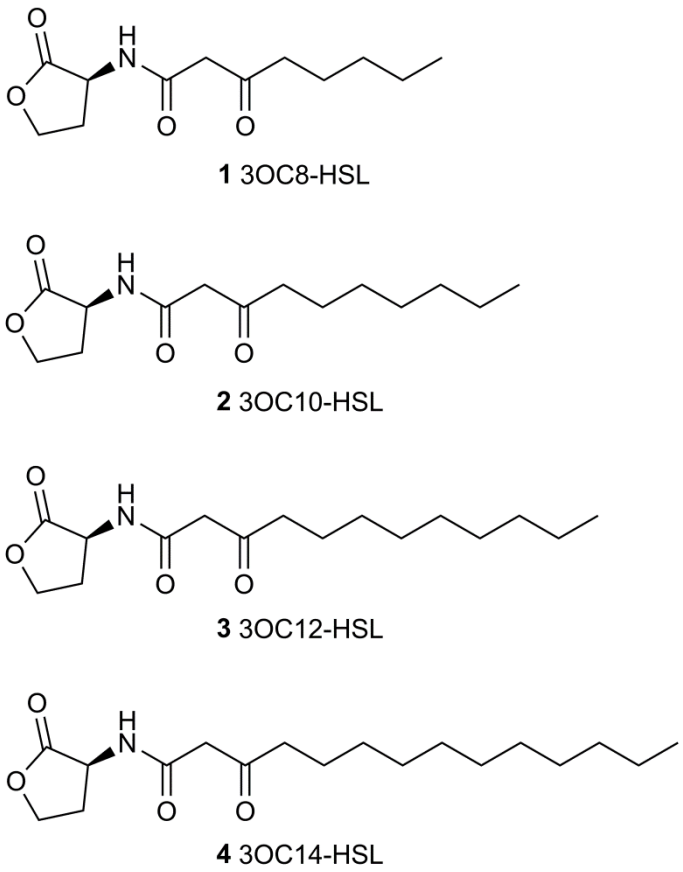

Figure 2.1 Structures of selected *N*-(3-oxoalkanoyl)-L-homoserine lactones.

N-(3-Oxododecanoyl)-L-homoserine lactone revealed a number of adverse effects reported in literature, e.g. biofilm formation,[14] resistance to antibiotics[16] or down-modulation of defence-relevant functions[18,25,26] such as an inhibition of stimulated T-cell proliferation and antigen presentation[3,18,25] as was induction of apoptosis.[27] In contrary, 3OC12-HSL might also support the immune system defence of the host.[19,20] The host defence against bacteria or bacterial biofilms relies on phagocytic cells, particularly on polymorphonuclear neutrophils (PMN), and their capacity to infiltrate into infected sites to recognize and eliminate pathogens. Accordingly, PMN known as the "first line defence" against bacterial infections, are equipped with receptors and adhesion molecules allowing directed migration towards an infected site, by recognizing chemotactic molecules.[28] As neutrophils are of major importance for the host defence against bacteria, their activation by *N*-(3-oxododecanoyl)-L-homoserine lactone could modulate the local host defence presuming that 3OC12-HSL is generated in sufficient concentrations *in vivo*.[22]

Since 3OC12-HSL enters mammalian cells and retains intracellular activity,[29] an intracellular receptor seems most likely, but an extracellular membrane-bound receptor cannot be

excluded. In this context, Jahoor *et al.*[30] obtained evidence that suggests possible binding of 3OC12-HSL to at least two isoforms of the peroxisome proliferator activated receptors (PPARs), the PPARγ and PPARβ/δ. Both of them belong to the nuclear hormone receptor family binding a range of endogenous and exogenous lipids and play important roles in inflammation and lipid metabolism. 3OC12-HSL may therefore modulate the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) signalling *via* the direct interaction with PPARs. Seabra *et al.*[31] identified calprotectin as a target, using an affinity matrix, although this calcium binding protein is unlikely to be the primary receptor for 3OC12-HSL.

The ability of QS signal molecules to interact with intracellular components must be preceded by an interaction with a cell membrane. Structure-activity assays of 3OC12-HSL with mouse spleenocytes revealed that optimal immune modulatory activity requires a C11 to C13 acyl chain, an intact homoserine lactone ring and L-configuration at the chiral centre (while the isomer with D-configuration is inactive). Thus lipophilicity is suggested as an important factor for QS immunosuppressive activity.[18]

The potential interactions of AHLs with many types of membrane have so far been mostly ignored, although a recent study by Lowery *et al.*[32] indicated that they may have effects on bacterial membrane permeability.[33] Based on these observations and known a broad biological activity of 3OC12-HSL in both pathogens and eukaryotic cells, Davis and co-workers[34] explored the interactions of long chain AHLs with simple membranes and T lymphocytes. They used the membrane dipole fluorescent sensor di-8-ANEPPS to characterise effects of AHL. The AHLs in micromolar concentration range led to significant modulation of the membrane dipole potential. Therefore, their study presents evidence that 3OC12-HSL and two close structural analogues, *N*-(3-oxodecanoyl)-L-homoserine lactone **2** (3OC10-HSL; Figure 2.1) and *N*-(3-oxotetradecanoyl)-L-homoserine lactone **4** (3OC14-HSL; Figure 2.1) are able to insert into the lipid bilayer in both artificial membrane and T-lymphocyte cell systems. The AHL molecules synthesized in this work were used to investigate the interaction of AHLs with model lipid membranes and the results are presented in section 3.1.

However, the detailed mechanism of interaction of AHL with potential receptors or mechanism of the ability of 3OC12-HSL to passively cross the eukaryotic cell membranes, is still under investigation.

2.2 Isotopic labelling in organic chemistry

2.2.1 The discovery, isolation and early applications of isotopes

The discovery and isolation of isotopes took place at the turn of the nineteenth and twentieth century. The first observations of radioactivity were reported in 1896 by Becquerel,[35] after the discovery of X-rays by Röntgen in 1895. In 1898 Maria Skłodowska-Curie and her husband Pierre Curie studied the uranium ore pitchblende, which led to the isolation of the radioactive elements polonium, an analogue of bismuth, and radium, obtained from the barium fraction.[36] Further discovery of radioactive elements were associated with thorium.

In 1899 Rutherford's investigations of radiation nature of these materials and its response to magnetic fields showed the presence of α- and β-rays.[35] The Curies detected more penetrating γ-rays in 1900 from the radiation of the radium atoms. The difference between α-, β- and γ-rays was in their deflection by electromagnetic fields, as well as in terms of their penetrating power. The radiation was detected at this time not only by photographic plates but also by the flashes produced by a phosphor zinc sulfide, which was a precursor of scintillation counter apparatus. The first versions of Geiger counter were introduced in 1908 and relied on the ionization of a low-pressure gas permitting discharge.[37] Subsequently, in 1911 the Wilson cloud chamber was introduced. This particle detector revealed the track of an α- and β-particle by the condensation of supersaturated moist air on an ionized gas.

The radioactive disintegration and radioactive decay ensued from the work of Rutherford, Soddy, Becquerel and Curies on the beginning of the twentieth century. The identification of disintegration or so-called decay series led to a non-radioactive end product, which is typically a lead. The atomic weight and isotopic distribution of the lead being produced from different decay series, is in a consequence, different when going from one source to another.

The analysis of these decay series led to the term *isotope* introduced by Margaret Todd and Frederick Soddy in 1913 in the meaning of atoms of the same element having different nuclear masses. Todd suggested that if each position in the periodic table was occupied by multiple entities - suitable name for such an entity would be "isotope" the Greek term for "at the same place".[38]

The first observation of different stable isotopes for an element was by Thomson in 1912, who was working on the composition of canal rays. Thomson channelled streams of neon ions in a cathode ray tube (a precursor of the mass spectrometer) through a magnetic and electric fields and measured its deflection by placing photographic plate in their path. He has observed appearance of two separate patches of light on the photographic plate, identified as neon-20 and neon-22.[39]

Subsequently, in 1919 Aston discovered different stable isotopes of various elements using a mass spectrometer,[40] what resulted in a rapid increase of the number of known isotopes in the 1920s. For example, Mulliken proved the existence of a number of isotopes of light elements by the detection of species such as ^{16}O-^{18}O, ^{15}N-^{16}O and ^{13}C-^{16}O. Moreover, Aston was able to fractionate these isotopes by careful distillation and by diffusion through a clay pipe, indicating the later method of separating isotopes *via* diffusion.

The existence of deuterium was predicted by Rutherford in 1920, but the isotope was not detected until 1932 by Urey, Brickwedde and Murphy. Kendall and Crittenden suggested in 1923 that isotopes might be separated by electrolysis, and in 1933 Lewis obtained deuterium oxide by electrolysis of water. At this time, a number of diverse methods of separation were explored, for example the distillation of water, the adsorption of hydrogen or water on charcoal, the diffusion of hydrogen through palladium or the reaction of water or acids with metals.[36]

The isotopes development during the 1930s paved the way for their applications as tracers, so the enrichment of stable isotopes and the production of radioactive isotopes of the lighter elements were of great interest. The first examples in which isotopic tracers were used in elucidation of organic reaction mechanisms and determination of biochemical pathways, were founds in 1930s.[36]

By the mid-1930s, over 100 new radioisotopes had been revealed. At that time, many of the physical phenomena had been discovered, which had greatly supported the development of the organic chemistry of isotopic labelling.

2.2.2 The definition, properties and applications of isotopes

Isotope is an atom with the same number of protons, but differing numbers of neutrons. Isotopes have the same atomic number but different mass number.[36]

Isotopes can be divided into:

1. Radioactive isotopes (heavier radioisotopes or radionuclides) – isotopes which are able to undergo radioactive decay
   - Primordial isotopes (primordial nuclides) - they are the long-lived fraction of radionuclides surviving in the primordial solar nebula through planet accretion until the present. The 33 primordial radionuclides represent nuclides of 27 separate elements (cadmium, neodymium, tellurium, uranium and samarium).[41]
2. Stable isotopes (lighter nuclides) - they have not been observed to decay (or with half-lives too long to be estimated so far)

Isotopic labelling has only little effect on physical properties of the labelled compounds (e.g. density, melting point, boiling point, magnetic moment). However, the number of neutrons has drastic effects on the nuclear properties, particularly on the stability of the nucleus.[42] Since a neutral atom has the same number of electrons as protons, different isotopes of a given element all have the same number of protons and share a similar electronic structure. Because the chemical behaviour of an atom is largely determined by its electronic structure, different isotopes exhibit nearly identical chemical behaviour. The main exception to this are changes in the reaction rate - the kinetic isotope effect: due to their larger masses, heavier isotopes tend to react more slowly than lighter isotopes of the same element. This is mostly pronounced for protium (^{1}H), deuterium (^{2}H) and tritium (^{3}H), since deuterium has twice the mass of protium, and tritium has a triple mass of protium.

The isotopic replacement modifies the spectroscopic properties of a molecule. Several forms of spectroscopy rely on the unique nuclear properties of specific isotopes, particularly in the infrared, the mass and the nuclear magnetic resonance spectra For example, NMR spectroscopy can be used only for isotopes with a nonzero nuclear spin. The most common isotopes used with NMR spectroscopy are ^{1}H, ^{15}N, ^{13}C, ^{19}F or ^{31}P.

Changes in the IR absorption of a compound arising from the replacement of specific hydrogen atoms by deuterium, have a great impact on the molecular vibrations and thus on

the spectrum. Isotopic substitutions of hydrogens change the ^{1}H NMR spectrum by removing various proton signals and couplings. Moreover, useful information about various inter- and intramolecular interactions can be evaluated. The position of deuterium and tritium can also be established from the ^{2}H and ^{3}H NMR spectra. The presence of deuterium also modifies the ^{13}C NMR spectra. Carbon-deuterium couplings remain in the proton noise-decoupled spectrum, affecting the intensity and multiplicity of the signals from carbon atoms bearing deuterium. Isotope shifts can also be detected. In biological systems, identification of resonances in the ^{1}H NMR spectrum by the site-specific deuterium labelling of proteins, may lead to important structural information. The deuteration has a specific effect also on the mass spectrum, particularly in elucidating the fragmentation pattern of biologically active compounds and in labelling internal standards for the quantitative analysis of components of complex mixtures by chromatographic methods coupled with mass spectrometry.[36]

In consequence, isotope-labelled compounds have several important applications. Compounds labelled with stable isotopes have an application in mechanistic studies[43] in organic chemistry, as well as in many other branches of science such as in the investigation of models of biological systems[44] or metabolism and in metabolism-mediated toxicity studies.[45] Stable isotopes found application also in genetics. In proteomics, the study of the full set of proteins expressed by a genome, identifying diseases biomarkers by use of isotope labelled amino acids (using ^{2}H, ^{13}C, ^{18}O or ^{15}N isotopes).[46]

Compounds labelled with radioactive isotopes are called radioactive tracers ("isotopic markers" or "isotopic labels"). They are applied in the analysis of drug metabolism or in biochemical assays in biochemistry to help understand chemical reactions and various interactions. With the information on the positions of the isotopes in the products (using e.g. NMR spectroscopy or mass spectrometry), the reaction pathway of the initial metabolites can be elucidated. Radioactive isotopes can be tested using the autoradiography of gels in gel electrophoresis. The radiation emitted by compounds containing radioactive isotopes causes darkens of a proper area of photographic film, recording the position of the labelled compounds relative to one another in the gel. In medicine, radioactive tracers are used to provide information about the functioning of a human's specific organs or to treat disease. Bones, thyroid, heart, liver and many other organs can be easily imaged, and disorders in their function revealed. In some cases radiation can be used to treat diseased organs, or tumours.

Moreover, in geology isotopic tracers are used to understand a wide array of processes in earth systems.[47]

2.2.3 The kinetic isotope effect (KIE)

The substitution of any atom by one of its isotopes at a reactive centre or at the adjacent to reactive centre may have an influence on the rate of reactions involving this centre. This influence is known as the kinetic isotope effect. When a carbon-hydrogen bond is replaced by carbon-deuterium bond, a reaction of a bond breaking or bond making between carbon and substituted deuterium, the reaction rate is changed. The significant effect on the reaction rate is only in case when the relevant C-H bond is being broken in the rate-determining step, which is known as the primary kinetic isotope effect.

For example for the reaction of ketones with bromine and sodium hydroxide forming a haloketone with the deuterated α-carbonyl positions, a large KIE of 5.56 was reported (Figure 2.2).[48] In this reaction the rate-limiting step is enolate formation by proton (deuterium) abstraction from the ketone by base. In this study the KIE is calculated from the reaction rate constants for regular 2,4-dimethyl-3-pentanone and its deuterated isomer by optical density measurements.

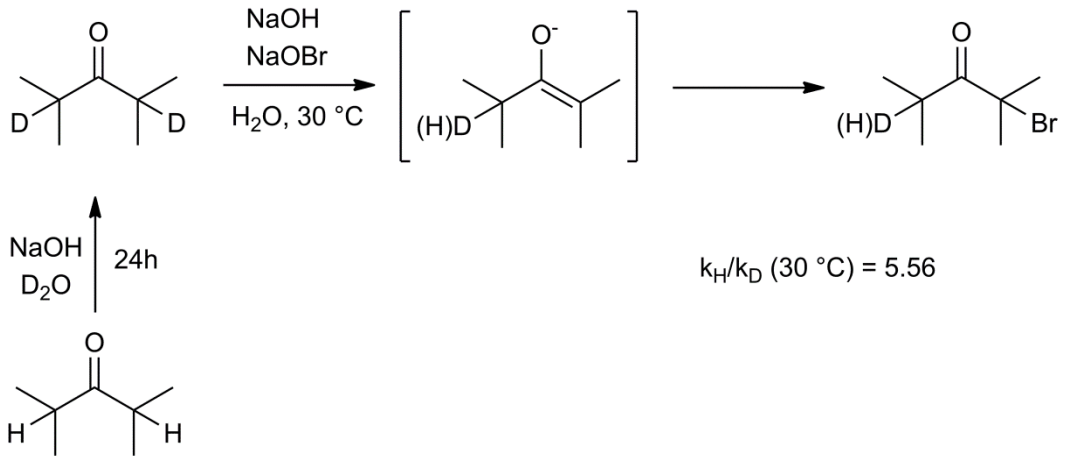

Figure 2.2 The reaction of ketones with bromine and sodium hydroxide forming a haloketone with the deuterated α-carbonyl positions.

In a primary isotope effect, the ratio k_H/k_D may be up to 8 and k_H/k_T up to 16, although they are usually considerably less.[36] The primary KIE has been put to synthetic use with deuterium being employed as a "protecting group" for carbon. By substituting deuterium with a

hydrogen at acidic positions of amides and carbamates, the usual regiochemical course of their reactions with alkyllithiums (ortholithiation vs. lateral lithiation vs. nucleophilic addition) can be overturned by the kinetic isotope effect. The deuterium substituent works here as a protecting group for carbon.[49]

In the secondary kinetic isotope effect, the reaction rate changes due to isotopic substitution at other than a site of bond breaking or bond making in the rate determining step of a mechanism. The replacement of a hydrogen by a deuterium at a bond which is not broken during the reaction brings a change in the reaction rate. The remote atom influences the internal vibrations of the system that - *via* changes in the zero point energy (ZPE) - affect the reaction free enthalpies and in consequence – the reaction rates. Moreover, isotopic labelling can influence on electronic effects such as induction, bond hybridization or hyperconjugation.

The steric kinetic isotope effect also does not involve bond breaking or formation. This effect is caused by different vibrational amplitudes of selected bonds of the isotopologues. Such an effect was observed for example in the racemization of 9,10-dihydro-4,5-dimethylphenanthrene (Figure 2.3).[50]

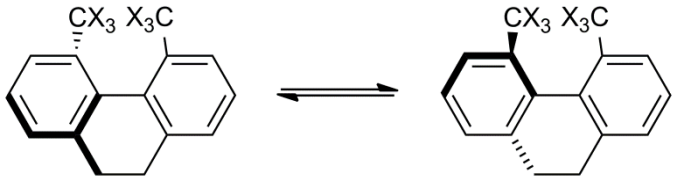

Figure 2.3 The steric KIE on the racemization of 9,10-dihydro-4,5-dimethylphenanthrene. X = H or D.

Lower amplitude of the vibration of C-D bonds as compared to C-H bonds results in a smaller steric hindrance of CD_3 groups. In a consequence, a deuterium isotopologue racemizes faster than the hydrogen one. This phenomenon is called steric isotope effect.

2.2.4 Labelling with deuterium and tritium

Due to the greatest differences in properties, the most important isotopes used in organic chemistry are the isotopes of hydrogen - ^{2}H (deuterium) and ^{3}H (tritium). Deuterium and tritium labelled reagents are relatively cheap, safe and commercially available. Labelling of the substrates with deuterium or tritium can provide very important mechanistic and

stereochemical information about chemical or enzymatic reaction pathways. Moreover, the isotopic substitution effect has an influence on the reaction rates (e.g. *via* KIE), indicating the rate-determining step in a multi-stage processes.

While deuterium is a stable isotope of hydrogen, tritium is a radioactive one, with a half-life of 12.26 years. Tritium is a weak β-emitter (maximum energy 0.016 MeV)[36] and decays according to equation:

$$^{3}_{1}H \rightarrow {}^{3}_{2}He + e^{-} + \tilde{\nu}$$

This low value of emitted energy, low toxicity, low cost, high specific activity and excellent autoradiographic properties make tritium a very convenient tracer for a laboratory use.[51]

There are four main methods for deuterium and tritium labelling of organic molecules:

1. Exchange reactions
2. Hydrogenolysis (deuterolysis and tritiolysis)
3. Reduction reactions
4. Incorporation of previously labelled fragments into the molecule

2.2.4.1 Exchange reactions

Exchange reactions in deuterium and tritium labelling could be divided into:

a) Heteroatom X-H exchange reaction
b) General C-H exchange reactions
c) Site-specific C-H exchange reactions
d) Exchange reactions of carbonyl compounds

In general, the exchange reactions relies on the exchange process of a deuterium or tritium from commercially available chemical reagents, such as deuterium and tritium labelled water, solvents, acids, or bases.

In heteroatom X-H exchange reactions, one of the simple exchange involve D_2O with solution of alcohol, acid, amine or amide in aprotic solvent, e.g. $CDCl_3$. However such a procedure may be suitable for the NMR scale, but not for preparative one. One of a common preparative method is based on the deuterolysis of the metal salt of an alcohol, where the sodium or

magnesium alkoxides are treated with deuterium oxide. The product – deuterated alcohol is then isolated by distillation.[36]

In "general C-H exchange reactions", the primary source of tritium labels were tritium gas and tritiated water. For example in the Wilzbach procedure,[52] a substrate is exposed to tritium gas for a long period (even a few weeks) and slow C-H exchange takes place. The reaction may be catalysed by charcoal or platinum or palladium catalyst. In the other method of generally labelling is application of a Lewis acid catalyst and tritiated water, which is useful mostly for the labelling of aromatic compounds.[36]

Site-specific C-H exchange reactions normally require acidic, basic, metal or organometallic catalysis.[36] However, a few C-H exchange reactions have been reported to take place without any catalyst. In an aromatic ring the protons will exchange with deuterium or tritium from ^2H_2O or ^3H_2O in the presence of a catalyst at sites and rates dependent on the activating or deactivating substituents effects. For example the novel method involving the usage of cyclooctadienyl (cod) iridium(I) penta-1,3-dionate as a catalyst, gave very efficient deuteration in the *ortho* position to the carbonyl group of a substituted acetophenone.[53]

The other example of a convenient regioselective deuteration, was reported by Kurita *et al*.[54] The Pd/C- or Pd/C–ethylenediamine complex [Pd/C(en)] catalysed efficient and regioselective hydrogen–deuterium (H-D) exchange reaction on the benzylic site, proceeded in D_2O in the presence of a small amount of H_2 gas (Figure 2.4).

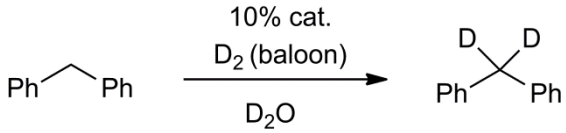

Figure 2.4 Heterogeneous palladium-catalyzed regioselective deuteration at the benzylic position.

The next class of the exchange reactions represents exchange reaction of carbonyl compounds. The synthesis of α-deuterated and tritiated carbonyl compounds takes place in either acidic or basic conditions, but mostly in the latter ones. Refluxing of some carbonyl substrates in deuterium oxide or in deuterated methanol with deuterium oxide may take 30 min to 1h, but in the other cases reaction may takes even several days.

One of the spectacular example of the deuterium exchange of active α-hydrogen atoms in the phase-transfer catalysis conditions, was introduced by Starks (Figure 2.5).[55]

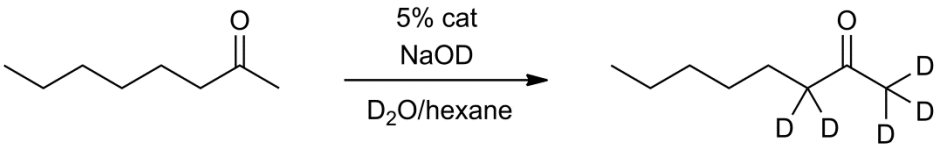

Figure 2.5 Deuterium exchange of active hydrogen atoms in the presence of phase-transfer catalyst.

The α-hydrogens of 2-octanone were exchanged with deuterium atoms in a solution of 5% NaOD in D_2O and 5% quaternary ammonium catalyst at 30 °C. Within 30 minutes complete equilibration of the hydrogen atoms on the 1- and 3-carbons of the ketone took place. This method is widely used for preparation of some deuterated solvents for NMR spectroscopy.

2.2.4.2 Hydrogenolysis (deuterolysis and tritiolysis)

Hydrogenolysis is a chemical reaction whereby a carbon–carbon or carbon–heteroatom single bond is cleaved by hydrogen. The heteroatom is usually halogen, oxygen, nitrogen or sulfur. The hydrogenolysis of aryl halides is a very important method for specific deuterium and tritium labelling of an aromatic ring. These reaction can be carried out using metal hydrides, such as sodium borotritide or lithium aluminium deuteride or by deuterium or tritium gas over palladium catalysts on carbon or charcoal. In general, the reactivity of aryl halides is in the order I > Br > C1> F. Electron-donating groups in a *para* position decrease the rate of reaction and electron-withdrawing groups - increase the rate of reaction. The steric effect, such as a presence of bulky substituents in the *ortho* position is known to increase the rate of hydrogenolysis.[56]

For instance, Adapa *et al*[57]. showed that the hydrogenolysis of anthracene halides by $LiAlD_4$ afforded site specifically monodeuterated products. However, the 100% of deuterium incorporation was achieved only when synthesis was followed by D_2O workup.

In the more recent work of Faucher *et al*.,[58] chemoselective hydrogenolysis, deuterolysis and tritiolysis of iodoarenes was presented. The selective catalytic hydrodehalogenation reaction using hydrogen (deuterium or tritium) gas and Pd/C was performed on several iodoarene derivatives, without reducing the carbonyl group or destruction of azido function.

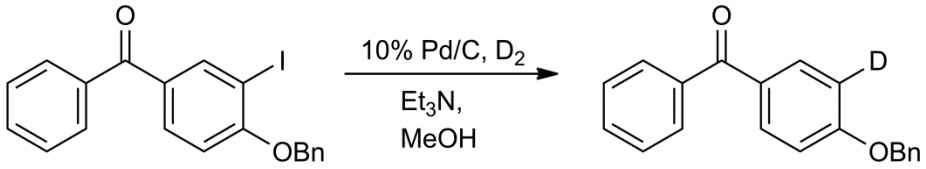

Figure 2.6 Hydrogenolysis of *p*-benzyloxy-*m*-iodobenzophenone (n = 1, 2 or 3).

An example of the hydrogenolysis of aliphatic substituents, is the nucleophilic substitution of a methane- or toluene-*p*-sulfonate by a hydride from sodium borohydride or lithium aluminium hydride. The label can be introduced stereospecifically, by either the reduction of the carbonyl compound to an alcohol or by the nucleophilic substitution of the sulfonate.[36]

2.2.4.3 Reduction reactions

One of the most significant method of deuterium and tritium labelling is the reduction of a functional group such as a carbonyl group, an epoxide, an alkyl halide, an alkene or an alkyne.

Reducing agents are represented by four classes of reagents, depending on their mechanism of introducing a deuterium or tritium label.[36]

a) Hydrides, such as sodium borodeuteride, sodium borotritide and lithium aluminium deuteride introduce deuterium and tritium as a nucleophile. Lithium, sodium and potassium borohydride were prepared by exchange reactions with deuterium and tritium gas at 250-300 °C. Whereas lithium borohydride undergo exchange with deuterium oxide, sodium borohydride does not.
b) The so-called "dissolving metal" group such as sodium dissolving ethanol-*d*, electrons are first delivered from the metal to the substrate, which is forming radical or anionic species. Then the reaction is completed by addition of deuterium.
c) In the catalytic group of reagents, deuterium or tritium is provided by the catalyst to an adsorbed substrate.
d) In the final family of reducing reagents, deuterium or tritium is transferred from a labelled reagent to the substrate, by a cyclic mechanism. One of the prominent example of such a reagent is labelled NADH or NADPH in enzymatic methods.

Reduction with $LiAlD_4$ and $NaBD_4$ have been extensively studied in the context of selective deuterium incorporation into different positions e. g. of hydrocarbon chain of fatty acids. This

method was applied in the synthesis of stearic-d_2 acid (Figure 2.7).[59] Selective reduction of ketone group with $NaBD_4$ formed an appropriate hydroxyl ester, which was in turned activated by tosyl chloride and cleaved by $LiAlD_4$. Resulted alcohol was deuterated at the 1 and 17 positions. The oxidation with chromium oxide led to desired product.

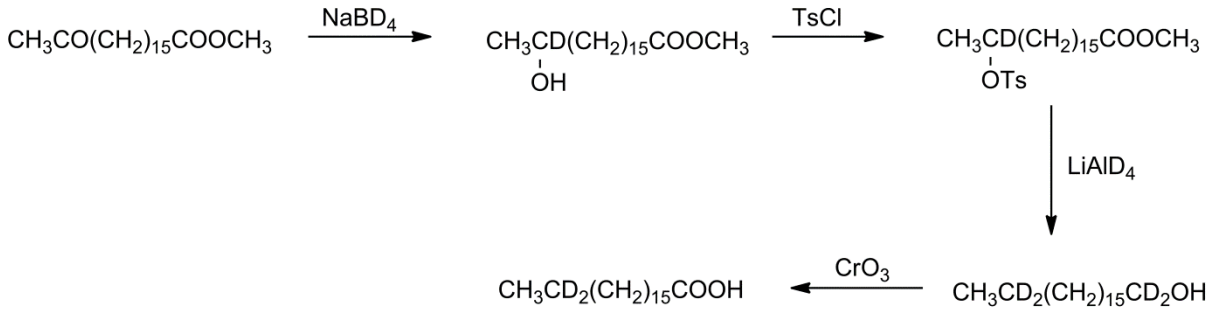

Figure 2.7 Reduction of oxo-ester by $NaBD_4$ and $LiAlD_4$.

The other method of labelling *via* reduction reaction is the reduction of alkenes and alkynes with hydrogen gas, in the presence of platinum group metals catalysts. For example reduction of cinnamic acid by catalytic hydrogen transfer from a donor such as labelled formate in the presence of Wilkinson's catalyst was demonstrated by Al-Qahtani *et al.*[60] Dideuteriocinnamic acid was obtained from cinnamic acid, by reduction using solid deuterium in the presence of Wilkinson's catalyst and three possible potassium-*d* formats: $[H_2O+DCO_2^-]$, $[D_2O+HCO_2^-]$, $[D_2O+DCO_2^-]$ or DCO_2D salt of tetramethylethylenediamine (TMEDA) on its own (Figure 2.8). The reaction was enhanced by microwave irradiation.

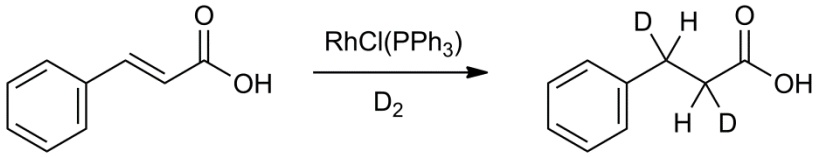

Figure 2.8 Deuterium labelling of cinnamic acid *via* catalytic hydrogenation of double bond.

In order to obtain stereospecifically labelled compounds, e.g. acrylic acids, as building blocks for the synthesis of more complex biologically active molecules, the partial hydrogenation of alkynes to *cis*-alkenes over Lindlar catalyst ($Pd/CaCO_3$) have been performed.[36] The variation of reaction conditions led to the formation of either the *E* or the *Z* geometric isomers.

Another classical example of regiospecific labelling method is the *cis*-Markovnikov hydration of an alkene by deuterated borane. This form alkylborane, in which the borane is attached to the less highly substituted carbon. Such a alkylborane constitutes very useful reagent, which

can react e.g. with alkaline hydrogen peroxide, to form an “ate” complex with electron-deficient borane. In a consequence of rearrangement and decomposition, the boron of the borane is replaced by hydroxyl group to give desired alcohol.[36]

However, most of above presented labelling methods require the usage of hydrogen gas, expensive metal based catalysts, aggressive reagents such as strong acids, bases, reducing or oxidizing agents and high temperatures or microwave irradiation, which is not agreed with the contemporary "green chemistry" idea. In particular hydrogen (deuterium or tritium) gas is undesired due to difficulty in handling, danger of explosion and radiation contamination in case of tritium gas, and environment contamination caused by post-synthetic wastes. Among a number of hydride reagents, such as lithium aluminium hydride ($LiAlH_4$), triethylsilane (Et_3SiH), sodium cyanoborohydride ($NaBH_3CN$), the sodium borohydride ($NaBH_4$) is especially valuable, due to its low cost, mild nature and facile in handling. However, its application was found to be limited mostly to reduction of polar, carbonyl-containing functional groups.[71]

In 1962 Brown[61] described reduction of simple alkenes by $NaBH_4$, which first reduced metal salts to active metals that in turn catalysed hydrolysis of sodium borohydride to generate hydrogen gas *in situ*. Followed, generated hydrogen gas reduced alkenes, under repeated catalysis of these reduced metals.[61,62] Subsequently, Brown and others studied extensively modifications of this method, including combination of sodium borohydride and other hydride reagents with simple and more complex salts of palladium, rhodium, platinum, osmium, nickel, indium and other.[63-69]

The effect of the nature of the active component and support on the activity of catalysts for the hydrolysis of $NaBH_4$ was also explored by Simagina *et al.*[70] It was found that the activity of the supported metals, in reduction of sodium borohydride decries in the order Rh > Pt ≈ Ru >> Pd regardless of the nature of the support (γ-Al_2O_3, a Sibunit® carbon material, or TiO_2). A nano-sized dispersed platinum metal powders were found to increase the rate of hydrogen generation dramatically. In 2009 Tran *et al.*[71] presented a modification of this method, which relied on direct use of palladium metal instead of metal salts and the addition of acetic acid, to catalyse hydrolysis of sodium borohydride.

Although a considerable amount of work was done to explore the selectivity of borohydride-metal hydrogenations, only little was done to expand application of this method for the

isotopic labelling. To the best of our knowledge, only one example of the use of potassium [^{3}H]borohydride in the catalytic hydrogenation of alkenes was described in the literature. In 1978 Schwarzmann showed a modification of this method, that relies on the reduction of unsaturated gangliosides and other sphingolipids by potassium borotritide dissolved in 1 M NaOH solution in the presence of palladium (II) chloride as a pre-catalyst. (Figure 2.9).[72] This method was applied also for catalytic reduction of the keto group of sphingosines by NaB^3H_4.[73]

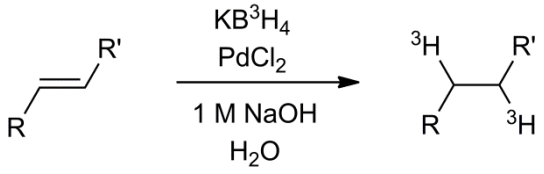

Figure 2.9 Catalytic reduction of the alkene moiety of sphingolipids by KB^3H_4. R, R' = alkyl chains.

However, none of precise deuterium labelling studies on this or related method have been reported up to date. Therefore the detailed investigation of deuterium and tritium labelling *via* modified catalytic reduction of double bond by sodium borodeuteride and sodium borotritide and their application in the isotopic labelling of *N*-acyl-L-homoserine lactones, is presented herein.

2.2.4.4 4. Incorporation of previously labelled fragments into the molecule

In principle, this type of labelling include all of reactions based on use of previously labelled substrates, such as deuterium and tritium labelled carboxylic acids or amino acids.

For example the deuterium labelled malonic acid, which was prepared from reaction of deuterium oxide and carbon suboxide (C_3O_2), undergo thermal decarboxylation and lead to formation of acetic acid-d_4.

2.3 Photoaffinity labelling agents

Affinity labelling is a biochemical technique of investigation of the structural and functional properties of biological systems. The method focuses on the labelling of an active site of proteins (e.g. receptors) by a small molecule, bearing a group which is capable of being specifically and bound to the active site, and the other group which is chemically reactive.[74-76] One of the most important variant of this method is *photoaffinity labelling*. In this method the group that is chemically unreactive in the dark, upon photolysis is converted to an extremely reactive intermediate that binds irreversibly to the biological receptor.[77] Photoactivatable reagents must possess a photoactive group and must be easily detectable by either having radioactive isotope or a fluorophore moiety. However, the latter contain usually several combined aromatic groups, which change the structure of the ligand dramatically. Therefore, the most convenient label is a radioactive isotope, particularly the tritium. This radioactive isotope of hydrogen, actually doesn't change the structure of the ligand, which makes it very specific and unique for a target receptor.

Moreover, these 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.[78]

Several photoactivatable groups are known, for example: azido, diazo, and azo groups, diazonium ions, benzophenone group or diazirines. A special class of the photoactivatable reagents constitutes an "intrinsically activated photoaffinity reagents."[79] These ligands in their "native" form can itself be covalently bonded to the receptor under the influence of photolysis. For example tritiated aromatic nitro compounds can act as photoaffinity agents, however it is difficult to state whether the nitro group effect in a direct or supportive photolysis role.[80]

The azides, particularly aryl azides were found to be very useful photoactive functions, for many radiolabeled ligands. The photolytic loss of nitrogen leads to reactive nitrene intermediate, which is involved in photolabeling of a biological target. The efficiency of irreversible coupling strongly depends on the nature of the adjacent amino acids in the receptor binding pocket.[81]

One of the prominent example of the synthesis of an azide photoaffinity label, was done by Filer *et al.*,[79] who synthesized alpha-2 adrenergic agonist [^{3}H]clonidine and its analogue [^{3}H]azidoclonidine.[82,83] First an analogue of ligand - unlabelled aryl amine was halogenated in *ortho* positions, nearby the amine. Then these halogenated sites were tritiated *via* catalytic reduction with tritium gas. The desired radiolabeled azide **5** was obtained from resulting aryl amine by mild diazotization and azide displacement (Figure 2.10). This synthetic approach was then often repeated for the synthesis of the other tritiated azides.

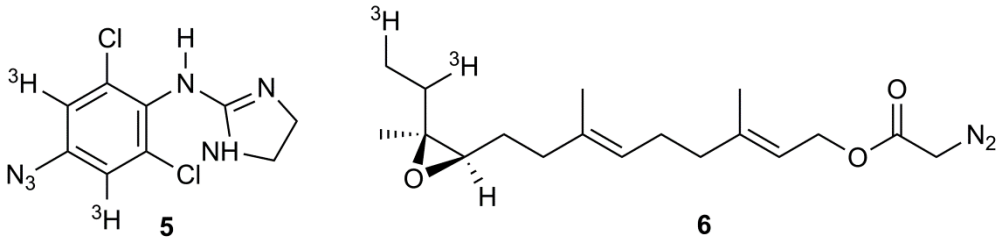

Figure 2.10 Structures of tritiated azido and diazo photoaffinity reagents.

The first report about the use of diazo group as a photopendant, was described by Westheimer *et al.* in 1962.[84] Diazo group undergo photolysis to a reactive carbene that can be added into the C-H, O-H, N-H or S-H bonds of proteins. Carbenes formed from diazoketones can also be converted to intermediate ketenes, through the Wolff rearrangement. These ketenes can in turn react with flanking nucleophilic protein receptor.

The work of Prestwich and co-workers on preparation of the tritiated juvenile hormone and other pheromone analogues was found to be the most significant in the area of tritiated diazoketone photoaffinity agents. Insect juvenile hormones belong to the group of sesquiterpenoids and contain an epoxide ring as well as one or several stereogenic centres. Their interaction with insect proteins include directing of biosynthesis, transport or other diverse cellular mechanisms of action. Prestwich synthesized hormone derivative **6** (Figure 2.10) in a few efficient steps.[85] The last - and crucial one - relied on formation of diazoketo ester and the mild Corey procedure with glyoxylic acid chloride tosylhydrazone.[86] This and other tritiated photoaffinity reagents have been studied in the context of juvenile hormone receptors in many insects.[87,88]

One of the most promising photoactive species bear diazirine group, that exhibits convenient photochemical properties as well as an excellent chemical stability. Diazirine is easier to photoactivate than the diazo function and upon irradiation is photolyzed to a carbene, which is

able to react with a full range of functional groups present in biological systems and insert to C-H bond. The diazirine group was first introduced as a photopendant function in 1973 by Knowles.[89]

Rousseau *et al.* has presented the great synthesis of three bifunctional 3-phenyl-3-(trifluoromethyl)diazirinyl building blocks and their tritiated analogues.[78] An interesting aspect of his method lies in a tritiation methodology. So far most of radiolabeling strategies of azides, diazo compounds or other diazirines were performed only before introducing of photoactive group, due to incompatibility of these reductible groups under the standard catalytic tritiation conditions. Tritium labelled 3-aryl-3-(trifluoromethyl) diazirines **7**-**9** (Figure 2.11) were prepared *via* tritiodeiodination reaction using tritium gas and Pd/C.[58,90-92] Hydrodeiodination of compounds **7**, **8**, and **9** was carried out in a chemoselective reaction step, in the presence of many other reducible groups because of the preferential regioselective adsorption of the aryl iodide on the catalyst. This method was also applied by Casida to prepare the ditritiated diazirine analogue as a photo probe for the GABA receptor.[93]

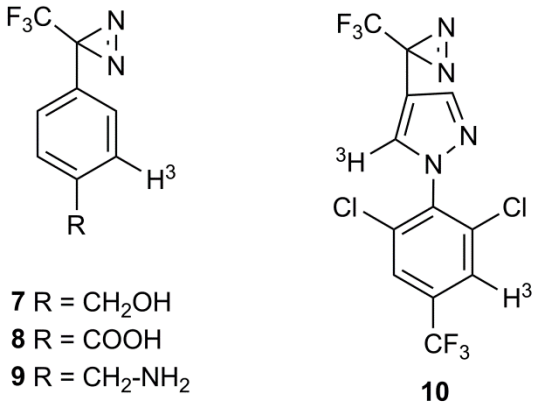

Figure 2.11 Structures of aromatic diazirine building blocks for photoaffinity labelling.

The three above nitrogen-contaning groups have been widely used as a photoaffinity pendant for many useful radiolabeled ligands. However, due to their polarity and chemical reactivity, they are not compatible in all cases. Therefore the benzophenone group was applied as a stable, lipophilic and easily tritiated molecule. Benzophenone can be photoactivated to form radicals at wavelengths that are not protein destructive.

One of a very effective approach to the synthesis of tritiated benzophenone photoaffinity agents, was the synthesis of tritium labelled 4-benzoyl-dihydrocinnamoyl group **11** (Figure 2.12). This compound can be set up by the catalytic tritiation of a precursor of 4-

benzoylcinnamic acid ester or by the esterification of an appropriate ligand with the activated ester.[94] Both of this methods were applied for the synthesis of many different tritiated biologically useful photoaffinity reagents.[95,96]

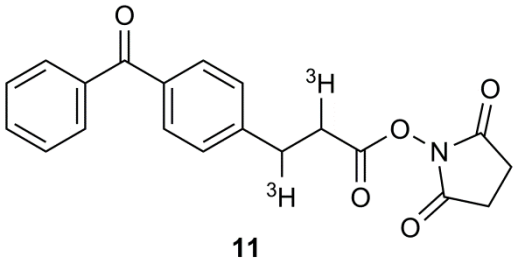

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Figure 2.12 Structure of the tritium labelled benzophenone photoaffinity reagent.

3 Results and discussion

3.1 Deuterium labelled AHLs: synthesis, structural studies and interaction with model cell membranes

Although a wide spectrum of biological effects caused by *N*-acyl-L-homoserine lactones was reported in details, interactions of AHLs with cell membranes were only rarely mentioned.[32,34] In order to study interactions between these signal molecules and model cell membranes, using vibrational sumfrequency-generation (SFG) spectroscopy, deuterium labelled AHLs were synthesized. To avoid overlapping of spectral bands and to obtain sufficient intensities of these signals, almost fully deuteration of acyl chain moiety was achieved. Moreover, with only one isomer of AHL being biologically active, detailed structural studies were performed to determine the conformation of the active isomer. For this reason, deuterium labelling *via* incorporation of previously labelled fragments of fatty acids was developed.

3.1.1 Synthesis of deuterium labelled *N*-acyl-L-homoserine lactones

The method used in this work is based on those described by Chhabra *et al.*[18] The reaction conditions were first optimised using unlabelled reagents. 5-Acyl Meldrum's acid derivatives **14a-e** were prepared in very good to excellent yields (Table 1) by 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) mediated condensation[97] of appropriate deuterated carboxylic acids **12a-e** with Meldrum's acid (2,2-dimethyl-1,3-dioxane-4,6-dione) **13**, in the presence of 4-(*N*,*N*-dimethylamino)pyridine (DMAP) (Figure 3.1).

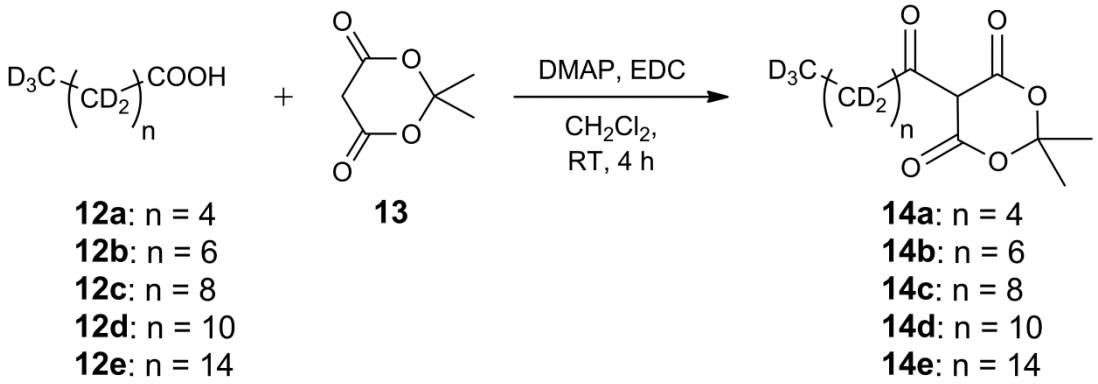


Figure 3.1 Acylation of Meldrum's acid **13** by deuterated fatty acids **12a-e**.

In comparison to the original procedure[18], the reaction time was reduced to 4-6 h to avoid a hydrogen–deuterium exchange. The isolated products were used without further purification.

Use of *N*,*N*′-dicyclohexylcarbodiimide (DCC) resulted in lower yields and the removal of the by-product - *N*,*N*′-dicyclohexylurea - was difficult, because of its solubility in organic solvents.

Table 1 Results of acylation of Meldrum's acid **13** by deuterated fatty acids **12a-e**.

Entry	n	Substrate[a]	Product[a]	Time (h)	Yield (%)	Deuterium content[b] (%) of isomer-d_n
1	4	**12a**	**14a**	4	90	61 d_{11} 39 d_{10}
2	6	**12b**	**14b**	5	92	74 d_{15} 26 d_{14}
3	8	**12c**	**14c**	6	98	76 d_{19} 24 d_{18}
4	10	**12d**	**14d**	4	83	83 d_{23} 17 d_{22}
5	14	**12e**	**14e**	5	77	66 d_{31} 34 d_{30}

[a] See Figure 3.1.[b] Determined by ESI-TOF MS.

In the second step, the amidation of **14a-d** with L-homoserine lactone (L-HSL) hydrobromide **15** and triethylamine afforded the desired deuterated *N*-(3-oxoalkanoyl)-L-homoserine lactones **16a-d** in yields from 53% to 74% (See Figure 3.2 and Table 2). The reaction of

substrate **14e** (the longest acyl chain with n = 14) resulted in mixture of products. The desired **16e** derivative was not isolated.

It is worth to mention that one or two of the deuterium atoms at the C′-4 position are lost, due to their acidic character (see Table 2 and ”Experimental” section).

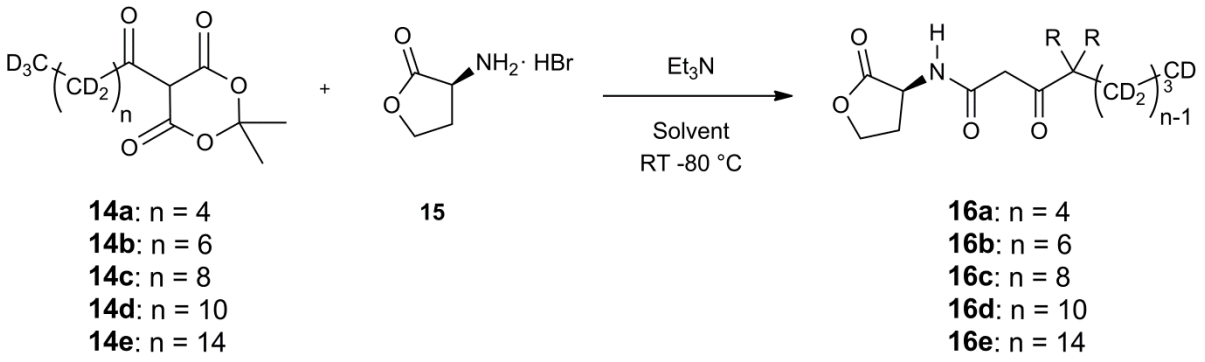


Figure 3.2 Amidation of **14a-e** with L-homoserine lactone (L-HSL) hydrobromide **15** (R = H or D).

Table 2 Results of the amidation of **14a-e**.

Entry	n	Substrate[a]	Product[a]	Solvent	Time (h)	Yield (%)	Deuterium content[b] (%)of isomer-d_n
1	4	**14a**	**16a**	EtOAc	16	70	10 d_{11} 20 d_{10} 70 d_9
2	6	**14b**	**16b**	CH_3CN	8	74	24 d_{13} 13 d_{12} 63 d_{11}
3	8	**14c**	**16c**	CH_3CN	22	60	7 d_{19} 25 d_{18} 68 d_{17}
4	10	**14d**	**16d**	CH_3CN	18	53	8 d_{23} 27 d_{22} 65 d_{21}
5	14	**14e**	**16e**	CH_3CN	17	-	-
6	14	**14e**	**16e**	EtOAc	24	-	-

[a] See Figure 3.2. [b] Determined by ESI-TOF MS.

The ESI-TOF MS studies on intermediates **14a-e** indicated only a minor loss of deuterium in the first step of the synthesis. The major loss of deuterium occurred in the amidation step (see

"Experimental" section). In case of the most biologically important compound **16c**, the ratio of [3OC12-d_{17}-HSL]:[3OC12-d_{18}-HSL]:[3OC12-d_{19}-HSL] was determined to be 68:25:7. For the other derivatives, similar deuterium distributions were found and the overall deuterium content in the molecule was sufficient for the further investigations (See Table 2).

3.1.2 Structural studies of deuterated AHLs

The structures of the representative products **16a**, **16c** and **16d** were confirmed by means of ^{1}H NMR, ^{13}C NMR, infrared and Raman spectroscopy. Moreover TLC, ESI-TOF MS, ESI-MS/MS, HRMS, elemental analysis and optical rotation were carried out (for details see "Experimental" section).

In the ^{1}H NMR spectra of **16c**, the absence of following signals was observed: methyl protons (δ~0.9 ppm, 3H, t, CH_3), methylene protons (δ~1.27 ppm, 12H, m, 6 x CH_2) and methylene protons (δ~1.59 ppm, 2H, m, CH_2CH_2CO)[18] due to deuteration of the chain of 3OC12-d_{17}-HSL **16c**. The singlet at 2.52 ppm (2H, CH_2CO) confirmed the loss of deuterium at the C′-4 position. ^{13}C NMR spectra showed the δ values of the deuterated carbons at 12.9 ppm (sept, J = 19.0 Hz, C′-12, CD_3), 21.3 ppm (quin, J = 19.0 Hz, C′-11, CD_2), 22.3 ppm (quin, J = 20.0 Hz, C′-5, CD_2), 22.6 ppm (quin, J = 20.0 Hz, C′-9, CD_2), 27.6 ppm (m, J = 19.0 Hz, C′- 6, C′-7, C′-8, CD_2), 30.4 ppm (quin, J = 19.0, C′-10, CD_2), 43.3 ppm (t, J = 19.3, C′-4, CHD) and 43.6 ppm (s, C′-4, CHD).

Further insight into the structure of **16c** was gained *via* IR and Raman spectroscopy. The analysis of experimental IR and Raman spectra was supported by theoretical calculations.[1] In Figure 3.3, experimental spectra are compared with those calculated by DFT. In the geometry optimizations, two structures were located — one with internal N-H···O hydrogen bond (1.95 Å) **16c$_2$** and a second without this hydrogen bond **16c$_1$**. The latter is less strained and is lower in energy of about 2 kcal/mol (including zero point energies). It is worth noting that both conformers were also obtained in case of **16a** and **16d** with exactly the same energy span. The calculated spectra for **16c$_1$** and **16c$_2$** are almost similar with the most significant difference being the position and intensity of the N–H stretching vibration. According to the calculated IR spectra for this mode we should expect a weak band around 3452 cm^{-1} or strong band around 3348 cm^{-1} for isomer **16c$_1$** and **16c$_2$**, respectively. The experimental signal is strong and appears at 3288 cm^{-1}. This suggests the presence of a hydrogen bond. Here, **16c$_2$**

represents the experimental band much better than **16c$_1$**. Also, the so-called amide II band (mainly N–H bending) is better reproduced by **16c$_2$**.

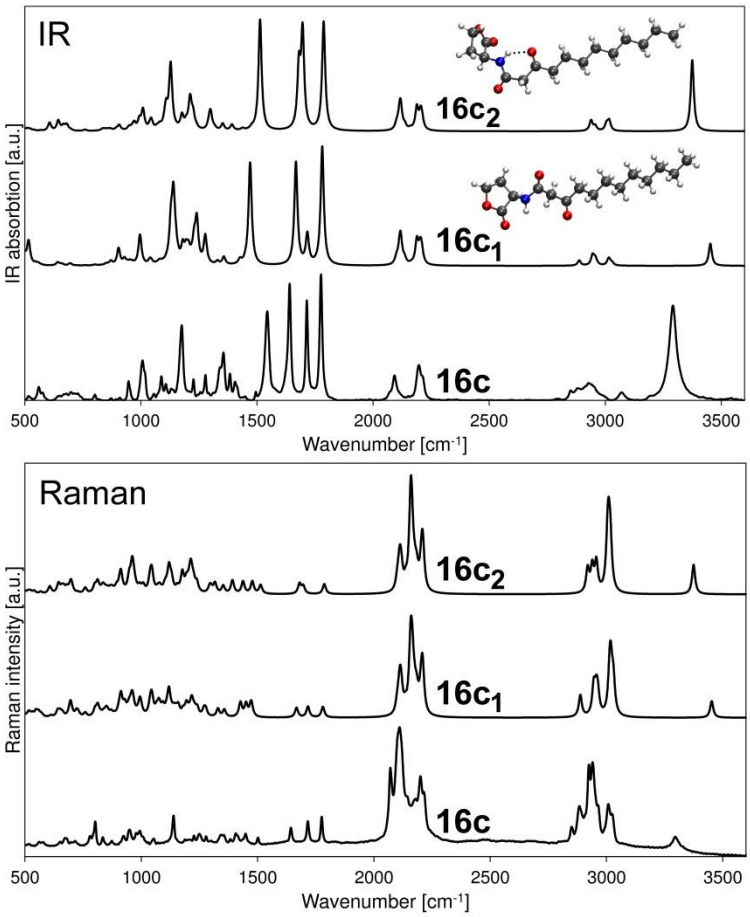


Figure 3.3 Comparison of experimental IR/Raman spectra of compound **16c** (bottom lines) with theoretical spectra of its two conformers **16c$_1$** and **16c$_2$**.

Experimentally, it was found at 1543 cm^{-1} while calculated values are 1513 cm^{-1} and 1470 cm^{-1} for **16c$_2$** and **16c$_1$**, respectively. The possible internal hydrogen bond involves a carbonyl group — its stretching vibration should therefore be also affected and accordingly shifted in the experimental spectrum. This band is found at 1714 cm^{-1} in experiment. Surprisingly, the **16c$_1$** structure without a hydrogen bond shows this band almost in the same position (1716 cm^{-1}) but for **16c$_2$** it appears at 1696 cm^{-1} and is very close to a typical amide I band (1680 cm^{-1}). Moreover, the relative intensity of the amide I band (exp. 1639 cm^{-1}) and the stretching of the carbonyl group (exp. 1774 cm^{-1}) is much better reproduced in case of conformer **16c$_1$**. We can therefore assume that the changes in vibrations involving N–H bond can be attributed to intermolecular interactions (intermolecular hydrogen bond) and that the measured structure is closer to that of isomer **16c$_1$**. The latter was used for assignment of the

experimental bands (see Table 3). IR and Raman spectra of **16a** and **16d** derivatives showed similar bands positions as in case of **16c** (Figure 3.4).

Table 3 Tentative band assignment for IR spectra of **16c** based on a theoretical spectrum of the geometry **16c$_1$**.

Wavenumber [cm^{-1}]	Tentative assignment
800	N-H bending (out-of-plane)
945	skeletal vibration (C-C-C lactone) + peptide bond
1005	skeletal rocking (chain)
1174	C-O-C symmetric stretch (lactone)
1228	skeletal vibration
1350–1273	CH_2 wagging + twisting (bending out-of-plane, lactone)
1408	C-N stretch
1543	NH bend in-plane (amide II band, 2° amide)
1640	C=O stretch (amide I band, intermolecular H-bonding
1714	C=O stretch (ketone)
1774	C=O stretch (lactone)
2092	CD_2 symmetric stretch
2196	CD_2 asymmetric stretch
2844	CH_2 symmetric stretch (lactone)
2911	CH_2 asymmetric stretch (lactone)
3057	CH stretch (tentatively O=C-CH_2-C=O or HO-C=CH-C=O, tautomer)
3288	NH stretch

The experimental Raman spectrum shows two weak bands at 2092 cm^{-1} and 2196 cm^{-1}. The positions of these vibrations in the simulated spectra are 2115 cm^{-1} and 2201 cm^{-1}. The first band corresponds primarily to the symmetric CD_2 stretching while the second results from the asymmetric CD_2 bond stretching. A strong, three-signal band was found in a similar region of the Raman spectra (exp. 2071 cm^{-1}, 2109 cm^{-1}, 2200 cm^{-1}) and is in agreement with the theoretical spectra (2110 cm^{-1}—symmetric CD_2, 2164 cm^{-1} and 2201 cm^{-1}—asymmetric CD_2).

Table 4 Tentative band assignment for experimental Raman spectra of **16c**.

Wavenumber [cm^{-1}]	Tentative assignment
570	C-C skeletal vibration
720–660	O=C-N bending vibration
801	N-H bend out-of-plane (amide IV band)
992–923	skeletal vibration (C-C-C ring)
1052	CH_2 rocking (bending in-plane, lactone)
1141	C-O-C stretch (lactone)
1226	skeletal vibration
1249	NH bending (amide III band)
1348–1275	CH_2 wagging + twisting (bending out-of-plane, lactone)
1408	C-N stretch
1444	CH_2 scissoring (bending in-plane, lactone)
1500	NH bend in-plane (amide II band, 2° amide)
1648	C=O stretch (amide I band, intermolecular H-bonding)
1714	C=O stretch (ketone)
1779	C=O stretch (lactone)
2071	CD_2 symmetric stretch
2109	CD_2 symmetric stretch
2200	CD_2 asymmetric stretch
2844	CH_2 symmetric stretch (lactone)
2945	CH_2 asymmetric stretch (lactone)
3299	NH stretch

The agreement between theoretical and experimental Raman spectra is satisfactory. However, the molecule can interact in the solid state with neighbouring molecules through dispersion and correlation interactions which play an important role in alkane–alkane chain interactions.[98] It has also been mentioned that the final product is composed of a non-equal mixture of isotopologues. In the region of about 3000 cm^{-1}, a relatively broad weak band was found suggesting the presence of some additional CH bonds in the molecule. The ability to distinguish between these bonds is limited both by precision of the Raman apparatus and theoretical accuracy which can be achieved for such relatively big molecules (see Table 4). However, the combination of theoretical and experimental IR and Raman spectroscopy allowed us to identify most probable structure (**16c$_1$**) of these important signal molecules.

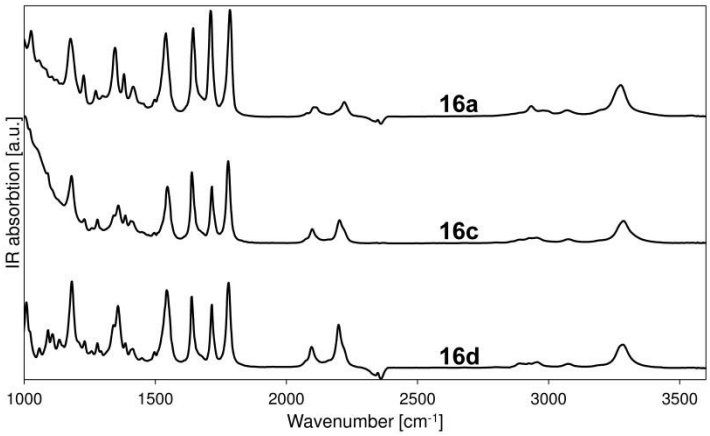


Figure 3.4 Comparison of IR spectra of **16a**, **16c**, **16d**.

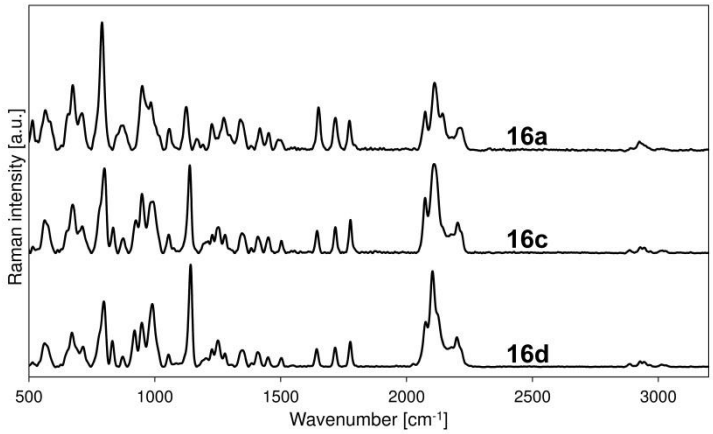


Figure 3.5 Comparison of Raman spectra of **16a**, **16c**, **16d**.

3.1.3 Interaction of AHL with model cell membranes

Sum-frequency-generation (SFG) spectroscopy was used to investigate the integration of deuterated AHLs in supported lipid bilayers (SLBs) as a model of cell membranes.[1,12] Supported lipid bilayers were produced *via* the method of vesicle fusion[99] from appropriate amounts of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) (POPG) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC). The presence of deuterated AHL alone does not lead to a SFG signal due to the isotropic arrangement in the vicinity of the (hydrophilic) SiO_2 surface. Only in the presence of both SLB and deuterated AHL can a reasonable SFG signal be detected in the CD vibrational region as a consequence of AHL inclusion (and subsequent symmetry break) in the SLB. Similar results were found for the other AHL derivatives with 8 and 14 carbon atoms in the aliphatic chain (accordingly **16a** and **16d**).[12] This unique surface specificity and sensitivity—unattainable *via* conventional linear

IR spectroscopic methods—allowed the detection of trace amounts of AHL integrated in the SLB.

These results demonstrate the ability to use SLBs as effective platforms to study the inclusion of deuterated AHLs in membranes, using SFG spectroscopy. Although the used model membranes differ from natural cell membranes, our results support that AHL is integrated in SLBs and indicates the ability of AHL to diffuse through these systems. The obtained signal strength suggests that the compound is integrated in a highly ordered manner.[1]

Moreover, all deuterated AHL derivatives integrate into the upper leaflet of the lipid bilayers in a well-ordered all-trans conformation with a mean orientation of the terminal CD_3 groups toward the surface (parallel to the surface normal). An increased amount of incorporated deuterium labelled AHLs was found for longer chain lengths **16d**, which may result from the decreased ratio of hydrophilic head to aliphatic tail, making it more favourable for short-chain **16a** to remain in solution. Flip-flop or translocation of AHL within the membrane was not observed for the conditions and lipid system used in these experiments.[12]

3.2 Deuterium and tritium labelling of AHLs *via* catalytic reduction of double bond by sodium borohydride

3.2.1 Deuterium labelling of *N*-acyl-L-homoserine lactones

Most of the isotopic labelling methods are based on the usage of deuterium or tritium gas, aggressive reagents, expensive catalysts or vast amounts of energy (temperature, microwave irradiation). In this work, the isotopic labelling of N-acyl-L-homoserine lactones was carried out by simple reduction of an unsaturated precursor by sodium borodeuteride (or sodium borotritide). The procedure used and optimised herein does not require sophisticated apparatuses or complicated work up.

The substrates for the labelling of **20a-d** were prepared *via* known procedures.[100,101,18] The commercially available terminally unsaturated carboxylic acids **17a-d** were first converted to corresponding acyl chlorides **19a-d** using oxalyl chloride with good to very good yields. The advantage of this method is the usage of oxalyl chloride. It is less toxic than thionyl chloride or phosphorus pentachloride utilized in standard procedures of synthesising acyl chlorides. The amidation reaction with L-homoserine lactone hydrobromide was first carried out with commercially available decanoyl chloride to yield *N*-decanoyl-L-homoserine lactone in 86%.

The intermediates **19a-d** reacted with L-homoserine lactone hydrobromide **15** in the presence of triethylamine to give the terminally unsaturated AHLs **20a-d** with very good yields (Figure 3.6 and Table 5).

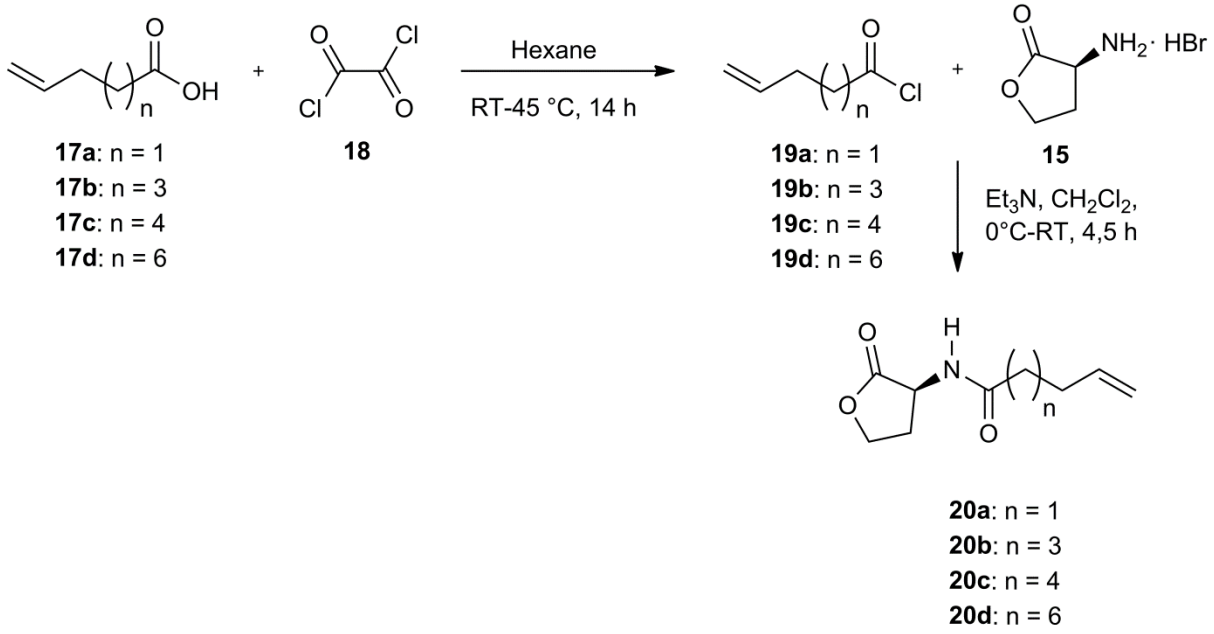


Figure 3.6 The synthesis of terminally unsaturated *N*-acyl-L-homoserine lactones **20a-d**.

Table 5 The results for the synthesis of terminally unsaturated *N*-acyl-L-homoserine lactones.

Entry	1	2	3	4	5	6	7	8
Products[a]	**19a**	**19b**	**19c**	**19d**	**20a**	**20b**	**20c**	**20d**
n	1	3	4	6	1	3	4	6
Time (h)	-	20	18	16	4.5	12	10	4.5
Yield (%)	Commercially available	99	30	68	55	15	85	90

[a] See Figure 3.6.

A method of deuterium labelling of terminally unsaturated AHLs *via* catalytic reduction of double bond by sodium borohydride was first tested on the model molecule – 9-decenoic acid **21** (Figure 3.7). The synthesis was performed in a quasi-solid state. Particular reagents were frozen in a liquid nitrogen layer-by-layer in the appropriate mixture of solvents in order to separate reagents.

In the first layer the terminally unsaturated substrate was dissolved in THF and frozen in liquid nitrogen. In the second layer a pre-catalyst - palladium (II) acetate - in THF, acetic acid and methanol were added and again frozen. In the next layer more THF was added and frozen, to isolate the catalyst solution from sodium borohydride. In the last layer, sodium

hydroxide water (or methanol) solution of sodium borohydride (sodium borodeuteride or sodium borotritide) was added and the reaction vessel was closed tightly. The reaction mixture was then allowed to come to the room temperature and the proper reaction was initiated (Figure 3.7 and Figure 3.8).

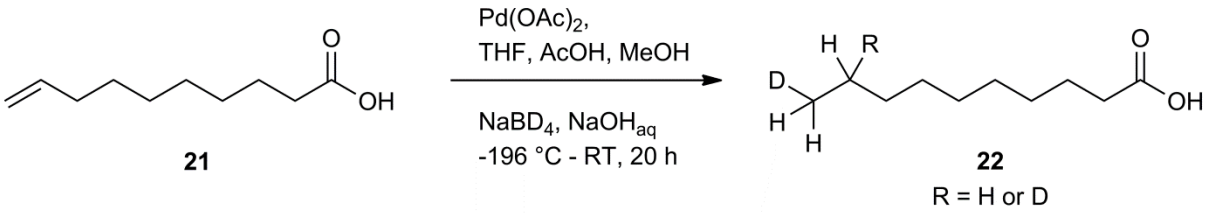


Figure 3.7 Deuterium labelling *via* catalytic reduction of double bond by sodium borohydride on the model alkene – 9-decenoic acid **21**.

In this reaction, the sodium borohydride is hydrolysed by acetic acid. Released hydrogen reduces palladium (II) acetate to the metallic palladium. The reaction is autocatalytic because formed palladium nanoparticles in turn support hydrolysis of sodium borohydride to generate hydrogen gas *in situ*. This gas is then utilised in the reduction of provided alkene.

First results gave mainly *mono*-deuterated isotopologue of decanoic-*d* acid **22** with following deuterium distribution: 32% d_2, 54% d_1, 14% d_0.

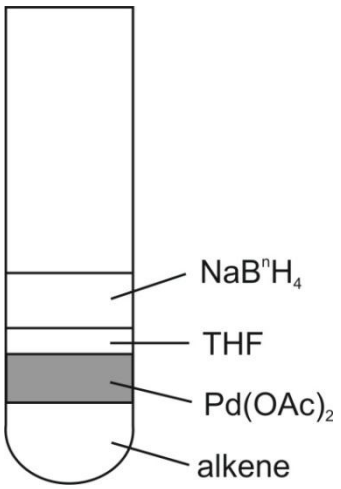
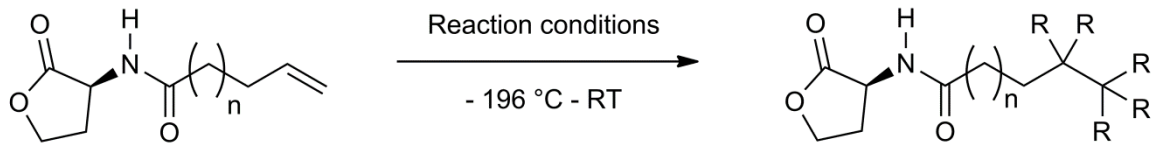


Figure 3.8 Layer-by-layer preparation of reaction mixture for catalytic reduction of alkenes by sodium borohydride in liquid nitrogen. n = 1, 2, 3.

The reaction was performed on four terminally unsaturated AHL derivatives **20a-d** with varying chain lengths. (Figure 3.9 and Table 6).



20a: n = 1
20b: n = 3
20c: n = 4
20d: n = 6

23a: n = 1
23b: n = 3
23c: n = 4
23d: n = 6

Figure 3.9 Deuterium labelling of terminally unsaturated AHLs **20a-d** through $Pd(OAc)_2$ catalysed reduction of double bond by $NaBD_4$ (See Table 6).

Table 6 Representative results of deuterium labelling of **20a-d**[a] *via* $Pd(OAc)_2$ catalysed reduction of double bond by $NaBD_4$.

Entry	n	Product	Reactions conditions	Comment	Time (h)	Yield (%)	Deuterium content[b] (%) of isomer-d_n
1	1	**23a**	0.2 equiv. $Pd(OAc)_2$, THF, AcOH, $MeOH/H_2O$, 1 equiv. $NaBD_4$, 1 M NaOHaq	-	24	80	6 d_5, 10 d_4, 22 d_3, 20 d_2, 24 d_1, 18 d_0
2	3	**23b**	0.2 equiv. $Pd(OAc)_2$, THF, AcOH, $MeOH/H_2O$, 1 equiv. $NaBD_4$, 1 M NaOHaq	-	22	83	6 d_4, 18 d_3, 35 d_2, 27 d_1, 14 d_0
3	4	**23c**	0.2 equiv. $Pd(OAc)_2$, THF, AcOH, $MeOH/H_2O$, 1 equiv. $NaBD_4$, 1 M NaOHaq	-	22	74	23 d_3, 36 d_2, 30 d_1, 11 d_0
4	6	**23d**	0.2 equiv. $Pd(OAc)_2$, THF, AcOH, $MeOH/H_2O$, 1 equiv. $NaBD_4$, 1 M NaOHaq	-	16	82	12 d_3, 30 d_2, 36 d_1, 22 d_0
5	6	**23d**	1 equiv. $NaBD_4$ in 1 M NaOHaq; 0.2 equiv. $Pd(OAc)_2$, THF, AcOH, $MeOH/H_2O$	1st layer: $NaBD_4$	17	89	7 d_5, 16 d_4,19 d_3, 26 d_2, 19 d_1, 13 d_0
6	6	**23d**	0.2 equiv. $Pd(OAc)_2$, THF, AcOH-d_4, 2 equiv. $NaBD_4$, 1 M NaOH in D_2O	Deuterated solvents	22	94	3 d_5, 12 d_4, 24 d_3, 33 d_2, 20 d_1, 8 d_0
7	6	**23d**	0.2 equiv. $Pd(OAc)_2$, THF, AcOH-d_4, $MeOD/D_2O$, 3 equiv. $NaBD_4$, 1 M NaOD in D_2O	Deuterated reagents	25	96	10 d_5, 15 d_4, 27 d_3, 18 d_2, 17 d_1, 13 d_0
8	6	**23d**	1 equiv. $Pd(OAc)_2$ in THF, AcOH-d_4; 2 equiv. $NaBD_4$, 1 M NaOH in D_2O	Deuterated solvents, 1 equiv. $Pd(OAc)_2$	17	94	6 d_5, 13 d_4, 19 d_3, 23 d_2, 27 d_1, 12 d_0

[a] See Figure 3.9. [b] Determined by ESI-TOF MS.

The first reactions on substrates **20a-d** with 0.2 equivalents of palladium (II) acetate and 1 equivalent of sodium borodeuteride were carried out over 16-24 hours and gave similar

distribution of isotopologues like the test reaction (Table 6, Entries 1-4). Mono- and di-deuterated isomers were observed almost in the equal ratio (20-30%) with very good yields (74-83%). Reversing of the layer system (starting from sodium borodeuteride frozen solution) decreased the selectivity (six isotopologues were observed).

The deuteration degree increased when deuterated reagents were used. However, because of the H-D exchange, especially from AcOH-d_4 or D_2O source, more isomers were observed (Table 6, Entries 6-8). Excess of sodium borohydride (2-3 equivalents) resulted in a higher number of heavier isotopologues (Table 6, Entries 6 and 7). Addition of excess of the palladium (II) acetate (one equivalent) did not have a significant effect on the deuterium distribution (Table 6, Entry 8). The length of the acyl chain was found not to influence on the reaction result (Table 6).

Further development of the method was carried out on the AHL derivative with the shortest acyl chain **20a** (Figure 3.9 and Table 7). Locating the substrate and catalyst solutions in one layer led to a failure of the reaction (Table 7, Entry 1). Time reduction (from 24 to 16 hours) under the same conditions (0.2 equivalents of catalyst, 1 equivalent of $NaBD_4$, non-deuterated solvents) showed similar deuterium distribution with almost the same yields (Table 7, Entries 2 and 3). The usage of deuterated acetic acid and sodium hydroxide in deuterated solvents (MeOD and D_2O) revealed a greater number of heavier isomers with excellent 95% yield (Table 7, Entry 4). A major excess of $NaBD_4$ (3-4 equivalents) caused dominance of isomer-d_3 accompanied mostly by their heavier isotopologues (Table 7, Entries 5 and 6).

The usage of deuterated solvents or excess of sodium borohydride causes the formation of heavier isomers (d_3 and higher). Incorporation of more than two deuterium atoms to the labelled compound is rather an advantage of the method, particularly in the context of its detectability in a biological material. However, from the chemical point of view, the labelling reaction is then less selective.

Table 7 Representative results of deuterium labelling of **20a**[a] *via* $Pd(OAc)_2$ catalysed reduction of double bond by $NaBD_4$.

Entry	Reactions conditions	Comment	Time (h)	Yield (%)	Deuterium content[b] (%) of isomer-d_n
1	0.2 equiv. $Pd(OAc)_2$, THF, AcOH, MeOH/H_2O, 0.5 equiv. $NaBD_4$, 1 M NaOHaq	Substrate was in one layer with catalyst	19	Failed	-
2	0.2 equiv. $Pd(OAc)_2$, THF, AcOH, MeOH/H_2O, 1 equiv. $NaBD_4$, 1 M NaOHaq	1 equiv. $NaBD_4$	24	80	6 d_5, 10 d_4, 22 d_3, 20 d_2, 24 d_1, 18 d_0
3	0.2 equiv. $Pd(OAc)_2$, THF, AcOH, MeOH/H_2O, 1 equiv. $NaBD_4$, 1 M NaOHaq	Time change	16	82	7 d_4, 14 d_3, 23 d_2, 35 d_1, 21 d_0
4	0.2 equiv. $Pd(OAc)_2$, THF, AcOH-d_4, MeOD/D_2O, 1 equiv. $NaBD_4$, 1 M NaOD in D_2O	Deuterated reagents	19	95	17 d_5, 21 d_4, 26 d_3, 21 d_2, 15 d_1
5	0.2 equiv. $Pd(OAc)_2$, THF, AcOH, MeOD/D_2O, 3 equiv. $NaBD_4$, 1 M NaOD in D_2O	3 equiv. $NaBD_4$	17	95	29 d_5, 17 d_4, 28 d_3, 17 d_2, 9 d_1
6	0.2 equiv. $Pd(OAc)_2$, THF, AcOH, MeOD/D_2O, 4 equiv. $NaBD_4$, 1 M NaOD in D_2O	4 equiv. $NaBD_4$	20	95	16 d_5, 20 d_4, 36 d_3, 20 d_2, 8 d_1
7	0.2 equiv. $Pd(OAc)_2$, THF, AcOH, MeOD, 2 equiv. $NaBD_4$, 30% NaOD in D_2O diluted in MeOD to 0.33 M	NaOD diluted with MeOD	17	86	13 d_4, 21 d_3, 54 d_2, 12 d_1
8	0.2 equiv. $Pd(OAc)_2$, THF, AcOH, MeOD, 2 equiv. $NaBD_4$, 0.33 M NaOD in MeOD	No H_2O or D_2O	21	70	16 d_4, 23 d_3, 44 d_2, 13 d_1, 4 d_0
9	0.2 equiv. $Pd(OAc)_2$, THF, MeOD, 2 equiv. $NaBD_4$, 30% NaOD in D_2O diluted in MeOD to 0.33 M	No AcOH	17	Failed	-
10	0.2 equiv. $Pd(OAc)_2$, THF, AcOH, MeOD, 2 equiv. $NaBD_4$, 30% NaOD in D_2O diluted in MeOD to 0.33 M	Reduced time	4.5	85	7 d_4, 19 d_3, 54 d_2, 20 d_1
11	0.2 equiv. $Pd(OAc)_2$, THF, AcOH, MeOD, 2 equiv. $NaBD_4$, 30% NaOD in D_2O diluted in MeOD to 0.33 M	Reduced time	1.5	63	8 d_4, 21 d_3, 48 d_2, 20 d_1, 3 d_0
12	0.2 equiv. $Pd(OAc)_2$, THF, AcOH, MeOD, 2 equiv. $NaBD_4$, 30% NaOD in D_2O diluted in MeOD to 0.33 M	Reduced time	0.25	61	12 d_4, 24 d_3, 49 d_2, 15 d_1
13	0.2 equiv. $Pd(OAc)_2$, THF, AcOH, MeOD, 2 equiv. $NaBD_4$, 30% NaOD in D_2O diluted in MeOD to 0.15 M	30% NaOD in D_2O diluted in MeOD to 0.15 M	2	80	13 d_4, 21 d_3, 54 d_2, 12 d_1
14	0.2 equiv. $Pd(OAc)_2$ in THF and MeOD; AcOH; 2 equiv. $NaBD_4$, 30% NaOD in D_2O diluted in MeOD to 0.15 M	AcOH was frozen as a separated layer	1	60	6 d_5, 17 d_4, 30 d_3, 22 d_2, 18 d_1, 7 d_0
15	0.2 equiv. $Pd(OAc)_2$ in THF, AcOH, MeOD; 2 equiv. $NaBD_4$ in MeOH	$NaBD_4$ in MeOH	2	55	9 d_5, 11 d_4, 14 d_3, 18 d_2, 18 d_1, 21 d_0, 9 substrate
16	0.2 equiv. $Pd(OAc)_2$ in THF, AcOH, MeOH; 4 equiv. $NaBD_4$ in MeOH	$NaBD_4$ in separated flask – gas provided by pipe to the flask with catalyst	20	52	7 d_5, 8 d_4, 22 d_3, 18 d_2, 19 d_1, 26 d_0
17	0.2 equiv. Pd/C in THF, AcOH; 2 equiv. $NaBD_4$ (powder)	Pd/C	1.5	80	13 d_2, 16 d_1, 11 d_0, 60 substrate
18	0.2 equiv. $Pd(PPh_3)_4$, THF, AcOH, MeOH 2 equiv. $NaBD_4$, 30% NaOD in D_2O diluted in MeOH to 0.33 M	$Pd(PPh_3)_4$	17	Failed	-

[a] See Figure 3.9.[b] Determined by ESI-TOF MS.

The selectivity of the reaction was increased by elimination of acidic deuterium atoms coming mostly from acetic acid-d_4. This was reached by dilution of the solution of NaOD in deuterated water with MeOD (to 0.33 M). Reducing the amount of sodium borodeuteride to 2 equivalents supported selectivity as well to yield isotopologue-d_2 in 54% of deuteration degree in the presence of only 3 more isomers (Table 7, Entry 7).

Total elimination of deuterated water resulted in the decrease of deuterium content (Table 7, Entry 8). Moreover, total elimination of acetic acid from the reaction medium, caused the reaction to fail (Table 7, Entry 9). This fact proved importance of acetic acid in the hydrolysis of sodium borohydride, which was discussed in section 2.2.4.3.

A considerable reduction of reaction time to 4.5 hours resulted in higher selectivity (Table 7, Entry 10). Further time decrease (up to 15 minutes) led to slightly lower deuteration degree and lower yield (Table 7, Entries 11 and 12).

Optimal time of the reaction was found to be 2 hours. Furthermore, additional dilution of sodium deuteroxide down to 0.15 M afforded the highest selectivity so far (Table 7, Entry 13). However, absolute removal of sodium hydroxide or sodium deuteroxide, caused minimal conversion of the substrate. This confirmed NaOH (water/methanol) solution to be a very convenient solvent system for sodium borohydride.

In order to make hydrolysis of sodium borodeuteride more effective, the acetic acid was frozen as a separate layer. Unfortunately, this change resulted in a less selective deuterium distribution (Table 7, Entry 14). Additionally, sodium borohydride is also known to undergo hydrolysis upon methanol treatment. This was tested on the **20a** substrate to give non total conversion of the substrate and with general lower deuterium content (Table 7, Entry 15).

The next modification tested was to keep sodium borohydride with methanol in a separate flask. The generated gas was then provided to the second flask with a solution of $Pd(OAc)_2$ by a Teflon pipe. After saturation of a pre-catalyst by deuterium gas, the substrate solution was added. The resulting deuterium content as well as yield of the reaction was still lower when compared to the previous ones (Table 7, Entry 16).

Usage of palladium (0) catalysts such as palladium on carbon or tetrakis(triphenylphosphine)palladium(0) were also not successful. In case of Pd/C, 60% of the substrate still remained in the reaction mixture. The products were obtained only with the

low deuterium content (Table 7, Entry 17). This confirmed the importance of the nano-size of palladium metal as was mentioned in the theoretical part of this work in section 2.2.4.3.[70] Reaction with $Pd(PPh_3)_4$ was unsuccessful (Table 7, Entry 18).

In summary, the optimal result was obtained by usage of 0.2 equiv. $Pd(OAc)_2$, THF, AcOH, MeOD, 2 equiv. $NaBD_4$, 30% NaOD in D_2O diluted in MeOD to 0.15 M in 2 hours (Table 7, Entry 13). It is worth to mention that a normal acid-base work-up lead to very pure products, which do not need any further purification.

3.2.2 Synthesis of highly biologically active, deuterium and tritium labelled *N*-(3-oxododecanoyl)-L-homoserine lactones

The uncommon reductions of the double bond using sodium borodeuteride or sodium borotritide in the presence of palladium (II) acetate were performed also on the highly biologically active, terminally unsaturated *N*-(3-oxododecanoyl)-L-homoserine lactone **26**. The first two steps of the synthesis of terminally unsaturated substrate were carried out according to the procedures described for the first method of deuterium labelling, reported above in section 3.1.1 (Figure 3.10).[18] Yields were satisfactory (55-60% see Table 8).

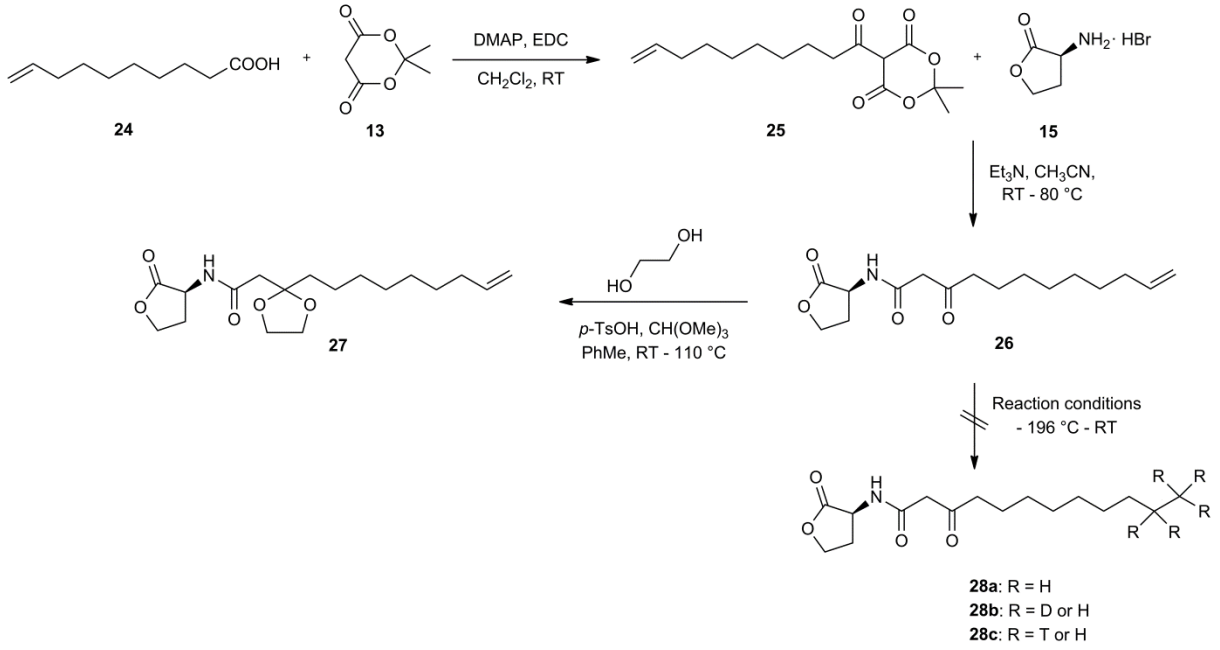


Figure 3.10 The synthesis of *N*-[3-(1', 3'-dioxolane)-11-dodecenoyl]-L-homoserine lactone **27** and attempts of reduction reaction on substrate **26**.

First experimental reduction reactions were carried out on *N*-(3-oxo-11-dodecenoyl)-L-homoserine lactone (Figure 3.10 and Table 9). Extending the time of the reaction, usage of deuterated reagents, excess of sodium borodeuteride or temperature variations, however, did not yield the desired product (Table 9). This result could most probably be explained by the presence of a ketone group which is known to undergo reduction to hydroxyl group, by sodium borohydride. Anyhow, the potential by-product *N*-(3-hydroxydodecanoyl)-L-homoserine lactone was not isolated from the mixture of products.

Table 8 Results of the synthesis of *N*-[3-(1', 3'-dioxolane)-11-dodecenoyl]-L-homoserine lactones.

Entry	Products[a]	Time (h)	T (° C)	Yield (%)
1	**25**	12	RT	60
2	**26**	5	RT – 80	55
3	**27**	25	RT - 110	64

[a] See Figure 3.10.

Therefore, acetal protection of the ketone group was performed by use of ethylene glycol and assistance of *p*-toluenesulfonic acid, with good yield (Figure 3.10 and Table 8).[102] The addition of trimethyl orthoformate, enabled removal of water without use of a Dean-Stark trap.[103]

Table 9 Results of attempts of reduction reaction on substrate **26**.

Entry	Product[a]	R	Reactions conditions	Comment	Time (h)	Yield (%)	Deuterium content (%)
1	**3**	H	0.2 equiv. $Pd(OAc)_2$, THF, AcOH, MeOH/H_2O, 1 equiv. $NaBH_4$, 1 M NaOHaq	-	4	Failed	-
2	**28b**	D	0.2 equiv. $Pd(OAc)_2$, THF, AcOH, MeOH/H_2O, 1 equiv. $NaBD_4$, 1 M NaOHaq	$NaBD_4$, extended time	24	Failed	-
3	**28b**	D	0.2 equiv. $Pd(OAc)_2$, THF, AcOH, MeOH/H_2O, 1 equiv. $NaBD_4$, 1 M NaOHaq, - 196 – 0 °C	- 196 – 0 °C	6	Failed	-
4	**28b**	D	0.2 equiv. $Pd(OAc)_2$ in THF and MeOD; AcOH-d_4; 2 equiv. $NaBD_4$, 30% NaOD in D_2O diluted in MeOD to 0.33 M	Deuterated reagents	18	Failed	-

[a] See Figure 3.10.

Intermediate **27** was in turn labelled with deuterium and tritium *via* catalytic reduction of double bond by sodium borodeuteride and sodium borotritide (Figure 3.11 and Table 10).

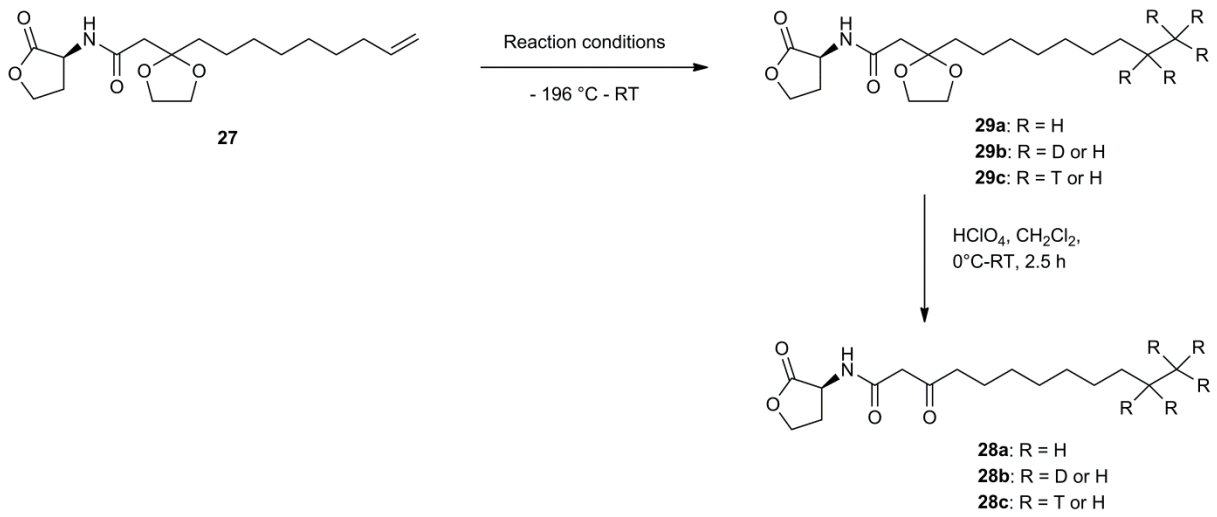


Figure 3.11 Reduction and deprotection reactions of *N*-[3-(1', 3'-dioxolane)-11-dodecenoyl]-L-homoserine lactone **27** (See Table 10).

In the first step, alkene **27** was reduced by non-labelled sodium borohydride, with very good 77% yield (Table 10, Entry 1). Deuteration of the compound **27** gave similar results as in case of products **23a-d**. Usage of 1 equivalent of sodium borodeuteride in the presence of non-labelled solvents, led mostly to the *mono*-deuterated isotopologue, with a very good 77% yield (Table 10, Entry 2).

Addition of other deuterated reagents resulted in larger number of heavier isomers, e.g. 31% of the isomer-d_3 was present in the product (Table 10, Entry 4). Application of more concentrated 2 M NaOH solution led to a failure of the reaction (Table 10, Entry 5). When the reaction was performed in smaller, micromolar scale, problems with reduction occurred. The solution was the use of 4 equivalents of sodium borodeuteride which afforded desired isotopologue-d_2 in 41% deuteration degree, even in small 10 μmol scale (Table 10, Entries 6 and 7).

The best result for the reduction with sodium borodeuteride was obtained in the similar conditions like for products **23a-d**. Dilution of sodium deuteroxide with MeOH to 0.33 M, allowed to obtain the desired double deuterated isomer with very good yield and 52% of deuterium content(Table 10, Entry 3). In all cases the deuteration-rate on the terminal carbon was found to be higher than on the preterminal carbon atom (See Section 4.2.2.5).

Tritium labelling of **27** was performed with the use of sodium borotritide in 0.33 M NaOH solution, with a very good 77% yield. Total radioactivity of tritiated product was 3.52 mCi

and specific activity 588.6 Ci/mol (Table 10, Entry 8). This reduction reaction was especially useful for tritium labelling because of easy handling of the solid sodium borotritide, in comparison to the tritium gas.

Table 10 Results of the reduction reaction on the acetal-protected substrate **27**.

Entry	Product[a]	R	Reactions conditions	Comment	Time (h)	Yield (%)	R content[b] (%)
1	**29a**	H	0.2 equiv. $Pd(OAc)_2$, THF, AcOH, MeOH, 4 equiv. $NaBH_4$, 0.33 M NaOHaq	-	13	77	-
2	**29b**	D	0.2 equiv. $Pd(OAc)_2$, THF, AcOH, MeOH, 1 equiv. $NaBD_4$, 1 M NaOHaq	-	17	77	7 d_3, 14 d_2, 53 d_1, 26 d_0
3	**29b**	D	0.2 equiv. $Pd(OAc)_2$, THF, AcOH, MeOH, 1 equiv. $NaBD_4$, 30% NaOD in D_2O diluted in MeOH to 0.33 M	30% NaOD in D_2O diluted in MeOH to 0.33 M	16.5	58	13 d_3, 52 d_2, 28 d_1, 7 d_0
4	**29b**	D	0.2 equiv. $Pd(OAc)_2$, THF, AcOH-d_4, MeOD, 1 equiv. $NaBD_4$, 30% NaOD in D_2O diluted in MeOD to 0.33 M	Deuterated reagents	17	60	13 d_5, 25 d_4, 31 d_3, 19 d_2, 8 d_1, 5 d_0
5	**29b**	D	0.2 equiv. $Pd(OAc)_2$, THF, AcOH, MeOH, 1 equiv. $NaBD_4$, 2 M NaOHaq	2 M NaOHaq	18	Failed	-
6	**29b**	D	0.2 equiv. $Pd(OAc)_2$, THF, AcOH, MeOH, 1 equiv. $NaBD_4$, 30% NaOD in D_2O diluted in MeOH to 0.33 M	Small scale (10 µmol)	16	Failed	-
7	**29b**	D	0.2 equiv. $Pd(OAc)_2$, THF, AcOH, MeOH 4 equiv. $NaBD_4$, 30% NaOD in D_2O diluted in MeOH to 0.33 M	Excess of $NaBD_4$ (4 equiv); Small scale	18	74	12 d_4, 24 d_3, 41 d_2, 17 d_1, 6 d_0
8	**29c**	T	0.2 equiv. $Pd(OAc)_2$, THF, AcOH, MeOH 1 equiv. $NaBT_4$, 0.33 M NaOHaq	Radioactivity = 3.52 mCi; Specific activity = 588.6 Ci/mol	18	77	84 t_3, 16 t_2

[a] See Figure 3.11. [b] Determined by ESI-TOF MS.

In the last step the acetal groups of all of deuterium-, tritium- and non-labelled derivatives **29a-c** were deprotected by perchloric acid to yield the corresponding ketones in excellent 90% (Figure 3.11).[104]

In summary, the synthesis of deuterium and tritium labelled AHLs *via* catalytic reduction of double bond by sodium borohydride, was proven to be an efficient, mild and facile labelling method. Such tritium labelled molecules can be helpful weapons to gain more insight into the mechanism of the crossing of cell membranes by 3OC12 HSL **3**.

3.3 Synthesis of isotope labelled and photoactivatable *N*-(3-oxododecanoyl)-L-homoserine lactone

3.3.1 Synthesis of *N*-(3-oxo-5-diazirinedodecanoyl)-L-homoserine lactone

The first step of the synthesis of *N*-(3-oxo-5-diazirinedodecanoyl)-L-homoserine lactone is based on the synthesis of β-keto-decanoic acid. For this purpose two methods were tested (Figure 3.12). In the first one, a bis(trimethylsilyl) malonate (BSM) **30** was acylated with octanoyl chloride **31** using a mild base such as triethylamine and lithium bromide, with a good yield (Table 11).[105] Complexation of a lithium cation by the BSM **30** increased the acidity of the α-carbon protons thus making them easier to be removed. In consequence the use of strong bases such as metal alkoxides, metal amides, or alkyl lithium reagents was avoided.[106]

In the second method, a two-step synthesis was started from an acylation of methyl acetate **33** by octanoyl chloride **31** in the presence of lithium diisopropylamide (LDA), with a very good yield (Figure 3.12 and Table 11).[107-109] The obtained methyl 3-oxodecanoate **34** was further hydrolysed under strong basic conditions. However, even usage of the strong base like a lithium hydroxide and heating under reflux over two days, yielded only 13% of the desired product (Figure 3.12 and Table 11). Thus, the first method was chosen as a more efficient and convenient way.

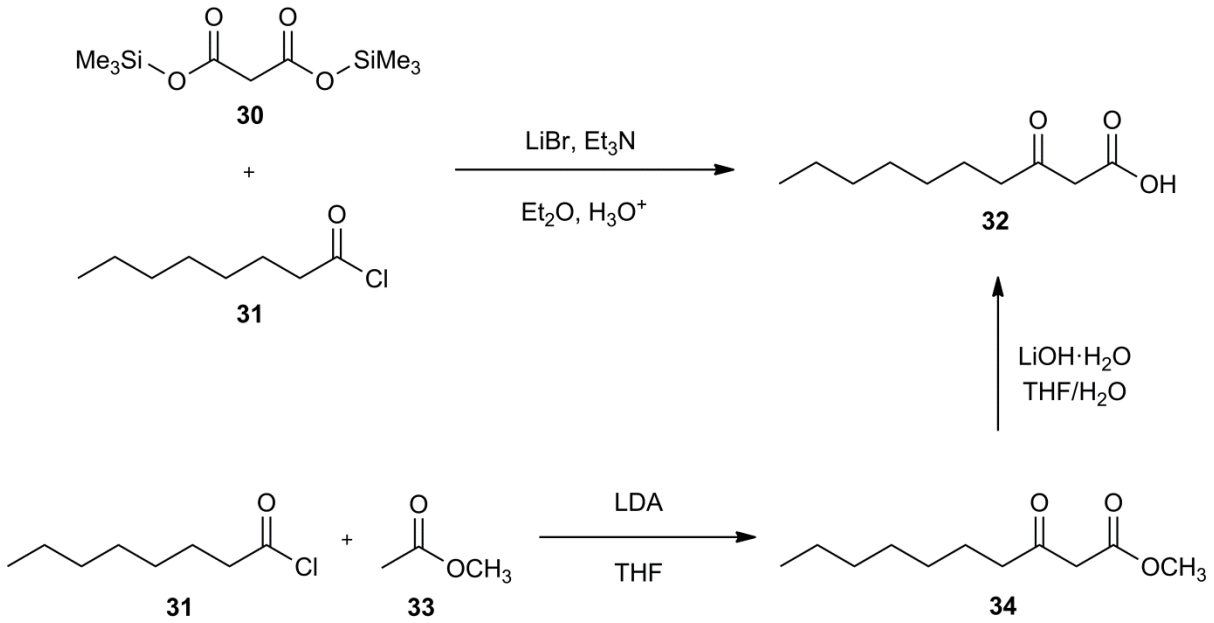


Figure 3.12 Two selected methods of the β-keto-decanoic acid **32** synthesis.

Table 11 Results of the synthesis of β-keto-decanoic acid.

Entry	Substrates[a]	Products[a]	Time (h)	T (° C)	Yield (%)
1	**30**	**32**	2	0 - RT	52
2	**33**	**34**	4.5	-78 - RT	80
3	**34**	**32**	48	RT - 60	13

[a] See Figure 3.12.

The diazirine group was chosen as the most convenient photoactivatable agent. Thus, 3-diazirine-decanoic acid **35** was prepared according to a procedure described by Husain *et al.*[110] β-Keto-decanoic acid **32** was treated with hydroxylamine-*O*-sulfonic acid in liquid ammonia to give an aziridine intermediate. Oxidation with iodine resulted in the formation of diazirine **35** with 30% yield (Figure 3.13, Table 12).[110,13]

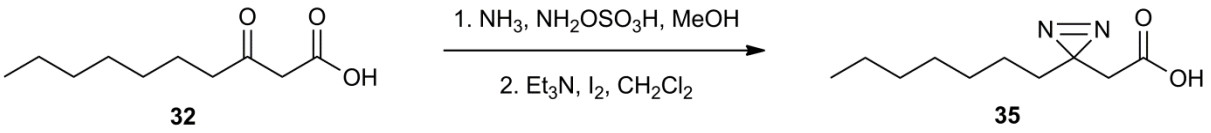


Figure 3.13 Formation of 3-diazirine-decanoic acid.

3-Diazirine-decanoic acid **36** was subsequently converted into an active ester of Meldrum's acid using procedure described above in section 3.1.1, in 60% yield (Figure 3.14 and Table 12).[18,1]

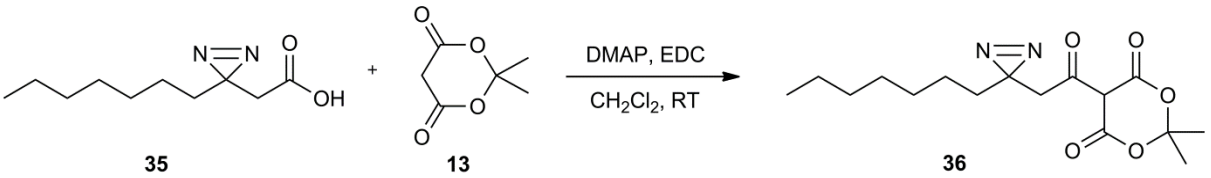


Figure 3.14 Synthesis of 1-oxo-3-diazirinedecanoyl Meldrum's acid **36**.

Unfortunately, several attempts of amidation of **36** under previously reported conditions[18,1] were not successful. Neither extended reaction time nor slightly raised temperature gave desired product **37** (Figure 3.15 and Table 12, Entries 3-5). It is noteworthy that the diazirine group is temperature sensitive, thus heating up to 80 °C led to decomposition of this moiety (Table 12, Entry 6).

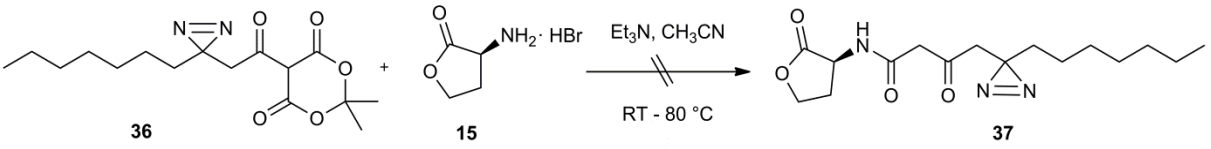


Figure 3.15 Attempt of the synthesis of *N*-(3-oxo-5-diazirinedodecanoyl)-L-homoserine lactone **37**.

Second trial of the activation of 3-diazirine-decanoic acid to an active hydroxysuccinimide ester resulted only in a mixture of the products (Figure 3.16 and Table 12).

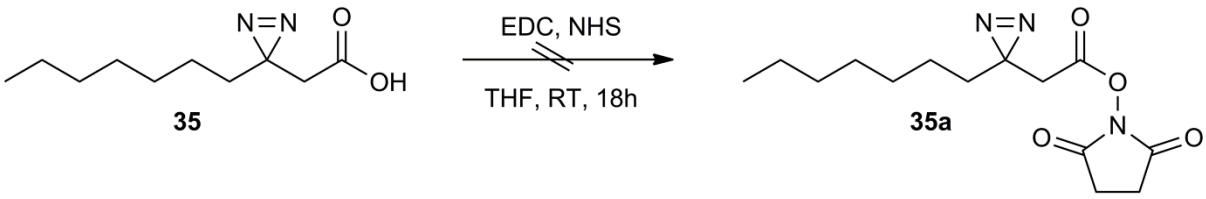


Figure 3.16 Attempt of the synthesis of hydroxysuccinimide ester of 3-diazirine-decanoic acid **35a**.

Table 12 Results of the synthesis of *N*-(3-oxo-5-diazirinedodecanoyl)-L-homoserine lactone **37** (with intermediates).

Entry	Substrates[a]	Products[a]	Time (h)	T (° C)	Yield (%)
1	**32**	**35**	24	-78 - RT	30
2	**35**	**36**	4	RT	60
3	**36**	**37**	5	RT	0
4	**36**	**37**	48	RT	0
5	**36**	**37**	24	RT - 60	0
6	**36**	**37**	24	RT - 80	0
7	**35**	**35a**	18	RT	0
7	**35**	**38**	24	0 - RT	20
8	**38**	**37**	16	RT	25

[a] See Figure 3.13, Figure 3.14, Figure 3.15, Figure 3.16 Figure 3.17 and Figure 3.18.

Next approach to the synthesis of **37** utilized the conversion of 3-diazirine-decanoic acid **35** to the corresponding *tert*-butyl active ester **38** (Figure 3.17 and Table 12, Entry 7) [13,111]

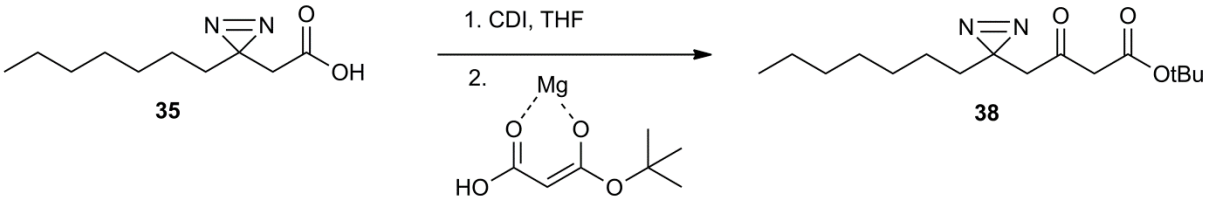


Figure 3.17 Synthesis of 1-*tert*-butyl-3-oxo-5-diazirine-dodecanoate. CDI =1,1' Carbonyldiimidazole.

Ester **38** was hydrolysed 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 **37** with 25% yield (Figure 3.18 and Table 12, Entry 8).

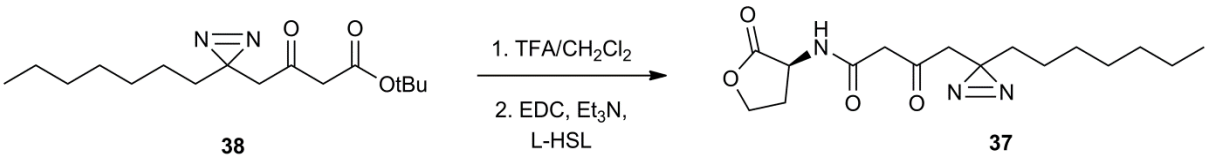


Figure 3.18 Synthesis of *N*-(3-oxo-5-diazirinedodecanoyl)-L-homoserine lactone **37**.

3.3.2 Deuterium and tritium labelling of AHLs *via* exchange reactions

Deuterium and tritium labelling of *N*-(3-oxo-5-diazirinedodecanoyl)-L-homoserine lactone **37** require mild reaction conditions. A strong base for instance can cleave lactone moiety and to high temperature can decompose diazirine.

Thus, the first method tested in this work is based on a post-synthetic hydrogen-deuterium (tritium)-exchange from the deuterated (tritiated) water in mild conditions.

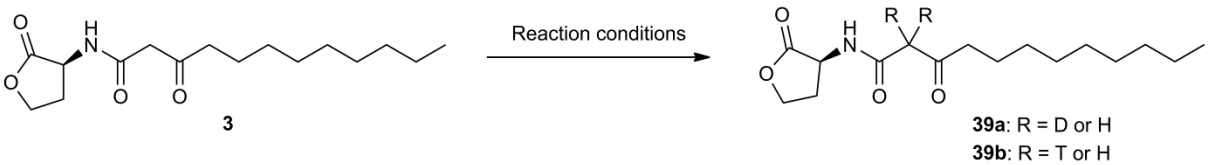


Figure 3.19 Deuterium and tritium labelling of AHLs *via* exchange reactions.

First experimental exchange reactions were carried out with *N*-(3-oxo-dodecanoyl)-L-homoserine lactone **3** using triethylamine and deuterium oxide. This reaction yielded only 31% of the mono-deuterated isomer. The deuteration degree was slightly increased by extension of the reaction time (See Figure 3.19 and Table 13, Entries 1 and 2).

The second approach relies on the application of the phase-transfer catalyst triethylbenzylammonium chloride (TEBA) in the solvents system consisting of a solution of 5% sodium deuteroxide and ethyl acetate (2.2.4.1).[55]

Table 13 Results of the deuterium and tritium labelling of **28a** and **37**.

Entry	Substrate[a]	Product[a]	Reactions conditions	Time (h)	Yield (%)	Deuterium content[b] (%) of isomer-d_n
1	**28a**	**39a**	Et_3N, D_2O, ACN	4	90	38 d_1, 62 d_0
2	**28a**	**39a**	Et_3N, D_2O, ACN	12	93	41 d_1, 59 d_0
3	**28a**	**39a**	5% NaOD in D_2O/EtOAc TEBA	2	92	44 d_1, 56 d_0
4	**28a**	**39a**	5% NaOD in D_2O/EtOAc, TEBA, 30 °C	24	93	51 d_1, 49 d_0
5	**28a**	**39a**	LiBr, Et_3N, D_2O, THF	24	93	11 d_2, 50 d_1, 39 d_0
6	**28a**	**39a**	LiBr, Et_3N, D_2O, THF	48	94	29 d_2, 56 d_1, 15 d_0
7	**28a**	**39a**	$Mg(OAc)_2$, Et_3N, D_2O, THF	25	96	27 d_3, 67 d_2, 6 d_1
8	**28a**	**39a**	$Mg(OAc)_2$, Et_3N, D_2O, THF	48	98	6 d_3, 79 d_2, 6 d_1
9	**28a**	**39b**	$Mg(OAc)_2$, Et_3N, HTO, THF	48	89	Radioactivity: 17.84 μCi; Specific activity: 0.66 Ci/mol
10	**37**	**40**	$Mg(OAc)_2$, Et_3N, HTO, THF	48	99	Radioactivity: 0.14 μCi; Specific activity: 0.50 Ci/mol

[a] See Figure 3.19.

However, even after two days of reaction time the deuterium content was not higher than 51% so in this system phase-transfer catalysis (PTC) conditions were not efficient.

Another concept of deuterium labelling of these molecules was inspired by previously demonstrated activation of the acidic protons in malonic esters, by metal salts.[105] Metal cations are chelated by the two carbonyl groups and the acidity of α-protons is enhanced. In this case lithium and magnesium salts gave similar good effect.

Application of lithium bromide to the labelling of the substrate **28a** increased the deuterium content up to 56%. The deuteration degree was still lower than expected, probably because of the low solubility of LiBr. Therefore, a magnesium(II) acetate was tested, resulting a very high deuterium incorporation, up to 79% of double deuterated isotopologue (Figure 3.19 and Table 13, Entries 5-8). This method was then applied in tritium labelling - firstly of the substrate **3** and next followed by the *N*-(3-oxo-5-diazirinedodecanoyl)-L-homoserine lactone **37**. As expected, very good yields and relatively good specific activities were obtained (Figure 3.19, Figure 3.20 and Table 13, Entries 9 and 10).

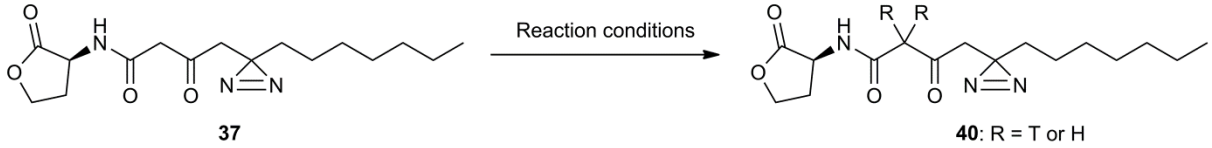


Figure 3.20 Tritium labelling of *N*-(3-oxo-5-diazirinedodecanoyl)-L-homoserine lactone **37**.

The *N*-(3-Oxo-5-diazirinedodecanoyl-[$^3H_2$])-L-homoserine lactone **40** possess a photoactivatable diazirine group and is easily detectable because it has the radioactive isotope. The diazirine moiety is located close to the hydrophilic part of the molecule, and affects the structure of a highly biologically active *N*-(3-oxododecanoyl)-L-homoserine lactones in a negligible way. Additional advantage includes introducing the tritium label in the last step of the synthesis, reducing the time of dealing with a radioactive compound. Such bifunctional molecule can be applied in photoaffinity labelling of a potential receptor for inter-kingdom signalling.

4 Experimental

4.1 General remarks

4.1.1 Materials and reagents

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 50 °C on 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 the Seebach solution (solution of molybdato phosphate: 5% phosphor molybdic acid in ethanol) and heated with a heat gun.[112] Normal-phase silica gel (Silica gel 60, 230-400 mesh, Merck) was used for flash chromatography.

4.1.2 Apparatus

4.1.2.1 Nuclear magnetic resonance (NMR) spectroscopy

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker-ACS-60, UltrashieldTM 500 Plus spectrometer at the room temperature. 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, quin = quintet, sept = septet, m = multiplet, dt = doublet of triplets, ddd = double doublet of doublets.

4.1.2.2 Infrared (IR) and Raman spectroscopy

IR and Raman spectra were recorded with the use of a Bruker Vertex 80 FTIR spectrometer (Bruker Optik GmbH, Ettlingen, Germany) with a single reflection "Golden Gate" diamond ATR sampling unit (Specac, UK). Additionally, Raman spectra were recorded with the use of a Bruker Senterra Raman microscope (Bruker Optics GmbH, Ettlingen, Germany), laser: excitation wavelength 785 nm, power 50 mw, 20x Objective (Olympus Mplan) = spot size 5

μm. IR spectra were recorded using a Bruker Hyperion 3000 FTIR Microscope, 15x reflection objective, spot size = 40μm. Absorption is reported as *v* values in cm^{-1}.

4.1.2.3 Mass spectrometry (ESI-TOF, ESI-MS/MS, HRMS)

Electrospray ionization time-of-flight (ESI-TOF) mass spectra were recorded on a ESI-TOF Mariner TM system (Applied Biosystems). ESI-MS/MS experiments were performed on the API 365 triple quadrupole mass spectrometer (PE Sciex, Concord, Ontario, Canada) using a turbo ion spray. In all ESI experiments samples were directly infused into the mass spectrometer using a Harvard syringe pump (Harvard Apparatus, South Natick, MA, USA). High-resolution mass spectra (HRMS) were determined on a Finnigan MAT90. The molecular fragments are quoted as a relation between mass and charge (*m/z*), the intensities as a percentage value relative to the intensity of the base signal (100%). The abbreviation $[M^+]$ refers to the molecular ion.

4.1.2.4 Ultraviolet–visible (UV-Vis) spectroscopy

UV-Vis spectroscopy was performed using (Agilent 8453 UV-Vis-Photospectrometer). Absorption was measured in tetrahydrofuran solution from 200 nm to 800 nm with a resolution of 1 nm in a 8,5 mm 1 ml UV micro cuvette (Brand GmbH, Wertheim, Germany) at the room temperature.

4.1.2.5 Optical rotation and elemental analysis (EA)

Optical rotations were measured in $CHCl_3$ on a Perkin–Elmer 241 polarimeter. $[\alpha]_D$ values are reported in 10^{-1} degrees cm^2 g^{-1} at Na, 589 nm. Elemental analysis (EA) was performed with Heraeus CHNO-Rapid and Elementar vario MICRO CUBE instruments.

4.1.2.6 Liquid Scintillation Counter measurements

Scintillation counting was carried out with a Wallac 1409 apparatus using a ‘‘me´lange scintillant III’’ cocktail from SDS company.

4.1.2.7 Analytical balance

Balance KERN ALJ310-4N, Kern & Sohn GmbH has been used.

4.2 Synthesis of *N*-acyl-L-homoserine lactones (AHLs) and their labelled derivatives

4.2.1 General procedures

4.2.1.1 General procedure for preparation of Meldrum's acid derivatives[18]

To a dry dichloromethane solution (10 mL per 1 mmol) containing 1 equiv. of the appropriate carboxylic acid derivative was added 4-(dimethylamino)pyridine (DMAP; 1.1 equiv.), 1.1 equiv. of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) or *N*,*N*'-dicyclohexylcarbodiimide (DCC) and Meldrum's acid (1 equiv.). The solution was stirred at room temperature overnight (16-20h). After this time the solvent 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 per 1 mmol), 1 M sodium hydrogen carbonate solution (20 mL per 1 mmol) and saturated sodium chloride solution (20 mL per 1 mmol). After drying over anhydrous sodium sulfate, the organic solution was concentrated to afford 1-oxoalkanoyl Meldrum's acid.

4.2.1.2 General procedure for preparation of *N*-(3-oxoalkanoyl)-L-homoserine lactones

4.2.1.2.1 Method A[18]

L-Homoserine lactone hydrobromide (1 equiv.) and triethylamine (1.2 equiv.) were added to a stirred solution of the appropriate acylated Meldrum´s acid (1 equiv.) in acetonitrile (20 mL per 1 mmol). The mixture was stirred at room temperature for 2 h, and then heated under reflux for 3 h (in case of the deuterated compounds, the reaction mixture was allowed to come to the room temperature and stirred overnight). After this time solvent was removed by rotary evaporation and any residue was redissolved in ethyl acetate. The organic solution was

sequentially washed with saturated $NaHCO_3$ and 1 M $KHSO_4$ solutions and brine. Combined aqueous layers were washed one more time with ethyl acetate. Combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude products were then purified by flash column chromatography (hexane/ethyl acetate 2:1–1:3) to give desired products.

4.2.1.2.2 Method B[13,110]

tert-Butyl-β-keto ester was stirred in trifluoroacetic acid (TFA)/dichloromethane (DCM) 1:1 mixture for 20 min. After solvent evaporation, the resulting β-keto acid (1 equiv.) was dissolved in 1,4-dioxane (2 mL per 0.19 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC; 1.4 equiv.), hydroxybenzotriazole (HOBt; 1 equiv.) and L-homoserine lactone hydrobromide (1 equiv.) were added alongside a few drops of water (1.6 mL per 0.19 mmol) at room temperature. Triethylamine (2 equiv.) was added and the solution was stirred at room temperature for 16 hours. The 1,4-dioxane was evaporated and the residue was solved with ethyl acetate, washed with water, dried over Na_2SO_4 and the solvent was removed by rotary evaporation. The crude product was purified by flash chromatography.

4.2.1.3 General procedure for preparation of *N*-acyl-L-homoserine lactones[18]

To a stirred solution of L-homoserine lactone hydrobromide (1 equiv.) in dichloromethane (20 mL per 1 mmol) at 0 °C was added and triethylamine (2.4 equiv.). The mixture was stirred at 0 °C for 0,5h when the decanoyl chloride (1 equiv.) was added dropwise over 5 min. The reaction mixture was allowed to come to the room temperature and was stirred for 4h. After this time the solution was 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 removed by rotary evaporation to leave the crude product that was purified by column chromatography on silica in ethyl acetate.

4.2.1.4 General procedure for preparation of deuterium and tritium labelled *N*-(3-oxoalkanoyl)-L-homoserine lactones

To a stirred solution of *N*-(3-oxododecanoyl)-L-homoserine lactone (1 equiv.) in tetrahydrofuran (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 room temperature for 24-48 h. After this time the solution was 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*.

4.2.1.5 General procedure for preparation of deuterium labelled *N*-acyl-L-homoserine lactones via catalytic reduction

The synthesis was performed in a quasi-solid state. Particular reagents were frozen in a liquid nitrogen layer-by-layer in the appropriate mixture of solvents in the screw cap test tubes.

4.2.1.5.1 Method A[72]

1st Layer: Terminally unsaturated carboxylic acid or *N*-acyl-L-homoserine lactone (1 equiv.) was dissolved in THF (0.5 mL per 0.1 mmol) and frozen in the liquid nitrogen. 2nd Layer: Then a palladium (II) acetate solution (0.2 equiv.) in THF (1 mL per 0.02 mmol), 300 μL of acetic acid (5.25 mmol per 0.02 mmol) and methanol or the mixture of methanol/water 4:1 (1.5 mL per 0.02 mmol) was added and again frozen. 3rd Layer: Next, more THF (0.5 mL per 0.1 mmol) was added and frozen. 4th Layer: Subsequently, 1 equiv. of sodium borohydride (sodium borodeuteride/sodium borotritide) was dissolved in 0.33-1 M water solution of sodium hydroxide (1 mL per 0.05 mmol) and closed tightly in the reaction vessel. The reaction mixture was allowed to come to the room temperature and was agitated for 16-24h. After that time the mixture was filtrated from black palladium residue and the solvent was removed by rotary evaporation. The residue was redissolved in ethyl acetate and washed sequentially with 1 M sodium hydrogen carbonate solution, 1 M potassium hydrogen sulfate solution and brine. The water solution was washed one more time with ethyl acetate. After

drying the organic solution over anhydrous sodium sulfate, the solvent was evaporated *in vacuo*.

4.2.1.5.2 Method B

1st Layer: Sodium borodeuteride (1 equiv.) was dissolved in 1 M solution of sodium hydroxide (1 mL per 0.1 mmol) and frozen in the liquid nitrogen. 2nd Layer: Next, more THF (0.5 mL per 0.1 mmol) was added and frozen. 3rd Layer: Subsequently, 0.2 equiv. of palladium (II) acetate solution in THF (1 mL per 0.02 mmol), acetic acid (300 µL per 0.1 mmol) and the mixture of methanol/water 4:1 (1 mL per 0.1 mmol) was added and again frozen. 4th Layer: Then *N*-(1-oxo-9-decenoyl)-L-homoserine lactone (1 equiv.) was dissolved in THF (2 mL per 0.1 mmol) and closed tightly in the reaction vessel. The reaction mixture was allowed to come to the room temperature and was agitated for 17h. After that time the mixture was filtrated from black palladium residue and the solvent was removed by rotary evaporation. The residue was redissolved in ethyl acetate and washed sequentially 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 removed by rotary evaporation.

4.2.1.5.3 Method C

1st Layer: *N*-(1-Oxo-9-decenoyl)-L-homoserine lactone (1 equiv.) was dissolved in THF (2 mL per 0.1 mmol) and frozen in the liquid nitrogen. 2nd Layer: Then the palladium (II) acetate solution (0.2 equiv.) in THF (2 mL per 0.02 mmol), 500 µL of acetic acid or acetic acid-d_4 (8.2 mmol per 0.02 mmol) and methanol-*d* or mixture of the methanol-*d*/D_2O 4:1 (1.25 mL per 0.1 mmol) was added and frozen. 3rd Layer: Next, more THF (1.5 mL per 0.1 mmol) was added and again frozen. 4th Layer: Subsequently 1-4 equiv. of sodium borodeuteride was dissolved in 0.15-0.33 M water solution of sodium hydroxide or sodium deuteroxide solution in D_2O or in methanol-*d* (1 mL per 0.05 mmol) and closed tightly in the reaction vessel. The reaction mixture was allowed to come to the room temperature and was agitated for 1-16h. After that time the mixture was filtrated from black palladium residue and the solvent was removed by rotary evaporation. The residue was redissolved in ethyl acetate and washed sequentially with 1 M sodium hydrogen carbonate solution, 1 M potassium hydrogen sulfate solution and saturated sodium chloride solution. The water solution was

washed one more time with ethyl acetate. After drying the organic solution over anhydrous sodium sulfate, the solvent was evaporated *in vacuo*.

4.2.1.6 General procedure for preparation of acyl chlorides[100]

To a solution of 9-decenoic acid (1 equiv.) in hexane (15 mL per 1 mmol) at room temperature, oxalyl chloride (2 equiv.) was added dropwise over 10 min, under nitrogen atmosphere. The reaction temperature was then increased to 40-45°C and the reaction was continued overnight. After this time the solvent was evaporated and the crude product was dried by a high vacuum pump to give 9-decenoyl chloride which was used without further purification in the next step.

4.2.1.7 General procedure for preparation of acetal protected *N*-(3-oxoalkanoyl)-L-homoserine lactones[102]

To a stirred solution of *N*-(3-oxo-11-dodecenoyl)-L-homoserine lactone (1 equiv.) in toluene (15 mL per 0.2 mmol), ethylene glycol (6 equiv.), *p*-toluenesulfonic acid (2.7 equiv.) and trimethylorthoformate (1 equiv.) were added. The mixture was stirred with heating under reflux for 5h and then continued at the room temperature overnight. After this time the solution was evaporated to dryness, and the residue was redissolved in ethyl acetate. The ethyl acetate solution was sequentially washed with 1M sodium hydroxide, 1M sodium hydrogen carbonate solution and brine. After drying over anhydrous sodium sulfate, the solvent was evaporated.

4.2.1.8 General procedure for deprotection of *N*-[3-(1', 3'-dioxolane)-alkanoyl]-L-homoserine lactones[104]

To an ice-cooled solution of *N*-[3-(1', 3'-dioxolane)-dodecanoyl-d_2]-L-homoserine lactone (1 equiv.) in dichloromethane (15 mL per 0.05 mmol), 60% perchloric acid solution (250 μL per 0.05 mmol) was added. The mixture was stirred at 0°C for 0.5 h and then at the room temperature for 2h. After this time the solution was evaporated to dryness, and the residue was redissolved in ethyl acetate. The ethyl acetate solution was washed with cold water and brine. After drying over anhydrous sodium sulfate, the solvent was evaporated.

4.2.1.9 General procedure for preparation of β-keto carboxylic acids

4.2.1.9.1 Method A[105]

1 equiv. of anhydrous lithium bromide (powder) was transferred to a 100 mL two neck flask that was fitted with an efficient stirrer, a septum inlet and a gas inlet tube with gas bubbler. Next, anhydrous diethyl ether (30 mL per 1 mmol) and 1.05 equiv. of bis(trimethylsilyl) malonate were added. 1.1 Equiv. of triethylamine was added dropwise and a precipitate was formed almost instantly. After stirring for 10 minutes, the flask was cooled to 0 °C and 1 equiv. of octanoyl chloride was added (dropwise, slowly). After stirring in room temperature for 1 hour, it was quenched with cold saturated aqueous $NaHCO_3$ (15 mL per 1 mmol) and stirred for 10 minutes in an ice bath. The aqueous layer was separated and was acidified to pH 2-3 by the dropwise addition of cold 1 M HCl. Resulting precipitate was extracted with ethyl acetate. The ether layer was washed several times with saturated aqueous $NaHCO_3$. After drying the combined organic solution over anhydrous sodium sulfate, the solvent was evaporated *in vacuo*.

4.2.1.9.2 Method B[107-109]

To a stirred solution of 1 equiv. of appropriate ester in a mixture of THF and water 1:1, 3 equiv. of $LiOH.H_2O$ were added. The reaction mixture was heated under reflux (2 x 12h) and then stirred at room temperature overnight (2 x). After that, the solution was cooled to 0 °C and carefully acidified to pH 2-3 with 1 M HCl. The reaction mixture was extracted with ethyl acetate. The combined extracts were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure.

4.2.1.10 General procedure for preparation of β-keto esters

4.2.1.10.1 Method A[107-109]

Methyl acetate (2 equiv.) was added to a stirred solution of lithium diisopropylamide (LDA) (2.0 M solution in THF, 2 equiv.) in dry THF (20 mL per 1 mmol) at -78 °C. The solution was stirred at -78 °C for 15 min. In next step the octanoyl chloride (1 equiv.) was added. The reaction was allowed to warm to room temperature and was stirred for 4 hours. THF was evaporated and reaction mixture was diluted with 1 M HCl, and extracted with ethyl acetate.

The combined organic extracts were dried over anhydrous Na_2SO_4 and the solvents were removed under reduced pressure.

4.2.1.10.2 Method B[13,110]

Solution 1: To a solution of appropriate carboxylic acid (1.1 equiv.) in dry THF (2.5 mL per 1.1 mmol) under nitrogen atmosphere, 1,1'-carbonyldiimidazole (CDI; 1.3 equiv.) was added at room temperature. The mixture was stirred at room temperature for 4 hours. Solution 2: To a solution of mono *tert*-butyl malonate (1.3 equiv.) in dry THF (2.5 mL) at 0 ºC under nitrogen atmosphere, isopropyl magnesium chloride (2 M in THF, 1.2 mL, 2.4 equiv.) was added dropwise. After 30 minutes at 0 ºC the solution was heated at 50 ºC for 30 minutes and cooled again to 0 ºC. The solution 1 was added via cannula. The mixture was allowed to warm to room temperature. After stirring for 16 hours, it was quenched with HCl 1 M (6 mL per 1.1 mmol). The aqueous phase was extracted with ethyl acetate. The combined organic layers were washed with $NaHCO_3$, brine, dried over Na_2SO_4 and concentrated under reduced pressure.

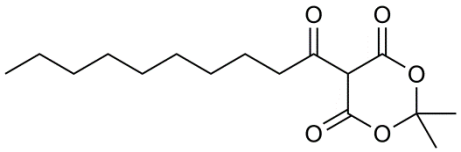
4.2.1.11 General procedure for preparation of 3-diazirine carboxylic acids[13,110]

Anhydrous ammonia (100 mL per 17 mmol) was condensed into a round bottomed flask. 3-Oxodecanoic acid (1 equiv.) was dissolved in a small amount of anhydrous methanol (10 mL per 17 mmol) and added to the flask. The mixture was stirred at -35-40 ºC for 5 h. The solution was cooled with dry ice, and a solution of hydroxylamine-*O*-sulfonic acid (1.1 equiv.) in anhydrous methanol (5 mL per 19 mmol) was added over a period of 30 min. The dry ice bath was removed, and the mixture was refluxed with stirring at -35 ºC for 1 h. The mixture was allowed to warm slowly to room temperature and stirred overnight. The ammonia was then allowed to evaporate. The resulting slurry was filtered and the filter cake washed with several portions of methanol. The combined solution was concentrated *in vacuo*. The crude aziridine residue was dissolved in dichloromethane (10 mL per 17 mmol) and treated with triethylamine (1.2 equiv.). A solution of iodine (1.3 equiv.) in dichloromethane (20 mL per 23 mmol) was slowly added with stirring until the appearance of a persistent orange-brown colour.

4.2.2 Synthesis and characterization of the target compounds

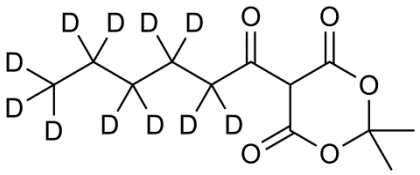
4.2.2.1 Synthesis and characterization of acylated Meldrum's acids and their deuterated derivatives

1-Oxo-decanoyl Meldrum's acid



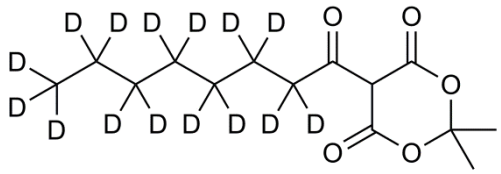
This compound was obtained according to procedure 4.2.1.1 from 344 mg of decanoic acid (2 mmol), 288 mg of Meldrum's acid (2 mmol), 268.5 mg of DMAP (2.2 mmol), 341 mg of EDC (2.2 mmol) in 20 mL of dry dichloromethane, as a yellow oil with an isolated yield of 582 mg (60%). R_f = 0.56 (hexane/ethyl acetate 3:1); ESI-TOF MS: m/z [M^+ - H] (%) = 297 (100), (M^+ - H, required m/z 297).

1-Oxo-hexanoyl-d_{11} Meldrum's acid **14a**



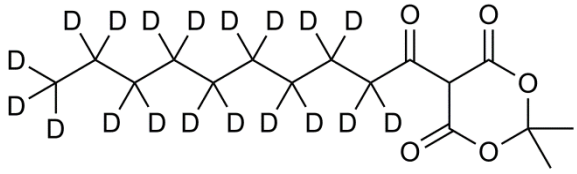
This compound was obtained according to procedure 4.2.1.1 from 95 mg of hexanoic-d_{11} acid (0.75 mmol), 108 mg of Meldrum's acid (0.75 mmol), 100 mg of DMAP (0.82 mmol) and 127 mg of EDC (0.82 mmol) in 20 mL of dry dichloromethane, as a yellow oil with an isolated yield of 170 mg (90%). R_f = 0.39 (hexane/ethyl acetate 3:1); ESI-TOF MS: m/z [M^+ - H] (%) = 252 (100), 251 (64) (M^+ - H, required m/z 252); Deuterium distribution: 61% d_{11}, 39% d_{10}.

1-Oxo-octanoyl-d_{15} Meldrum's acid **14b**



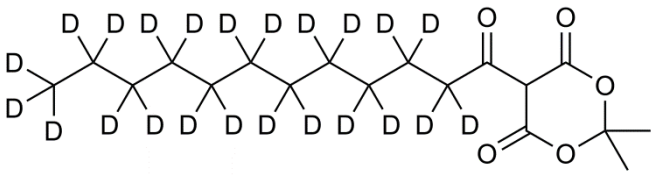
This compound was obtained according to procedure 4.2.1.1 from 446 µL octanoic-d_{15} acid (1.57 mmol), 226 mg of Meldrum's acid (1.57 mmol), 268 mg of DMAP (1.72 mmol) and 268 mg of EDC (1.72 mmol) in 10 mL of dry dichloromethane as a yellow oil with an isolated yield of 410 mg (92%). R_f = 0.43 (hexane/ethyl acetate 3:1); ESI-TOF MS: m/z [M^+ - H] (%) = 284 (100), 283 (35) (M^+ - H, required m/z 284); Deuterium distribution: 74% d_{15}, 26% d_{14}.

1-Oxo-decanoyl-d_{19} Meldrum's acid **14c**



This compound was obtained according to procedure 4.2.1.1 from 382 mg of decanoic-d_{19} acid (2 mmol), 288 mg of Meldrum's acid (2 mmol), 268 mg of DMAP (2.2 mmol) and 341 mg of EDC (2.2 mmol) in 20 mL of dry dichloromethane as a yellow oil with an isolated yield of 627 mg (98%). R_f = 0.53 (hexane/ethyl acetate 3:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.71 (s, 6 H, 2 x CH_3), 2.52 (s, 0.32 H, CHD), 3.20 (s, 1 H, COCHCO) ppm; ESI-TOF MS: m/z [M^+ - H] (%) = 316 (100), 315 (32) (M^+ - H, required m/z 316); Deuterium distribution: 76% d_{19}, 24% d_{18}.

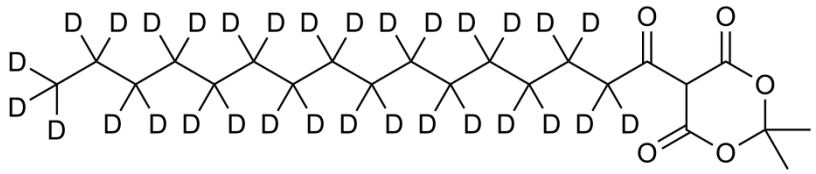
1-Oxo-dodecanoyl-d_{23} Meldrum's acid **14d**



This compound was obtained according to procedure 4.2.1.1 from 223 mg of dodecanoic-d_{23} acid (1 mmol), 144 mg of Meldrum's acid (1 mmol), 134 mg of DMAP (1.1 mmol), 170 mg of EDC (1.1 mmol) in 20 mL of dry dichloromethane, as a yellow oil with an isolated yield of

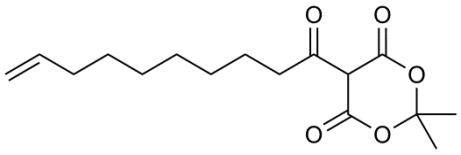
289 mg (83%). R_f = 0.69 (hexane/ethyl acetate 3:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.72 (s, 6 H, 2 x CH_3), 2.51 (s, 0.21 H, CHD), 3.40 (s, 1 H, COCHCO) ppm; ESI-TOF MS: m/z [M^+ - H] (%) = 348 (100), 347 (21) (M^+ - H, requires m/z 348); Deuterium distribution: 83% d_{23}, 17% d_{22}.

1-Oxo-hexadecanoyl-d_{31} Meldrum's acid **14e**



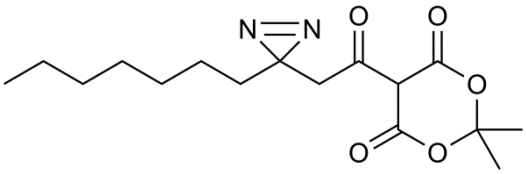
This compound was obtained according to procedure 4.2.1.1 from 35 mg of pentadecanoic-d_{31} acid (0.125 mmol), 18 mg of Meldrum's acid (0.125 mmol), 17 mg of DMAP (0.137 mmol) and 31 mg of EDC (0.137 mmol) in 15 mL of dry dichloromethane as a yellow oil with an isolated yield of 36 mg (77%). R_f = 0.78 (hexane/ethyl acetate 3:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.74 (s, 6 H, 2 x CH_3), 2.20 (s, 0.5 H, CHD), 3.10 (s, 1 H, COCHCO) ppm; ESI-TOF MS: m/z [M^+ - H] (%) = 412 (100), 411 (50) (M^+ - H, requires m/z 412); Deuterium distribution: 66% d_{31}, 34% d_{30}.

1-Oxo-9-decenoyl Meldrum's acid **25**



This compound was obtained according to procedure 4.2.1.1 from 406.6 µL of 9-decenoic acid (2 mmol), 288 mg of Meldrum's acid (2 mmol), 268.5 mg of DMAP (2.2 mmol) and 341 mg of EDC (2.2 mmol) in 20 mL of dry dichloromethane as a yellow oil with an isolated yield of 355 mg (60%). R_f = 0.66 (hexane/ethyl acetate 2:3); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.30-1.34 (m, 10 H, 5 x CH_2), 1.60-1.62 (m, 6 H, 2 x CH_3), 2.0-2.20 (m, 2 H, CH_2=CHC$\underline{H}_2$), 2.36-2.39 (m, 2 H, CH_2CO), 4.96 (dd, ^1J = 14.0 Hz, ^2J = 18.5 Hz, 2 H, C$\underline{H}_2$=CHCH_2), 5.79-5.82 (m, 2 H, CH_2=C$\underline{H}CH_2$) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 22.1, 25.9 (2C), 28.9, 29.1 (2C), 33.7, 40.1, 41.8, 101.7, 114.1, 139, 166.7, 166.8, 201 ppm; ESI-TOF MS: m/z [M^+ - H] (%) = 295 (100), (M^+ - H, required m/z 295).

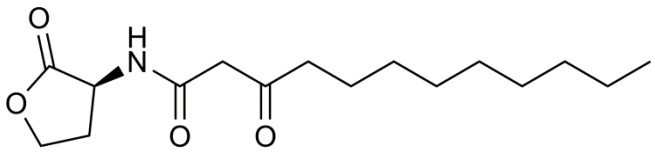
1-Oxo-3-diazirinedecanoyl Meldrum's acid **36**



This compound was obtained according to procedure 4.2.1.1 from 33 mg of 3-diazirinedecanoic acid (0.17 mmol), 24 mg of Meldrum's acid (0.17 mmol), 22 mg of DMAP (0.18 mmol) and 35 mg of EDC (0.18 mmol) in 30 mL of dry dichloromethane as an orange-brown oil with an isolated yield of 32 mg (60%). R*f* = 0.38 (hexane/ethyl acetate 1:5); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.87 (t, ^{1}J = 6.9 Hz, 3 H, CH_3), 1.00-1.20 (m, 2 H, $C\underline{H}_2CH_2CN_2$), 1.12-1.21 (m, 8 H, 4 x CH_2), 1.40-1.58 (m, 2 H, $C\underline{H}_2CN_2$), 1.68 (s, 6 H, 2 x CH_3), 3.02 (s, 2 H, $CN_2C\underline{H}_2CO$), 3.95 (s, 1 H, $COC\underline{H}CO$) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.1 (s, C'-10, CH_3), 22.6 (s, C'-9, CH_2), 23.9 (s, C'-8, CH_2), 25.9 (s, C'-7, CH_2), 26.0 (s, C-4, 2 x CH_3), 29.0 (s, C'-6, CH_2), 29.9 (s, C'-5, CH_2), 31.7 (s, C'-4, CH_2), 32.4 (s, C'-2, CH_2), 33.7 (s, C-1, CH), 39.6 (s, C'-3, CN_2), 101.7 (s, C-3, 2 x CH_3), 175.1 (s, C-1, $(COO\underline{C})_2$), 206.3 (s, C'-1, C=O) ppm; IR (ATR), υ = 2917, 1720, 1687, 1370, 1222, 1035, cm^{-1}; Raman, υ = 2893, 1686, 1592, 1298, 1067, 889 cm^{-1}; UV (THF): λ_{max} = 270, ε = 244 M^{-1} cm^{-1}, 343 nm, ε = 90 M^{-1} cm-1; ESI-TOF MS: m/z [M^+ - H] (%) = 323 (100), (M^+ - H, required m/z 323).

4.2.2.2 Synthesis and characterization of *N*-(3-oxoalkanoyl)-L-homoserine lactones and their deuterium and photoactivatable AHL derivatives

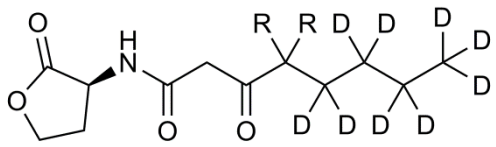
N-(3-Oxododecanoyl)-L-homoserine lactone (3OC12-HSL; **3***)*



This compound was obtained according to procedure 4.2.1.2.1 Method A from 1031 mg of 1-oxodecanoyl Meldrum's acid (3.46 mmol), 476 mg of L-homoserine lactone hydrochloride (3.46 mmol) and 595 µL of triethylamine (4.15 mmol), in 20 mL of acetonitrile. The crude residue was purified by flash column chromatography (hexane/ethyl acetate 2:1–1:3) to give desired product as a white solid with an isolated yield of 515 mg (50%). Mp 84 °C; R_f = 0.53

(hexane/ethyl acetate 1:3); ^{1}H NMR[113] (500 MHz, $CDCl_3$): δ = 0.88 (t, *J* = 6.5 Hz, 3 H, CH_3), 1.22-1.40 (m, 12 H, 6 x CH_2), 1.45-1.59 (m, 2 H, C$\underline{H}_2$CH_2CO), 2.22-2.31 (m, 1 H, 3α-H), 2.52 (t, ^{1}J = 7.4 Hz, 2 H, C$\underline{H}_2$CO), 2.72-2.81 (m, 1 H, 3β-H), 3.47 (s, 2 H, COC$\underline{H}_2$CO), 4.28 (m, 1 H, 4α-H), 4.48 (td, ^{1}J = 9.1 Hz, ^{2}J = 1.3 Hz, 1 H, 4β-H), 4.56-4.71 (m, 1 H, 2-H), 7.69-7.71 (m, 1 H, NH) ppm; ESI-TOF MS: m/z [M^+ - H] (%) = 316 (100), 315 (32) (M^+ - H, required m/z 316).

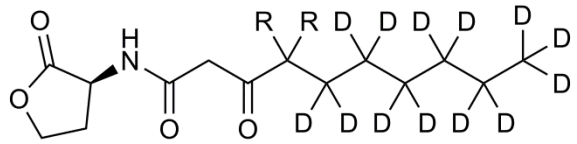
N-(3-Oxooctanoyl-d_9)-L-homoserine lactone **16a**



This compound was obtained according to procedure 4.2.1.2.1 Method A from 201 mg of 1-oxo-hexanoyl-d_{11} Meldrum's acid (0.8 mmol), 146 mg of L-homoserine lactone hydrochloride (0.8 mmol) and 133 μL of triethylamine (0.96 mmol) in 20 mL of acetonitrile. The crude residue was purified by flash column chromatography (hexane/ethyl acetate 2:1–1:3) to give desired product as a white solid with an isolated yield of 140 mg (70%). Mp[18] 82 °C R_f = 0.25 (hexane/ethyl acetate 1:4); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.26 (dddd, ^{1}J = 12.2 Hz, ^{2}J = 11.3 Hz, ^{3}J = 8.9 Hz, ^{4}J = 3.5 Hz, 1 H, 3α-H), 2.52 (m, 1 H, C$\underline{H}$DCO), 2.76 (dddd, ^{1}J = 12.5 Hz, ^{2}J = 8.6 Hz, ^{3}J = 6.1 Hz, ^{4}J = 1.3 Hz, 1 H, 3β-H), 3.48 (s, 2 H, COC$\underline{H}_2$CO), 4.28 (ddd, ^{1}J = 11.0 Hz, ^{2}J = 9.3 Hz, ^{3}J = 6.1 Hz, 1 H, 4α-H), 4.49 (td, ^{1}J = 9.1 Hz, ^{2}J = 1.3 Hz, 1 H, 4β-H), 4.61 (ddd, ^{1}J = 11.5 Hz, ^{2}J = 8.8 Hz, ^{3}J = 6.7 Hz, 1 H, 2-H), 7.70 (d, ^{1}J = 5.7 Hz, 1 H, NH) ppm; ^{13}C NMR[113] (125 MHz, $CDCl_3$): δ = 12.7 (sept, ^{1}J = 19.0 Hz, C'-8, CD_3), 21.0 (quin, ^{1}J = 18.9 Hz, C'-7, CD_2), 22.1 (quin, ^{1}J = 19.6 Hz, C'-6, CD_2), 29.4 (s, C-3, CH_2), 29.9 (m, ^{1}J = 18.9, C'-5, CD_2), 43.1 (t, ^{1}J = 19.2, C'-4, CHD), 43.4 (s, C'-4, CHD), 48.6 (s, C-2, CH), 48.9 (s, C'-2, CO$\underline{C}H_2$CO), 65.9 (s, C-4, $\underline{C}H_2$CO), 166.6 (s, C'-1, C=O), 175.1 (s, C-1, C=O), 206.4 (s, C'-3, C=O) ppm; IR (ATR),[114] υ = 3284, 3084, 2959, 2924, 2898, 2224, 2110, 1783, 1720, 1643, 1545, 1349, 1220, 1183, 1003, 946, 802 cm^{-1}; Raman,[115] υ = 2921, 2200, 2122, 2078, 1782, 1713, 1645, 1497, 1454, 1415, 1341, 1273, 1221, 1121, 1056, 996, 876, 786, 680, 564 cm^{-1}; $[\alpha]^{20}_D$ = +10.5 (c 0.25 $CHCl_3$);[114] ESI-TOF MS: m/z [M^+ + H] (%) = 251 (100), 252 (28), 253 (14) (M^+ + H, required m/z 251); ESI-MS/MS: m/z [%] = 251 (100) [M^+ + H], 150 (47) [M^+ + H – $C_4H_7O_2N^+$], 102 (67) [$C_4H_8O_2N$+], 74 (19), 56 (14); HRMS ($C_{12}H_{10}D_9NO_4$):

calc: 250.1882 found: 250.1879; EA ($C_{12}H_{10}D_9NO_4$): calc: C, 57.57; H, 7.65; N, 5.59; found: C, 57.48; H, 7.66; N, 5.35; Deuterium distribution: 10% d_{11}, 20% d_{10}, 70% d_9.

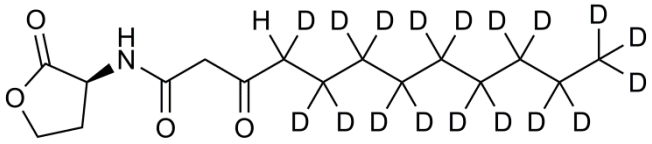
N-(3-Oxodecanoyl-d_{13})-L-homoserine lactone **16b**



R = H or D

This compound was obtained according to procedure 4.2.1.2.1 Method A from 407 mg of 1-oxooctanoyl-d_{15} Meldrum's acid (1.43 mmol), 260 mg of L-homoserine lactone hydrochloride (1.43 mmol) and 239 µL of triethylamine (1.72 mmol), in 25 mL of acetonitrile, as a white solid with an isolated yield of 259 mg (64%); R_f = 0.35 (hexane/ethyl acetate 1:4); ESI-TOF MS: m/z [M^+ + H] (%) = 283 (100), 285 (38), 284 (20) (M^+ + H, required m/z 285); Deuterium distribution: 24% d_{13}, 13% d_{12}, 63% d_{11}.

N-(3-Oxododecanoyl-d_{17})-L-homoserine lactone **16c**

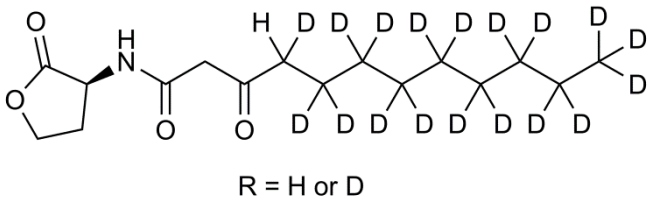


R = H or D

This compound was obtained according to procedure 4.2.1.2.1 Method A from 253 mg of 1-oxo-decanoyl-d_{19} Meldrum's acid (0.8 mmol), 143 mg of L-homoserine lactone hydrochloride (0.8 mmol) and 133 µL of triethylamine (0.96 mmol), in 20 mL of acetonitrile. The crude residue was purified by flash column chromatography (hexane/ethyl acetate 2:3) to give desired product as a white solid with an isolated yield of 150 mg (60%). Mp[18] 88 °C [5]; R_f = 0.27 (hexane/ethyl acetate 2:3). ^{1}H NMR[113] (500 MHz, $CDCl_3$): δ = 2.25 (dddd, ^1J = 12.3 Hz, ^2J = 11.3 Hz, ^3J = 8.9 Hz, ^4J = 3.5 Hz, 1 H, 3α-H), 2.52 (m, 1 H, C<u>H</u>DCO), 2.77 (dddd, ^1J = 12.3 Hz, ^2J = 8.7 Hz, ^3J = 6.0 Hz, ^4J = 1.3 Hz, 1 H, 3β-H), 3.48 (s, 2 H, COC<u>H$_2$</u>CO), 4.29 (ddd, ^1J = 11.0 Hz, ^2J = 9.3 Hz, ^3J = 6.1 Hz, 1 H, 4α-H), 4.49 (td, ^1J = 9.1 Hz, ^2J = 1.3 Hz, 1 H, 4β-H), 4.61 (ddd, ^1J = 11.5 Hz, ^2J = 9.0 Hz, ^3J = 6.7 Hz, 1 H, 2-H), 7.69 (d, ^1J = 5.7, 1 H, NH) ppm; ^{13}C NMR[113] (125 MHz, $CDCl_3$): δ = 12.9 (sept, ^1J = 19.0 Hz, C'-12, CD_3), 21.3 (quin, ^1J = 19.0 Hz, C'-11, CD_2), 22.3 (quin, ^1J = 20.0 Hz, C'-5, CD_2), 22.6 (quin, ^1J = 20.0

Hz, C'-10, CD_2), 27.6 (m, ^1J = 19.0 Hz, C'-6, C'-7, C'-8, CD_2), 29.7 (s, C-4, CH_2), 30.4 (quin, ^1J = 19.0, C'-9, CD_2), 43.3 (t, ^1J = 19.3, C'-4, CHD), 43.6 (s, C'-4, CHD), 48.3 (s, C'-2, CO<u>C</u>H_2CO), 49.0 (s, C-2, CH), 65.9 (s, C-4, CH_2CO), 166.5 (s, C'-1, C=O), 175.0 (s, C-1, C=O), 206.5 (s, C'-3, C=O) ppm; IR (ATR),[114] υ = 3288, 3057, 2911, 2844, 2196, 2092, 1774, 1714, 1640, 1543, 1350, 1273, 1228, 1174, 1005, 945, 800 cm^{-1}; Raman,[115] υ = 3299, 3074, 2945, 2844, 2200, 2109, 2071, 1779, 1714, 1648, 1500, 1444, 1408, 1348, 1275, 1249, 1226, 1141, 1052, 992–923, 801, 720, 660, 570 cm^{-1}; $[\alpha]^{20}_D$ = +18.0 (c 0.1 $CHCl_3$);[114] ESI-TOF MS: m/z [M^++H] (%) = 315 (100), 316 (36), 317 (10) (M^++H, required m/z 315); ESI-MS/MS: m/z [%] = 315 (100) [M^++H], 214 (43) [M^++H – $C_4H_7O_2N$+], 102 (47) [$C_4H_8O_2N^+$], 74 (24), 56 (14); HRMS ($C_{16}H_{10}D_{17}NO_4$): calc: 314.3012 found: 314.3009; EA ($C_{16}H_{10}D_{17}NO_4$): calc: C, 61.10; H, 8.59; N, 4.45; found: C, 60.87; H, 8.51; N, 4.33; Deuterium distribution: 7% d_{19}, 25% d_{18}, 68% d_{17}.

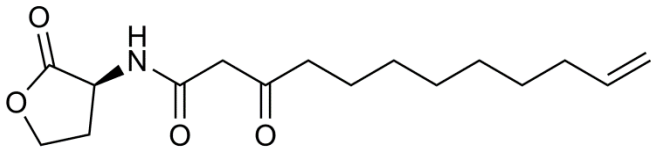
N-(3-Oxotetradecanoyl-d_{21})-L-homoserine lactone **16d**



This compound was obtained according to procedure 4.2.1.2.1 Method A from 279 mg of 1-oxo-dodecanoyl-d_{23} Meldrum's acid (0.8 mmol), 143 mg of L-homoserine lactone hydrochloride (0.8 mmol) and 133 µL of triethylamine (0.96 mmol), in 20 mL of acetonitrile. The crude residue was purified by flash column chromatography (hexane/ethyl acetate 1:4) to give desired product as a white solid with an isolated yield of 146 mg (53%). Mp[18] 94 °C; R_f = 0.25 (hexane/ethyl acetate 1:4). ^{1}H NMR[113] (500 MHz, $CDCl_3$): δ = 2.25 (dddd, ^1J = 11.9 Hz, ^2J = 11.4 Hz, ^3J = 8.9 Hz, ^4J = 3.3 Hz, 1 H, 3α-H), 2.52 (m, 1 H, CHDCO), 2.77 (dddd, ^1J = 12.5 Hz, ^2J = 8.7 Hz, ^3J = 6.1 Hz, ^4J = 1.1 Hz, 1 H, 3β-H), 3.48 (s, 2 H, COC<u>H</u>$_2$CO), 4.29 (ddd, ^1J = 11.0 Hz, ^2J = 9.3 Hz, ^3J = 6.1 Hz, 1 H, 4α-H), 4.49 (td, ^1J = 9.1 Hz, ^2J = 1.0 Hz, 1 H, 4β-H), 4.61 (ddd, ^1J = 11.5 Hz, ^2J = 8.7 Hz, ^3J = 6.7 Hz, 1 H, 2-H), 7.70 (d, ^1J = 5.7, 1 H, NH) ppm; ^{13}C NMR[113] (125 MHz, $CDCl_3$): δ = 13.0 (sept, ^1J = 19 Hz, C'-14, CD_3), 21.4 (quin, ^1J = 19.1 Hz, C'-13, CD_2), 23.0 (quin, ^1J = 19.6 Hz, C'-12, CD_2), 23.7 (quin, ^1J = 19.4 Hz, C'-11, CD_2), 28.2 (m, ^1J = 19 Hz, C'-7, C'-8, C'-9, CD_2), 29.8 (s, C-3, CH_2), 30.6 (quin, ^1J = 18.5, C'-10, CD_2), 33.4 (quin, ^1J = 19.3, C'-5, C'6, CD_2), 43.3 (t, ^1J = 19.1, C'-4, CHD), 43.6 (s, C'-4, CHD), 48.3 (s, C-2, CH), 49.1 (s, C'-2, CO<u>C</u>H_2CO), 65.9 (s, C-4, <u>C</u>H_2CO),

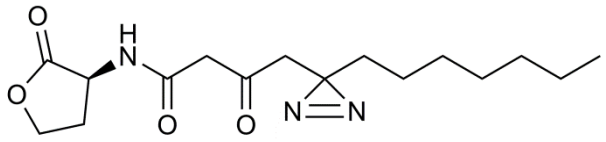
166.9 (s, C'-1, C=O), 179.5 (s, C-1, C=O), 209.8 (s, C'-3, C=O) ppm; IR (ATR),[114] υ = 3285, 3080, 2946, 2887, 2198, 2105, 1779, 1715, 1640, 1544, 1354–1279, 1181, 1006, 949, 802 cm^{-1}; Raman,[115] υ = 2943, 2209, 2100, 2078, 1776, 1716, 1645, 1505, 1411, 1408, 1346, 1276, 1251, 1142, 1051, 987, 919, 822, 797, 671, 567 cm^{-1}; $[\alpha]^{20}_{D}$ = +7.0 (c 0.25 $CHCl_3$);[114] ESI-TOF MS: m/z [M^++H] (%) = 347 (100), 348 (42), 349 (12) (M^++H), requires m/z 347); ESI-MS/MS: m/z [%] = 347 (100) [M^++H], 246 (57) [M^++H – $C_4H_7O_2N^+$], 102 (64) [$C_4H_8O_2N^+$], 74 (29), 56 (10); HRMS ($C_{18}H_{10}D_{21}NO_4$): calc. 346.3574 found: 346.3573; EA ($C_{18}H_{10}D_{21}NO_4$): calc: C, 62.38; H, 9.01; N, 4.04; found: C, 62.17; H, 9.02; N, 3.79. Deuterium distribution: 8% d_{23}, 27% d_{22}, 65% d_{21}.

N-(3-Oxo-9-dodecenoyl)-L-homoserine lactone **26**



This compound was obtained according to procedure 4.2.1.2.1 Method A from 384 mg of 1-oxo-9-decenoyl Meldrum's acid (1.29 mmol), 235 mg of L-homoserine lactone hydrochloride (1.29 mmol) and 222.2 µL of triethylamine (1.55 mmol), in 30 mL of acetonitrile. The crude residue was purified by flash column chromatography (hexane/ethyl acetate 2:3) to give desired product as a white solid with an isolated yield of 211 mg (55%).; R_f = 0.34 (hexane/ethyl acetate 2:3). ^{1}H NMR[113] (500 MHz, $CDCl_3$): δ = 1.24-1.39 (m, 8 H, 4 x CH_2), 1.58-1.66 (m, 2 H, $COCH_2C\underline{H_2}$), 2.06 (dd, ^1J = 14.45 Hz, ^2J = 6.86 Hz, 2 H, CH_2=$CH_2C\underline{H_2}$), 2.26 (dddd, ^1J = 11.6 Hz, ^2J = 11.0, ^3J = 8.9 Hz, ^4J = 3.4 Hz, 1 H, 3α-H), 2.54 (t, ^1J = 7.4 Hz, 2 H, $COCH_2COC\underline{H_2}$), 2.77 (dddd, ^1J = 13.2 Hz, ^2J = 8.7 Hz, ^3J = 5.8 Hz, ^4J = 1.3 Hz, 1 H, 3β-H), 3.48 (s, 2 H, $COC\underline{H_2}CO$), 4.29 (ddd, ^1J = 11.0 Hz, ^2J = 9.3 Hz, ^3J = 4.9 Hz, 1 H, 4α-H), 4.49 (td, ^1J = 9.1 Hz, ^2J = 1.2 Hz, 1 H, 4β-H), 4.61 (ddd, ^1J = 11.5 Hz, ^2J = 8.7 Hz, ^3J = 6.7 Hz, 1 H, 2-H), 4.92-5.12 (m, 2 H, $C\underline{H_2}$=$CHCH_2$), 5.79-5.98 (m, 1 H, CH_2=$C\underline{H}CH_2$), 7.68 (d, ^1J = 5.6 Hz, 1 H, NH) ppm; ^{13}C NMR[113] (125 MHz, $CDCl_3$): δ = 23.3 (s, C'-9, CH_2), 28.8-28.9 (m, C'-8, CH_2), 29.2 (s, C'-7, CH_2), 29.8 (s, C'-6, CH_2), 33.7 (s, C'-10, CH_2), 43.9 (s, C'-4, CH_2), 48.1 (s, C-2, CH), 49.0 (s, C'-2, CH_2), 65.8 (s, C-4,CH_2), 114.2 (s, C'-12, CH_2), 139.0 (s, C'-11, CH), 166.3 (s, C'-1, C=O), 174.8 (s, C-1, C=O), 206.5 (s, C'-3, C=O) ppm; ESI-TOF MS: m/z [M^+ + H] (%) = 296 (100), (M^+ + H, required m/z 296).

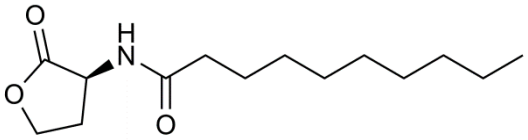
N-(3-Oxo-5-diazirinedodecanoyl)-L-homoserine lactone **37**



This compound was obtained according to procedure 4.2.1.2.2 Method B from 7.4 mg of 1-*tert*-butyl-3-oxo-5-diazirine-dodecanoate (0.025 mmol), 6.7 mg of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC; 0.035 mmol), 3.4 mg of hydroxybenzotriazole (HOBt; 0.025 mmol), 4.5 mg of L-homoserine lactone hydrobromide (0.025 mmol), 7 µL of triethylamine (0.05 mmol) and 540 µL mixture of TFA/DCM 1:1 in 3 mL of 1,4-dioxane and 1.6 mL water. The crude residue was purified by flash column chromatography (hexane/ethyl acetate 1:3) to give desired product as a yellow oil with an isolated yield of 2 mg (25%). R_f = 0.66 (hexane/ethyl acetate 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, C$\underline{H}_2$CH_2CN_2), 1.22-1.39 (m, 8 H, 4 x C$\underline{H}_2$), 1.50 (t, ^1J = 8.1 Hz, 2 H, C$\underline{H}_2$CN_2), 2.18-2.31 (m, 1 H, 3α-H), 2.49 (s, 2 H, COC$\underline{H}_2$CN_2), 2.70-2.89 (m, 1 H, 3β-H), 3.51 (s, 2 H, COC$\underline{H}_2$CO), 4.12-4.30 (m, 1 H, 4α-H), 4.51 (td, ^1J = 8.9 Hz, ^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 [M^+ + H] (%) = 346 (100), (M^+ + Na, required m/z 346).

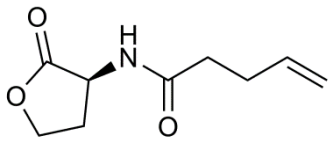
4.2.2.3 Synthesis and characterization of *N*-acyl-L-homoserine lactones

N-Decanoyl-L-homoserine lactone



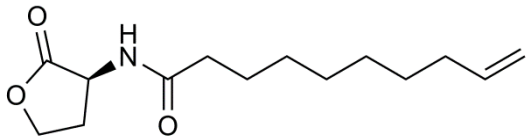
The target product was obtained according to procedure 4.2.1.3 from 206 µL of decanoyl chloride (1 mmol), 182 mg of L-homoserine lactone hydrochloride (1 mmol) and 333 µL of triethylamine (2.4 mmol), in 20 mL of dichloromethane, as a white solid with an isolated yield of 220 mg (86%); R_f = 0.59 (hexane/ethyl acetate 1:4). ^{1}H NMR[18] (500 MHz, $CDCl_3$): δ = 0.79-0.90 (m, 3 H, CH_3), 1.16-1.30 (m, 12 H, 6 x CH_2), 1.45-1.60 (m, 2 H, $COCH_2C\underline{H_2}$), 2.10-2.22 (m, 1 H, 3α-H), 2.21 (t, ^1J = 7.4 Hz, 2 H, $COC\underline{H_2}$), 2.70-2.89 (m, 1 H, 3β-H), 4.09-4.28 (m, 1 H, 4α-H), 4.30-4.46 (m, 1 H, 4β-H), 4.50-4.68 (m, 1 H, 2-H), 5.90-6.01 (m, 1 H, NH) ppm; ESI-TOF MS: m/z [M^+ + H] (%) = 256 (100), (M^+ + H, required m/z 256).

N-(1-Oxo-4-pentenoyl)-L-homoserine lactone **20a**



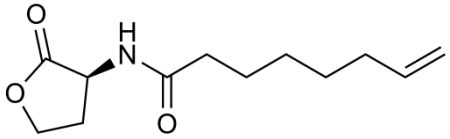
This compound was obtained according to procedure 4.2.1.3 from 109.8 µL of pentenoyl chloride (1 mmol), 182 mg of L-homoserine lactone hydrochloride (1 mmol) and 333 µL of triethylamine (2.4 mmol), in 20 mL of dichloromethane, as a white solid with an isolated yield of 100 mg (55%).; R_f = 0.30 (hexane/ethyl acetate 2:3). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.50-1.69 (m, 2 H, $COCH_2C\underline{H_2}$), 2.12 (dddd, ^1J = 19.5 Hz, ^2J = 11.6 Hz, ^3J = 8.8, ^4J = 4.9, 1 H, 3α-H), 2.29-2.40 (m, 2 H, $COC\underline{H_2}$), 2.88 (ddd, ^1J = 13.9 Hz, ^2J = 8.4 Hz, ^3J = 5.83 Hz, 1 H, 3β-H), 4.28 (ddd, ^1J = 11.3 Hz, ^2J = 9.4 Hz, ^3J = 5.8 Hz, 1 H, 4α-H), 4.38-4.49 (m, 1 H, 4β-H), 4.50-4.68 (m, 1 H, 2-H), 5.06 (dd, ^1J = 12.9 Hz, ^2J = 6.6 Hz, 2 H, $C\underline{H_2}$=$CHCH_2$), 5.70-5.90 (m, 1 H, CH_2=$C\underline{H}CH_2$), 5.91-6.19 (m, 1 H, NH) ppm; $[\alpha]^{20}_D$ = +22.0 (c 0.18 $CHCl_3$); ESI-TOF MS: m/z [M^+ + H] (%) = 184 (100), (M^+ + H, required m/z 184).

N-(1-Oxo-9-decenoyl)-L-homoserine lactone **20d**



This compound was obtained according to procedure 4.2.1.3 from 260 μL of 9-decenoyl chloride (1.27 mmol), 321 of mg L-homoserine lactone hydrochloride (1.27 mmol) and 423 μL of triethylamine (3.05 mmol), in 20 mL of dichloromethane, as a white solid with an isolated yield of 288 mg (90%).; R_f = 0.49 (hexane/ethyl acetate 2:3). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.20-1.39 (m, 8 H, $COCH_2CH_2(C\underline{H}_2)_4$), 1.50-1.1.68 (m, 2 H, $COCH_2C\underline{H}_2$), 2.06 (dd, ^1J = 13.9 Hz, ^2J = 7.3 Hz, 2 H, $COC\underline{H}_2$), 2.18 (dddd, ^1J = 18.4 Hz, ^2J = 11.5 Hz, ^3J = 8.8 Hz, ^2J = 4.8 Hz, 1 H, 3α-H), 2.27 (dt, ^1J = 7.3Hz, ^2J = 1.4 Hz, 2 H, CH_2=CHC$\underline{H}_2$), 2.91 (ddd, ^1J = 13.9 Hz, ^2J = 8.1 Hz, ^3J = 5.8 Hz, 1 H, 3β-H), 4.3 (ddd, ^1J = 11.3 Hz, ^2J = 9.4 Hz, ^3J = 5.9 Hz, 1 H, 4α-H), 4.49 (dt, ^1J = 9.2 Hz, ^2J = 0.9 Hz, 1 H, 4β-H), 4.59 (ddd,^{1} J = 11.6 Hz, ^2J = 8.6 Hz, ^3J = 5.8 Hz, 1 H, 2-H), 4.80-4.98 (m, 2 H, $C\underline{H}_2$=CH_2CH_2), 5.70-5.89 (m, 1 H, CH_2=C$\underline{H}CH_2$), 6.07 (d, ^1J = 4.5 Hz, 1 H, NH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 25.4 (s, C'-3, CH_2), 28.8 (m, C'-4, CH_2), 28.9 (m, C'-5, CH_2), 29.3 (m, C'-4, CH_2) 30.6 (s, C-3, CH_2), 33.7 (s, C'-8, CH_2), 36.2 (s, C'-2, CH_2), 49.2 (s, C-2, CH_2), 66.2 (s, C-4, CH_2), 114.2 (s, C'-10, CH_2), 139.1 (s, C'-9,CH), 173.8 (s, C'-1, C=O), 175.7 (s, C-1, C=O), ppm; ESI-TOF MS: m/z [M^+ + H] (%) = 254 (100), (M^+ + H, required m/z 254).

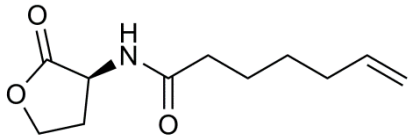
N-(1-Oxo-7-octenoyl)-L-homoserine lactone **20c**



This compound was obtained according to procedure 4.2.1.3 from 96 mg of 7-octenoyl chloride (0.6 mmol), 109 mg of L-homoserine lactone hydrochloride (0.6 mmol) and 199 μL of triethylamine (1.44 mmol), in 20 mL of dichloromethane, as a white solid with an isolated yield of 115 mg (85%).; R_f = 0.39 (hexane/ethyl acetate 2:3). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.35-1.50 (m, 4 H, 2 x $C\underline{H}_2$), 1.56-1.69 (m, 2 H, $COCH_2C\underline{H}_2$), 2.01-2.16 (m, 2 H, $COC\underline{H}_2$), 2.19-2.38 (dddd,^1J = 18.3 Hz, ^2J = 11.4 Hz, ^3J = 8.8 Hz, ^4J = 4.1 Hz, 1 H, 3α-H), 2.27 (dt, ^1J = 7.4 Hz, ^2J = 1.8 Hz, 2 H, CH_2=CHC$\underline{H}_2$), 2.82 (ddd, ^1J = 12.9 Hz, ^2J = 8.6 Hz, ^3J = 5.9 Hz, 1 H, 3β-H), 4.32 (ddd, ^1J = 11.2 Hz, ^2J = 9.3 Hz, ^3J = 5.9 Hz, 1 H, 4α-H), 4.49 (dt, ^1J = 9.1

Hz, ^{2}J = 1.0 Hz, 1 H, 4β-H), 4.63 (ddd, ^{1}J = 11.5 Hz, ^{2}J = 8.6 Hz, ^{3}J = 6.2 Hz, 1 H, 2-H), 4.90-5.10 (m, 2 H, C$\underline{H_2}$=CH_2CH_2), 5.70-5.91 (m, 1 H, CH_2=C$\underline{H}$$CH_2$), 6.41 (d, ^{1}J = 5.7 Hz, 1 H, NH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 24.6 (s, C'-3, CH_2), 28.5 (m, C'-4, CH_2), 28.8 (m, C'-5, CH_2) 30.3 (s, C-3, CH_2), 33.5 (s, C'-6, CH_2), 36.0 (s, C'-2, CH_2), 49.2 (s, C-2, CH_2), 66.2 (s, C-4, CH_2), 114.4 (s, C'-8, CH_2), 138.7 (s, C'-7,CH), 174.0 (s, C'-1, C=O), 175.8 (s, C-1, C=O), ppm; ESI-TOF MS: m/z [M^+ + H] (%) = 226 (100), (M^+ + H, required m/z 226).

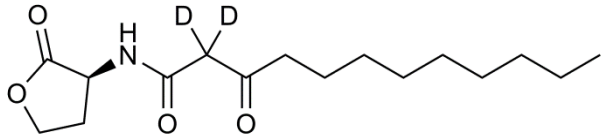
N-(1-Oxo-6-heptenoyl)-L-homoserine lactone **20b**



This compound was obtained according to procedure 4.2.1.3 from 256 mg of 6-heptenoyl chloride (2 mmol), 364 mg of L-homoserine lactone hydrochloride (2 mmol) and 666 µL of triethylamine (4.8 mmol), in 20 mL of dichloromethane as a white solid with an isolated yield of 62 mg (15%).; R_f = 0.31 (hexane/ethyl acetate 2:3). ESI-TOF MS: m/z [M^+ + H] (%) = 212 (100), (M^+ + H, required m/z 212).

4.2.2.4 Synthesis and characterization of *N*-(3-oxoalkanoyl-d_n)-L-homoserine lactones

N-(3-Oxododecanoyl-d_2)-L-homoserine lactone **39a**

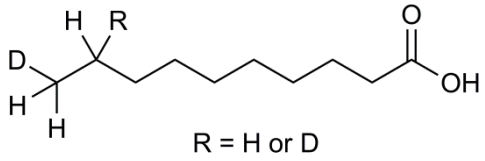


This compound was obtained according to procedure 4.2.1.4 from 11 mg of *N*-(3-oxododecanoyl)-L-homoserine lactone (0.037 mmol), 8 mg of magnesium acetate tetrahydrate (0.037 mmol), 41 mL of deuterium oxide (56 mmol) and 6 µL of triethylamine (0.044 mmol), in 1 mL of tetrahydrofuran. The crude residue was purified by recrystallization (ethyl acetate with a few drops of hexane) to give the desired product as a white solid with an isolated yield of 11 mg (99%); R_f = 0.51 (hexane/ethyl acetate 1:3); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.87 (t, ^{1}J = 6.5 Hz, 3 H, CH_3), 1.19-1.31 (m, 12 H, 6 x CH_2), 1.50-1.68 (m, 2 H, C$\underline{H_2}$$CH_2$CO),

2.12-2.30 (m, 1 H, 3α-H), 2.52 (t, ^{1}J = 7.3 Hz, 2 H, C<u>H</u>$_2$CO), 2.60-2.80 (m, 1 H, 3β-H), 3.47 (s, 0.2 H, COC<u>H</u>DCO), 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 (quin, ^{1}J = 20.1 Hz, C'-2, CD_2), 43.9 (s, C'-4, CH_2), 49.0 (s, C-2, CH_2), 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 [M^+ + Na] (%) = 322 (100), 323 (40), 321 (9) (M^+ + Na, required m/z 322); Deuterium distribution: 79% d_2, 15% d_3, 6% d_1.

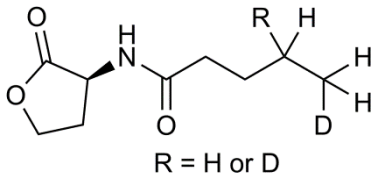
4.2.2.5 Synthesis and characterization of deuterium labelled carboxylic acids and *N*-acyl-L-homoserine lactones via catalytic reduction

Decanoic-d acid **22**



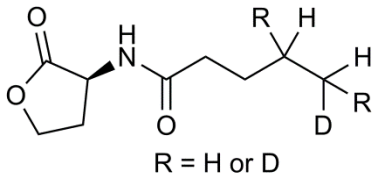
This compound was obtained according to procedure 4.2.1.5.1 Method A from 18.5 µL of 9-decenoic acid (0.1 mmol), 4 mg of palladium (II) acetate (0.02 mmol), 300 µL of acetic acid (5.25 mmol) in 2.5 mL of tetrahydrofuran and 1.5 mL of the mixture of methanol/water 4:1, followed by 4 mg of sodium borodeuteride (0.1 mmol), which was dissolved in 1 M water solution of sodium hydroxide (1 mL), as a yellow oil with an isolated yield of 16 mg (94%). R_f = 0.66 (hexane/ethyl acetate 5:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.90 (t, ^{1}J = 6.9 Hz, 2 H, CDH_2), 1.10-1.31 (m, 11.5 H, C<u>DH + 5 x CH$_2$</u>), 1.55-1.75 (m, 2 H, C<u>H$_2$</u>CH_2COOH), 2.37 (t, ^{1}J = 7.5 Hz, 2 H, C<u>H$_2$</u>COOH) ppm; ESI-TOF MS: m/z [M^+ - H] (%) = 171 (100), 172 (60), 170 (25) (M^+ - H, required m/z 172). Deuterium distribution: 32% d_2, 54% d_1, 14% d_0.

N-(Pentanoyl-d)-L-homoserine lactone **23a** *(Method A)*



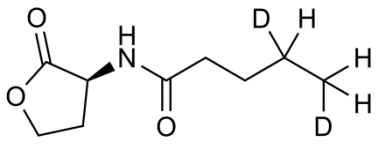
This compound was obtained according to procedure 4.2.1.5.1 Method A from 18 mg of *N*-1-oxo-4-pentenoyl-L-homoserine lactone (0.1 mmol), 4 mg of palladium (II) acetate (0.02 mmol), 300 μL of acetic acid (5.25 mmol) in 2.5 mL of tetrahydrofuran and 1.5 mL of the mixture of methanol/water 4:1, followed by 4 mg of sodium borodeuteride (0.1 mmol), which was dissolved in 1M water solution of sodium hydroxide (1 mL). The crude residue was purified by flash column chromatography (hexane/ethyl acetate 2:3) to give the desired product as a white solid with an isolated yield of 46 mg (82%) R_f = 0.2 (hexane/ethyl acetate 2:3); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.92 (t, ^1J = 7.4 Hz, 2 H, $CD\underline{H_2}$), 1.36 (m, 1.65 H, $CD\underline{H}CDH_2$), 1.50-1.69 (m, 2 H, $C\underline{H_2}CH_2CO$), 2.20 (dddd, ^1J = 20.3 Hz, ^2J = 12.4 Hz, ^3J = 8.9, ^4J = 4.8 Hz, 1 H, 3α-H), 2.25 (t, ^1J = 7.2 Hz, 2 H, $COC\underline{H_2}$), 2.80 (ddd, ^1J = 13.2 Hz, ^2J = 8.7 Hz, ^3J = 3.9 Hz, 1 H, 3β-H), 4.31 (ddd, ^1J = 11.2 Hz, ^2J = 9.3 Hz, ^3J = 5.9 Hz, 1 H, 4α-H), 4.48 (td, ^1J = 9.1 Hz, ^2J = 1.2 Hz, 1 H, 4β-H), 4.61 (ddd, ^1J = 11.5 Hz, ^2J = 8.7 Hz, ^3J = 6.5 Hz, 1 H, 2-H), 6.44 (d, ^1J = 5.43 Hz, 1 H, NH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.6 (quin, ^1J = 19.3 Hz, C'-5, CHD_2), 22.0 (t, ^1J = 19.48 Hz, C'-4, CHD), 27.5 (s, C'-3, CH_2), 30.3 (s, C-3, CH_2), 35.8 (s, C'-2, CH_2), 49.2 (s, C-2, CH_2), 66.2 (s, C-4, CH_2), 174 (s, C'-1, C=O), 175.8 (s, C-1, C=O) ppm; IR (ATR), υ = 3313, 2933, 1778, 1640, 1542, 1360, 1171, 1009 cm^{-1}; Raman, υ = 3322, 2935, 2147, 1775, 1648, 1500, 1453, 1358, 1280, 1227, 949, 797 cm^{-1}; ESI-TOF MS: m/z [M^+ + H] (%) = 187 (100), 188 (68), 186 (62), 189 (41), 190 (19) (M^+ + H, required m/z 188); ESI-MS/MS: m/z [%] = 187 (100) [M^+ + H], 86 (49) [M^+ + H – $C_4H_7O_2N^+$], 102 (67) [$C_4H_8O_2N$+], 74 (30), 56 (20); HRMS ($C_9H_{14}DNO_3$): calc: 186.1882, found. 186.1879; EA ($C_9H_{14}DNO_3$): calc: C, 58.05; H, 8.66; N, 7.52; found: C, 58.9; H, 8.13; N, 7.04; Deuterium distribution: 7% d_4, 14% d_3, 23% d_2, 35% d_1, 21% d_0.

N-(pentanoyl-d_3)-L-homoserine lactone **23a** *(Method C)*



This compound was obtained according to procedure 4.2.1.5.3 Method C from 36 mg of *N*-1-oxo-4-pentenoyl-L-homoserine lactone (0.2 mmol), 8 mg of palladium (II) acetate (0.04 mmol), 300 μL of acetic acid (5.25 mmol) in 6.5 mL of tetrahydrofuran and 1.25 mL of the mixture of methanol-*d*/D_2O 4:1, followed by 16 mg of sodium borodeuteride (0.38 mmol), which was dissolved in 0.33M sodium deuteroxide solution in D_2O (2 mL). The crude residue was purified by flash column chromatography (hexane/ethyl acetate 2:3) to give desired product as a white solid with an isolated yield of 35 mg (95%) R_f = 0.3 (hexane/ethyl acetate 2:3); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.80-1.10 (m,, 1.6 H, CD$\underline{H}_2$), 1.22-1.42 (m, 0.5 H, CD$\underline{H}$CDH_2), 1.50-1.70 (m, 2 H, C$\underline{H}_2$CH_2CO), 2.13 (dddd, ^1J = 20.4 Hz, ^2J = 11.5 Hz, ^3J = 8.8, ^4J = 5.1 Hz, 1H, 3α-H), 2.19-2.49 (m, 2 H, COC$\underline{H}_2$), 2.86 (ddd, ^1J = 13.9 Hz, ^2J = 8.6 Hz, ^3J = 5.9 Hz, 1 H, 3β-H), 4.29 (ddd, ^1J = 11.3 Hz, ^2J = 9.3 Hz, ^3J = 5.9 Hz, 1 H, 4α-H), 4.47 (td, ^1J = 9.0 Hz, ^2J = 1.0 Hz, 1 H, 4β-H), 4.55 (ddd, ^1J = 11.6 Hz, ^2J = 8.6 Hz, ^3J = 5.8 Hz, 1 H, 2-H), 5.90-6.11 (m, 1 H, NH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.4 (quin, ^1J = 19.3 Hz, C'-5, CHD_2), 22.2 (t, ^1J = 20.8 Hz, C'-4, CHD), 27.5, 30.6, 35.9, 49.3, 66.1, 173.8, 175.6 ppm; ESI-TOF MS: m/z [M^+ + H] (%) = 189 (100), 190 (80), 188 (79), 191 (64), 187 (59) (M^+ + H, required m/z 188); Deuterium distribution: 17% d_5, 21% d_4, 26% d_3, 21% d_2, 15% d_1.

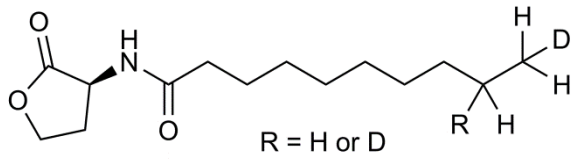
N-(pentanoyl-d_2)-L-homoserine lactone **23a** *(Method C)*



This compound was obtained according to procedure 4.2.1.5.3 Method C from 18 mg of *N*-1-oxo-4-pentenoyl-L-homoserine lactone (0.1 mmol), 4 mg of palladium (II) acetate (0.02 mmol), 300 μL of acetic acid (5.25 mmol) in 5 mL of tetrahydrofuran and 1.25 mL of the methanol-*d*, followed by 8 mg of sodium borodeuteride (0.2 mmol), which was dissolved in 2 mL of 0.33M sodium deuteroxide solution (30% sodium deuteroxide in D_2O diluted with

methanol-*d* to 0.33M). The crude residue was purified by flash column chromatography (hexane/ethyl acetate 2:3) to give desired product as a white solid with an isolated yield of 16 mg (86%) R_f = 0.2 (hexane/ethyl acetate 2:3); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.80-1.10 (m, 1.8 H, CD$\underline{H}_2$), 1.22-1.39 (m, 1 H, CD$\underline{H}$CH_2), 1.50-1.69 (m, 2 H, C$\underline{H}_2$CH_2CO), 2.13 (dddd, ^1J = 20.3 Hz, ^2J = 11.5 Hz, ^3J = 8.8, ^4J = 5.0 Hz, 1 H, 3α-H), 2.18-2.38 (m, 2 H, COC$\underline{H}_2$), 2.86 (ddd, ^1J = 13.9 Hz, ^2J = 8.6 Hz, ^3J = 5.8 Hz, 1 H, 3β-H), 4.28 (ddd, ^1J = 11.3 Hz, ^2J = 9.3 Hz, ^3J = 5.9 Hz, 1 H, 4α-H), 4.47 (td, ^1J = 9.0 Hz, ^2J = 1.0 Hz, 1 H, 4β-H), 4.55 (ddd, ^1J = 11.6 Hz, ^2J = 8.6 Hz, ^3J = 5.8 Hz, 1 H, 2-H), 6.00-6.19 (m, 1 H, NH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.5 (quin, ^1J = 19.1 Hz, C'-5, CHD_2), 22.1 (t, ^1J = 19.1 Hz, C'-4, CHD), 27.4, 30.7, 35.9, 49.3, 66.1, 173.7, 175.5 ppm; ESI-TOF MS: m/z [M^+ + H] (%) = 188 (100), 189 (39), 190 (24), 187 (22) (M^+ + H, required m/z 188); Deuterium distribution: 13% d_4, 21% d_3, 54% d_2, 12% d_1.

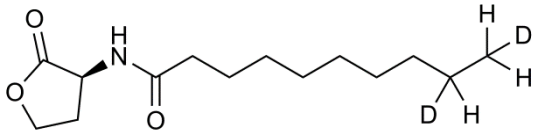
N-(1-Oxodecanoyl-d)-L-homoserine lactone **23d** *(Method A)*



This compound was obtained according to procedure 4.2.1.5.1 Method A from 50 mg of *N*-(1-oxo-9-decenoyl)-L-homoserine lactone (0.2 mmol), 48 mg of palladium (II) acetate (0.04 mmol), 600 μL of acetic acid (10.5 mmol) in 5.5 mL of tetrahydrofuran and 2 mL of the mixture of methanol/water 4:1, followed by 8 mg of sodium borodeuteride (0.2 mmol), which was dissolved in 1M water solution of sodium hydroxide (3 mL). Desired product was obtained as a white solid with an isolated yield of 46 mg (82%) R_f = 0.59 (hexane/ethyl acetate 1:4); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.80-1.01 (m, 2 H, CD$\underline{H}_2$), 1.20-1.38 (m, 11.5 H, CDH_2CD$\underline{H}$(C$\underline{H}_2$)$_5$), 1.55-1.70 (m, 2 H, C$\underline{H}_2$CH_2CO), 2.16 (dddd, ^1J = 20.3 Hz, ^2J = 11.5 Hz, ^3J = 8.8, ^4J = 4.7 Hz, 1 H, 3α-H), 2.15-2.21 (m, 2 H, COC$\underline{H}_2$), 2.89 (ddd, ^1J = 14.2 Hz, ^2J = 8.6 Hz, ^3J = 5.9 Hz, 1 H, 3β-H), 4.32 (ddd, ^1J = 11.3 Hz, ^2J = 9.3 Hz, ^3J = 5.9 Hz, 1 H, 4α-H), 4.48 (td, ^1J = 9.0 Hz, ^2J = 0.9 Hz, 1 H, 4β-H), 4.61 (ddd, ^1J = 11.6 Hz, ^2J = 8.6 Hz, ^3J = 5.9 Hz, 1 H, 2-H), 6.19 (d, ^1J = 5.2 Hz, 1 H, NH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.9 (quin, ^1J = 19.6 Hz, C'-10, CHD_2), 22.6 (t, ^1J = 19.5 Hz, C'-9, CHD), 25.5 (s, C'-8, CH_2), 29.1 (m, C'-7, CH_2), 29.5 (m, C'-6, CH_2), 29.9 (m, C'-5, CH_2), 30.3 (s, C'-4, CH_2), 31.2 (s, C'-3, CH_2), 33.9 (s, C-3, CH_2), 36.2 (s, C'-2, CH_2), 49.2 (s, C-2, CH_2), 66.2 (s, C-4, CH_2), 174 (s,

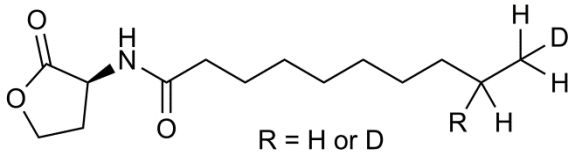
C'-1, C=O), 175.7 (s, C-1, C=O) ppm; ESI-TOF MS: m/z [M^+ + H] (%) = 257 (100), 258 (83), 256 (60), 259 (36) (M^+ + H, required m/z 258). Deuterium distribution: 12% d_3, 30% d_2, 36% d_1, 22% d_0.

N-(1-Oxodecanoyl-d_2)-L-homoserine lactone **23d** *(Method B)*



This compound was obtained according to procedure 4.2.1.5.2 Method B from 18 mg of *N*-(1-oxo-9-decenoyl)-L-homoserine lactone (0.071 mmol), 3 mg of palladium (II) acetate (0.014 mmol), 300 μL of acetic acid (5.25 mmol) in 3.5 mL of tetrahydrofuran and 1 mL of the mixture of methanol/water 4:1, followed by 3 mg of sodium borodeuteride (0.071 mmol), which was dissolved in 1M water solution of sodium hydroxide (1 mL). Desired product was obtained as a white solid with an isolated yield of 16 mg (89%) R_f = 0.59 (hexane/ethyl acetate 1:4); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.80-1.11 (m, 2.6 H, $CD\underline{H}_2$), 1.18-1.31 (m, 11.6 H, $CDH_2CD\underline{H}(C\underline{H}_2)_5$), 1.54-1.72 (m, 2 H, $C\underline{H}_2CH_2CO$), 2.18 (dddd, ^1J = 20.3 Hz, ^2J = 11.5 Hz, ^3J = 8.8 H, 3α-H), 2.18-2.39 (m, 2 H, $COC\underline{H}_2$), 2.86 (ddd, ^1J = 13.2 Hz, ^2J = 8.6 Hz, ^3J = 6.0 Hz, 1 H, 3β-H), 4.31 (ddd, ^1J = 11.3 Hz, ^2J = 9.3 Hz, ^3J = 5.9 Hz, 1 H, 4α-H), 4.46 (td, ^1J = 9.1 Hz, ^2J = 0.9 Hz, 1 H, 4β-H), 4.62 (ddd, ^1J = 11.6 Hz, ^2J = 8.6 Hz, ^3J = 6.1 Hz, 1 H, 2-H), 6.20-6.40 (m, 1 H, NH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.2 (quin, ^1J = 19.5 Hz, C'-10, CH_2D), 22.7 (t, ^1J = 19.0 Hz, C'-9, CHD), 25.5, 29.0, 29.3, 29.5, 30.5, 31.8, 34.2, 36.2, 49.3, 66.2, 174.2, 176.8 ppm; ESI-TOF MS: m/z [M^+ + H] (%) = 258 (100), 259 (73), 257 (72), 260 (60), 256 (49), 261 (28) (M^+ + H, required m/z 258). Deuterium distribution: 7% d_5, 16% d_4,19% d_3, 26% d_2, 19% d_1, 13% d_0.

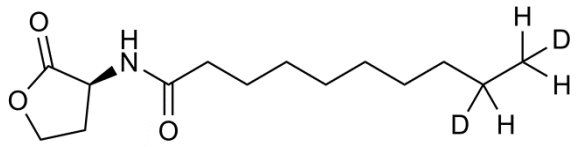
N-(1-Oxodecanoyl-d)-L-homoserine lactone **23d** *(Method C)*



This compound was obtained according to procedure 4.2.1.5.3 Method C from 18 mg of *N*-(1-oxo-9-decenoyl)-L-homoserine lactone (0.071 mmol), 3 mg of palladium (II) acetate (0.014 mmol) and 500 μL of acetic acid-d_4 (8.2 mmol) in 5.5 mL of tetrahydrofuran, followed

by 3 mg of sodium borodeuteride (0.071 mmol), which was dissolved in 1 M water solution of sodium hydroxide (1 mL). The desired product was obtained as a white solid with an isolated yield of 17 mg (94%) R_f = 0.59 (hexane/ethyl acetate 1:4); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.77-0.98 (m, 2.34 H, $CD\underline{H}_2$), 1.16-1.35 (m, 11.83 H, $CDH_2CD\underline{H}(C\underline{H}_2)_5$), 1.50-1.70 (m, 2 H, $C\underline{H}_2CH_2CO$), 2.17 (dddd, ^1J = 20.4 Hz, ^2J = 11.5 Hz, ^3J = 8.8 Hz, ^4J = 5.2 Hz, 1 H, 3α-H), 2.15-2.30 (m, 2 H, $COC\underline{H}_2$), 2.87 (ddd, ^1J = 14.1 Hz, ^2J = 8.5 Hz, ^3J = 5.8 Hz, 1 H, 3β-H), 4.29 (ddd, ^1J = 11.3 Hz, ^2J = 9.3 Hz, ^3J = 5.9 Hz, 1 H, 4α-H), 4.46 (td, ^1J = 9.1 Hz, ^2J = 1.0 Hz, 1 H, 4β-H), 4.58 (ddd, ^1J = 11.6 Hz, ^2J = 8.6 Hz, ^3J = 5.9 Hz, 1 H, 2-H), 6.18 (d, ^1J = 4.9 Hz, 1 H, NH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.1 (quin, ^1J = 19.0 Hz, C'-10, CHD_2), 22.5 (t, ^1J = 19.8 Hz, C'-9, CHD), 25.5, 29.1-29.4, 30.6, 31.8, 34.2, 36.2, 49.3, 66.2, 173.9, 175.7 ppm; ESI-TOF MS: m/z [M^+ + H] (%) = 279 (100), 280 (83), 281 (70), 282 (47), 278 (45), 283 (20), (M^+ + Na, required m/z 280). Deuterium distribution: 6% d_5, 13% d_4,19% d_3, 23% d_2, 27% d_1, 12% d_0.

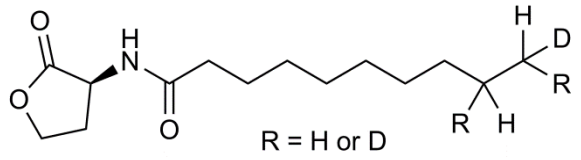
N-(1-Oxodecanoyl-d_2)-L-homoserine lactone **23d** *(Method C)*



This compound was obtained according to procedure 4.2.1.5.3 Method C from 18 mg of *N*-(1-oxo-9-decenoyl)-L-homoserine lactone (0.071 mmol), 3 mg of palladium (II) acetate (0.014 mmol), 500 μL of acetic acid-d_4 (8.2 mmol) in 5.5 mL of tetrahydrofuran, followed by 6 mg of sodium borodeuteride (0.142 mmol), which was dissolved in 1 M solution of sodium hydroxide (1 mL) in D_2O. The desired product was obtained as a white solid with an isolated yield of 17 mg (94%) R_f = 0.59 (hexane/ethyl acetate 1:4); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.87 (m, 2.14 H, $CD\underline{H}_2$), 1.15-1.35 (m, 11 H, $CDH_2CD\underline{H}(C\underline{H}_2)_5$), 1.50-1.71 (m, 2 H, $C\underline{H}_2CH_2CO$), 2.12 (dddd, ^1J = 20.4 Hz, ^2J = 11.5 Hz, ^3J = 8.9, ^4J = 5.5 Hz, 1 H, 3α-H), 2.10-2.21 (m, 2 H, $COC\underline{H}_2$), 2.86 (ddd, ^1J = 14.0 Hz, ^2J = 8.6 Hz, ^3J = 5.8 Hz, 1 H, 3β-H), 4.29 (ddd, ^1J = 11.3 Hz, ^2J = 9.3 Hz, ^3J = 5.8 Hz, 1 H, 4α-H), 4.47 (td, ^1J = 8.9 Hz, ^2J = 0.9 Hz, 1 H, 4β-H), 4.56 (ddd, ^1J = 11.6 Hz, ^2J = 8.6 Hz, ^3J = 5.8 Hz, 1 H, 2-H), 6.07 (d, ^1J = 4.6 Hz, 1 H, NH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.8 (quin, ^1J = 19.2 Hz, C'-10, CHD_2), 22.5 (t, ^1J = 19.8 Hz, C'-9, CHD), 25.4, 29.0, 29.2, 29.4, 30.3, 30.7, 34.2, 36.2, 49.3, 66.1, 173.9, 175.6 ppm; ESI-TOF MS: m/z [M^+ + H] (%) = 280 (100), 281 (73), 279 (60), 282 (37),

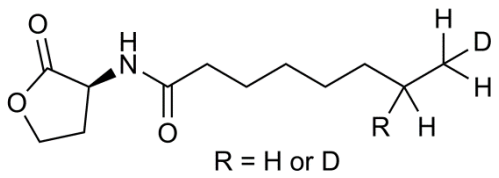
278 (25), 283 (10), (M^+ + Na, required m/z 280). Deuterium distribution: 3% d_5, 12% d_4,24% d_3, 33% d_2, 20% d_1, 8% d_0.

N-(1-Oxodecanoyl-d_3)-L-homoserine lactone (Method C) **23d**



This compound was obtained according to procedure 4.2.1.5.3 Method C from 18 mg of *N*-(1-oxo-9-decenoyl)-L-homoserine lactone (0.071 mmol), 3 mg of palladium (II) acetate (0.014 mmol), 500 μL of acetic acid-d_4 (8.2 mmol) in 5.5 mL of tetrahydrofuran and mixture of the methanol-d/D_2O 4:1 (1.25 mL), followed by 9 mg of sodium borodeuteride (0.21 mmol), which was dissolved in 1M solution of sodium deuteroxide (2 mL) in D_2O. The desired product was obtained as a white solid with an isolated yield of 17 mg (96%) R_f = 0.59 (hexane/ethyl acetate 1:4); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.69-0.89 (m, 1.5 H, C<u>D</u>H$_2$), 1.12-1.32 (m, 11 H, CDH$_2$CD<u>H + 5 x C</u>H$_2$), 1.52-1.70 (m, 2 H, C<u>H$_2$</u>CH$_2$CO), 2.13 (dddd, ^1J = 20.4 Hz, ^2J = 11.5 Hz, ^3J = 8.9, ^4J = 4.9 Hz, 1 H, 3α-H), 2.16-2.30 (m, 2 H, COC<u>H$_2$</u>), 2.86 (ddd, ^1J = 14.3 Hz, ^2J = 8.6 Hz, ^3J = 5.9 Hz, 1 H, 3β-H), 4.29 (ddd, ^1J = 11.3 Hz, ^2J = 9.3 Hz, ^2J = 5.8 Hz, 1 H, 4α-H), 4.46 (td, ^1J = 9.2 Hz, ^2J = 1.0 Hz, 1 H, 4β-H), 4.57 (ddd, ^1J = 11.6 Hz, ^2J = 8.6 Hz, ^3J = 5.8 Hz, 1 H, 2-H), 6.20 (d, ^1J = 4.7 Hz, 1 H, NH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.9 (quin, ^1J = 19.6 Hz, C'-10, CHD_2), 22.6 (t, ^1J = 19.9 Hz, C'-9, CHD), 25.4, 29.1-29.4, 30.3, 30.6, 34.2, 36.2, 49.3, 66.2, 174.0, 175.7 ppm; ESI-TOF MS: m/z [M^+ + H] (%) = 259 (100), 258 (69), 257 (62), 260 (55), 256 (49), 261 (39) (M^+ + H, required m/z 258). Deuterium distribution: 10% d_5, 15% d_4, 27% d_3, 18% d_2, 17% d_1, 13% d_0.

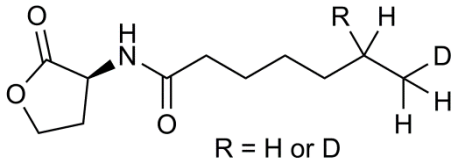
N-(1-Oxooctanoyl-d_2)-L-homoserine lactone **23c**



This compound was obtained according to procedure 4.2.1.5.1 Method A from 56 mg of *N*-(1-oxo-7-octenoyl)-L-homoserine lactone (0.25 mmol), 11 mg of palladium(II) acetate (0.05 mmol), 700 μL of acetic acid (12.2 mmol) in 6.5 mL of tetrahydrofuran and 2.5 mL of the mixture of methanol/water 4:1, followed by 1 mg of sodium borodeuteride (0.25 mmol),

which was dissolved in 1 M, water solution of sodium hydroxide (4 mL). The desired product was obtained as a white solid with an isolated yield of 41 mg (74%) R_f = 0.49 (hexane/ethyl acetate 1:4); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.79-0.96 (m, 2 H, $CD\underline{H_2}$), 1.20-1.39 (m, 7.5 H, $CDH_2CD\underline{H}$ + 3 x $C\underline{H_2}$), 1.40-1.58 (m, 2 H, $C\underline{H_2}CH_2CO$), 2.19 (dddd, ^1J = 20.4 Hz, ^2J = 11.5 Hz, ^3J = 8.9 Hz, ^4J = 4.4 Hz, 1 H, 3α-H), 2.15-2.31 (m, 2 H, $COC\underline{H_2}$), 2.88 (ddd, ^1J = 13.3 Hz, ^2J = 8.6 Hz, ^3J = 5.9 Hz, 1 H, 3β-H), 4.32 (ddd, ^1J = 11.3 Hz, ^2J = 9.3 Hz, ^3J = 5.9 Hz, 1 H, 4α-H), 4.50 (td, ^1J = 9.1 Hz, ^2J = 1.0 Hz, 1 H, 4β-H), 4.62 (ddd, ^1J = 11.5 Hz, ^2J = 8.5 Hz, ^3J = 6.0 Hz, 1 H, 2-H), 6.27 (d, ^1J = 4.8 Hz, 1 H, NH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.9 (quin, ^1J = 20.6 Hz, C'-8, CHD_2), 22.6 (t, ^1J = 19.3 Hz, C'-7, CHD), 24.2 (s, C'-6, CH_2), 28.9 (m, C'-5, CH_2), 30.3 (s, C'-4, CH_2), 31.0 (s, C'-3, CH_2), 33.9 (s, C-3, CH_2), 36.2 (s, C'-2, CH_2), 49.3 (s, C-2, CH_2), 66.2 (s, C-4, CH_2), 174.0 (s, C'-1, C=O), 175.7 (s, C-1, C=O) ppm; ESI-TOF MS: m/z [M^+ + H] (%) = 230 (100), 229 (83), 231 (62), 228 (31) (M^+ + H, required m/z 230). Deuterium distribution: 23% d_3, 36% d_2, 30% d_1, 11% d_0.

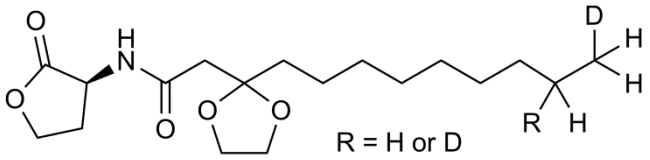
N-(1-Oxoheptanoyl-d_2)-L-homoserine lactone **23b**



This compound was obtained according to procedure 4.2.1.5.1 Method A from 42 mg of *N*-(1-oxo-6-heptenoyl)-L-homoserine lactone (0.2 mmol), 8 mg of palladium (II) acetate (0.04 mmol), 600 μL of acetic acid (10.5 mmol) in 6 mL of tetrahydrofuran and 2.5 mL of the mixture of methanol/water 4:1, followed by 8 mg of sodium borodeuteride (0.2 mmol), which was dissolved in 1 M, water solution of sodium hydroxide (42 mL). The desired product was obtained as a white solid with an isolated yield of 36 mg (84%) R_f = 0.38 (hexane/ethyl acetate 1:4); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.70-0.89 (m, 2 H, $CD\underline{H_2}$), 1.19-1.40 (m, 5.5 H, $CDH_2CD\underline{H}$ + 2 x $C\underline{H_2}$), 1.51-1.69 (m, 2 H, $C\underline{H_2}CH_2CO$), 2.17 (dddd, ^1J = 20.4 Hz, ^2J = 11.5 Hz, ^3J = 8.8 Hz, ^4J = 4.5 Hz, 1 H, 3α-H), 2.25 (m, 2 H, $COC\underline{H_2}$), 2.86 (ddd, ^1J = 13.9 Hz, ^2J = 8.5 Hz, ^3J = 5.8 Hz, 1 H, 3β-H), 4.30 (ddd, ^1J = 11.3 Hz, ^2J = 9.3 Hz, ^3J = 5.9 Hz, 1 H, 4α-H), 4.50 (td, ^1J = 8.9 Hz, ^2J = 0.8 Hz, 1 H, 4β-H), 4.60 (ddd, ^1J = 11.6 Hz, ^2J = 8.6 Hz, ^3J = 6.3 Hz, 1 H, 2-H), 6.25 (d, ^1J = 4.4 Hz, 1 H, NH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.8 (quin, ^1J = 19.8 Hz, C'-7, CHD_2), 22.2 (t, ^1J = 19.1 Hz, C'-6, CHD), 25.4 (s, C'-5, CH_2), 30.3 (s, C'-4, CH_2), 31.5 (s, C'-3, CH_2), 33.9 (s, C-3, CH_2), 36.7 (s, C'-2, CH_2), 49.2 (s, C-2,

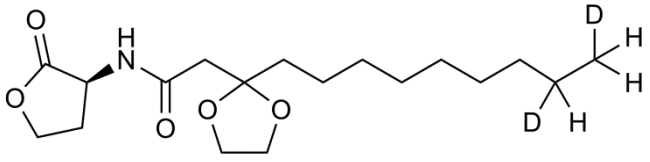
CH_2), 66.2 (s, C-4, CH_2), 174.0 (s, C'-1, C=O), 175.7 (s, C-1, C=O) ppm; ESI-TOF MS: m/z [M^+ + H] (%) = 216 (100), 215 (79), 217 (53), 214 (41), 218 (15) (M^+ + H, required m/z 216). Deuterium distribution: 6% d_4, 18% d_3, 35% d_2, 27% d_1, 14% d_0.

N-[3-(1', 3'-Dioxolane)-dodecanoyl-d]-L-homoserine lactone **29b** *(Method A)*



This compound was obtained according to procedure 4.2.1.5.1 Method A from 8 mg of *N*-[3-(1', 3'-dioxolane)-11-dodecenoyl]-L-homoserine lactone (0.23 mmol), 15 mg of palladium (II) acetate (0.023 mmol), 300 μL of acetic acid (4.9 mmol) in the mixture of 1.8 mL tetrahydrofuran and 0.5 mL methanol, followed by 3 mg of sodium borodeuteride (92 μmol), which was dissolved in 2 mL of 0.33 M water solution of sodium hydroxide. Desired product was obtained as a white solid with an isolated yield of 6 mg (77%) R_f = 0.54 (hexane/ethyl acetate 1:3); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.71-0.90 (m, 2 H, $CD\underline{H_2}$), 1.10-1.29 (m, 14 H, 7 x $C\underline{H_2}$), 1.51-1.69 (m, 2 H, $CH_3(CH_2)_7C\underline{H_2}$), 2.10 (dddd, ^1J = 20.3 Hz, ^2J = 11.4 Hz, ^3J = 8.8 Hz, ^4J = 4.9 Hz. 1 H, 3α-H), 2.65 (s, 2 H, $COC\underline{H_2}$), 2.80 (ddd, ^1J = 14.2 Hz, ^2J = 8.6 Hz, ^3J = 5.9 Hz, 1 H, 3β-H), 4.10 (m, 4 H, -OCH_2CH_2O-) 4.27 (ddd, ^1J = 11.2 Hz, ^2J = 9.3 Hz, ^3J = 5.9 Hz, 1 H, 4α-H), 4.48 (dt, ^1J = 9.1 Hz, ^2J = 1.1 Hz, 1 H, 4β-H), 4.59 (ddd, ^1J = 11.6 Hz, ^2J = 8.6 Hz, ^3J = 6.3 Hz, 1 H, 2-H), 7.01 (d, ^1J = 6.5 Hz, 1 H, NH) ppm; ESI-TOF MS: m/z [M^+ + H] (%) = 343 (100), 342 (50), 344 (26), 345 (13) (M^+ + H, required m/z 344). Deuterium distribution: 7% d_3, 14% d_2, 53% d_1, 26% d_0.

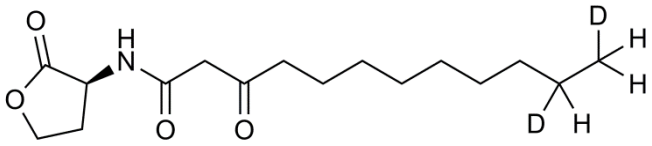
N-[3-(1', 3'-Dioxolane)-dodecanoyl-d_2]-L-homoserine lactone **29b** *(Method C)*



This compound was obtained according to procedure 4.2.1.5.3 Method C from 33 mg of *N*-[3-(1', 3'-dioxolane)-11-dodecenoyl]-L-homoserine lactone (0.097 mmol), 8 mg of palladium (II) acetate (0.04 mmol), 500 μL of acetic acid (8.2 mmol) in the mixture of 4.5 mL tetrahydrofuran and 1 mL methanol, followed by 4 mg of sodium borodeuteride (0.097 mmol), which was dissolved in 2 mL of 0.33M sodium deuteroxide solution (30% sodium

deuteroxide in D_2O diluted with methanol-*d* to 0.33 M). The crude residue was purified by flash column chromatography (hexane/ethyl acetate 1:2) to give desired product as a white solid with an isolated yield of 19 mg (58%) R_f = 0.57 (hexane/ethyl acetate 1:3); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.75-0.94 (m, 2 H, $CD\underline{H}_2$), 1.15-1.35 (m, 13 H, $CDH_2CD\underline{H}$ + 6 x $C\underline{H}_2$), 1.56-1.77 (m, 2 H, $CDH(CH_2)_6C\underline{H}_2$), 2.14 (dddd, ^1J = 20.3 Hz, ^2J = 11.4 Hz, ^3J = 8.8 Hz, ^4J = 4.8 Hz, 1 H, 3α-H), 2.65 (s, 2 H, $COC\underline{H}_2$), 2.80 (ddd, ^1J = 14.3 Hz, ^2J = 8.6 Hz, ^3J = 5.9 Hz, 1 H, 3β-H), 4.01-4.19 (m, 4 H, -OCH_2CH_2O-) 4.27 (ddd, ^1J = 11.2 Hz, ^2J = 9.3 Hz, ^3J = 5.9 Hz, 1 H, 4α-H), 4.47 (dt, ^1J = 9.1 Hz, 1.1 Hz, 1 H, 4β-H), 4.58 (ddd, ^1J = 11.6 Hz, ^2J = 8.6 Hz, ^2J = 6.3 Hz, 1 H, 2-H), 7.01 (d, ^1J = 6.1 Hz, 1 H, NH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.9 (quin, ^1J = 19.2 Hz, C'-12, CH_2D), 22.5 (t, ^1J = 19.1 Hz, C'-11, CHD), 23.7 (s, C'-10, CH_2), 28.8 (m, C'-9, CH_2), 29.1 (m, C'-8, CH_2), 29.3 (m, C'-7, CH_2), 29.9 (m, C'-6, CH_2), 30.4 (s, C-3, CH_2), 37.5 (s, C'-4, CH_2),44.2 (s, C'-2, CH_2), 49.2 (s, C-2, CH_2), 64.9 (s, -$OCCH_2CH_2O$-, CH_2), 65.1 (s, -$OCCH_2CH_2O$-, CH_2), 65.9 (s, C-4, CH_2), 109.6 (s, C'-3, C), 169.8 (s, C'-1, C=O), 175.2 (s, C-1, C=O) ppm; ESI-TOF MS: m/z [M^+ + H] (%) = 344 (100), 343 (54), 345 (25), 342 (14) (M^+ + H, required m/z 344). Deuterium distribution: 13% d_3, 52% d_2, 28% d_1, 7% d_0.

N-(3-Oxododecanoyl-d_2)-L-homoserine lactone **28b**

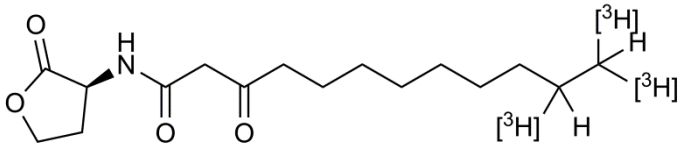


This compound was obtained according to procedure 4.2.1.8 from 17 mg of *N*-[3-(1', 3'-dioxolane)-dodecanoyl-d_2]-L-homoserine lactone (0.05 mmol) and 60% perchloric acid solution (250 μL per 0.05 mmol) in 15 mL of dichloromethane. The crude residue was purified by flash column chromatography (hexane/ethyl acetate 2:1–1:3) to give desired product as a white solid with an isolated yield of 13 mg (90%); R_f = 0.54 (hexane/ethyl acetate 1:3); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.72-0.97 (m, 2 H, $CD\underline{H}_2$), 1.16-1.31 (m, 11 H, $CDH_2CD\underline{H}(C\underline{H}_2)_5$), 1.49-1.69 (m, 2 H, $COCH_2C\underline{H}_2$), 2.24 (dddd, ^1J = 20.3 Hz, ^2J = 12.4 Hz, ^3J = 8.9 Hz, ^4J = 1 H, 3α-H), 2.53 (t, ^1J = 7.4 Hz, 2 H, $COC\underline{H}_2$), 2.76 (ddd, ^1J = 14.6 Hz, ^2J = 8.7 Hz, ^3J = 6.0 Hz, 1 H, 3β-H), 3.47 (s, 2 H, $COC\underline{H}_2CO$), 4.28 (ddd, ^1J = 11.0 Hz, ^2J = 9.3 Hz, ^3J = 6.0 Hz, 1 H, 4α-H), 4.48 (dt, ^1J = 9.1 Hz, ^2J = 1.3 Hz, 1 H, 4β-H), 4.59 (ddd, ^1J = 11.5 Hz, ^2J = 8.7 Hz, ^3J = 6.7 Hz, 1 H, 2-H), 7.68 (d, ^1J = 5.7 Hz, 1 H, NH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.8 (quin, ^1J = 19.3 Hz, C'-12, CH_2D), 22.5 (t, ^1J = 19.1 Hz, C'-11,

CHD), 23.4 (s, C'-10, CH_2), 28.9 (m, C'-9, CH_2), 29.1 (m, C'-8, CH_2), 29.4 (m, C'-7, CH_2), 29.9 (m, C'-6, CH_2), 30.1 (s, C-3, CH_2), 43.9 (s, C'-4, CH_2),47.9 (s, C'-2, CH_2), 49.0 (s, C-2, CH_2), 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 [M^+ + H] (%) = 322 (100), 321 (54), 323 (25), 320 (14) (M^+ + Na, required m/z 322). Deuterium distribution: 13% d_3, 52% d_2, 28% d_1, 7% d_0.

4.2.2.6 Synthesis and characterisation of tritium labelled *N*-(3-oxoalkanoyl)-L-homoserine lactones

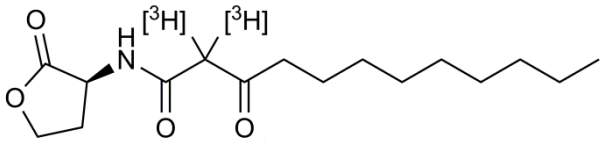
N-(3-Oxododecanoyl-[$^3H_3$])-L-homoserine lactone **28c**



This compound was obtained in 2 steps according to procedure 4.2.1.5.3 Method A and procedure 4.2.1.8 from 2.6 mg of *N*-[3-(1', 3'-dioxolane)-11-dodecenoyl]-L-homoserine lactone (7.63 µmol), 1.7 mg of palladium (II) acetate (7.63 µmol), 50 µL of acetic acid (0.82 mmol) in the mixture of 477 µL tetrahydrofuran and 99 µL methanol, followed by 0.288 mg of sodium borohydride-[^{3}H] (7.63 µmol, 100 mCi, 3.7 GBq), which was dissolved in 160 µL of 0.33 M sodium hydroxide solution.

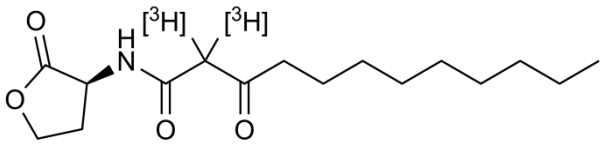
Deprotection of acetal group was performed by use of 3.81 µL perchloric acid in 2 mL of dichloromethane. The crude residue was purified by preparative TLC (hexane/ethyl acetate 1:4) to give desired product as a white solid with an isolated yield of 1.8 mg (77%); Radioactivity = 3.52 mCi (130.45 MBq); Specific activity = 588.6 Ci/mol; R_f = 0.50 (hexane/ethyl acetate 1:4); ESI-TOF MS: m/z [M^+ + H] (%) = 304 (100), 302 (19), (M^+ + H, required m/z 304); m/z [M^++H_2O] (%) = 322 (100), 320 (19), (M^+ + H_2O, required m/z 322). Tritium distribution: 84% t_3, 16% t_2.

N-(3-Oxododecanoyl-[$^3H_2$])-L-homoserine lactone **39b** *(4.2.1.5.3 Method A)*



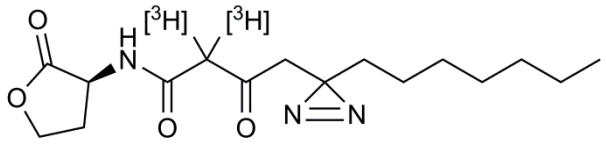
This compound was obtained in 2 steps according to procedure 4.2.1.5.3 Method A and procedure 4.2.1.8 from 0.5 mg of *N*-[3-(1', 3'-dioxolane)-11-dodecenoyl-[$^3H]_2$]-L-homoserine lactone (1.47 μmol), 0.5 mg of palladium (II) acetate (1.47 μmol), 20 μL of acetic acid (0.33 mmol) in the mixture of 140 μL tetrahydrofuran and 40 μL methanol, followed by 0.22 mg of sodium borohydride (5.86 μmol), which was dissolved in 130 μL of 0.33M sodium hydroxide solution. Deprotection of acetal group was performed by use of 2 μL of perchloric acid in 500 mL of dichloromethane. Desired product was obtained as a white solid with an isolated yield of about 0.4 mg (90%); Radioactivity = 0.84 mCi (31.05 MBq); Specific activity = 571.5 Ci/mol; R_f = 0.50 (hexane/ethyl acetate 1:4); ESI-TOF MS: m/z [M^+ + H] (%) = 320 (100), 318 (19), 322 (16) ($M^+ + H_2O$, required m/z 320).

N-(3-Oxododecanoyl-[$^3H_2$])-L-homoserine lactone **39b** *(4.2.1.4)*



This compound was obtained according to procedure 4.2.1.4 from 10 mg of *N*-(3-oxo-dodecanoyl)-L-homoserine lactone (33.6 μmol), 7 mg of magnesium acetate tetrahydrate (33.6 μmol), 900 μL of tritium oxide (50 mmol, 30.26 μCi, 1.12 MBq) and 5.5 μL of triethylamine (40 μmol), in 1 mL of tetrahydrofuran. Desired product was obtained as a white solid with an isolated yield of 8 mg (89%); Radioactivity = 17.84 μCi (0.66 MBq); Specific activity = 0.66 Ci/mol; R_f = 0.51 (hexane/ethyl acetate 1:3).

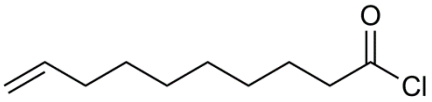
N-(3-Oxo-5-diazirinedodecanoyl-[$^3H_2$])-L-homoserine lactone **40**



This compound was obtained according to procedure 4.2.1.4 from 1 mg of *N*-(3-oxo-5-diazirinedodecanoyl)-L-homoserine lactone (3 μmol), 1 mg of magnesium acetate tetrahydrate (4 μmol), 100 μL of tritium oxide (5.5 mmol, 3.36 μCi, 120 kBq) and 1 μL of triethylamine (7 μmol), in 100 μL of tetrahydrofuran. The crude residue was purified by recrystallization (ethyl acetate with a few drops of hexane) to give desired product as a white solid with an isolated yield of 1 mg (99%); Radioactivity = 0.14 μCi (5.2 kBq); Specific activity = 0.5 Ci/mol; R_f = 0.65 (hexane/ethyl acetate 1:3).

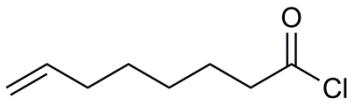
4.2.2.7 Synthesis and characterisation of acyl chlorides

9-Decenoyl chloride **19d**



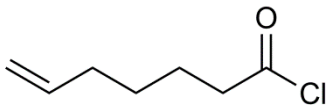
This compound was obtained according to procedure 4.2.1.6 from 316.6 µL of 9-decenoic acid (2 mmol), 343 µL of oxalyl chloride (4 mmol) in 30 mL of hexane, as a yellow liquid with an isolated yield of 257 mg (68%). R_f = 0.76 (hexane/ethyl acetate 5:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.25-1.44 (m, 8 H, 4 x $\underline{CH_2}$), 1.65-1.69 (m, 2 H, $C\underline{H_2}CH_2COCl$), 1.71-1.76 (m, 2 H, CH_2=CHC$\underline{H_2}$), 1.97-2.26 (m, 2 H, $C\underline{H_2}COCl$), 4.94-5.03 (dd, ^1J = 17.2 Hz, ^2J = 2.1 Hz, 2 H, $C\underline{H_2}$=$CHCH_2$), 5.77-5.86 (m, 1 H, CH_2=C$\underline{H}CH_2$) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 24.2 (s, C-3), 28.3 (s, C-4), 28.8 (m, C-5), 29.0 (m, C-6), 29.5 (m, C-7, CH_2), 33.7 (m, C-8), 47.1 (s, C-2), 114.2 (s, C-10), 138.9 (s, C-9), 169.6 (s, C-1) ppm; ESI-TOF MS: m/z [M^+ - H] (%) = 211 (100), (M^+ + Na, required m/z 211).

7-Octenoyl chloride **19c**



This compound was obtained according to procedure 4.2.1.6 from 307 µL of 7-octenoic acid (2 mmol), 343 µL of oxalyl chloride (4 mmol) in 30 mL of hexane, as a yellow liquid with an isolated yield of 96 mg (30%). R_f = 0.52 (hexane/ethyl acetate 5:1); ESI-TOF MS: m/z [M^+ - H] (%) = 183 (100), (M^+ + Na, required m/z 183).

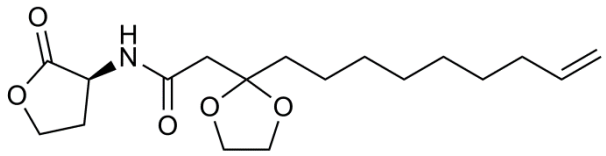
6-Heptenoyl chloride **19b**



This compound was obtained according to procedure 4.2.1.6 from 271 µL of 6-heptenoic acid (2 mmol), 343 µL of oxalyl chloride (4 mmol) in 20 mL of hexane, as a yellow liquid with an isolated yield of 291 mg (99%). R_f = 0.42 (hexane/ethyl acetate 5:1); ESI-TOF MS: m/z [M^+ - H] (%) = 169 (100), (M^+ + Na, required m/z 169).

4.2.2.8 Synthesis and characterisation of acetal protected *N*-acyl-L-homoserine lactones

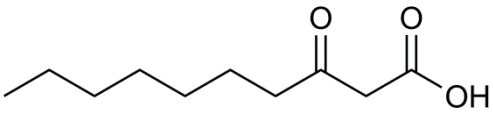
N-[3-(1', 3'-Dioxolane)-11-dodecenoyl]-L-homoserine lactone **27**



This compound was obtained according to procedure 4.2.1.7 from 137 mg of *N*-(3-oxo-11-dodecenoyl)-L-homoserine lactone (0.464 mmol), 154.5 μL of ethylene glycol (2.78 mmol), 30 mg of *p*-toluenesulfonic acid (0.174 mmol) and 50.5 μL of trimethyl orthoformate (0.464 mmol), in 25 mL of toluene. The crude residue was purified by flash column chromatography (hexane/ethyl acetate 2:1–1:2) to give desired product as a white solid with an isolated yield of 140 mg (64%). R_f = 0.36 (hexane/ethyl acetate 1:3); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.20-1.39 (m, 10 H, 5 x C$\underline{H}_2$), 1.59-1.79 (m, 2 H, CH_2=CHCH_2(CH_2)$_5$C$\underline{H}_2$), 1.90-2.18 (m, 2 H, CH_2=CHC$\underline{H}_2$(CH_2)$_5$), 2.16 (dddd, ^1J = 20.4 Hz, ^2J = 11.4 Hz, ^3J = 8.9 Hz, ^4J = 5.1 Hz, 1 H, 3α-H), 2.67 (s, 2 H, COC$\underline{H}_2$), 2.82 (ddd, ^1J = 14.0 Hz, ^2J = 8.5 Hz, ^3J = 5.9 Hz, 1 H, 3β-H), 4.10 (m, 4 H, -OCH_2CH_2O-) 4.29 (ddd, ^1J = 11.2 Hz, ^2J = 9.3 Hz, ^3J = 5.9 Hz, 1 H, 4α-H), 4.48 (dt, ^1J = 9.1 Hz, ^2J = 0.9 Hz, 1 H, 4β-H), 4.59 (ddd, ^1J = 11.6 Hz, ^2J = 8.6 Hz, ^3J = 6.3 Hz, 1 H, 2-H), 4.86-5.10 (m, 2 H, C$\underline{H}_2$=CHCH_2), 5.71-5.92 (m, 1 H, CH_2=C$\underline{H}$CH_2), 7.01 (d, ^1J = 5.9 Hz, 1 H, NH) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 23.6 (s, C'-9, CH_2), 28.8 (m, C'-8, CH_2), 29.2 (m, C'-7, CH_2), 29.6 (m, C'-6, CH_2), 29.9 (m, C'-5, CH_2), 30.3 (s, C-3, CH_2), 33.7 (s, C'-10, CH_2), 37.5 (s, C'-4, CH_2),44.2 (s, C'-2, CH_2), 49.2 (s, C-2, CH_2), 64.5 (s, -OCCH_2CH_2O-, CH_2), 65.1 (s, -OCCH_2CH_2O-, CH_2), 66.9 (s, C-4, CH_2), 109.6 (s, C'-3, C), 114.2 (s, C'-12, CH_2), 139.2 (s, C'-11,CH), 169.8 (s, C'-1, C=O), 175.2 (s, C-1, C=O), ppm; ESI-TOF MS: m/z [M^+ + H] (%) = 340 (100), (M^+ + H, required m/z 340).

4.2.2.9 Synthesis and characterisation of β-keto carboxylic acids

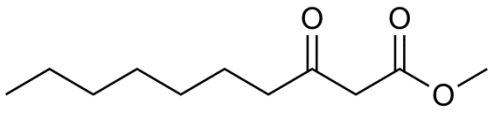
3-Oxodecanoic acid **32**



This compound was obtained according to procedure 4.2.1.9.1 Method A from 170.7 µL of octanoyl chloride (1 mmol), 95 mg of lithium bromide (1.1 mmol), 267.7 µL of bis(trimethylsilyl) malonate (1.05 mmol) and 152.5 µL of triethylamine (1.1 mmol), in 30 mL of dry diethyl ether. The desired product was obtained as a white solid with an isolated yield of 96 mg (52%). R_f = 0.58 (hexane/ethyl acetate 1:10); ^{1}H NMR[107,116] (500 MHz, $CDCl_3$): δ = 0.91 (t, ^1J = 6.7 Hz, 3 H, CH_3), 1.21-1.40 (m, 8 H, $CH_3(C\underline{H}_2)_4$), 1.54-1.69 (m, 2 H, $C\underline{H}_2CH_2CO$), 2.58 (t, ^1J = 7.4 Hz, 2 H, $C\underline{H}_2CO$), 3.54 (s, 2 H, $COC\underline{H}_2CO$) ppm; ^{13}C NMR[116] (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 [M^+ + H] (%) = 187 (100), (M^+ + H, required m/z 187).

4.2.2.10 Synthesis and characterisation of β-keto esters

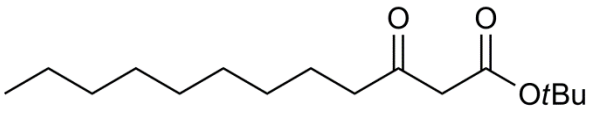
Methyl 3-oxodecanoate **34**



This compound was obtained according to procedure 4.2.1.10.1 Method A from 79.3 µL of methyl acetate (1 mmol), 500 µL of lithium diisopropylamide (LDA) (2.0 M solution in THF, 1 mmol) and 85 µL of octanoyl chloride (0.5 mmol) in 20 mL of dry tetrahydrofuran. The crude residue was purified by flash column chromatography (hexane/ethyl acetate 9:1) to give desired product as a pale yellow oil with an isolated yield of 80 mg (80%). R_f = 0.68 (hexane/ethyl acetate 1:10); ^{1}H NMR[116] (500 MHz, $CDCl_3$): δ = 0.89 (t, ^1J = 6.8 Hz, 3 H, CH_3), 1.18-1.29 (m, 8 H, $CH_3(C\underline{H}_2)_4$), 1.51-1.70 (m, 2 H, $C\underline{H}_2CH_2CO$), 2.54 (t, ^1J = 7.4 Hz, 2 H, $C\underline{H}_2CO$), 3.46 (s, 2 H, $COC\underline{H}_2CO$), 3.75 (s, 3 H, $COOC\underline{H}_3$) ppm; ^{13}C NMR[116] (125 MHz, $CDCl_3$): δ = 14.1 (s, C-10, CH_3), 22.6 (s, C-9, CH_2), 23.5 (s, C-8, CH_2), 28.9 (m, C-7, CH_2),

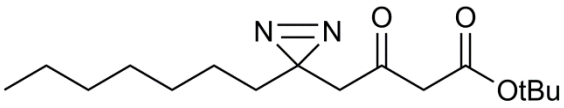
29.7 (m, C-6, CH_2), 31.6 (s, C-5, CH_2), 43.1 (s, C-4, CH_2), 49.0 (s, C-2, CH_2), 52.3 (s, C'-1, O$\underline{C}H_3$), 167.7 (s, C-1, C=O), 202.8 (s, C-3, C=O) ppm; ESI-TOF MS: m/z [M^+ + H] (%) = 201 (100), (M^+ + H, required m/z 201).

1-tert-Butyl-3-oxododecanoate



This compound was obtained according to procedure 4.2.1.10.2 Method B from 189 mg of decanoic acid (1.1 mmol), 210 mg of 1,1'-carbonyldiimidazole (CDI; 1.3 mmol), 192.3 µL of mono *tert*-butyl malonate (1.3 mmol) and 1.2 mL of isopropyl magnesium chloride (2 M in THF, 2.4 mmol) in 5 mL of dry tetrahydrofuran. The crude residue was purified by flash column chromatography (hexane/ethyl acetate 10:1) to give desired product as yellow oil with an isolated yield of 89 mg (30%). R_f = 0.61 (hexane/ethyl acetate 1:10); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.89 (t, ^1J = 6.9 Hz, 3 H, CH_3), 1.17-1.27 (m, 12 H, 6 x C$\underline{H}_2$), 1.48 (s, 9 H, 3 x CH_3), 1.50-1.72 (m, 2 H, C$\underline{H}_2$CH_2CO), 2.53 (t, ^1J = 7.4 Hz, 2 H, C$\underline{H}_2$CO), 3.35 (s, 2 H, COC$\underline{H}_2$CO) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.1 (s, C-12, CH_3), 22.7 (s, C-11, CH_2), 23.5 (s, C-10, CH_2), 27.9 (s, 3 x $\underline{C}H_3$), 29.1 (m, C-9, CH_2), 29.3 (m, C-8, CH_2), 29.9 (m, C-7, CH_2), 31.8 (s, C-6, CH_2), 42.9 (s, C-5, CH_2), 44.3 (s, C-4, CH_2), 50.7 (s, C-2, CH_2), 81.8 (s, $\underline{C}(CH_3)_3$), 166.6 (s, C-1, C=O), 203.5 (s, C-3, C=O) ppm; ESI-TOF MS: m/z [M^+ + H] (%) = 271 (100), (M^+ + H, required m/z 271).

1-tert-Butyl-3-oxo-5-diazirine-dodecanoate **38**

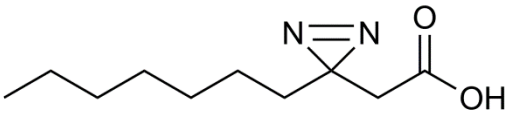


This compound was obtained according to procedure 4.2.1.10.2 Method B from 34 mg of 3-diazirine-decanoic acid (0.17 mmol), 33 mg of 1,1'-carbonyldiimidazole (CDI; 0.2 mmol), 32 µL of mono *tert*-butyl malonate (0.2 mmol) and 187 µL of isopropyl magnesium chloride (2 M in THF, 0.37 mmol) in 5 mL of dry tetrahydrofuran. The crude residue was purified by flash column chromatography (hexane/ethyl acetate 10:1) to give desired product as orange-brown oil with an isolated yield of 10 mg (20%). R_f = 0.47 (hexane/ethyl acetate 1:10); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.89 (t, ^1J = 6.9 Hz, 3 H, C$\underline{H}_3$), 0.91-1.12 (m, 2 H, C$\underline{H}_2$CN_2), 1.14-1.32 (m, 10 H, 5 x C$\underline{H}_2$), 1.48 (s, 9 H, 3 x C$\underline{H}_3$), 2.47 (s, 2 H, CN_2C$\underline{H}_2$CO),

3.38 (s, 2 H, COC<u>H</u>$_2$CO) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.1 (s, C-12, <u>C</u>H$_3$), 22.6 (s, C-11, <u>C</u>H$_2$), 23.7 (s, C-10, <u>C</u>H$_2$), 27.9 (s, 3 x <u>C</u>H$_3$), 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, <u>C</u>(CH$_3$)$_3$), 165.8 (s, C-1, C=O), 199.3 (s, C-3, C=O) ppm; ESI-TOF MS: m/z [M$^+$ + H] (%) = 319 (100), (M$^+$ + Na, required m/z 319).

4.2.2.11 Synthesis and characterisation of β-diazirine carboxylic acids

3-Diazirine-decanoic acid **35**



This compound was obtained according to procedure 4.2.1.11 from 3.2 g of 3-oxodecanoic acid (17.2 mmol), 100 mL of anhydrous ammonia, 2.22 g of hydroxylamine-*O*-sulfonic acid (19.6 mmol), 2.96 mL of triethylamine (21.3 mmol) and 5.8 g of iodine (23 mmol) in 20 mL of dry methanol and 25 mL of dichloromethane. The crude residue was purified by flash column chromatography (hexane/ethyl acetate 1:1) to give desired product as an orange-brown oil with an isolated yield of 1.02 g (30%). R_f = 0.39 (hexane/ethyl acetate 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, C<u>H</u>$_2$(CH$_2$)$_4$), 1.15-1.26 (m, 8 H, CH$_3$(C<u>H</u>$_2$)$_4$), 1.55 (t, ^1J = 7.8 Hz, 2 H, C<u>H</u>$_2$CN$_2$), 2.33 (s, 2 H, CN$_2$C<u>H</u>$_2$CO), 10.10 (s, 1 H, COO<u>H</u>) 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), υ = 2980, 1710, 1657, 1330, 1218, 1055, cm^{-1}; Raman, υ = 2899, 1693, 1592, 1258, 1045, 881 cm^{-1}; UV (THF): λ_{max} = 343 nm, ε = 69 M^{-1} cm^{-1}; ESI-TOF MS: m/z [M$^+$ - H] (%) = 197 (100), (M$^+$ - H, required m/z 197).

5 Summary

In this thesis three challenging problems concerning the synthesis of isotope labelled and photoactivatable *N*-acyl-L-homoserine lactones were studied. Obtained molecules are easy to track in biological materials like membranes or cells. The first project focused on the synthesis of deuterium labelled AHLs with vary chain lengths. Starting from readily available deuterated fatty acids, a two-step procedure towards deuterated *N*-acyl-L-homoserine lactones (AHLs) was accomplished. It includes the acylation of Meldrum's acid, followed by the amidation. Advantages include facile scalability, easy work-up and good yields. Furthermore, these procedures are simple, safe and efficient. In addition, the combination of theoretical and experimental IR and Raman spectroscopy allowed us to identify most probable structures of these important signal molecules. Interactions of the newly synthesized deuterated AHLs with model cell membranes were explored using sum-frequency-generation spectroscopy. We found that all AHLs are well-ordered within the supported lipid bilayers (SLBs) in a preferentially all-trans conformation of the deuterated alkyl chain and integrated into the upper leaflet of the SLB with the methyl terminal groups pointing downward. Within the SLBs system described above, no flip-flop or translocation of AHLs from the upper leaflet to the lower one could be observed. Thus, these results suggest a possible localisation in the membrane of a potential receptor of inter-kingdom signalling.

Next, a novel method of deuterium and tritium labelling of AHLs was explored in details. Substrates for reduction experiments were prepared from terminally unsaturated carboxylic acids, which were first converted to the appropriate acid chlorides using oxalyl chloride and then reacted with L-homoserine lactone hydrobromide in the presence of triethylamine to give the terminally unsaturated *N*-acyl-L-homoserine lactones. *N*-(3-Oxoalkanoyl)-L-homoserine lactones were prepared according to the procedures described for the first method of deuterium labelling, reported above. Then, the acetal protection of ketone group with the ethylene glycol, led to desired products. The uncommon palladium (II) acetate catalysed reduction of a double bond by sodium borohydride was proven to be mild, efficient and convenient labelling method. We took advantage of an interesting frozen layer-by-layer system that ensured proper control of the reaction. In contrary to the most of labelling methods, this procedure is “gas-free” and, in a consequence, safe and environmentally friendly. Moreover, this process does not require usage of expensive catalysts, aggressive reagents or high energy consumption (e.g. high temperature, microwave irradiation). Several

deuterium and tritium labelled AHLs were obtained in this way with excellent yields and good deuteration degrees. Particularly tritium labelled AHLs were applied in biological investigations of mechanism of the ability of AHL to cross the cell membranes.

The last topic covered by this work, combines all the experience from previously studied systems in order to synthesise photoactivatable and isotopically modified *N*-acyl-L-homoserine lactones. The diazirine group was chosen as a most convenient photoactive group that can be exceptionally easily activated by UV irradiation. The first part of the synthesis of *N*-(3-oxo-5-diazirinedodecanoyl)-L-homoserine lactone involved a synthesis of β-keto-decanoic acid intermediate. In the first step a bis(trimethylsilyl) malonate (BSM) was acylated by an octanoyl chloride in the presence of triethylamine and lithium bromide. The latter activated acidic position of BSM, so the strong bases were avoided. Subsequently, a reaction with hydroxylamine-*O*-sulfonic acid in liquid ammonia and oxidation with iodine gave the 3-diazirine-decanoic acid. The conversion to *tert*-butyl active ester followed by hydrolysis with trifluoroacetic acid and EDC-mediated condensation with L-homoserine lactone gave desired *N*-(3-oxo-5-diazirinedodecanoyl)-L-homoserine lactone afterwards. The new method of deuterium and tritium labelling *via* exchange reaction was designed because this photoactive AHL derivative is temperature sensitive and cannot be treated with strong bases. Thus, the mild post-synthetic hydrogen isotopes exchange was developed using deuterium (tritium) water, triethylamine and magnesium acetate. Magnesium ions act here in a similar way like Li ions do in case of BSM, i.e. by enhancing the acidity of the α-protons. In consequence, the efficient isotopic exchange in the last step of the synthesis was possible even in the mild basic conditions with excellent yields and very good deuterium/tritium content. Such a tritium enriched *N*-(3-oxo-5-diazirinedodecanoyl)-L-homoserine lactone can be further applied in photoaffinity labelling of a potential receptor responsible for inter-kingdom signalling.

Recently, a broad spectrum of novel methods for investigation of biological systems appeared including functionalization of biological active compounds and their immobilisation on the surface. However, most of these methods change the structures of biomolecules dramatically. Usually only one isomer of a given molecule is biologically active. Therefore, the isotopic substitution seems to be the most reliable method because it does not affect the structure. Although many of isotopic labelling methods were developed, still only very few of them do not require a deuterium/tritium gas, aggressive reagents or expensive metal catalysts. Thus, novel methods developed in this work are gas-free, mild, easy in handle and efficient.

Deuterium and tritium labelled AHLs obtained *via* these procedures will constitute a key in elucidation of the mechanism for this unexplored communication between eukaryotic and prokaryotic worlds. In perspective, this will be a big step in immunology, particularly in our defence against chronic, incurable bacterial infections.

6 Abbreviations list

3OC10-HSL	*N*-(3-oxodecanoyl)-L-homoserine lactone
3OC12-HSL	*N*-(3-oxododecanoyl)-L-homoserine lactone
3OC14-HSL	*N*-(3-oxotetradecanoyl)-L-homoserine lactone
AHLs	*N*-acyl--homoserine lactones
BSM	Bis(trimethylsilyl) malonate
CDI	1,1'-Carbonyldiimidazole
DCC	*N*,*N*′-Dicyclohexylcarbodiimide
DMAP	4-(*N*,*N*-Dimethylamino)pyridine
EA	Elemental analysis
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
Equiv.	Equivalent
ESI-TOF	Electrospray ionization time-of-flight
HOBt	Hydroxybenzotriazole
HRMS	High-resolution mass spectra
IR	Infrared spectroscopy
KIE	Kinetic isotope effect
LDA	Lithium diisopropylamide
L-HSL	L-Homoserine lactone hydrobromide
MS	Mass spectrometry
NHS	*N*-Hydroxysuccinimide
NMR	Nuclear magnetic resonance
$Pd(OAc)_2$	Palladium(II) acetate
PMN	Human polymorphonuclear neutrophils
p-TsOH	*p*-Toluenesulfonic acid
QS	Quorum-sensing
SFG	Sumfrequency-generation
SLBs	Supported lipid bilayers
TEBA	Triethylbenzylammonium chloride
UV-vis	Ultraviolet–visible

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1. C. Barth, D. Jakubczyk, A. Kubas, F. Anastassacos, G. Brenner-Weiß, K. Fink, U. Schepers, S. Bräse, P. Koelsch *Inter-kingdom Signaling: Integration, Conformation and Orientation of N-Acyl-L-Homoserine Lactones in Supported Lipid Bilayers* Langmuir **2012**, 28(22): 8456

2. D. Jakubczyk, C. Barth, A. Kubas, F. Anastassacos, P. Koelsch, K. Fink, U. Schepers, G. Brenner-Weiß, S. Bräse *Deuterium-labelled N-acyl-L-homoserine lactones (AHLs)—inter-kingdom signalling molecules—synthesis, structural studies, and interactions with model lipid membranes* Anal. Bioanal. Chem. **2012**, 404, 473

8 List of scientific publications

1. C. Barth, D. Jakubczyk, A. Kubas, F. Anastassacos, G. Brenner-Weiß, K. Fink, U. Schepers, S. Bräse, P. Koelsch *Inter-kingdom Signaling: Integration, Conformation and Orientation of N-Acyl-L-Homoserine Lactones in Supported Lipid Bilayers* Langmuir **2012**, 28(22): 8456

2. D. Jakubczyk, C. Barth, A. Kubas, F. Anastassacos, P. Koelsch, K. Fink, U. Schepers, G. Brenner-Weiß, S. Bräse *Deuterium-labelled N-acyl-L-homoserine lactones (AHLs)—inter-kingdom signalling molecules—synthesis, structural studies, and interactions with model lipid membranes* Anal. Bioanal. Chem. **2012**, 404, 473

9 Curriculum Vitae

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