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Published on: 01 Aug 2016 - RSC Advances (The Royal Society of Chemistry)

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Synthesis and analysis of stable isotope-labelled *N*-acyl homoserine lactones

Ewout Ruysbergh, Christian V. Stevens, Norbert De Kimpe and Sven Mangelinckx*

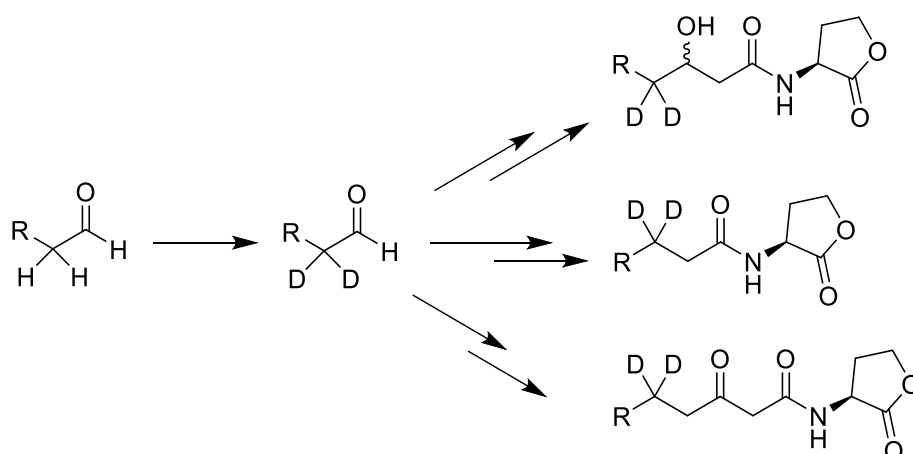
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Received: / Accepted: / Published:

Keywords: AHL, quorum sensing, isotope labelling, tetramic acid, isotope dilution, GC-MS

Graphical abstract



Abstract

Aliphatic aldehydes were deuterated at the α -position via a base-catalyzed exchange reaction with D₂O. These deuterated building blocks were used for the synthesis of labelled analogues of quorum sensing signal molecules belonging to the three major classes of naturally occurring *N*-acylated homoserine lactones (AHLs), with the label on a non-enolizable and therefore stable position. Besides the application of these stable isotope-labelled AHLs as labelled standard for analysis via isotope dilution mass spectrometry, these compounds can be used to study the metabolic fate of the fatty acid tail of the AHL-molecule. These isotope-labelled compounds were fully characterized and used to synthesize the deuterated analogues of two commonly occurring AHL-degradation products, a tetramic acid and a ring opened *N*-acyl homoserine.

Introduction

For many years, researchers thought of bacteria as individual cells, unable to interact with one another. However, this view changed dramatically with the discovery of population density dependent light production by the marine bacterium *Vibrio fischeri*.¹ Because this type of gene regulation is linked to the population density or quorum, the term 'quorum sensing' (QS) was used to describe this phenomenon.² To communicate with each other, bacteria rely on small, diffusible molecules. Since these molecules can upregulate their own production, they are commonly referred to as autoinducers. In Gram-negative bacteria these autoinducers are *N*-acylated homoserine lactones (AHLs).^{3, 4} The structure of an AHL consists of a homoserine lactone ring coupled with a variable *N*-acyl chain. This acyl chain can vary in length from four to eighteen carbon atoms, and the chain can contain an oxo or hydroxy group at the third carbon of the acyl chain. AHLs with an unsaturated or branched side chain have also been detected in nature.⁵⁻⁷ Very recently, a new class of AHLs, containing a *p*-coumaryl or a cinnamoyl group instead of an acyl chain, was discovered in nature, extending the range of possible occurring AHLs.^{8, 9} Many diverse bacterial phenotypes such as bioluminescence, enzyme secretion, virulence expression and biofilm formation are regulated by QS.³ Besides intraspecies communication, AHL-molecules are also involved in interspecies and even interkingdom signaling.¹⁰⁻¹²

Although the AHL-concentration inside a biofilm can reach as high as 600 μM , only concentrations in the nM range are encountered in the effluent.¹³ To detect these trace amounts of AHLs, sensitive detection methods are needed. One possible method is via the application of biosensors, such as *Agrobacterium tumefaciens* NT1,¹⁴ *Chromobacterium violaceum* CV026¹⁵ and *Escherichia coli* JB523.¹⁶ The detection relies on a phenotypic response such as enzyme secretion, pigment production or bioluminescence when the threshold concentration of AHLs is exceeded.¹⁷ Although detection of sub-picomole amounts of AHLs is feasible, the possibility of artefacts, such as medium-derived diketopiperazines,¹⁸ and the need to use multiple biosensor strains in parallel for complete coverage of all AHLs, has prompted researchers to develop multiple techniques for instrumental AHL-detection and identification.¹⁹⁻²³ To obtain reliable results and exact quantification, an internal standard needs to be included in these analyses. The use of odd-chain AHLs as internal standard was abandoned after the discovery of odd-chain AHL-producing bacteria.^{24, 25} Several groups have reported the synthesis of isotope-labelled AHLs **1-10** (Figure 1) which can be used as internal standards.^{13, 26-33} In the case of isotope dilution mass spectrometry (IDMS), the spike compound should be chemically identical with the analyte. As the isotope-labelled compound acts as an internal standard, compensating for losses during sample isolation and analysis, the mass difference between the isotope and the analyte should not be too high, to avoid possible isotope effects.³⁴ Besides the application as standard, isotope-labelled molecules are often used in absorption, distribution, metabolism and excretion (ADME) studies and to study reaction mechanisms.³⁵ Hereby, mostly a 1:1 mixture of non-labelled and isotope-labelled compounds is used, to create a recognizable mass spectral ion pattern to study the *in vitro*

and *in vivo* metabolic dispersion.³⁵⁻³⁸ However, no methodology is available to synthesize AHLs belonging to the major different classes with an isotope label in the acyl chain, starting from one, easily available deuterated building block.

In this work, a convenient synthesis of several deuterium-labelled AHLs is reported. D₂O is used to introduce the deuterium label in aldehydes, which are used as building blocks to synthesize AHLs belonging to the three major classes of naturally occurring AHLs.

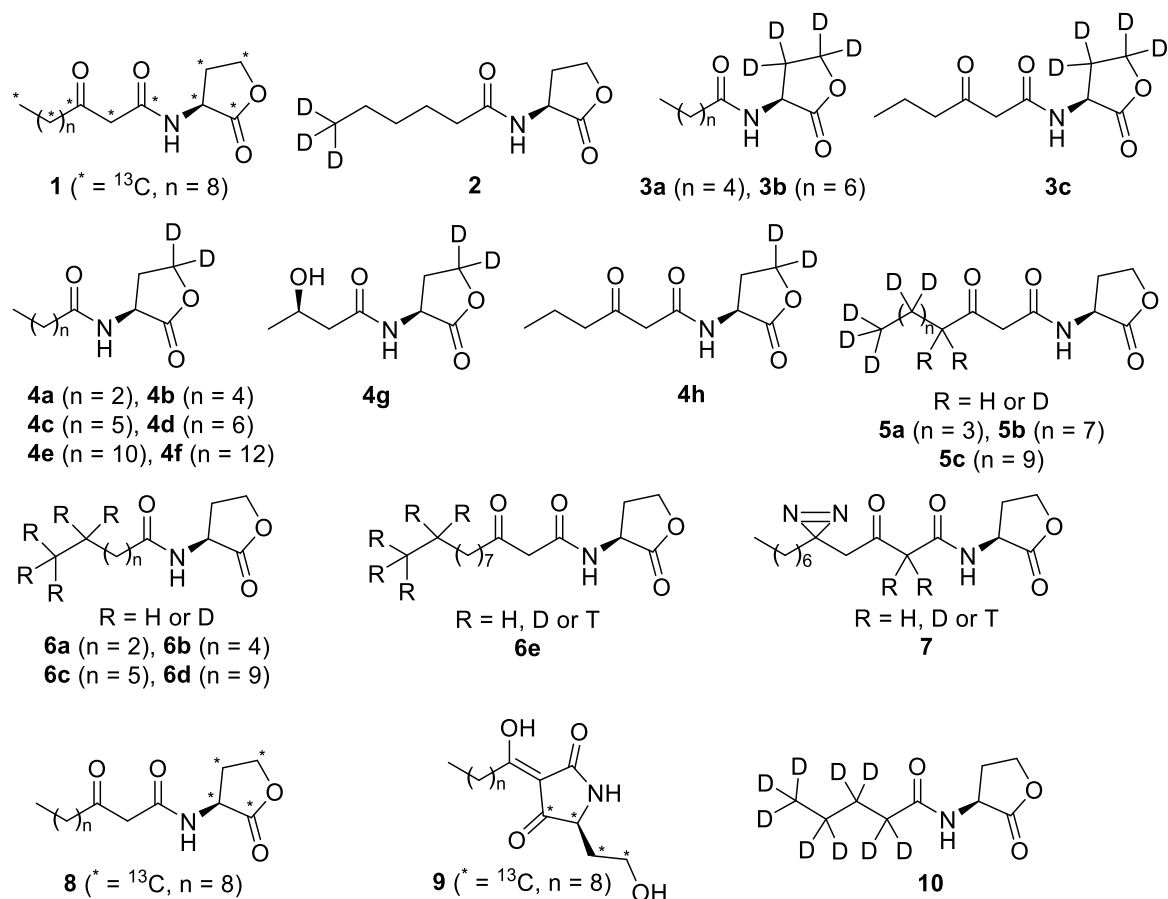


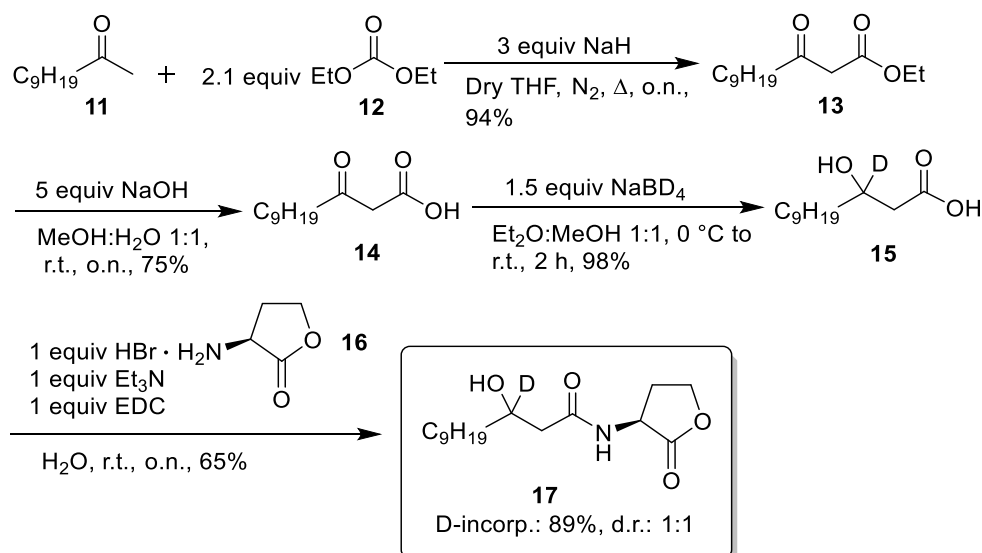
Figure 1: Isotope-labelled AHL-analogues **1**,¹³ **2**,²⁶ **3a-c**,²⁷ **4a-h**,²⁸ **5a-c**,²⁹ **6a-e**,³⁰ **7**,³¹ **8**,⁹³² and **10**³³ described in the literature.

Results and discussion

Since secondary metabolites are quite expensive to synthesize in terms of energy and used materials, it is tempting to speculate that certain elements of these metabolites are reused. Several enzymes are known that are able to degrade AHLs.³⁹ Acylases catalyze the hydrolysis of the amide bond, thereby releasing a fatty acid and homoserine lactone.⁴⁰⁻⁴² Haloperoxidases are able to halogenate *N*-(3-oxoacyl)-*L*-homoserine lactones in a hydrogen peroxide-dependent manner.⁴³⁻⁴⁵ Subsequent cleavage of the halogenated acyl chain yields *N*-(α,α -dihaloacetyl)-*L*-homoserine lactone and a fatty acid.⁴⁴ Therefore, to study the metabolic fate of the fatty acid side chains, we decided to include the isotope label in the acyl side chain of the AHL-molecule. When the isotope label is included in the lactone ring (analogues **3** and **4**), all classes of AHLs can easily be accessed.^{27, 28} However, studies to

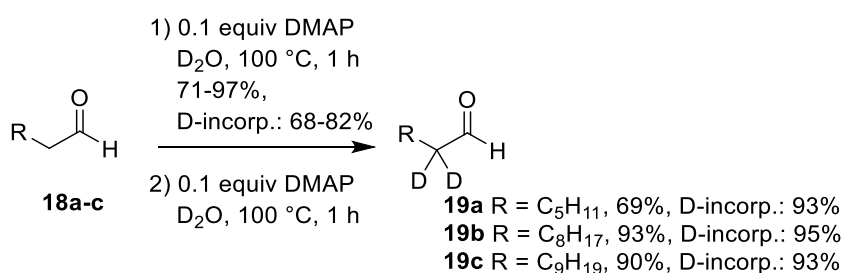
investigate the metabolization of the fatty acid side chain are impossible with these analogues. To include the label in the side chain (analogues **2**, **5** and **10**), most synthetic routes rely on the use of rather expensive deuterated fatty acids.^{26, 29, 33} A metal-catalyzed reduction of terminally unsaturated AHLs with sodium borodeuteride to include the isotope label (analogues **6**), has been reported as well.³⁰

To the best of our knowledge, no analogues of *N*-(3-hydroxyacyl)-L-homoserine lactones with an isotope label in the acyl chain have been synthesized yet. The synthesis of this type of compounds was therefore our first goal. In a first approach, β -ketoester **13** was synthesized in excellent yield by reacting undecanone **11** with diethyl carbonate **12** in the presence of sodium hydride, followed by a sodium hydroxide-mediated hydrolysis to yield 3-oxododecanoic acid **14** (Scheme 1).⁴⁶ The deuterium label was introduced via a reduction of the keto function with sodium borodeuteride to give deuterated β -hydroxy fatty acid **15** with a deuterium incorporation of 89% at the carbon atom bearing the hydroxy moiety.⁴⁷ 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)-mediated coupling with L-homoserine lactone hydrobromide **16** yielded the desired *N*-(3-hydroxydodecanoyl-[3-²H])-L-homoserine lactone **17**.⁴⁸ Only the class of AHLs bearing the hydroxy group is accessible via this route. The introduction of only one deuterium atom causes a mass shift of one unit. The natural presence of the heavy isotopes of mainly carbon, oxygen, nitrogen and hydrogen, gives rise to the presence of a mass (M+1) one unit higher than the expected parental mass (M). The abundance of this M+1 can be as high as 20% of the parental mass M for molecules with a molecular weight of 300 Da, hindering the application of these monodeuterated molecules as standards, but they can be useful to study the fragmentation patterns during GC/MS-analysis. Although M+2 is naturally present as well, even for the AHLs with the highest mass, the abundance never exceeds 3% of the presence of the parental mass M, resulting in less interference with peaks of a dideuterated labeled compound. Noteworthy, dideuterated AHL-analogues **4a-h** were applied as internal standards in an isotope dilution tandem mass spectrometric method.²⁸ Therefore, a more general route was developed to gain access to the dideuterated analogues of all different types of AHLs.



Scheme 1: Synthesis of *N*-(3-hydroxydodecanoyl-[3-²H])-L-homoserine lactone **17**. The deuterium content was determined by ESI-MS.

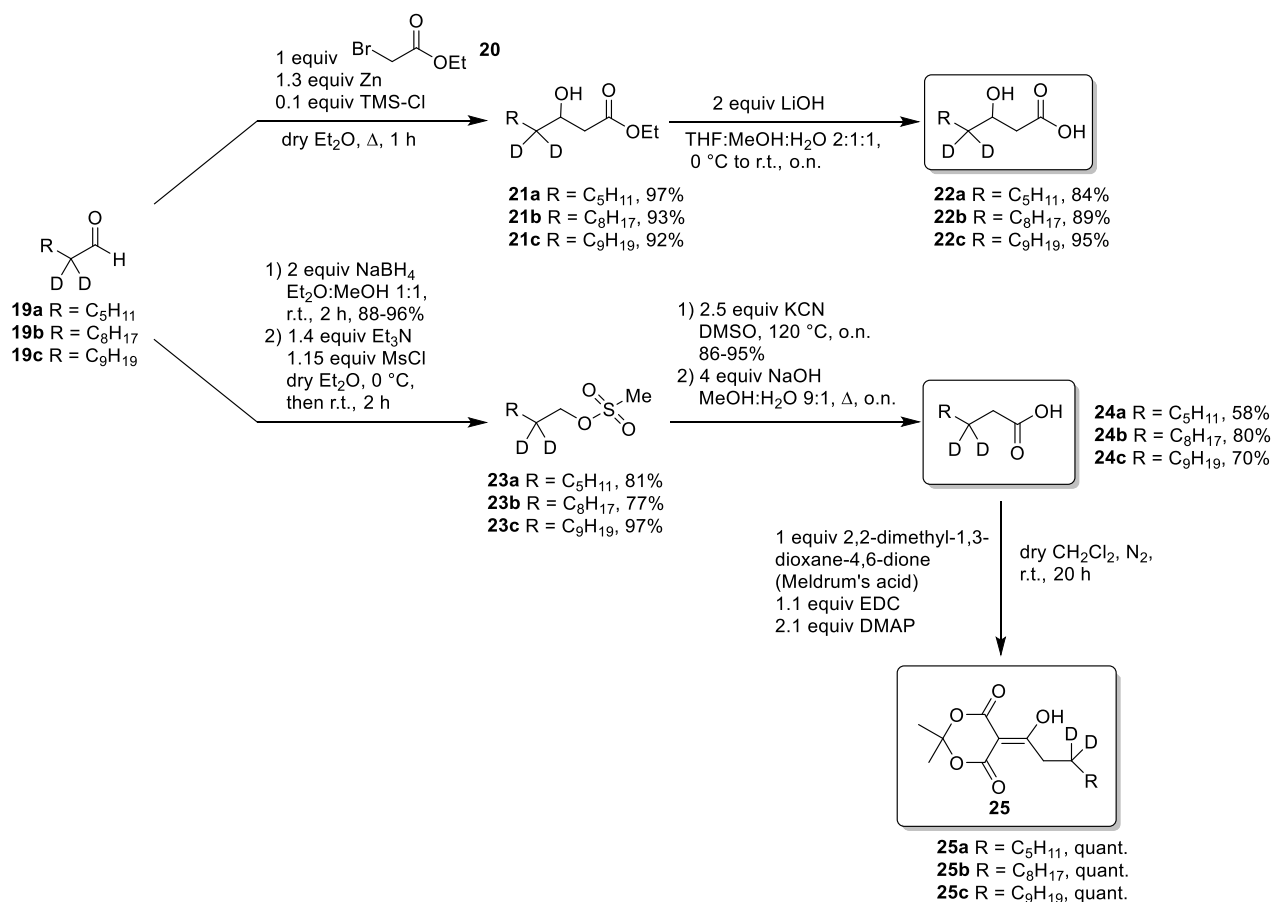
Aliphatic aldehydes **18a-c** were deuterated at the α -position via a 4-(*N,N*-dimethylamino)pyridine (DMAP)-catalyzed H/D-exchange reaction with D_2O (Scheme 2).⁴⁹ This mild catalysis only allows selective deuterium introduction at the acidic α -position. Due to the formation of HDO and H_2O during this exchange reaction, complete deuteration is impossible. Therefore, to get a higher degree of deuterium incorporation than 68-82%, obtained after one iteration, a second treatment with D_2O was applied, yielding aldehydes **19a-c** with a satisfactory deuterium incorporation of 93-95%. To achieve a deuterium incorporation higher than 95%, a third iteration with D_2O should be included. However, as in IDMS, one is rather interested in the alteration of the ratio of two isotopes (labelled compared to non-labelled), the incomplete deuterium incorporation will not hinder the applicability, as long as the isotope ratio of the standard is determined in a parallel analysis.^{34, 50, 51}



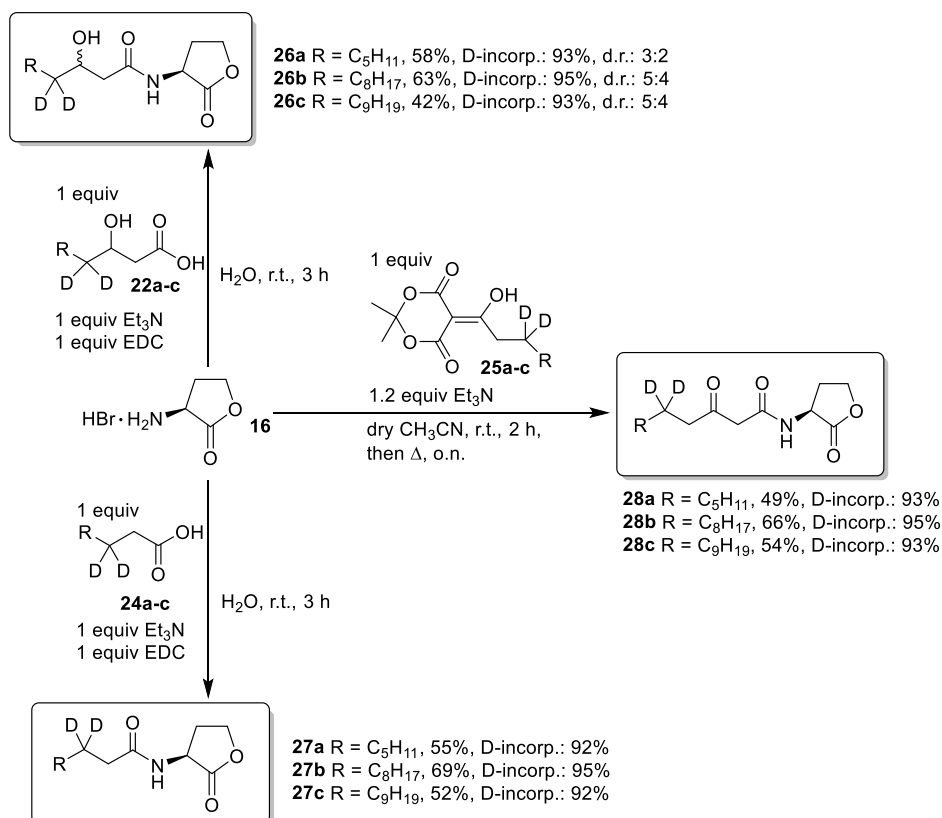
Scheme 2: Synthesis of α,α -dideuterated aldehydes **19a-c** as deuterated building blocks. The deuterium content was determined via ^1H NMR.

These α,α -dideuterated aldehydes **19a-c** were transformed to the corresponding β -hydroxy esters **21a-c** via a Reformatsky reaction with ethyl bromoacetate **20** (Scheme 3). Hydrolysis of β -hydroxy esters **21a-c** gave the [4-²H₂]- β -hydroxy fatty acids **22a-c** in excellent yields.⁵² Alternatively, further elaboration of these deuterated aldehydes **19a-c** towards unfunctionalized fatty acids **24a-c** was also possible. Because the α -position of a carbonyl

group is quite acidic, a chain extension was needed to shift the deuterium label to the β -position of the carbonyl and avoid deuterium loss due to a deuterium-hydrogen exchange. The labelled aldehydes **19a-c** were reduced to the alcohols after reaction with two equivalents of sodium borohydride in ethereal methanol. Mesylation,⁵³ followed by substitution with potassium cyanide and alkaline hydrolysis yielded the [3-²H₂]-fatty acids **24a-c** (Scheme 3).⁵⁴ These labelled fatty acids **24a-c** were coupled with L-homoserine lactone hydrobromide **16** to give *N*-(acyl-[3-²H₂])-L-homoserine lactones **27a-c** (Scheme 4). A similar reaction with [4-²H₂]- β -hydroxy fatty acids **22a-c** gave *N*-(3-hydroxyacyl-[4-²H₂])-L-homoserine lactones **26a-c**, as a mixture of diastereomers. As expected, no separation of both diastereomers was observed during both HPLC and GC-analysis, allowing this mixture to be used as such as labelled spike for IDMS, utilizing a common analytical equipment. For the *N*-(3-oxoacyl-[5-²H₂])-L-homoserine lactones **28a-c**, [3-²H₂]-fatty acids **24a-c** were reacted with Meldrum's acid to introduce the oxo functionality (Scheme 3), followed by amidation with L-homoserine lactone hydrobromide **16** (Scheme 4).⁵⁵ No significant loss of deuterium was observed during this synthetic route.



Scheme 3: Transformation of α,α -dideuterated aldehydes **19a-c** into deuterated fatty acid derivatives **22a-c**, **24a-c** and **25a-c**.



Scheme 4: Synthesis of *N*-(3-hydroxyacyl-[4-²H₂])-L-homoserine lactones **26a-c**, *N*-(acyl-[3-²H₂])-L-homoserine lactones **27a-c** and *N*-(3-oxoacyl-[5-²H₂])-L-homoserine lactones **28a-c** starting from deuterated precursors **22a-c**, **24a-c** and **25a-c**. The deuterium content was determined by ESI-MS.

This methodology allows the convenient and straightforward synthesis of AHLs with different functionalities, starting from one easily available deuterated building block without the use of deuterium gas or expensive catalysts. The presence of two deuterium atoms causes a mass shift of two units, making these isotope-labelled analogues easily distinguishable from the naturally occurring ¹³C-containing AHLs, with a mass one unit higher than the regular AHLs. All deuterated AHLs **26a-c**, **27a-c** and **28a-c** were fully characterized.

The degree of deuterium incorporation in the synthesized AHLs **17**, **26a-c**, **27a-c** and **28a-c** was determined via HPLC-MS. To confirm the position of the isotope label, ¹H and ¹³C NMR spectra were recorded and compared with those of the unlabelled AHLs. The presence of deuterium atoms at the β-position of the acyl chain in *N*-(acyl-[3-²H₂])-L-homoserine lactones **27a-c** was confirmed by the transformation of the signal of the α-protons of the acyl chain from a triplet to a singlet and the disappearance of the quintet at δ 1.64 ppm (in CDCl₃). A similar alteration was observed in the spectra of *N*-(3-oxoacyl-[5-²H₂])-L-homoserine lactones **28a-c**. For *N*-(3-hydroxydodecanoyl-[3-²H])-L-homoserine lactone **17**, the signal at δ 4.0 ppm (CDCl₃), linked with the *CHOH*-unit logically disappeared, while for *N*-(3-hydroxyacyl-[4-²H₂])-L-homoserine lactones **26a-c** the signal at δ 1.45 ppm (CDCl₃) was absent. The expected quintet associated with a dideuterated carbon atom, was not always clearly visible in the ¹³C NMR spectrum due to excessive signal splitting and increased relaxation times.⁵⁶ A small isotope effect, resulting

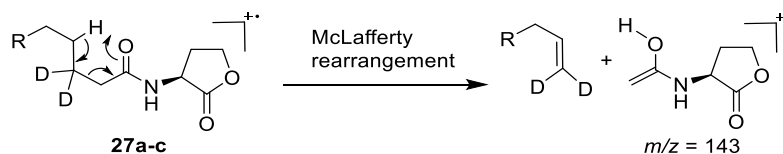
in an upfield shift of 0.1 to 0.2 ppm, was observed for the flanking carbons, while this was negligible for the other carbon atoms.

Several methods have been developed to analyze AHLs with GC-MS.^{13, 19, 57} Therefore, the suitability of the synthesized AHLs for this type of analysis was evaluated by studying the electron impact (EI) mass fragmentation pattern (Table 1). The retention times of labelled and unlabelled AHL were identical.

Table 1: GC-MS data of discussed AHL-molecules. EI MS spectra can be found in SI.

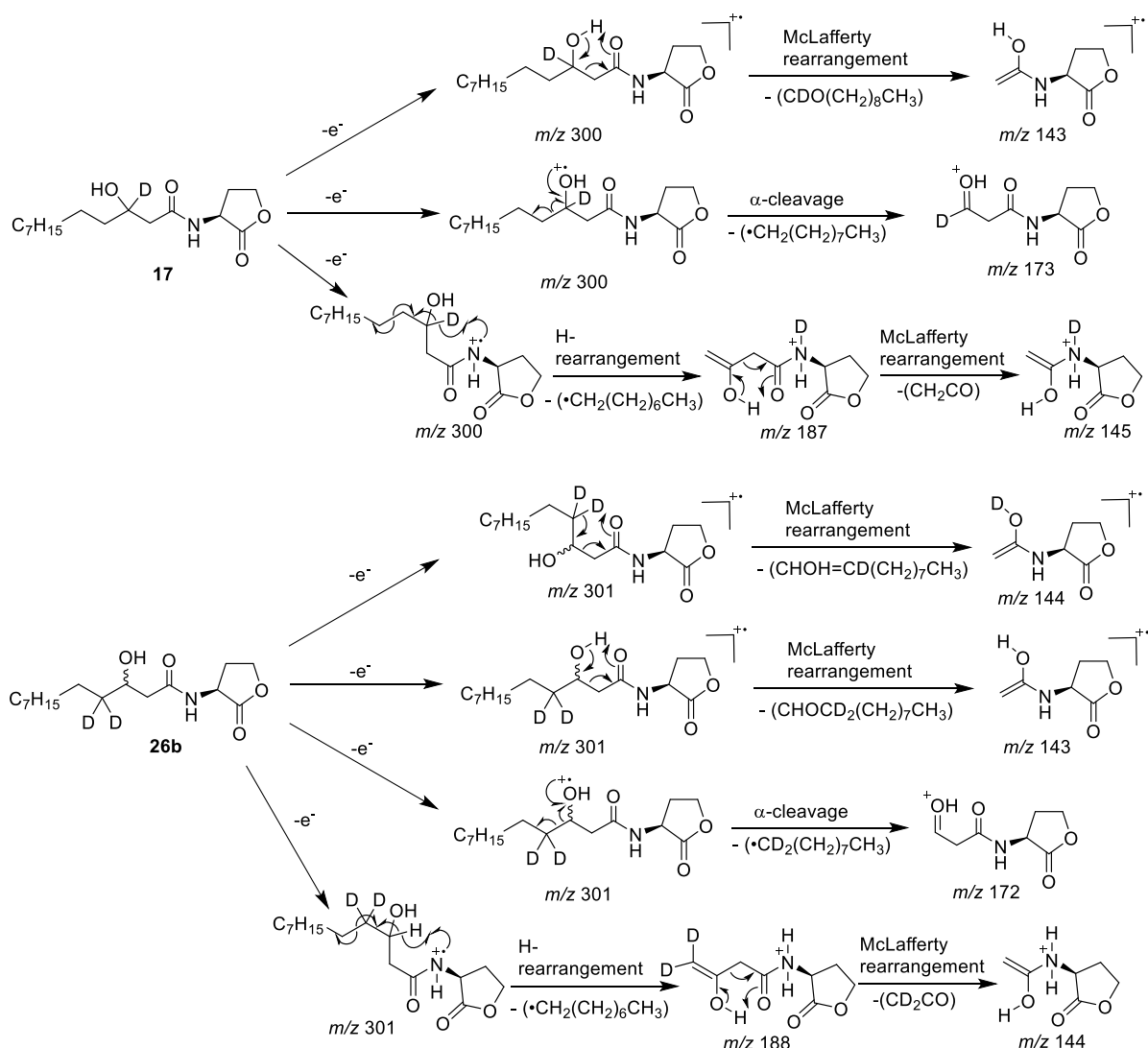
AHL	EI-MS <i>m/z</i> (rel. int.)
<i>N</i> -Dodecanoyl-L-homoserine lactone	283 [M] (4), 156 (21), 143 (100), 125 (17), 102 (18), 83 (15), 57 (41), 55 (19), 43 (21), 41 (17)
<i>N</i> -(Dodecanoyl-[3- ² H ₂])-L-homoserine lactone 27c	285 [M] (4), 158 (15), 143 (100), 125 (17), 102 (14), 83 (13), 57 (44), 55 (11), 43 (21), 41 (14)
<i>N</i> -(3-Hydroxydodecanoyl)-L-homoserine lactone	299 [M] (2), 281 (3), 214 (11), 197 (19), 172 (100), 144 (18), 143 (40), 125 (11), 102 (85), 83 (18), 69 (12), 57 (51), 56 (19), 55 (21), 43 (36), 41 (21)
<i>N</i> -(3-Hydroxydodecanoyl-[3- ² H])-L-homoserine lactone 17	300 [M] (2), 282 (3), 215 (8), 197 (16), 173 (100), 145 (15), 143 (46), 125 (13), 102 (86), 83 (16), 69 (12), 57 (55), 56 (28), 55 (19), 43 (37), 41 (22)
<i>N</i> -(3-Hydroxydodecanoyl-[4- ² H ₂])-L-homoserine lactone 26b	301 [M] (2), 283 (2), 216 (11), 199 (17), 172 (100), 144 (24), 143 (28), 125 (9), 102 (79), 83 (12), 69 (9), 57 (48), 56 (22), 55 (14), 43 (33), 41 (17)
<i>N</i> -(3-Hydroxydodecanoyl)-L-homoserine lactone + BSTFA-TMCS	371 [M+TMS] (1), 356 (100), 244 (40), 228 (10), 215 (5), 200 (14), 186 (8), 174 (8), 160 (6), 143 (23), 130 (11), 103 (6), 83 (9), 73 (27), 57 (5), 55 (5), 43 (7), 41 (6)
<i>N</i> -(3-Hydroxydodecanoyl-[3- ² H])-L-homoserine lactone 17 + BSTFA-TMCS	372 [M+TMS] (1), 357 (100), 245 (42), 229 (10), 215 (6), 200 (14), 187 (7), 175 (6), 160 (7), 144 (18), 130 (13), 103 (6), 84 (6), 75 (16), 73 (33), 57 (5), 55 (5), 43 (8), 41 (6)
<i>N</i> -(3-Hydroxydodecanoyl-[4- ² H ₂])-L-homoserine lactone 26b + BSTFA-TMCS	373 [M+TMS] (1), 358 (100), 244 (40), 228 (11), 215 (5), 200 (14), 186 (8), 174 (8), 160 (6), 143 (18), 130 (9), 103 (6), 85 (6), 73 (31), 57 (5), 55 (4), 43 (7), 41 (6)
<i>N</i> -(3-Oxodecanoyl)-L-homoserine lactone + BSTFA-TMCS	341 [M+TMS] (11), 326 (72), 270 (21), 257 (49), 241 (94), 215 (17), 169 (65), 150 (25), 125 (15), 73 (100), 57 (11), 43 (12)
<i>N</i> -(3-Oxodecanoyl-[5- ² H ₂])-L-homoserine lactone 28a + BSTFA-TMCS	343 [M+TMS] (9), 328 (60), 272 (16), 257 (44), 243 (81), 215 (16), 171 (57), 152 (24), 125 (12), 73 (100), 57 (11), 43 (15)

The characteristic mass fragment of *N*-acyl homoserine lactones is situated at m/z 143, corresponding to a McLafferty rearrangement. Since the synthesized deuterium-labelled derivatives **27a-c** have the deuterium atoms located at the β -carbon, the label is lost and the ion with m/z 143 is formed as well (Scheme 5). To distinguish between the native and labelled AHLs, the higher mass fragments, corresponding to different alkyl chain losses, but retaining the isotope label, should be targeted. The ion with m/z 158 (corresponding to m/z 156 in unlabelled AHLs) was detected with sufficient intensity in all *N*-(acyl-[3- 2 H $_2$])-L-homoserine lactones **27a-c** and can be used in a SIM-method to detect only labelled analogues. In all compounds analyzed, the molecular ion was detected, albeit with low intensities.



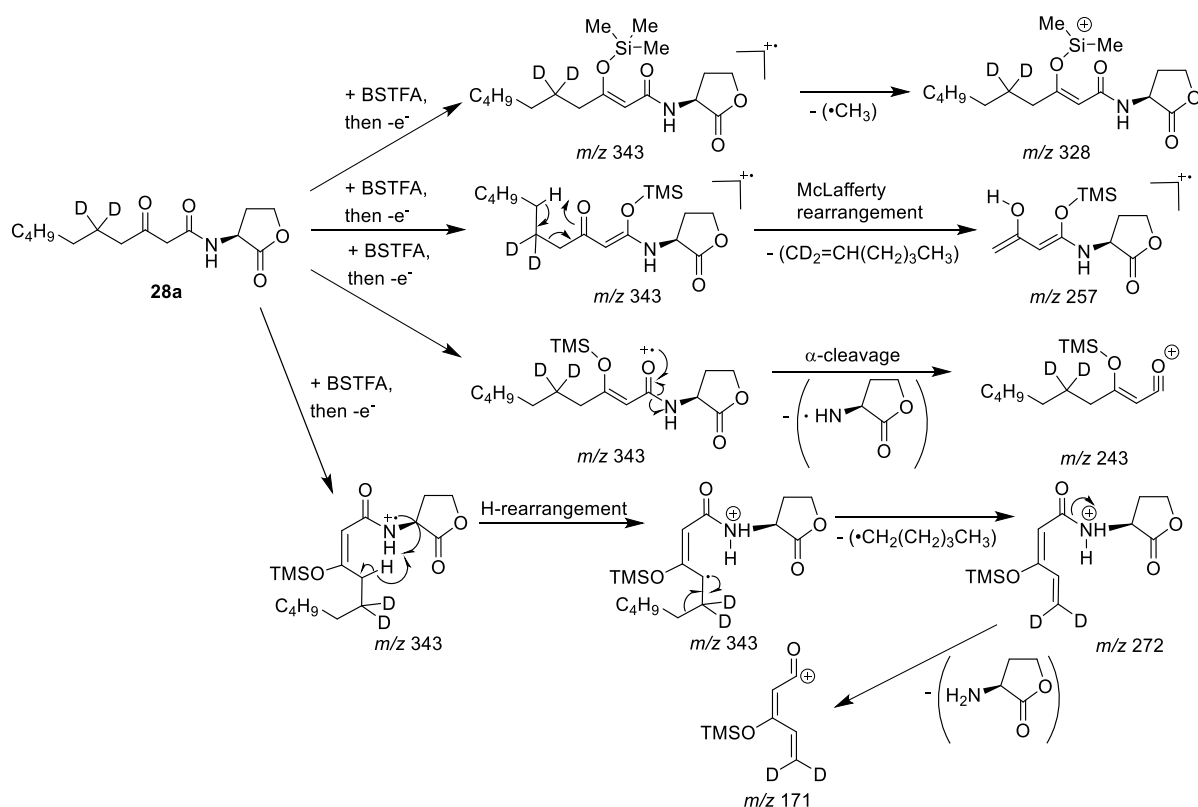
Scheme 5: Main mass fragment formed via a McLafferty rearrangement during EI-ionization induced fragmentation of *N*-(acyl-[3- 2 H $_2$])-L-homoserine lactones **27a-c**.

The characteristic ion formed during fragmentation of *N*-(3-hydroxyacyl)-L-homoserine lactones is located at m/z 172. This fragment arises from an α -cleavage next to the hydroxy group. Not surprisingly, this fragment can be observed in both unlabelled and γ -dilabelled *N*-(3-hydroxyacyl)-L-homoserine lactones **26a-c** (Scheme 6). However, in case of the monolabelled analogue **17**, with the deuterium label at the β -position, a fragment with one mass unit higher (m/z 173) results. Another characteristic ion is formed by the McLafferty rearrangement. While the mass fragment of m/z 143 is observed in all *N*-(3-hydroxyacyl)-L-homoserine lactones, the ion with m/z 144 is observed with an elevated intensity in case of the *N*-(3-hydroxyacyl-[4- 2 H $_2$])-L-homoserine lactones **26a-c**. This can be explained via an alternative McLafferty rearrangement, in which not the proton of the hydroxy group is abstracted, but rather the γ -proton (or deuterium in case of analogues **26a-c**) of the alkyl chain, causing a mass shift of one unit for analogues **26a-c**. As only a minor (37-43%) increase in abundance of m/z 144 is observed, a preference towards abstraction of the hydroxy proton can be concluded. Unfortunately, an alternative fragmentation, a hydrogen rearrangement followed by a McLafferty rearrangement, also leads to m/z 144 in both the unlabelled and dilabelled analogues, rendering this mass fragment not very useful to be targeted in a SIM-method. In the case of the monolabelled analogue **17** this fragmentation leads to a fragment with m/z 145, indicating that mainly the β -hydrogen (or deuterium) atom is abstracted (Scheme 6). Once again, the molecular ion itself was detected, albeit with low intensities.



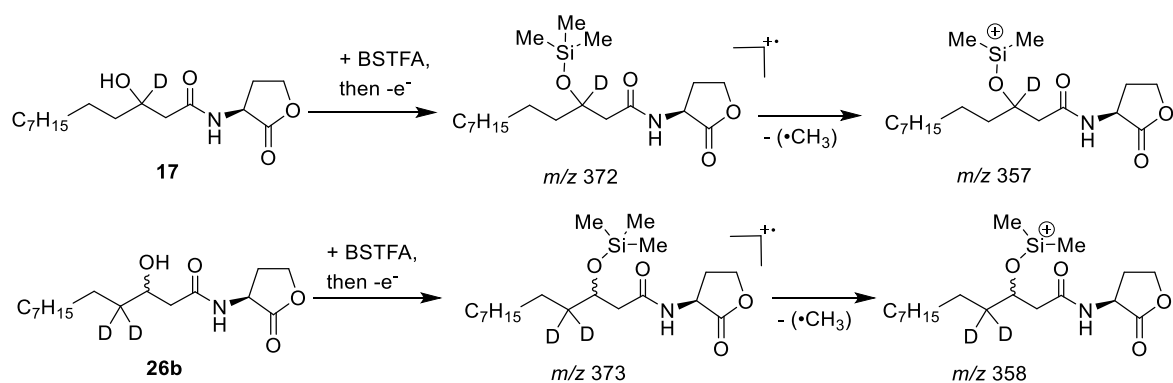
Scheme 6: Main mass fragments formed during the fragmentation of isotope-labelled *N*-(3-hydroxyacyl)-*L*-homoserine lactones **17** and **26b**.

Due to the lability of *N*-(3-oxoacyl)-*L*-homoserine lactones during chromatographic analysis, a derivatization step needs to be included. When *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) is used as silylation agent, detection via GC-MS becomes possible. The molecular ion and the mass fragment resulting from the loss of one methyl group are detected with satisfying intensities. The ion at m/z 257 is formed via a McLafferty rearrangement (Scheme 7). α -Cleavage, with loss of the lactone unit, results in an alkyl chain fragment with m/z 243 for *N*-(3-oxodecanoyl)-*L*-homoserine lactone **28a**. A possible marker fragment with m/z 171, common to all *N*-(3-oxoacyl-[5- 2 H $_2$])-*L*-homoserine lactones **28a-c** and with full retention of the isotope label, is obtained after a H-rearrangement, followed by the expulsion of a neutral homoserine lactone molecule.



Scheme 7: Formalisms for the main mass fragments formed during the fragmentation of isotope-labelled *N*-(3-oxodecanoyl)-L-homoserine lactone **28a** after derivatization with BSTFA.

When labelled *N*-(3-hydroxyacyl)-L-homoserine lactones **17** and **26a-c** are treated with BSTFA, the fragment observed with the highest intensity is the silylated molecule minus a methyl group (Scheme 8). Due to the high intensity and full retention of the isotope label, this fragment can be used to detect specific labelled *N*-(3-hydroxyacyl)-L-homoserine lactones **17** and **26a-c** in a reliable manner.



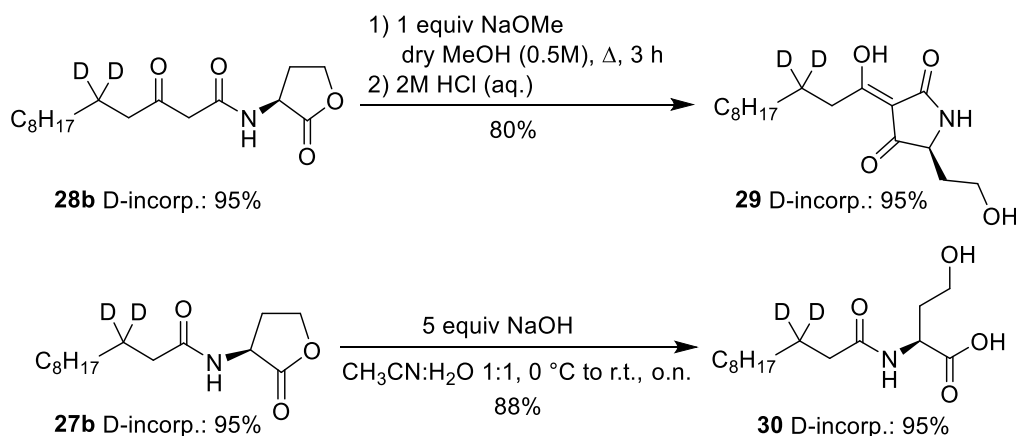
Scheme 8: Main mass fragments formed during the fragmentation of isotope-labelled *N*-(3-hydroxyacyl)-L-homoserine lactones **17** and **26b** after derivatization with BSTFA.

The presence of deuterium atoms is also obvious from the infrared spectrum by the occurrence of CD₂-vibrations. The small peaks around 2200 and 2100 cm⁻¹ correspond to

antisymmetric and symmetric CD₂-vibrations. These signals can be used to study the integration of deuterated AHLs in supported bilayers.^{29, 58}

To demonstrate and further extend the possible applications of these deuterated compounds, two AHL-degradation products, which occur in nature, were synthesized. The hydrolysis of the lactone ring of AHLs towards *N*-acyl homoserines, occurs in a temperature, pH and chain length dependent way and results in a loss of QS-activity.^{59, 60} *N*-(3-Oxoacyl)-L-homoserine lactones can form another type of degradation product. Via an intramolecular Claisen-type rearrangement, a tetramic acid can be formed. Fascinating biological properties such as antibacterial activity and iron chelation are associated with this type of molecules.⁶¹ Methods have been developed to detect both degradation products as well.^{32, 62} However, to the best of our knowledge, no deuterium-labelled *N*-acyl homoserines nor deuterated tetramic acids have been reported yet, although these compounds could be used as internal standards for IDMS.

The corresponding tetramic acid **29** and the ring opened product **30**, were synthesized in good yield and full retention of the deuterium label was observed during these transformations (Scheme 9).



Scheme 9: Synthesis of deuterated analogues tetramic acid **29** and ring opened AHL **30**. The deuterium content was determined by ESI-MS.

Conclusion

An easy, reliable manner to make suitable, deuterated standards of AHL-molecules belonging to all three important classes of AHLs is presented, starting from a cheap and commercially available deuterium source. Deuterium loss via D/H-exchange is avoided by the introduction of the isotope label on a non-enolizable position. All synthesized analogues were fully characterized. These deuterated analogues of the major classes of AHLs can also be used to synthesize the labelled analogues of the naturally occurring degradation products, without a significant loss of deuterium. These labelled AHLs and degradation products can be used to study the metabolization of AHLs in nature.

Acknowledgements

The authors thank Ghent University (BOF 14/DOC_V/285) for financial support of this research.

Experimental section

General

Solvents and chemicals used were bought from commercial suppliers and used as such, unless stated otherwise. Sodium borodeuteride (98% D) and D₂O (99.9% D) were purchased from Sigma-Aldrich. ¹H (400 MHz) and ¹³C (100.6 MHz) NMR spectra were recorded on a Bruker Avance III Nano-bay 400 at room temperature. IR spectra were recorded in neat form with a Perkin-Elmer Spectrum One FTIR spectrometer. High-resolution mass spectra were determined with an Agilent 1100 series HPLC coupled to an Agilent 6210 TOF mass spectrometer, equipped with an ESI/APCI multimode source. GC spectra were recorded on a Agilent HP 6890 series with a DB-5 MS fused-silica capillary column (30 m; 0.25 mm, film thickness = 0.25 μm). Electron Impact (EI) mass spectra were obtained with an Agilent HP 5973 series MSD mass spectrometer.

GC-MS conditions

GC-MS analyses were carried out according to the procedure developed by Cataldi *et al.* with minor modifications.¹⁹ Conditions applied were: inlet pressure 77.1 kPa; He: 23.3 mL/min; injection volume: 1 μL; injector temperature: 250 °C; transfer line: 300 °C; electron energy: 70 eV. The oven temperature program was: initial temperature and hold for 5 min at 100 °C, then increasing at a ramp of 10 °C per min to 300 °C. The injector was operated in splitless mode (60 s valve time). The carrier gas used was helium with a constant flow of 1 mL/min. AHLs were dissolved in dichloromethane. For the derivatization, 100 μL of 99:1 BSTFA-TMCS [*N,O*-bis(trimethylsilyl) trifluoroacetamide-trimethylchlorosilane] was added to 100 μL of dichloromethane containing the AHL and the mixture was allowed to stand for 10 min at room temperature prior to injection.

Synthesis of deuterium-labelled analogues

Synthesis of [3-²H]-3-hydroxydodecanoic acid **15**

Diethyl carbonate **12** (2.1 equiv, 22 mmol, 2.5 mL) and sodium hydride (60% dispersion in mineral oil, 3 equiv, 30 mmol, 1.2 g) were stirred at reflux in 20 mL of dry THF under a nitrogen atmosphere. Undecanone **11** (1 equiv, 10 mmol, 2.1 mL) was added dropwise and the resulting mixture was stirred at reflux overnight. After cooling down, the suspension was poured into a mixture of saturated aq. NH₄Cl, 5% aq. HCl and ice. Extraction (three times 50 mL of diethyl ether) and washing of the combined organic phases, followed by drying over MgSO₄ and removal of the solvent *in vacuo*, afforded ethyl 3-oxododecanoate **13** as a yellow oil in 94% yield. This crude β-ketoester **13** was used for the next step without further purification.

To a stirred solution of ethyl 3-oxododecanoate **13** (1 equiv, 8.5 mmol, 2.0 g) in 25 mL of methanol was added 22 mL of 2M NaOH (5 equiv, 44 mmol, 1.7 g). The resulting mixture was stirred overnight at room temperature, after which it was extracted with 50 mL of diethyl ether. The organic phase was discarded and the aqueous phase was acidified to pH 2 by addition of 2M HCl, and extracted three times with 50 mL of diethyl ether. The combined organic phases were dried over MgSO₄ and the solvent was removed *in vacuo*. Recrystallization from hexane afforded 3-oxododecanoic acid **14** as a colorless powder (1.4 g, 75%).

Sodium borodeuteride (1.5 equiv, 7.5 mmol, 0.31 g) was added to a solution of 3-oxododecanoic acid **14** (1 equiv, 5 mmol, 1.1 g) in diethyl ether:methanol 1:1 (10 mL) at 0 °C. The ice bath was removed and the mixture was stirred at room temperature for 2 h. The mixture was diluted with 10 mL of 2M HCl and 10 mL of diethyl ether. After phase separation, the aqueous phase was extracted twice with 10 mL of diethyl ether. The combined extracts were washed with 20 mL of brine and dried over MgSO₄. Filtration and evaporation of the solvent *in vacuo* yielded pure [3-²H]-3-hydroxydodecanoic acid **15** (98%, 1.1 g) as a colorless powder.

[3-²H]-3-Hydroxydodecanoic acid **15** (89% D) ¹H NMR (400 MHz, CDCl₃): δ = 0.88 (3H, t, *J* = 6.9 Hz, CH₃), 1.20-1.61 (16H, m, (CH₂)₈CH₃), 2.47 (1H, d, *J* = 16.6 Hz, CHH'COOH), 2.58 (1H, d, *J* = 16.6 Hz, CHH'COOH), 4.00-4.11 (0.1H, m, CHOH) ppm. ¹³C NMR (100 MHz, CDCl₃): δ = 14.1 (CH₃), 22.7, 25.4, 29.3, 29.5, 29.6 (2C), 31.9, 36.3 ((CH₂)₈CH₃), 41.0 (CH₂COOH), 67.4-68.3 (m, CDOH), 68.2 (CHOH), 177.8 (COOH) ppm. MS (ESI): *m/z* (%): 218 (M+H⁺, 7), 235 (M+NH₄⁺, 100). HRMS mass expected: C₁₂H₂₂DO₃⁻ 216.1715; found: 216.1721. IR (cm⁻¹): ν_{max} 913, 1226, 1680, 2130, 2848, 2915, 2953, 3050, 3534. Melting Point: 72-74 °C. Colorless powder. Yield: 98%.

General procedure for the synthesis of deuterated aldehydes **19a-c**

As described by Ariza *et al.*,⁴⁹ freshly distilled aldehyde **18a-c** (1 equiv, 13 mmol), 4-(*N,N*-dimethylamino)pyridine (0.1 equiv, 1.3 mmol, 0.16 g) and D₂O (10 equiv, 130 mmol, 2.3 mL) were added to a septum sealed flask and heated at 100 °C for 1 h. Then 20 mL of dichloromethane and 5 mL of 1M aq. HCl were added. The organic phase was washed with 15 mL of saturated aq. NaHCO₃ and 15 mL of brine. Drying over MgSO₄, filtration and removal of the solvent *in vacuo* yielded the deuterated aldehyde **19a-c** (71-97% yield). To obtain a higher degree of deuterium incorporation, the obtained deuterated aldehyde was subjected to a second iteration with fresh D₂O.

General procedure for the synthesis of deuterated β-hydroxy fatty acids **22a-c**

To a stirred suspension of activated zinc dust (acid-washed and dried) (1.3 equiv, 6.5 mmol, 0.43 g) in 20 mL of anhydrous diethyl ether in a flame-dried bulb, was added chlorotrimethylsilane (0.1 equiv, 0.5 mmol, 65 μL). The mixture was stirred at room temperature for 15 min and then heated at reflux for 10 min. The suspension was allowed to cool down and a mixture of ethyl bromoacetate **20** (1 equiv, 5 mmol, 0.57 mL) and deuterated

aldehyde **19a-c** (1 equiv, 5 mmol) was added dropwise in such a pace that a gentle reflux was obtained. The resulting mixture was further heated at reflux for 1 h, after which it was cooled down and 20 mL of 2M aq. HCl was added. After stirring for 15 min, the layers were separated and the aqueous phase was extracted two times with 20 mL of diethyl ether. The combined organic phases were washed with 20 mL of saturated aq. NaHCO₃ and 20 mL of brine. Drying over MgSO₄ and removal of the solvent *in vacuo* yielded the desired β -hydroxy ethyl ester **21a-c** as a colorless oil in 92-97% yield.

The obtained ester **21a-c** was dissolved in 30 mL of a 2:1 mixture of tetrahydrofuran:methanol and placed in an ice bath. Then, 10 mL of 1M aq. LiOH (2 equiv, 10 mmol, 0.24 g) was added and the stirring continued for 2 h at 0 °C. Subsequently, the ice bath was removed and the hydrolysis continued at room temperature overnight. The mixture was extracted once with 20 mL of diethyl ether to remove unreacted ester. The aqueous phase was acidified with 2M aq. HCl to pH 2 and extracted three times with 20 mL of diethyl ether. Drying over MgSO₄, filtration and rotary evaporation *in vacuo* of the solvent yielded crude β -hydroxy fatty acid **22a-c**. Recrystallization from diethyl ether gave the pure fatty acid **22a-c** as a colorless powder (84-95% yield).

[4-²H₂]-3-Hydroxynonanoic acid **22a** (93% D) ¹H NMR (400 MHz, CDCl₃): δ = 0.88 (3H, t, *J* = 6.8 Hz, CH₃), 1.21-1.50 (8H, m, (CH₂)₄CH₃), 2.48 (1H, dd, *J* = 16.6 Hz, 8.8 Hz, CHH'COOH), 2.58 (1H, dd, *J* = 16.6 Hz, 3.1 Hz, CHH'COOH), 4.03 (1H, dd, *J* = 8.8 Hz, 3.1 Hz, CHOH) ppm. ¹³C NMR (100 MHz, CDCl₃): δ = 14.1 (CH₃), 22.6, 25.2, 29.1, 31.8 ((CH₂)₄CH₃), 35.7 (quintet, *J*_{C-D} = 19.4 Hz, CD₂), 41.0 (CH₂COOH), 68.0 (CHOH), 178.0 (COOH) ppm. MS (ESI): *m/z* (%): 176 (M+H⁺, 26), 194 (M+NH₄⁺, 100). HRMS mass expected: C₉H₁₆D₂O₃⁻ 175.1309; found: 175.1311. IR (cm⁻¹): ν_{\max} 913, 1218, 1681, 2103, 2174, 2856, 2916, 2952, 3050, 3536. Melting Point: 61-63 °C. Colorless powder. Yield: 84%.

[4-²H₂]-3-Hydroxydodecanoic acid **22b** (95% D) ¹H NMR (400 MHz, CDCl₃): δ = 0.87 (3H, t, *J* = 7.0 Hz, CH₃), 1.21-1.50 (14H, m, (CH₂)₇CH₃), 2.48 (1H, dd, *J* = 16.5 Hz, 8.9 Hz, CHH'COOH), 2.58 (1H, dd, *J* = 16.5 Hz, 3.2 Hz, CHH'COOH), 4.05 (1H, dd, *J* = 8.9 Hz, 3.2 Hz, CHOH) ppm. ¹³C NMR (100 MHz, CDCl₃): δ = 14.1 (CH₃), 22.7, 25.3, 29.3, 29.4, 29.5, 29.6, 31.9 ((CH₂)₇CH₃), 35.7 (quintet, *J*_{C-D} = 19.8 Hz, CD₂), 41.1 (CH₂COOH), 68.0 (CHOH), 178.0 (COOH) ppm. MS (ESI): *m/z* (%): 201 (M-H₂O, 80), 219 (M+H⁺, 6), 241 (M+Na, 100). HRMS mass expected: C₁₂H₂₂D₂O₃NH₄⁺ 236.2189; found: 236.2181. IR (cm⁻¹): ν_{\max} 914, 1228, 1677, 2102, 2179, 2848, 2915, 2953, 3063, 3534. Melting Point: 73-74 °C. Colorless powder. Yield: 89%.

[4-²H₂]-3-Hydroxytridecanoic acid **22c** (93% D) ¹H NMR (400 MHz, CDCl₃): δ = 0.88 (3H, t, *J* = 6.8 Hz, CH₃), 1.20-1.48 (16H, m, (CH₂)₈CH₃), 2.47 (1H, dd, *J* = 16.6 Hz, 8.9 Hz, CHH'COOH), 2.58 (1H, dd, *J* = 16.5 Hz, 3.1 Hz, CHH'COOH), 4.02 (1H, dd, *J* = 8.9 Hz, 3.1 Hz, CHOH) ppm. ¹³C NMR (100 MHz, CDCl₃): δ = 14.1 (CH₃), 22.7, 25.2, 29.3, 29.4, 29.56, 29.58, 29.60, 31.9 ((CH₂)₈CH₃), 35.4-36.2 (m, CD₂), 41.0 (CH₂COOH), 67.9 (CHOH), 178.0 (COOH) ppm. MS (ESI): *m/z* (%): 233 (M+H⁺, 7), 250 (M+NH₄⁺, 100). HRMS mass expected: C₁₃H₂₄D₂O₃NH₄⁺ 250.2346; found:

250.2340. IR (cm⁻¹): ν_{\max} 915, 1219, 1681, 2100, 2182, 2847, 2914, 2952, 3031, 3534. Melting Point: 78-80 °C. Colorless powder. Yield: 95%.

General procedure for the synthesis of deuterated fatty acids **24a-c**

α,α -Dideuterated aldehyde **19a-c** (1 equiv, 10 mmol) was dissolved in 20 mL of a 1:1 methanol:diethyl ether mixture whereafter sodium borohydride (2 equiv, 20 mmol, 0.76 g) was added portion-wise. The reaction was stirred for 2 h at room temperature and quenched by the dropwise addition of water. The mixture was acidified by the addition of 2M aq. HCl and the aqueous layer was extracted three times with 20 mL of ethyl acetate. The combined organic phases were washed with 30 mL of brine and dried over MgSO₄. Removal of the drying agent by filtration and evaporation of the solvent *in vacuo* gave the β,β -dideuterated alcohol (88-96% yield).

The obtained crude β,β -dideuterated alcohol (1 equiv, 10 mmol) was dissolved in 30 mL of dry diethyl ether and triethylamine (1.4 equiv, 14 mmol, 2.0 mL) was added. The resulting solution was placed in an ice bath and methanesulfonyl chloride (1.15 equiv, 11.5 mmol, 1.1 mL) was added dropwise. The stirring was continued for 2 h at room temperature, then 25 mL of water was added to the resulting turbid suspension. The phases were separated and the organic phase was washed with 15 mL of saturated aq. NaHCO₃. Drying over MgSO₄, filtration and removal of the solvent *in vacuo* gave the desired alkyl methanesulfonate **23a-c** (77-96% yield). To obtain the alkyl nitrile, the mesylate **23a-c** (1 equiv, 10 mmol) was dissolved in dimethyl sulfoxide (30 mL), followed by the addition of potassium cyanide (2.5 equiv, 25 mmol, 1.63 g). Stirring overnight at 120 °C resulted in the formation of a thick precipitate. The reaction mixture was cooled down and partitioned between water (20 mL) and diethyl ether (20 mL). The aqueous layer was extracted three times with diethyl ether (3 x 20 mL) and the combined organic phases were dried over MgSO₄. Removal of the solvent *in vacuo* yielded the alkyl nitriles (86-95% yield).

To a solution of the nitrile (1 equiv, 10 mmol) in 40 mL of methanol was added 4 mL of 10M aq. NaOH (4 equiv, 40 mmol, 1.60 g) and the resulting mixture was stirred at reflux overnight. After cooling down, methanol was removed under reduced pressure and the remaining heterogeneous mixture was heated at reflux for a further 2 h. The suspension was extracted with 20 mL of diethyl ether to remove impurities and unreacted nitrile, followed by acidification with 2M aq. HCl and extraction with diethyl ether (3 x 50 mL). Drying over MgSO₄ and removal of the solvent *in vacuo* yielded the desired deuterium-labelled fatty acids **24a-c** (58-80% yield).

[3-²H₂]-Octanoic acid **24a** (93% D) ¹H NMR (400 MHz, CDCl₃): δ = 0.88 (3H, t, J = 6.9 Hz, CH₃), 1.20-1.38 (8H, m, (CH₂)₄CH₃), 2.33 (2H, s, CH₂COOH), 11.56 (1H, br s, COOH) ppm. ¹³C NMR (100 MHz, CDCl₃): δ = 14.1 (CH₃), 22.6, 28.80, 28.84, 31.6 ((CH₂)₄CH₃), 23.5-24.5 (m, CD₂), 34.0 (CH₂COOH), 180.6 (COOH) ppm. IR (cm⁻¹): ν_{\max} 934, 1230, 1275, 1412, 1705, 2857, 2925. Colorless oil. Yield: 58%.

[3-²H₂]-Undecanoic acid **24b** (95% D) ¹H NMR (400 MHz, CDCl₃): δ = 0.88 (3H, t, *J* = 6.9 Hz, CH₃), 1.18-1.37 (14H, m, (CH₂)₇CH₃), 2.33 (2H, s, CH₂COOH), 11.31 (1H, br s, COOH) ppm. ¹³C NMR (100 MHz, CDCl₃): δ = 14.1 (CH₃), 22.7, 28.9, 29.2, 29.3, 29.4, 29.6, 31.9 ((CH₂)₇CH₃), 23.5-24.7 (m, CD₂), 33.9 (CH₂COOH), 180.0 (COOH) ppm. IR (cm⁻¹): ν_{max} 927, 1252, 1692, 2111, 2201, 2850, 2918, 2955, 3019. Colorless oil. Yield: 80%.

[3-²H₂]-Dodecanoic acid **24c** (92% D) ¹H NMR (400 MHz, CDCl₃): δ = 0.88 (3H, t, *J* = 6.6 Hz, CH₃), 1.18-1.37 (16H, m, (CH₂)₈CH₃), 2.32 (2H, s, CH₂COOH), 9.34 (1H, br s, COOH) ppm. ¹³C NMR (100 MHz, CDCl₃): δ = 14.1 (CH₃), 22.7, 28.9, 29.2, 29.3, 29.4, 29.6 (2C), 31.9 ((CH₂)₈CH₃), 23.6-24.8 (m, CD₂), 34.0 (CH₂COOH), 179.9 (COOH) ppm. IR (cm⁻¹): ν_{max} 931, 1275, 1429, 1695, 2848, 2912. Colorless powder. Melting Point: 40-41 °C. Yield: 70%.

General procedure for the synthesis of deuterated AHLs **17**, **26a-c** and **27a-c**

The deuterated analogues of *N*-acyl-L-homoserine lactones and *N*-(3-hydroxyacyl)-L-homoserine lactones were synthesized following the procedure described by Chhabra *et al.*⁴⁸ Triethylamine (1 equiv, 2 mmol, 0.28 mL) was added to a stirred solution of L-homoserine lactone hydrobromide **16** (1 equiv, 2 mmol, 0.36 g) in 5 mL of water, followed by the addition of the appropriate deuterated fatty acid (**15**, **22a-c** or **24a-c**, 1 equiv, 2 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1 equiv, 2 mmol, 0.38 g). After stirring for 3 h or overnight at room temperature, 15 mL of water was added and the aqueous phase was extracted three times with 20 mL of ethyl acetate. The combined organic phases were washed with 30 mL of saturated aq. NaHCO₃ and 30 mL of brine. Drying over MgSO₄, followed by filtration and evaporation of the solvent *in vacuo* gave the crude product. Purification via column chromatography on silica gel (ethyl acetate/petroleum ether 3:2 to 4:1) yielded the desired deuterated AHLs **17**, **26a-c** and **27a-c** as a colorless powder.

N-(3-Hydroxydodecanoyl-[3-²H])-L-homoserine lactone **17** (89% D) ¹H NMR (400 MHz, CDCl₃): δ = 0.88 (3H, t, *J* = 6.9 Hz, CH₃, isomer 1 and 2), 1.19-1.57 (16H, m, (CH₂)₈CH₃, isomer 1 and 2), 2.13-2.26 (1H, m, OCH₂CHH' isomer 1 and 2), 2.33 (0.5H, d, *J* = 15.4 Hz, CHH'CDOH isomer 1), 2.35 (0.5H, d, *J* = 15.4 Hz, CHH'CDOH isomer 2), 2.44 (0.5H, d, *J* = 15.4 Hz, CHH'CDOH isomer 2), 2.46 (0.5H, d, *J* = 15.4 Hz, CHH'CDOH isomer 1), 2.81 (1H, dddd, *J* = 12.5 Hz, 8.6 Hz, 5.9 Hz, 1.2 Hz, OCH₂CHH' isomer 1 and 2), 3.13 (1H, br s, OH isomer 1 and 2), 4.0 (0.1H, m, CHOH isomer 1 and 2), 4.29 (1H, ddd, *J* = 11.2 Hz, 9.3 Hz, 6.0 Hz, OCHH' isomer 1 and 2), 4.480 (0.5H, td, *J* = 9.1 Hz, 1.3 Hz, OCHH' isomer 1), 4.483 (0.5H, td, *J* = 9.1 Hz, 1.4 Hz, OCHH' isomer 2), 4.56 (0.5H, ddd, *J* = 11.7 Hz, 8.6 Hz, 6.2 Hz, CHN isomer 1), 4.59 (0.5H, ddd, *J* = 11.8 Hz, 8.7 Hz, 6.3 Hz, CHN isomer 2), 6.60 (0.5H, d, *J* = 5.8 Hz, NH isomer 1), 6.65 (0.5H, d, *J* = 5.7 Hz, NH isomer 2) ppm. ¹³C NMR (100 MHz, CDCl₃): δ = 14.1 (CH₃, isomer 1 and 2), 22.7, 25.5, 29.3, 29.5, 29.56, 29.59, 31.9, 36.8, 36.9 ((CH₂)₈CH₃, isomer 1 and 2), 29.9 (CH₂CHN isomer 1), 30.0 (CH₂CHN isomer 2), 42.5 (CH₂CONH isomer 1), 42.6 (CH₂CONH isomer 2), 49.0 (CHN isomer 1), 49.2 (CHN isomer 2), 66.1 (CH₂O isomer 1), 66.2 (CH₂O isomer 2), 68.2 (t, *J*_{C-D} = 21.6 Hz, CDOH isomer 1), 68.3 (t, *J*_{C-D} = 21.7 Hz, CDOH isomer 2), 68.6 (COH isomer 1 unlabelled), 68.7 (COH isomer 2 unlabelled), 173.0 (CONH isomer 1), 173.1 (CONH isomer 2), 175.70 (COO isomer 1), 175.74 (COO isomer 2) ppm. MS (ESI): *m/z* (%): 301 (M+H⁺, 100). HRMS mass expected: C₁₆H₂₉DNO₄H⁺ 301.2232; found: 301.2237. IR (cm⁻¹

¹): ν_{\max} 951, 1008, 1178, 1548, 1627, 1768, 2850, 2919, 3315, 3494. Chromatography: EtOAc: Petroleum ether 4:1 R_f = 0.16. Melting Point: 107-108 °C. Colorless powder. Yield: 65%.

N-(3-Hydroxynonanoyl-[4-²H₂])-L-homoserine lactone **26a** (93% D) ¹H NMR (400 MHz, CDCl₃): δ = 0.88 (3H, t, J = 6.8 Hz, CH₃, isomer 1 and 2), 1.20-1.45 (8H, m, (CH₂)₄CH₃, isomer 1 and 2), 2.13-2.26 (1H, m, OCH₂CHH' isomer 1 and 2), 2.33 (0.4H, dd, J = 15.5 Hz, 8.9 Hz, CHH'CHOH isomer 1), 2.35 (0.6H, dd, J = 15.5 Hz, 8.9 Hz, CHH'CHOH isomer 2), 2.44 (0.6H, dd, J = 15.5 Hz, 2.8 Hz, CHH'CHOH isomer 2), 2.46 (0.4H, dd, J = 15.5 Hz, 2.8 Hz, CHH'CHOH isomer 1), 2.82 (1H, dddd, J = 12.6 Hz, 8.6 Hz, 5.9 Hz, 1.1 Hz, OCH₂CHH' isomer 1 and 2), 3.10 (1H, br s, OH isomer 1 and 2), 3.99-4.06 (1H, m, CHOH isomer 1 and 2), 4.29 (1H, ddd, J = 11.3 Hz, 9.3 Hz, 5.9 Hz, OCHH' isomer 1 and 2), 4.45-4.51 (1H, m, OCHH' isomer 1 and 2), 4.56 (0.4H, ddd, J = 11.5 Hz, 8.7 Hz, 6.1 Hz, CHN isomer 1), 4.58 (0.6H, ddd, J = 11.6 Hz, 8.7 Hz, 6.2 Hz, CHN isomer 2), 6.55 (0.4H, d, J = 5.6 Hz, NH isomer 1), 6.60 (0.6H, d, J = 5.6 Hz, NH isomer 2) ppm. ¹³C NMR (100 MHz, CDCl₃): δ = 14.1 (CH₃, isomer 1 and 2), 22.6, 25.3, 29.1, 31.8 ((CH₂)₄CH₃, isomer 1 and 2), 29.7 (CH₂CHN isomer 1), 29.8 (CH₂CHN isomer 2), 35.8-36.7 (m, CD₂, isomer 1 and 2), 42.6 (CH₂CONH isomer 1), 42.7 (CH₂CONH isomer 2), 48.9 (CHN isomer 1), 49.1 (CHN isomer 2), 66.15 (CH₂O isomer 1), 66.18 (CH₂O isomer 2), 68.5 (COH isomer 1), 68.6 (COH isomer 2), 173.0 (CONH isomer 1), 173.1 (CONH isomer 2), 175.8 (COO isomer 1), 175.9 (COO isomer 2) ppm. MS (ESI): m/z (%): 260 (M+H⁺, 100). HRMS mass expected: C₁₃H₂₁D₂NO₄H⁺ 260.1831; found: 260.1832. IR (cm⁻¹): ν_{\max} 950, 1012, 1174, 1382, 1543, 1645, 1768, 2103, 2184, 2855, 2920, 2956, 3302. Chromatography: EtOAc:Petroleum ether 4:1 R_f = 0.18. Melting Point: 97-98 °C. Colorless powder. Yield: 58%.

N-(3-Hydroxydodecanoyl-[4-²H₂])-L-homoserine lactone **26b** (95% D) ¹H NMR (400 MHz, CDCl₃): δ = 0.88 (3H, t, J = 6.9 Hz, CH₃, isomer 1 and 2), 1.19-1.43 (14H, m, (CH₂)₇CH₃, isomer 1 and 2), 2.13-2.26 (1H, m, OCH₂CHH' isomer 1 and 2), 2.33 (0.44H, dd, J = 15.3 Hz, 8.9 Hz, CHH'CHOH isomer 1), 2.35 (0.56H, dd, J = 15.4 Hz, 8.9 Hz, CHH'CHOH isomer 2), 2.43 (0.44H, dd, J = 15.4 Hz, 2.8 Hz, CHH'CHOH isomer 2), 2.46 (0.44H, dd, J = 15.4 Hz, 2.7 Hz, CHH'CHOH isomer 1), 2.77-2.86 (1H, m, OCH₂CHH' isomer 1 and 2), 3.11 (1H, br s, OH isomer 1 and 2), 3.98-4.05 (1H, m, CHOH isomer 1 and 2), 4.29 (1H, ddd, J = 11.2 Hz, 9.3 Hz, 5.9 Hz, OCHH' isomer 1 and 2), 4.43-4.51 (1H, m, OCHH' isomer 1 and 2), 4.56 (0.44H, ddd, J = 11.6 Hz, 8.7 Hz, 6.1 Hz, CHN isomer 1), 4.59 (0.56H, ddd, J = 11.6 Hz, 8.8 Hz, 6.1 Hz, CHN isomer 2), 6.53-6.64 (1H, m, NH isomer 1 and 2) ppm. ¹³C NMR (100 MHz, CDCl₃): δ = 14.1 (CH₃, isomer 1 and 2), 22.7, 25.3, 29.3, 29.5, 29.56, 29.60, 31.9 ((CH₂)₇CH₃, isomer 1 and 2), 29.98 (CH₂CHN isomer 1), 30.04 (CH₂CHN isomer 2), 35.8-36.8 (m, CD₂, isomer 1 and 2), 42.5 (CH₂CONH isomer 1), 42.6 (CH₂CONH isomer 2), 49.0 (CHN isomer 1), 49.2 (CHN isomer 2), 66.1 (CH₂O isomer 1), 66.2 (CH₂O isomer 2), 68.5 (COH isomer 1), 68.6 (COH isomer 2), 173.01 (CONH isomer 1), 173.05 (CONH isomer 2), 175.67 (COO isomer 1), 175.75 (COO isomer 2) ppm. MS (ESI): m/z (%): 302 (M+H⁺, 100). HRMS mass expected: C₁₆H₂₇D₂NO₄H⁺ 302.2300; found: 302.2300. IR (cm⁻¹): ν_{\max} 952, 1014, 1177, 1382, 1549, 1625, 1766, 2082, 2185, 2851, 2919, 2956, 3326, 3461. Chromatography: EtOAc:Petroleum ether 4:1 R_f = 0.15. Melting Point: 107-108 °C. Colorless powder. Yield: 63%.

N-(3-Hydroxytridecanoyl-[4-²H₂])-L-homoserine lactone **26c** (93% D) ¹H NMR (400 MHz, CDCl₃): δ = 0.88 (3H, t, J = 6.9 Hz, CH₃, isomer 1 and 2), 1.19-1.43 (16H, m, (CH₂)₈CH₃, isomer 1 and 2), 2.13-2.25 (1H, m, OCH₂CHH' isomer 1 and 2), 2.33 (0.44H, dd, J = 15.5 Hz, 9.0 Hz, CHH'CHOH isomer 1),

2.35 (0.56H, dd, $J = 15.4$ Hz, 8.7 Hz, $\text{CHH}'\text{CHOH}_{\text{isomer 2}}$), 2.43 (0.54H, dd, $J = 15.4$ Hz, 2.8 Hz, $\text{CHH}'\text{CHOH}_{\text{isomer 2}}$), 2.46 (0.44H, dd, $J = 15.3$ Hz, 2.8 Hz, $\text{CHH}'\text{CHOH}_{\text{isomer 1}}$), 2.79-2.87 (1H, m, $\text{OCH}_2\text{CHH}'_{\text{isomer 1 and 2}}$), 3.04 (1H, br s, $\text{OH}_{\text{isomer 1 and 2}}$), 3.98-4.04 (1H, m, $\text{CHOH}_{\text{isomer 1 and 2}}$), 4.29 (1H, ddd, $J = 11.2$ Hz, 9.3 Hz, 5.9 Hz, $\text{OCHH}'_{\text{isomer 1 and 2}}$), 4.45-4.51 (1H, m, $\text{OCHH}'_{\text{isomer 1 and 2}}$), 4.56 (0.44H, ddd, $J = 11.6$ Hz, 8.6 Hz, 5.9 Hz, $\text{CHN}_{\text{isomer 1}}$), 4.57 (0.56H, ddd, $J = 11.6$ Hz, 8.6 Hz, 5.7 Hz, $\text{CHN}_{\text{isomer 2}}$), 6.46-6.57 (1H, m, $\text{NH}_{\text{isomer 1 and 2}}$) ppm. ^{13}C NMR (100 MHz, CDCl_3): $\delta = 14.1$ (CH_3 , isomer 1 and 2), 22.7, 25.3, 29.3, 29.5, 29.6 (3C), 31.9 ($(\text{CH}_2)_8\text{CH}_3$, isomer 1 and 2), 29.9 ($\text{CH}_2\text{CHN}_{\text{isomer 1}}$), 30.0 ($\text{CH}_2\text{CHN}_{\text{isomer 2}}$), 35.7-36.8 (m, CD_2 , isomer 1 and 2), 42.5 ($\text{CH}_2\text{CONH}_{\text{isomer 1}}$), 42.6 ($\text{CH}_2\text{CONH}_{\text{isomer 2}}$), 49.0 ($\text{CHN}_{\text{isomer 1}}$), 49.1 ($\text{CHN}_{\text{isomer 2}}$), 66.1 ($\text{CH}_2\text{O}_{\text{isomer 1}}$), 66.2 ($\text{CH}_2\text{O}_{\text{isomer 2}}$), 68.5 ($\text{COH}_{\text{isomer 1}}$), 68.6 ($\text{COH}_{\text{isomer 2}}$), 173.02 ($\text{CONH}_{\text{isomer 1}}$), 173.05 ($\text{CONH}_{\text{isomer 2}}$), 175.7 ($\text{COO}_{\text{isomer 1 and 2}}$) ppm. MS (ESI): m/z (%): 316 ($\text{M}+\text{H}^+$, 100). HRMS mass expected: $\text{C}_{17}\text{H}_{29}\text{D}_2\text{NO}_4\text{H}^+$ 316.2457; found: 316.2448. IR (cm^{-1}): ν_{max} 948, 1022, 1177, 1382, 1549, 1641, 1770, 2102, 2177, 2849, 2917, 2953, 3312, 3490. Chromatography: EtOAc:Petroleum ether 4:1 $R_f = 0.16$. Melting Point: 110-112 °C. Colorless powder. Yield: 42%.

N-(Octanoyl-[3- $^2\text{H}_2$])-L-homoserine lactone **27a** (92% D) ^1H NMR (400 MHz, CDCl_3): $\delta = 0.88$ (3H, t, $J = 6.9$ Hz, CH_3), 1.20-1.36 (8H, m, $(\text{CH}_2)_4\text{CH}_3$), 2.12 (1H, dddd, $J = 12.5$ Hz, 11.5 Hz, 11.5 Hz, 8.8 Hz, $\text{OCH}_2\text{CHH}'$), 2.23 (2H, s, CH_2CONH), 2.88 (1H, dddd, $J = 12.5$ Hz, 8.5 Hz, 5.8 Hz, 1.2 Hz, $\text{OCH}_2\text{CHH}'$), 4.28 (1H, ddd, $J = 11.3$ Hz, 9.3 Hz, 5.8 Hz, OCHH'), 4.47 (1H, td, $J = 9.1$ Hz, 1.0 Hz, OCHH'), 4.54 (1H, ddd, $J = 11.6$ Hz, 8.5 Hz, 5.6 Hz, CHN), 5.94 (1H, s, NH) ppm. ^{13}C NMR (100 MHz, CDCl_3): $\delta = 14.1$ (CH_3), 22.6, 28.9, 29.0, 31.6 ($(\text{CH}_2)_4\text{CH}_3$), 24.4-25.4 (m, CD_2), 30.6 (CH_2CHN), 36.0 (CH_2CONH), 49.2 (CHN), 66.1 (CH_2O), 173.8 (CONH), 175.7 (COO) ppm. MS (ESI): m/z (%): 230 ($\text{M}+\text{H}^+$, 100). HRMS mass expected: $\text{C}_{12}\text{H}_{19}\text{D}_2\text{NO}_3\text{H}^+$ 230.1725; found: 230.1709. IR (cm^{-1}): ν_{max} 1010, 1170, 1545, 1643, 1774, 2107, 2194, 2852, 2920, 3314. Chromatography: EtOAc:Petroleum ether 3:2 $R_f = 0.19$. Melting Point: 130-132 °C. Colorless powder. Yield: 55%.

N-(Undecanoyl-[3- $^2\text{H}_2$])-L-homoserine lactone **27b** (95% D) ^1H NMR (400 MHz, CDCl_3): $\delta = 0.88$ (3H, t, $J = 6.9$ Hz, CH_3), 1.21-1.32 (14H, m, $(\text{CH}_2)_7\text{CH}_3$), 2.12 (1H, dddd, $J = 12.5$ Hz, 11.5 Hz, 11.5 Hz, 8.8 Hz, $\text{OCH}_2\text{CHH}'$), 2.23 (2H, s, CH_2CONH), 2.88 (1H, dddd, $J = 12.6$ Hz, 8.5 Hz, 5.8 Hz, 1.1 Hz, $\text{OCH}_2\text{CHH}'$), 4.29 (1H, ddd, $J = 11.3$ Hz, 9.3 Hz, 5.9 Hz, OCHH'), 4.47 (1H, td, $J = 9.1$ Hz, 1.0 Hz, OCHH'), 4.54 (1H, ddd, $J = 11.6$ Hz, 8.5 Hz, 5.6 Hz, CHN), 5.96 (1H, d, $J = 5.6$ Hz, NH) ppm. ^{13}C NMR (100 MHz, CDCl_3): $\delta = 14.1$ (CH_3), 22.7, 29.0, 29.28, 29.30, 29.5, 29.6, 31.9 ($(\text{CH}_2)_7\text{CH}_3$), 24.7 (quintet, $J_{\text{C-D}} = 19.2$ Hz, CD_2), 30.5 (CH_2CHN), 36.0 (CH_2CONH), 49.2 (CHN), 66.1 (CH_2O), 173.8 (CONH), 175.7 (COO) ppm. MS (ESI): m/z (%): 272 ($\text{M}+\text{H}^+$, 100). HRMS mass expected: $\text{C}_{15}\text{H}_{25}\text{D}_2\text{NO}_3\text{H}^+$ 272.2195; found: 272.2182. IR (cm^{-1}): ν_{max} 1010, 1171, 1547, 1643, 1779, 2113, 2205, 2851, 2919, 3315. Chromatography: EtOAc:Petroleum ether 3:2 $R_f = 0.21$. Melting Point: 135-136 °C. Colorless powder. Yield: 69%.

N-(Dodecanoyl-[3- $^2\text{H}_2$])-L-homoserine lactone **27c** (92% D) ^1H NMR (400 MHz, CDCl_3): $\delta = 0.88$ (3H, t, $J = 6.9$ Hz, CH_3), 1.19-1.35 (16H, m, $(\text{CH}_2)_8\text{CH}_3$), 2.12 (1H, dddd, $J = 12.5$ Hz, 11.5 Hz, 11.5 Hz, 8.8 Hz, $\text{OCH}_2\text{CHH}'$), 2.23 (2H, s, CH_2CONH), 2.88 (1H, dddd, $J = 12.6$ Hz, 8.5 Hz, 5.8 Hz, 1.1

Hz, OCH₂CHH'), 4.28 (1H, ddd, *J* = 11.3 Hz, 9.3 Hz, 5.8 Hz, OCHH'), 4.47 (1H, td, *J* = 9.1 Hz, 1.0 Hz, OCHH'), 4.53 (1H, ddd, *J* = 11.6 Hz, 8.5 Hz, 5.6 Hz, CHN), 5.93 (1H, d, *J* = 4.8 Hz, NH) ppm. ¹³C NMR (100 MHz, CDCl₃): δ = 14.1 (CH₃), 22.7, 29.0, 29.27, 29.32, 29.5, 29.6 (2C), 31.9 ((CH₂)₈CH₃), 24.3-25.4 (m, CD₂), 30.4 (CH₂CHN), 36.0 (CH₂CONH), 49.1 (CHN), 66.1 (CH₂O), 173.8 (CONH), 175.8 (COO) ppm. MS (ESI): *m/z* (%): 286 (M+H⁺, 100). HRMS mass expected: C₁₆H₂₇D₂NO₃H⁺ 286.2351; found: 286.2335. IR (cm⁻¹): ν_{max} 1010, 1171, 1546, 1643, 1775, 2110, 2194, 2850, 2918, 3315. Chromatography: EtOAc:Petroleum ether 3:2 R_f = 0.23. Melting Point: 137-139 °C. Colorless powder. Yield: 52%.

General procedure for the synthesis of deuterated AHLs 28a-c

To a stirred solution of β,β-dideuterated fatty acid **24a-c** (1 equiv, 2 mmol) in 20 mL of dry dichloromethane, was added 4-(*N,N*-dimethylamino)pyridine (2.1 equiv, 4.2 mmol, 0.51 g), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.1 equiv, 2.2 mmol, 0.42 g) and Meldrum's acid (1 equiv, 2 mmol, 0.29 g). The resulting clear yellow solution was stirred for 20 h at room temperature. The solvent was removed by rotary evaporation *in vacuo*, the residue was redissolved in ethyl acetate (50 mL) and washed with 30 mL of 2M aq. HCl. Drying over MgSO₄, followed by filtration to remove the drying agent and evaporation of the solvent *in vacuo* yielded acylated Meldrum's acid **25a-c** quantitatively, which was used without further purification. The deuterium-labelled, acylated Meldrum's acid **25a-c** was dissolved in acetonitrile (30 mL), after which triethylamine (1.2 equiv, 2.4 mmol, 0.33 mL) and L-homoserine lactone hydrobromide **16** (1 equiv, 2 mmol, 0.36 g) were added. The mixture was stirred at room temperature for 2 h and then heated at reflux overnight. The solvent was removed *in vacuo* and the resulting residue was dissolved in 30 mL of ethyl acetate and 30 mL of water. After separation of both layers, the aqueous phase was extracted two times with 30 mL of ethyl acetate. The combined organic phases were washed with 60 mL of saturated aq. NaHCO₃ and 60 mL of brine. Drying over MgSO₄ and removal of the solvent *in vacuo* yielded crude deuterated AHL **28a-c**. Purification via column chromatography on silica gel (ethyl acetate/petroleum ether 3:2 to 4:1) gave the pure product as a colorless powder.

N-(3-Oxodecanoyl-[5-²H₂])-L-homoserine lactone **28a** (93% D) ¹H NMR (400 MHz, CDCl₃): δ = 0.88 (3H, t, *J* = 6.8 Hz, CH₃), 1.20-1.32 (8H, m, (CH₂)₄CH₃), 2.23 (1H, dddd, *J* = 12.5 Hz, 11.3 Hz, 11.3 Hz, 8.9 Hz, OCH₂CHH'), 2.51 (2H, s, CH₂CONH), 2.76 (1H, dddd, *J* = 12.6 Hz, 8.7 Hz, 6.0 Hz, 1.4 Hz, OCH₂CHH'), 3.47 (2H, s, COCH₂CO), 4.28 (1H, ddd, *J* = 11.0 Hz, 9.3 Hz, 6.1 Hz, OCHH'), 4.47 (1H, td, *J* = 9.1 Hz, 1.3 Hz, OCHH'), 4.59 (1H, ddd, *J* = 11.5 Hz, 8.7 Hz, 6.6 Hz, CHN), 7.66 (1H, d, *J* = 5.6 Hz, NH) ppm. ¹³C NMR (100 MHz, CDCl₃): δ = 14.1 (CH₃), 22.6, 28.7, 28.9, 31.6 ((CH₂)₄CH₃), 22.2-23.4 (m, CD₂), 29.6 (CH₂CHN), 43.6 (CD₂CH₂CO), 48.4 (COCH₂CO), 49.0 (CHN), 65.9 (CH₂O), 166.5 (CONH), 175.0 (COO), 206.5 (CH₂COCH₂) ppm. MS (ESI): *m/z* (%): 272 (M+H⁺, 100), 289 (M+NH₄⁺, 20). HRMS mass expected: C₁₄H₂₁D₂NO₄H⁺ 272.1831; found: 272.1833. IR (cm⁻¹): ν_{max} 1010, 1023, 1165, 1356, 1548, 1646, 1712, 1776, 2112, 2193, 2851, 2918, 2957, 3081, 3284. Chromatography: EtOAc:Petroleum ether 3:2 R_f = 0.21. Melting Point: 81-82 °C. Colorless powder. Yield: 49%.

N-(3-Oxotridecanoyl-[5-²H₂])-L-homoserine lactone **28b** (95% D) ¹H NMR (400 MHz, CDCl₃): δ = 0.88 (3H, t, *J* = 6.8 Hz, CH₃), 1.19-1.33 (14H, m, (CH₂)₇CH₃), 2.24 (1H, dddd, *J* = 12.5 Hz, 11.3 Hz, 11.3 Hz, 9.0 Hz, OCH₂CHH'), 2.51 (2H, s, CH₂CONH), 2.76 (1H, dddd, *J* = 12.5 Hz, 8.7 Hz, 6.1 Hz, 1.4 Hz, OCH₂CHH'), 3.47 (2H, s, COCH₂CO), 4.28 (1H, ddd, *J* = 11.0 Hz, 9.3 Hz, 6.1 Hz, OCHH'), 4.48 (1H, td, *J* = 9.1 Hz, 1.3 Hz, OCHH'), 4.60 (1H, ddd, *J* = 11.5 Hz, 8.7 Hz, 6.6 Hz, CHN), 7.67 (1H, d, *J* = 5.7 Hz, NH) ppm. ¹³C NMR (100 MHz, CDCl₃): δ = 14.1 (CH₃), 22.7, 28.8, 29.3, 29.4, 29.5, 29.6, 31.9 ((CH₂)₇CH₃), 22.2-23.4 (m, CD₂), 29.7 (CH₂CHN), 43.7 (CD₂CH₂CO), 48.2 (COCH₂CO), 49.0 (CHN), 65.9 (CH₂O), 166.4 (CONH), 174.7 (COO), 206.6 (CH₂COCH₂) ppm. MS (ESI): *m/z* (%): 314 (M+H⁺, 100), 331 (M+NH₄⁺, 21). HRMS mass expected: C₁₇H₂₇D₂NO₄H⁺ 314.2295; found: 314.2305. IR (cm⁻¹): ν_{max} 1006, 1016, 1176, 1356, 1544, 1642, 1716, 1776, 2092, 2164, 2850, 2919, 3071, 3294. Chromatography: EtOAc:Petroleum ether 4:1 R_f = 0.37. Melting Point: 86-88 °C. Colorless powder. Yield: 66%.

N-(3-Oxotetradecanoyl-[5-²H₂])-L-homoserine lactone **28c** (93 %D) ¹H NMR (400 MHz, CDCl₃): δ = 0.88 (3H, t, *J* = 6.9 Hz, CH₃), 1.20-1.32 (16H, m, (CH₂)₈CH₃), 2.23 (1H, dddd, *J* = 12.5 Hz, 11.3 Hz, 11.3 Hz, 9.0 Hz, OCH₂CHH'), 2.51 (2H, s, CH₂CONH), 2.76 (1H, dddd, *J* = 12.6 Hz, 8.7 Hz, 6.1 Hz, 1.4 Hz, OCH₂CHH'), 3.47 (2H, s, COCH₂CO), 4.28 (1H, ddd, *J* = 11.1 Hz, 9.3 Hz, 6.1 Hz, OCHH'), 4.48 (1H, td, *J* = 9.1 Hz, 1.3 Hz, OCHH'), 4.59 (1H, ddd, *J* = 11.5 Hz, 8.7 Hz, 6.6 Hz, CHN), 7.66 (1H, d, *J* = 5.4 Hz, NH) ppm. ¹³C NMR (100 MHz, CDCl₃): δ = 14.1 (CH₃), 22.7, 28.8, 29.30, 29.32, 29.4, 29.6, 29.8, 31.9 ((CH₂)₈CH₃), 22.3-23.2 (m, CD₂), 29.6 (CH₂CHN), 43.7 (CD₂CH₂CO), 48.2 (COCH₂CO), 49.0 (CHN), 65.9 (CH₂O), 166.4 (CONH), 174.9 (COO), 206.6 (CH₂COCH₂) ppm. MS (ESI): *m/z* (%): 328 (M+H⁺, 100), 345 (M+NH₄⁺, 19). HRMS mass expected: C₁₈H₂₉D₂NO₄H⁺ 328.2457; found: 328.2446. IR (cm⁻¹): ν_{max} 1007, 1016, 1176, 1356, 1544, 1641, 1716, 1775, 2101, 2192, 2850, 2918, 2954, 3073, 3294. Chromatography: EtOAc:Petroleum ether 3:2 R_f = 0.24. Melting Point: 94-95 °C. Colorless powder. Yield: 54%.

Synthesis of tetramic acid **29**

313 mg of *N*-(3-oxotridecanoyl-[5-²H₂])-L-homoserine lactone **28b** (1 equiv, 1 mmol) was dissolved in 3 mL of dry methanol under a nitrogen atmosphere. Then 1 equiv of NaOMe in methanol (0.5M, 54 mg in 2 mL) was added and the mixture was stirred at reflux for 3 h. Subsequently, the reaction mixture was poured into 10 mL of water and extracted with 10 mL of diethyl ether. The organic phase was discarded and the aqueous phase was acidified with 2M aq. HCl and extracted three times with 10 mL of diethyl ether. Drying of the combined organic phases over MgSO₄, evaporation of the solvent *in vacuo* and recrystallization from hexane yielded 250 mg of tetramic acid **29** as a colorless powder.

3-(1-Hydroxyundecylidene-[5-²H₂])-5-(2-hydroxyethyl)pyrrolidine-2,4-dione **29** (95% D) ¹H NMR (400 MHz, CD₃OD): δ = 0.88 (3H, t, *J* = 6.8 Hz, CH₃), 1.20-1.37 (14H, m, (CH₂)₇CH₃), 1.68-1.79 (1H, m, HOCH₂CHH'), 1.96-2.05 (1H, m, HOCH₂CHH'), 2.80 (2H, s, CH₂CONH), 3.62-3.73 (2H, m, HOCH₂), 3.96 (1H, m, CHN) ppm. ¹³C NMR (100 MHz, CD₃OD): δ = 14.4 (CH₃), 23.7, 30.1, 30.3, 30.4, 30.6, 30.7, 33.1 ((CH₂)₇CH₃), 26.2 (quintet, *J*_{C-D} = 19.1 Hz, CD₂), 33.9 (CD₂CH₂CO), 35.7 (CH₂CHN), 59.3 (CH₂OH), 60.0-61.5 (m, CHN), 101.5-102.3 (m, COCCO), 175.5-177.1 (m,

CONH), 191.0-191.6 (m, CD₂CH₂CHOH), 197.8-198.5 (m, NHCHCO) ppm. MS (ESI): *m/z* (%): 314 (M+H⁺, 100). HRMS mass expected: C₁₇H₂₇D₂NO₄H⁺ 314.2295; found: 314.2297. Melting Point: 80-81 °C. Colorless powder. Yield: 80%.

Synthesis of ring opened AHL 30

200 mg of *N*-(undecanoyl-[3-²H₂])-L-homoserine lactone **27b** (1 equiv, 0.74 mmol) was dissolved in 2 mL of acetonitrile at 0 °C. Then 2 mL of 2M aq. NaOH (5 equiv, 3.7 mmol, 0.15 g) was added and the mixture was stirred overnight at room temperature. The reaction mixture was extracted with 5 mL of ethyl acetate. The organic phase was discarded and the aqueous phase acidified to pH 2 with 2M aq. HCl. Extraction with ethyl acetate (3 x 5 mL), followed by drying over MgSO₄ and evaporation of the combined organic phases *in vacuo* and recrystallization from acetone gave 188 mg of ring opened AHL **30** as a colorless powder.

N-(Undecanoyl-[3-²H₂])-L-homoserine **30** (95% D) ¹H NMR (400 MHz, CD₃OD): δ = 0.91 (3H, t, *J* = 6.8 Hz, CH₃), 1.23-1.40 (14H, m, (CH₂)₇CH₃), 1.81-1.91 (1H, m, HOCH₂CHH'), 2.05-2.15 (1H, m, HOCH₂CHH'), 2.24 (2H, s, CH₂CONH), 3.57-3.71 (2H, m, HOCH₂), 4.52 (1H, dd, *J* = 9.3 Hz, 4.6 Hz, CHN) ppm. ¹³C NMR (100 MHz, CD₃OD): δ = 14.4 (CH₃), 23.7, 30.1, 30.43, 30.46, 30.65, 30.71, 33.1 ((CH₂)₇CH₃), 26.0-27.0 (m, CD₂), 35.3 (CH₂CHN), 36.7 (CH₂CONH), 50.9 (CHN), 59.3 (CH₂OH), 175.5 (CONH), 176.5 (COOH) ppm. MS (ESI): *m/z* (%): 290 (M+H⁺, 100), 312 (M+Na, 38). HRMS mass expected: C₁₅H₂₇D₂NO₄H⁺ 290.2295; found: 290.2296. Melting Point: 114-115 °C. Colorless powder. Yield: 88%.

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