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Title: Bacterial medium chain 3-hydroxy fatty acid metabolites trigger immunity in Arabidopsis plants

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Short title: Arabidopsis senses bacterial metabolites

One Sentence Summary: Bacterial medium chain 3-hydroxy fatty acid metabolites, but not lipopolysaccharide, elicit immunity in *Arabidopsis thaliana*.

Abstract: In plants, cell-surface immune receptors sense molecular non-self-signatures. Lipid A of Gram-negative bacterial lipopolysaccharide is considered such a non-self-signature. The receptor kinase LORE mediates plant immune responses to *Pseudomonas* and *Xanthomonas*, but not enterobacterial, lipid A/lipopolysaccharide preparations. Here, we demonstrate that synthetic and bacterial, lipopolysaccharide-co-purified medium chain 3-hydroxy fatty acid (mc-3-OH-FA) metabolites elicit LORE-dependent immunity. Mc-3-OH-FAs are sensed in a chain length- and hydroxylation-specific manner with free (*R*)-3-hydroxydecanoic acid ((*R*)-3-OH-C10:0) representing the strongest immune elicitor. By contrast, bacterial compounds comprising mc-3-OH-acyl building blocks but being devoid of free mc-3-OH-FAs, including lipid A/lipopolysaccharide, rhamnolipids, lipopeptides, and acyl-homoserine-lactones do not trigger LORE-dependent responses. Hence, plants sense low-complexity bacterial metabolites to trigger immune responses.

Main Text: Plant cell-surface pattern recognition receptors sense characteristic microbe-/pathogen-associated and host-derived damage-associated molecular patterns (MAMPs/PAMPs and DAMPs) and activate pattern-triggered immunity (1). Lipopolysaccharide, a glycolipid consisting of lipid A, core oligosaccharide, and O-polysaccharide is the major outer membrane component of most Gram-negative bacteria (2). In humans and mammals, lipid A triggers inflammation and pyroptosis, potentially culminating in life-threatening sepsis (2, 3). Lipopolysaccharide also elicits defence responses in various plant species (4). In *Arabidopsis thaliana* (hereafter *Arabidopsis*) and other crucifers, lipopolysaccharide preparations from *Pseudomonas* spp. and *Xanthomonas campestris*, but not from *Escherichia coli*, *Salmonella enterica*, or *Burkholderia* spp., activate characteristic immune responses via the plant-specific

bulb-type lectin receptor kinase LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION/S-DOMAIN-1-29 (LORE/SD1-29) (5). *Pseudomonas aeruginosa* lipid A is sufficient to trigger immunity, whereas removal of the ester-linked acyl chains from lipid A abolishes its activity (5). Thus, lipid A with a distinctive acylation pattern appeared to be critical for immune sensing in Arabidopsis.

To decipher the structural features of LORE-dependent lipid A sensing, we screened lipopolysaccharide with different acylation patterns from various bacterial species and *P. aeruginosa* lipid A biosynthesis mutants for activation of cytosolic calcium ($[Ca^{2+}]_{cyt}$) signalling as indicator of an immune response in Arabidopsis (table S1; fig. S1–S4). Structurally, all tested, elicitor-active lipopolysaccharide preparations comprise a medium-length acyl chain, mostly 3-OH-decanoyl, at positions C-3 and/or C-3' of lipid A, whereas lipopolysaccharide with 3-OH-tetradecanoyl chains at these positions was inactive. This suggests that a 3-OH-decanoyl chain is a key structural feature for LORE-dependent lipopolysaccharide sensing.

Therefore, synthetic 3-OH-FAs of varying chain length (table S2) were tested for activation of Arabidopsis immune responses. Free synthetic 3-OH-C10:0 (**1**; see table S2 for assignment of compound numbers; fig. 1A) elicits $[Ca^{2+}]_{cyt}$ elevations comparable to *Pseudomonas* lipopolysaccharide preparations (fig. 1B; fig. S5A). Medium-chain (mc) 3-OH-FAs (bearing 8–12 carbon atoms, **1–5**), particularly 3-OH-C10:0 (**1**), but not long-chain (lc) 3-OH-FAs (13–16 carbon atoms, **6–8**) activated LORE-dependent $[Ca^{2+}]_{cyt}$ signalling, biphasic production of reactive oxygen species (ROS), transcript accumulation of defence-related genes, and phosphorylation of mitogen-activated protein kinases (fig. 1C,D; fig. S5B–F). In plants, local MAMP application induces systemic resistance to pathogen infection (6). Wild-type Arabidopsis plants pre-treated with 3-OH-C10:0 (**1**) were more resistant to *P. syringae* infection, but this systemic resistance was not

activated in *Arabidopsis lore-1* mutants or when 3-OH-C14:0 (**7**) was used (fig. 1E; fig. S5G). Solanaceous *Nicotiana benthamiana* plants are insensitive to *Pseudomonas* lipopolysaccharide, however gain responsiveness upon transient expression of a LORE-GFP fusion (**5**). Mc-3-OH-FAs (but not lc-3-OH-FAs) also induced ROS production in *N. benthamiana* leaves expressing LORE-GFP, but not leaves expressing a kinase-inactive control (fig. 1F; fig. S5H). LORE ectodomain expressed and purified from insect cells directly binds 3-OH-C10:0 (**1**) *in vitro*. Under these conditions the observed binding affinity was relatively low however (fig. 1G; fig. S6). Taken together, this supports that LORE is a receptor for mc-3-OH-FAs.

Next, we tested whether a 3-hydroxyl and/or a carboxyl group are molecular determinants for activating LORE-mediated immunity (fig. 2). Compared to mc-3-OH-FAs (**1–5**), mc-2-OH-FAs (**11–13**) and non-hydroxylated mc-FAs (**15–17**) induced only limited immune responses in *Arabidopsis* and LORE-GFP-expressing *N. benthamiana* (fig. 2A,B; fig. S7A–E). Non-hydroxylated or Δ^2 -unsaturated (**19,20**) mc-FAs induced weak LORE-independent responses (fig. 2A,B; fig. S7A–F). 4-OH-C10:0 (**21**) and 5-OH-C10:0 (**22**) did not elicit LORE-dependent ROS production (fig. 2C; fig. S7G). 3-Oxodecanoic acid (3-oxo-C10:0, **23**) or its keto-enol tautomer 3-OH-decenoic acid, induced a moderate LORE-dependent $[Ca^{2+}]_{cyt}$ response (fig. 2D; fig. S7H). Modification of the 3-hydroxyl group of 3-OH-C10:0 (**1**) with a methyl ether (**24**) or acetyl ester (**25**) or substitution with chlorine (**27**) abolished elicitor activity (fig. 2D,E; fig. S7H,J). The synthetic 3-OH-FAs used here are racemates, but *Pseudomonas* lipopolysaccharide comprises typically (*R*)-3-OH-decanoyl as primary ester-linked acyl chains (**7**) (table S1; fig. S1,S2). (*R*)-3-OH-C10:0 (**28**) induced stronger immune signalling than (*S*)-3-OH-C10:0 (**29**) (fig. 2F; fig. S8). Collectively, a free 3-hydroxyl group, preferably in the (*R*)-configuration, is critical for LORE-mediated immune sensing. 3-Decanol (**31**) or 1,3-decandiol (**32**) were inactive (fig. 2G;

fig. S9A,B), indicating that the carboxyl function is also required for activating LORE-mediated immunity. Ester- or amide-linked moieties at the carboxyl group of 3-OH-C10:0 (**26**, **33–44**) gradually impaired elicitor activity with increasing hydrophobicity and size, with bulky moieties, such as *tert*-butyl (**35**) or glucosamine (**43**), rendering the 3-OH-C10:0 derivatives inactive (fig. 2H–K; fig. S9B–E). Thus, mc-3-OH-FAs are the minimal motif for LORE-mediated immune activation with free (*R*)-3-OH-C10:0 (**28**) showing the strongest activity in Arabidopsis.

The latter indicates that free mc-3-OH-FAs are sensed by Arabidopsis rather than lipid A-bound mc-3-OH-acyl chains. Indeed, unbound 3-OH-C10:0 (**1**) was detected and quantified by means of UHPLC-MS/MS and stable isotope dilution analysis (SIDA-UHPLC-MS/MS_{MRM}) in all lipopolysaccharide/lipid A preparations that activated LORE-dependent immunity (table S3; fig. S10). As this includes samples from different sources, including commercial suppliers, or isolated by different multi-step procedures (table S3), mc-3-OH-FAs appear to co-purify with lipopolysaccharide/lipid A. Detergent-based repurification depleted unbound mc-3-OH-FAs from lipopolysaccharide samples (table S3; fig. S10,S11). Mc-3-OH-FA-depleted lipopolysaccharide samples did not activate immune signalling, whereas typical responses were induced by subsequent application of synthetic 3-OH-C10:0 (**1**) or the peptide MAMP flg22 (**8**) (fig. 3A,B; fig. S12,S13). Mc-3-OH-FA-depleted lipopolysaccharide samples also did not induce systemic resistance to *P. syringae* infection in Arabidopsis (fig. 3C). This hints at a separate source of free, elicitor-active mc-3-OH-FAs in bacteria rather than rapid release of mc-3-OH-FAs from lipopolysaccharide in the plant apoplast.

Several bacterial compounds, such as polyhydroxyalkanoates (**9**), rhamnolipids (**10**), lipopeptides (**11**), and *N*-acyl-homoserine-lactones (**12**) comprise 3-OH-acyl moieties, in *Pseudomonas* commonly 3-OH-decanoyl moieties (**11**, **12**). 3-OH-decanoyl-comprising rhamnolipids (**13**) or

synthetic 3-OH-C10:0- (**45**) and 3-oxo-C10:0-homoserine-lactones (**46**) did not activate LORE-mediated immune signalling in *Arabidopsis* (fig. 3D,E; fig. S14). Massetolide A, a 3-OH-decanoyl-containing lipopeptide (**14**), triggered LORE-independent ROS production and a weak LORE-dependent $[Ca^{2+}]_{\text{cyt}}$ elevation (fig. 3D; fig. S14A,B), most likely caused by the 3-OH-C10:0 (**1**) contamination detected in this sample (table S3; fig. S10). Taken together this suggests, that the receptor kinase LORE senses mc-3-OH-FAs in their free form, but not 3-OH-decanoyl-comprising bacterial compounds.

Pseudomonas releases free (*R*)-3-OH-C10:0 (**28**) during synthesis of penta-acylated lipid A through PagL-catalysed (*R*)-3-OH-C10:0 removal from hexa-acylated lipid A in the outer membrane (15, 16). However, lipopolysaccharide preparations from *Pseudomonas* Δ pagL mutants still contain free 3-OH-C10:0 (**1**) and activate LORE-mediated immunity (table S1,S3; fig. S3,S4F,G), indicating additional sources of free mc-3-OH-FAs in bacteria. Acyl carrier protein (ACP)- and coenzyme A (CoA)-bound mc-3-OH-FAs are intermediates of fatty acid metabolism. Free mc-3-OH-FAs may be released from ACP/CoA-precursors through thioesterase activities (17) or non-enzymatically upon cell lysis in bacteria as well as in plants. In crucifers, mc-3-OH-FAs released upon tissue damage could function as DAMPs. Outer membrane vesicles, constantly released by bacteria (18), may deliver membrane-located or intracellular mc-3-OH-FAs. Firmicutes (19), honey bees (20), and leaf-cutter ants (21) also produce free mc-3-OH-FAs. Thus, mc-3-OH-FAs could be delivered through multiple metabolic sources. We propose that plants evolved to sense a ubiquitous low-complexity metabolite instead of different, complex microbial end products.

Humans, animals, and fungi sense metabolites, including various hydroxy fatty acids, through G-protein-coupled receptors (GPCRs) (22-24). The pro-inflammatory receptor GPR84 expressed in

leukocytes senses mc-FAs and their 2- or 3-hydroxylated forms (22, 25). GPR84 may sense mc-FAs and mc-2/3-OH-FAs of pathogenic or commensal bacteria in addition to endogenous metabolites. Therefore, our finding that lipopolysaccharide, rhamnolipid, and lipopeptide preparations from certain bacterial species may contain free mc-3-OH-FAs (table S3) is relevant to immune studies in humans and mammals, as complete removal of highly elicitor-active compounds from such biological samples is difficult (26, 27). In conclusion, both plants and animals may monitor their microbial environments by sensing low-complexity microbial metabolites through cell-surface receptors (22, 23, 28).

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Gregor Mendel Institute (Y.B.). E.S.L is supported by a Hertha Firnberg Programme post-doctoral fellowship (T-947) from the FWF Austrian Science Fund. **Author contributions:** Detailed author contributions are provided in the supplement. **Competing interests:** Technical University of Munich has filed a patent application to inventors A.K., C.D., L.R., M.S, R.H., T.H., and S.R. The authors declare no financial conflicts of interest in relation to this work. **Data and materials availability:** All data are available in the manuscript or the supplementary material. *A. thaliana* Col-0 expressing pCaMV35S-apoaequorin in the cytosol (Col-0^{AEQ}) are available from Marc Knight under a material agreement with Durham University.

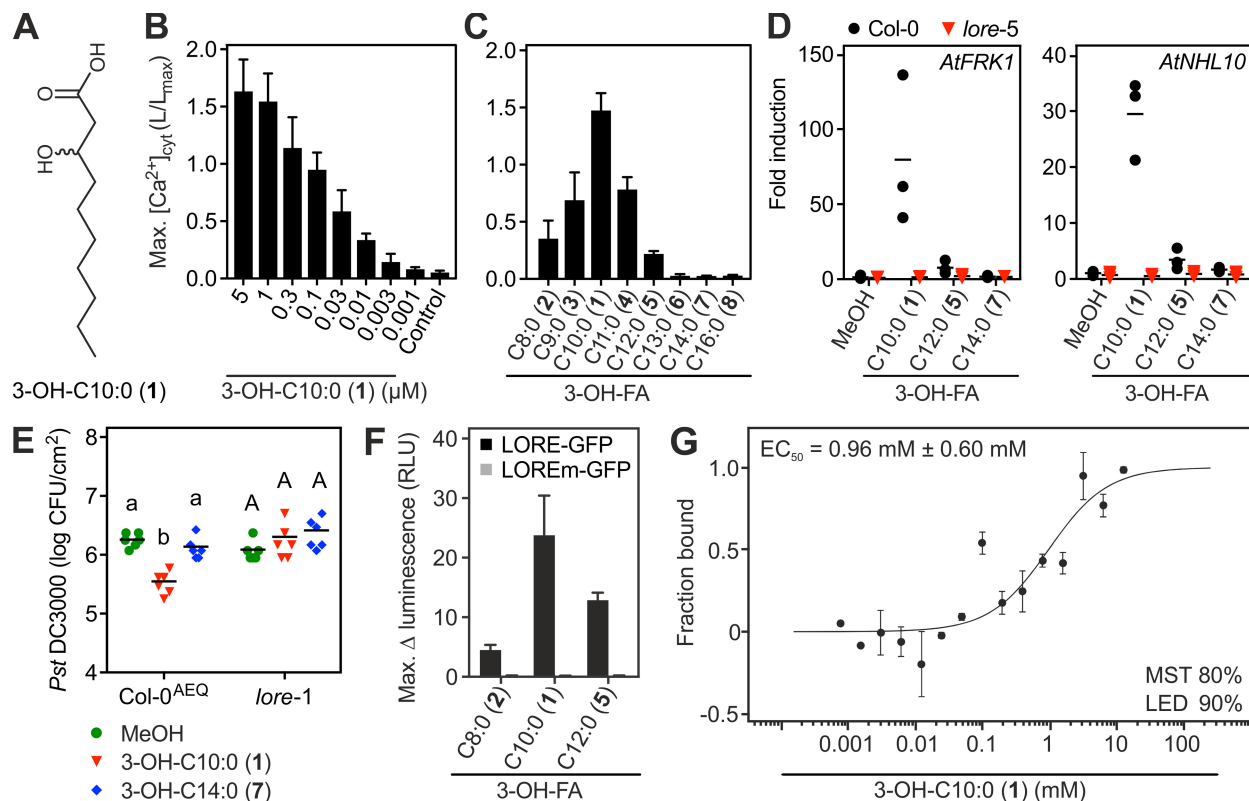
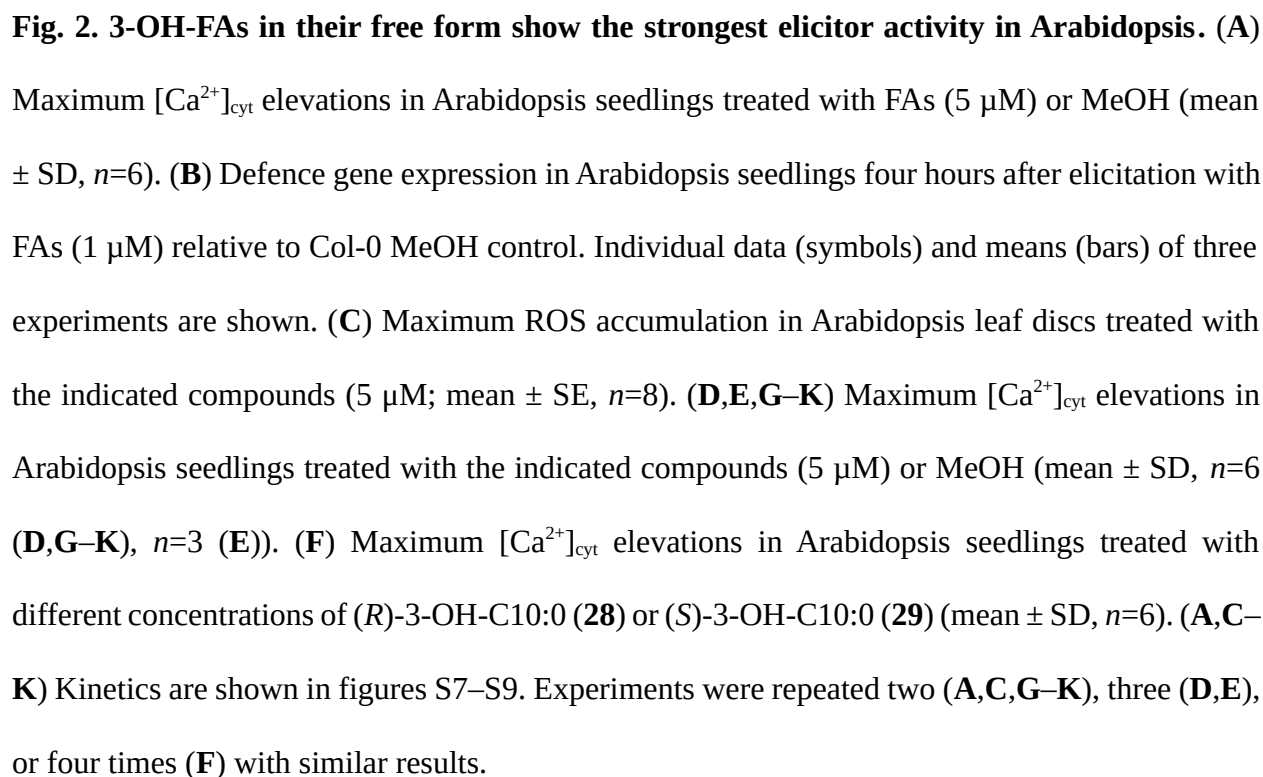


Fig. 1. 3-OH-FAs trigger LORE-dependent immune responses in a chain length-specific manner. (A) Chemical structure of 3-OH-C10:0 (1). (B,C) Maximum $[Ca^{2+}]_{cyt}$ elevations in Arabidopsis Col-0^{AEQ} seedlings treated with (B) different concentrations of 3-OH-C10:0 (1, stock concentration determined by quantitative NMR) or (C) 1 μ M 3-OH-FAs of various chain length (mean $\pm$ SD, $n=12$ (B), $n=3$ (C)). Kinetics are shown in figures S5A,B. (D) Defence gene expression in Arabidopsis seedlings four hours after elicitation with 3-OH-FAs (1 μ M) relative to Col-0 MeOH control. Individual data (symbols) and means (bars) of three experiments are shown. (E) *Pst* DC3000 titre four days post infection in Arabidopsis plants pre-treated with 10 μ M 3-OH-FAs or MeOH for three days before infection. Individual data (symbols) and means (bars) of two experiments are shown ($n=6$). Different letters indicate significant differences (one-way ANOVA with Tukey's post-hoc test applied separately to Col-0^{AEQ} (small letters) and *lore-1* (capital letters)),

$p<0.01$). Similar results with two days pre-treatment (fig. S5G). **(F)** Maximum ROS accumulation in leaf discs from *N. benthamiana* transiently expressing LORE-GFP or kinase-inactive LOREm-GFP elicited with 1 μ M 3-OH-FAs (mean $\pm$ SE, $n=8$). **(G)** Quantification of binding between 3-OH-C10:0 (**1**) and fluorescently His-tag-labelled LORE ectodomain by microscale thermophoresis (MST). Measurements were performed at an LED power of 90% and MST power of 80%. Data points indicate the fraction of labelled LORE ectodomain bound to 3-OH-C10:0 (**1**) (mean $\pm$ SE, $n=4$). Full figure including controls and additional data with alternatively labelled LORE ectodomain are provided in figure S6. Experiments were repeated two (**F,G**), three (**B**), or four times (**C**) with similar results.



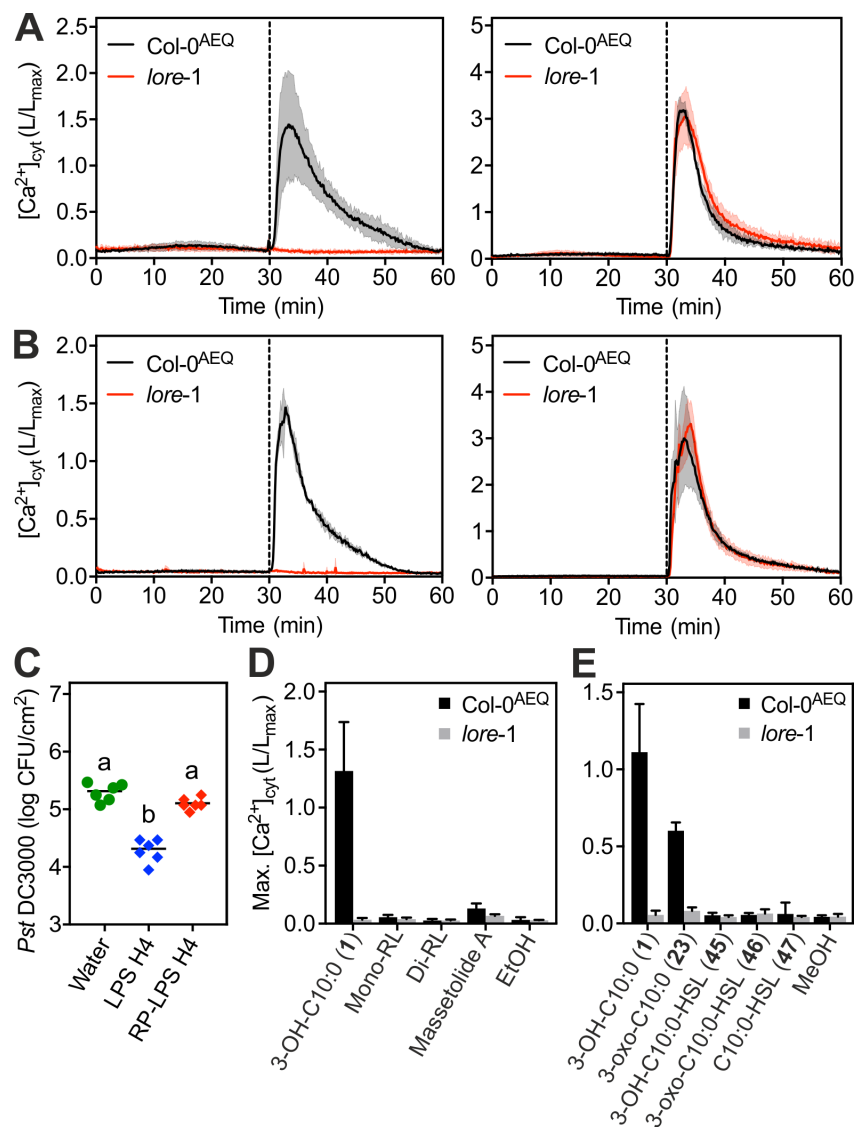


Fig. 3. Lipopolysaccharide, rhamnolipid, lipopeptide, and homoserine-lactone comprising 3-OH-acyl building blocks, but devoid of free 3-OH-C10:0 (1), do not activate LORE-mediated immune signalling. (A,B) $[Ca^{2+}]_{cyt}$ kinetics in Arabidopsis seedlings treated with (A) *P. cichorii* S400/S200 lipopolysaccharide (50 µg/mL; DOC-GPC pool 3; table S3; fig. S10,S11A-C) or (B) heat-detergent-repurified *P. aeruginosa* H4 lipopolysaccharide (100 µg/mL; table S3; fig. S10,S11D). After 30 min, synthetic 3-OH-C10:0 (1) (left panel) or flg22 (right panel) was added and measurements continued for 30 min (mean ± SD, $n=3$ each). Full figures including controls and additional repurified lipopolysaccharide pools are provided in figures S12 and S13A,B. (C) *Pst* DC3000 titre four days post infection in Arabidopsis plants pre-treated with *P. aeruginosa* H4 (25 µg/mL; LPS H4) or heat-detergent-repurified *P. aeruginosa* H4 lipopolysaccharide (25 µg/mL; RP-LPS H4; table S3; fig. S11D) or water for three days before infection. Individual data (symbols) and means (bars) of two experiments are shown ($n=6$). Different letters indicate significant differences (one-way ANOVA with Tukey's post-hoc test, $p<0.01$). (D,E) Maximum $[Ca^{2+}]_{cyt}$ elevations in Arabidopsis seedlings treated with the indicated compounds (5 µM; table S3) or solvent control (mean ± SD, $n=6$). Kinetics are shown in figure S14. RL, rhamnolipid; HSL, homoserine-lactone. Experiments were repeated two (D,E) or three times (A,B) with similar results.

Supplementary Materials:

Materials and Methods

Figures S1–S14

Tables S1–S3

References (29–89)

Author contributions

Supplementary Materials are provided in a separate file.



Supplementary Materials for

Bacterial medium chain 3-hydroxy fatty acid metabolites trigger immunity in Arabidopsis plants

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This PDF file includes:

Materials and Methods
Figs. S1 to S14
Tables S1 to S3
Author contributions

Other Supplementary Materials for this manuscript includes the following:

None

Materials and Methods

Chemicals

All chemicals including deuterated solvents (MeOD and CDCl₃) for NMR spectroscopy were purchased from Sigma-Aldrich (Taufkirchen, Germany) unless noted otherwise (e.g. cf. table S2). Ethyl 3-oxodecanoate was purchased from Carbosynth (Compton, United Kingdom). Hexane and ethylacetate (EtOAc) were of analytical grade and were used as obtained from VWR (Darmstadt, Germany). While solvents for HPLC separation (acetonitrile) were of HPLC grade (Mallinckrodt Baker, Griesheim, Germany), solvents used in LC-TOF-MS and LC-MS/MS analysis were of LC-MS grade (Honeywell, Seelze, Germany). Column chromatography was performed by using silica gel (60 Å porosity, 40-100 µm, Sigma-Aldrich, Taufkirchen, Germany). Ultrapure water for HPLC separation and mass spectrometry was purified by means of a Milli-Q water advantage A 10 water system (Millipore, Molsheim, France). Millipore-grade water was used for all experiments unless stated otherwise.

Plant material and growth conditions

A. thaliana Col-0 expressing pCaMV35S-apoaequorin in the cytosol (Col-0^{AEQ}) was obtained from M. Knight (29). Mutant lines *lore-1*, *lore-2*, complementation lines CL #1 and #2, and T-DNA insertion lines *lore-5* (SAIL_857_E06, alias *sd1-29*), *lore-5*^{AEQ} (alias *sd1-29*^{AEQ}) were described previously (5). For growth in liquid medium, surface-sterilized Arabidopsis seeds were stratified at 4 °C for ≥2 days and grown in liquid MS medium (0.5 × Murashige & Skoog medium including vitamins (Duchefa), 0.25% sucrose, 1 mM MES, pH 5.7) in 24-well plates (~15 seedlings/well) under long day (16 h light) conditions at 20 °C/22 °C (night/day) (30). Experimental Arabidopsis plants were grown on potting soil (ED73, Einheitserde) with vermiculite (5:1) in climate chambers under short day (8 h light) conditions at 20 °C/22 °C (night/day), and *N. benthamiana* under long day (16 h light) conditions at 21 °C/23 °C (night/day) in 55–60% relative humidity.

Aequorin luminescence measurements

Aequorin luminescence measurements were performed essentially as described (30). In short, 8- to 10-days-old liquid-grown apoaequorin-expressing seedlings were placed individually in 96-well plates in water containing 5–10 µM coelenterazine-h (p.j.k. GmbH) in the dark overnight. Luminescence was recorded by scanning two rows in 10 sec intervals using a Luminoskan Ascent 2.1 (Thermo Scientific) luminometer. Remaining aequorin was discharged by addition of 150 µL 2 M CaCl₂ with 20% EtOH per well. [Ca²⁺]_{cyt} concentrations were calculated as L/L_{max} (luminescence counts per sec/total luminescence counts remaining).

ROS detection in Arabidopsis leaves

Short-term ROS production was monitored as described (5) in 3 mm leaf discs from 6–8 weeks-old soil-grown plants in 100 µL 5 µM L-012 (WAKO chemicals) and 2 µg/mL horseradish peroxidase (Type II, Roche) in water. For experiments including 4-OH-10:0 (**21**) and 5-OH-10:0 (**22**), 10 mM Tris/HCl pH 8 was added to the L-012/horseradish peroxidase mix. Long-term ROS production was monitored in 3 mm leaf discs from 6–8 weeks-old soil-grown plants in 200 µL 200 µM luminol (luminol sodium salt, Sigma-Aldrich) and 10 µg/mL horseradish peroxidase (Type II, Roche) similarly as described (31). Luminescence was recorded as relative light units (RLU) in 1 min intervals using a Luminoskan Ascent 2.1 (Thermo Scientific) or a Tecan F200 luminometer. After 10 min background reading, elicitors were added to the final concentrations indicated and luminescence readings continued for 45–60 min. For assessment of long-time ROS production readings were further continued for 40 h in 5 min intervals. Data are depicted after normalization to average ROS levels 5 min before elicitor application and subtraction of water or MeOH controls that were included for each genotype on the same plate.

Detection of peroxidase activity in Arabidopsis leaves

Peroxidase secretion and activity was monitored as described (32) with minor modifications. Briefly, 3 mm leaf discs from 8–10 weeks-old soil-grown plants were incubated in 200 μ L 0.5 $\times$ MS medium for 1 h. Medium was replaced by 60 μ L elicitor solutions (diluted in 0.5 $\times$ MS medium) and incubated for 24 h in the dark. 50 μ L were withdrawn and mixed with 50 μ L 5-aminosalicylic acid solution (1 mg/mL, pH 6.0, Sigma-Aldrich) with 0.01% hydrogen peroxide. The reaction was stopped by adding 20 μ L 2 M sodium hydroxide solution and absorbance at 600 nm was assessed. Fold change was calculated and statistically analysed using two-way ANOVA with Tukey's post-hoc test (confidence level 0.95, letter threshold $p < 0.05$) after normal distribution of the data was confirmed by Shapiro-Wilk normality test.

Immunoblot analysis of MAPK activation

MAPK activation was elicited in 14-days-old liquid-grown seedlings as described (5) by adding elicitors to the final concentrations indicated. Seedlings were harvested at the indicated time points, frozen in liquid nitrogen and homogenized using a bead mill (TissueLyser II, Qiagen). Proteins were extracted in kinase extraction buffer containing 50 mM Tris/HCl pH 7.5, 100 mM NaCl, 20 mM EGTA, 30 mM beta-glycerophosphate, 30 mM 4-*p*-nitrophenylphosphate, 4 mM NaF, 4 mM Na₃VO₄, 4 mM Na₂MoO₄, 10 mM DTT, 0.2% Tween-20, and 1 $\times$ plant protease inhibitor cocktail P9599 (Sigma-Aldrich). 50 μ g total protein (according to Bradford Protein Assay, Bio-Rad) was separated by standard SDS polyacrylamide gel-electrophoresis (Mini-PROTEAN Tetra Cell, Bio-Rad) and blotted onto nitrocellulose membrane (AmershamTM ProtranTM 0.2 μ m NC, GE Healthcare) using a Trans-Blot[®] SD Semi-Dry Transfer Cell (Bio-Rad). Membranes were blocked in Protein-Free TBS Blocking Buffer (PierceTM) and immunostained with anti-phospho-MPK antibody (anti-phospho-p44/42-ERK, 1:1,000; Cell Signaling Technology #9101) and anti-rabbit-IgG-peroxidase conjugate (A9169, 1:50,000; Sigma-Aldrich) using SuperSignalTM West Dura Extended Duration Substrate (Thermo Scientific). Chemiluminescence was detected using a Fusion SL camera (Vilber Lourmat). Membranes were stained with Amido black to assess equal protein loading.

Gene expression analysis

12-days-old liquid-grown seedlings were equilibrated in fresh MS medium for two days before treatment with 1 μ M of the indicated elicitors or the respective amount of MeOH as control in MS medium. Seedlings were harvested at four hours, frozen in liquid nitrogen and homogenized using a bead mill (TissueLyser II, Qiagen). Total RNA was extracted using the conventional Trizol method. 2 μ g total RNA was digested with DNaseI (Thermo Scientific) and reverse-transcribed with oligo(dT)₁₈ and Revert Aid[®] reverse transcriptase (Thermo Scientific) according to the manufacturers' instructions. Gene expression was determined by quantitative PCR with primers described previously for *AtFRK1* (33), *AtNHL10* (34) and *AtUBQ5* (35). Quantitative real-time PCR was performed on an AriaMx Real-time PCR System G8830A (Agilent Technologies Inc.) according to manufacturer's instructions (3 min at 95 $^{\circ}$ C, 40 cycles of 5 sec at 95 $^{\circ}$ C, 20 sec at 60 $^{\circ}$ C, and 20 sec at 72 $^{\circ}$ C) using the Maxima SYBR Green-ROX qPCR Master Mix (Thermo Scientific; 1 μ L of 1:10 diluted cDNA in 10 μ L reaction volume) and analysed using the AriaMx Software V1.3 (Agilent). Primer efficiencies were determined according to manufacturer's instructions and found to be close to 100%. Amplification specificity was analysed by non-template controls and primer dissociation curves. Biological replicate samples were analysed in triplicates side-by-side in one run for each gene. Expression levels of *AtFRK1* and *AtNHL10* were normalized to *AtUBQ5* expression levels and fold changes relatively to the expression level of the Col-0 MeOH control calculated by the $2^{-\Delta\Delta C_t}$ method as described (36).

Induced resistance assay

10 μ M 3-OH-FAs, 25 μ g/mL lipopolysaccharide preparations, or the respective amount of MeOH (for 3-OH-FAs) or water (for lipopolysaccharide preparations) as control, were syringe-infiltrated into the first two true leaves of 4- to 5-week-old Arabidopsis plants as described (37). After two or three days *Pst* DC3000 was syringe-infiltrated into leaves 3 and 4 of the pre-treated plants at 1×10^5 CFU/mL. Bacterial growth in infected leaves was determined 4 days later as described (38). Statistical analysis (one-way analysis of variance, ANOVA, with Tukey's multiple comparison post-hoc test; $p < 0.01$) was applied separately for wild type and mutant plants using GraphPad Prism 7.0.

Transient expression in tobacco

Overnight cultures of *Agrobacterium tumefaciens* GV3101 carrying *LORE-GFP* or kinase-inactive *LOREm-GFP* (K516A) constructs (5) or the p19 suppressor of silencing (39) were grown in AB medium supplemented with 100 μ M acetosyringone (40). Bacteria were harvested by centrifugation (2,500g for 2 min), washed twice with infiltration medium (10 mM MgCl₂, 10 mM MES pH 5.7, 150 μ M acetosyringone), and resuspended in infiltration medium to an absorbance of 0.05 at 600 nm. Bacteria carrying *LORE-GFP* or *LOREm-GFP* constructs were each mixed 1:1 with p19 cultures and syringe-infiltrated into halves of the same leaves of 6–8 weeks-old *N. benthamiana* plants. Leaf discs (3 mm) were cut and used for ROS detection two days after infiltration as described above.

LORE ectodomain expression and purification

For the LORE expression in High Five (Hi5) cells, the ectodomain of LORE (LORE^{ECD}) was inserted into the baculovirus transfer vector pMelBac B1 (Invitrogen). The LORE^{ECD} (SSPLSIRQTLSSPGGFYELGFFSPNNTQNQYVGIWFKKIVPRVVVWVANRDTPVTSSA ANLTISSNGSLILLDGKQDVIWSTGKAFTSNKCHAELLDTGNFVVIDDVSGNKLWQSF EHLGNTMLPQSSLMYDTSNGKKRVLTWWSNSDPSPGEFSLEITPQIPTQGLIRRGSPV YWRCGPWAKTRFSGISGIDASYVSPFSVVQDTAAGTGSFSYSTLRNYNLSYVTLTPEG KMKILWDDGNNWKLHLSLPENPCDLYGRCPYGLCVRSDPPKCECLKGFVPKSDDEE WGKGNWTS GCVRRTKLSCQAKSSMKTQGKDTDIFYRMTDVKTPDLHQFASFLNAE QCYQGCLGNCCTAFAYISGIGCLVWNGELADTVQFLSSGEFLFIRLASSELAGSSRR K) was cloned between the existing honey bee melittin signal sequence and the C-terminal Strep II-9x histidine tag. All clones were verified by sequencing. Cloning primers (forward 5'-GGTCGTATACATTTCTTACATCTATGCGTCAAGTCCTTTGTCAATAAGACAACTC -3'; reverse 5'-GCACCCTGGAAGTACAGGTTCTCCTTCCTTCGACTGCTTCC-3') were designed to have an amplification part homologous to the desired boundaries of LORE ectodomain and extension for Gibson assembly strategy as recommended by the manufacturer (Gibson Assembly® Master Mix E2611, New England Biolabs).

The C-ter StrepII-9xHis fused LORE^{ECD} was produced by secreted expression in baculovirus-infected insect cells, harvested 72 h post-infection and purified by Ni-NTA affinity chromatography (Qiagen). The samples were then run onto a Superdex 200 10/300, GL (GE Healthcare) pre-equilibrated with 50 mM NaH₂PO₄/Na₂HPO₄ pH 7.5, 500 mM NaCl, 5% Glycerol, 1 mM DTT, and 0.05% Tween-20. Protein purity was checked by SDS-PAGE. The protein was subsequently concentrated to ~0.210 mg/mL via centrifugal filtration with a 5 kDa filter (Sartorius). Identity of the LORE^{ECD} was further confirmed by mass spectrometry and anti-His immunoblots.

Microscale thermophoresis (MST) binding assays

Prior to MST assays the LORE^{ECD} was labelled with a fluorescent dye by using a Monolith His-tag Labelling Kit RED-tris-NTA 2nd Generation (His-tag, MO-L018, NanoTemper) or Monolith Protein Labelling Kit RED-NHS 2nd Generation (Amine Reactive, MO-L011,

NanoTemper). The fluorescently labelled LORE^{ECD} was kept at constant concentrations: 22 nM using the His-tag labelling kit or 41.5 nM using the reactive amine NHS labelling kit, in a buffer containing 50 mM NaH₂PO₄/Na₂HPO₄ pH 7.5, 500 mM NaCl, 5% Glycerol and 0.05% Tween, whereas varying compound (3-OH-C10:0 (**1**), DSF (**9**), and sebacic acid (**10**)) concentrations were added. Approximately 4–6 μ L of each sample was loaded in premium capillaries (Monolith NT.115 Premium Capillaries, MO-K025, NanoTemper). Measurements were performed at room temperature in a Monolith NT.115 instrument at a constant LED power of 90% for His-tag labelled LORE^{ECD} or 60% for primary amine NHS labelled LORE^{ECD} and varying MST power of 40%, 60%, and 80%. Measurements were performed repeatedly on independent protein preparations to ensure reproducibility. The data were analysed by plotting compound concentrations against changes of initial fluorescence of MST-phase1 (His-tag labelled LORE^{ECD}) or thermophoresis (primary amine NHS labelled LORE^{ECD}). Curve fitting was performed by using the MO.Affinity Analysis v2.2.6 (NanoTemper). The EC₅₀ values were calculated and are indicated in mM. The standard error of the regression fit is $X^2=24.93$ for fig. 1G/fig. S6A upper panel, $X^2=18.49$ for fig. S6B upper panel, and $X^2=214.40$ for fig. S6B lower panel.

Genetic knockout of *pagL* in *P. syringae* pv. *tomato* DC3000

Knockout plasmids were constructed using a golden gate-compatible pGGKO-blue plasmid derived from pK18mobsacB (41). Flanking sequences (flank A: 550 bp, flank B: 553 bp) up and downstream of *pagL* (PSPTO_5636) were PCR-amplified from genomic DNA of *P. syringae* pv. *tomato* DC3000 (*Pst*) and inserted into the pGGKO-blue backbone by golden gate cloning using *BpiI*. A gentamicin resistance cassette (Gm^R) was amplified from plasmid pPS856 (42) and inserted between the flanking sequences (flank A forward primer 5'-TTTGAAGACTGTCGAGCCCTCAGATTTCGTCAAC-3' and reverse primer 5'-TTTGAAGACGCGGCCGCCATGGTGGATTCGCCGGT-3'; flank B forward primer 5'-TTTGAAGACGCGGCCGCTGATTTGACTGGCACTTGTGC-3' and reverse primer 5'-TTTGAAGACGTCTAGGGAAGTGATGCTTATCACCG-3'). Mutants of *Pst* were generated similar to the method described by (41). Briefly, knockout plasmids were transferred to *Pst* via triparental mating with *E. coli* HB101 (carrying plasmid pRK2013) as helper strain and *E. coli* DH5 α (carrying the knockout plasmid) as donor. Sucrose counter selection was used to screen cells which underwent complete homologous recombination replacing the target gene with the gentamicin resistance cassette. Mutants were verified by PCR and sequencing of the amplicon (forward primer 5'-GGGCTGGTCGAGCTGATCGAG-3', reverse primer 5'-TGCTCGACCTGCGCAGC-3').

Preparation of bacterial compounds

Flg22 (QRLSTGSRINSAKDDAAGLQIA) (43) peptide was described previously (5). Lipopolysaccharide, rhamnolipids (RL), and lipopeptide preparations are summarized in table S3. Some preparations were obtained from commercial suppliers or were kind donations (as indicated). Preparation of lipopolysaccharide from *E. coli* KPM 53 and purification of lipid A, core oligosaccharide, and core-lipid A backbone oligosaccharide with amide-bound fatty acids (LPS-OH) of *Pa* H4 was described previously (5). Preparation of rhamnolipid (RL) mono-RL (R-10,10) and di-RL (RR-10,10) was described previously (13, 44). Unpublished lipopolysaccharide and lipid A preparations and repurification of lipopolysaccharide preparations are described below.

Preparation and purification of lipopolysaccharide of *P. syringae* pv. *tomato* DC3000.

Bacteria were grown in King's B medium at 26 °C under shaking (230 rpm) to an absorbance of ~1.0 at 600 nm, harvested by centrifugation (3,000g) for 20 min at 4 °C, resuspended in a small volume of water, boiled for 45 min, and lyophilized. The bacterial mass was washed with ethanol, acetone (twice), and diethyl ether, and dried (recovery, 7.25 g). The pellet was

resuspended in water (15 mg/mL), sequentially treated overnight at room temperature with DNase/RNase and proteinase K (100 μ L of 10 mg/mL solutions per gram dry weight for each enzyme), then underwent dialysis (14-kDa cut-off) and lyophilization. For hot phenol-water extraction (PW extraction) (45), bacteria were resuspended in 45% aqueous phenol (10 mL per g bacteria) with an Ultra-Turrax and stirred for 20 min at 68 °C. After centrifugation (5,600g) for 20 min at 4 °C, the upper water phase was collected. The extraction was repeated with the same volume of water as had been collected. Combined water phases and the phenolic phase were dialyzed against deionized water at 4 °C (14-kDa cut-off) and lyophilized. Prior to lyophilization, the dialyzed phenolic phase (PP) was centrifuged (600g for 5 min at 20 °C) and divided into supernatant (sup) and sediment (sed). Lipopolysaccharide recovered from the water phase (0.39 g; *Pst* WP) was used as such, whereas a phenol-chloroform-petroleum ether extraction (46) was performed with the material of the PP (1.27 g PP-sup; 1.54 g PP-sed). Each was resuspended in phenol(90%)-chloroform-petroleum ether(40–60 °C) (2:5:8 (vol/vol/vol); ~80 mg/mL), then the respective suspension was stirred for 30 min at room temperature and centrifuged (5,800g) for 20 min at 20 °C. The supernatant was collected and the extraction was repeated twice. Combined supernatants were evaporated in vacuum until phenol crystallization began (at room temperature and normal pressure). Lipopolysaccharide from PP-supernatant was precipitated overnight at 4 °C with 2 mL water and collected by centrifugation (7,650g) for 30 min at 4 °C (*Pst* PP-sup sed1). To the supernatant, 12.5 mL ethanol were added, lipopolysaccharide was precipitated and collected by centrifugation (7,650g) for 30 min at 4 °C (*Pst* PP-sup sed2). Since the supernatant after centrifugation was still turbid, another centrifugation (20,000g for 1 h at 20 °C) was performed and the sediment (*Pst* PP-sup sed3) was collected. Lipopolysaccharide from PP-sed was precipitated as described for PP-sup resulting in an aqueous precipitate (*Pst* PP-sed sed1) and an ethanol precipitate (*Pst* PP-sed sed2). Aqueous precipitates were washed twice with 80% phenol (centrifugation at 4,400g for 20 min at 4 °C) and three times with acetone, ethanol precipitates only three times with acetone (centrifugation at 4,400g for 20 min at 20 °C) and dried. This resulted in the following yields: *Pst* PP-sup sed1: 5.7 mg; *Pst* PP-sup sed2: 111.2 mg; *Pst* PP-sup sed3: 92.2 mg; *Pst* PP-sed1: 4.3 mg; *Pst* PP-sed2: 14.0 mg.

Preparation and purification of lipopolysaccharide of P. syringae pv. tomato DC3000 Δ pagL::Gm^R

Bacteria were grown in King's B medium at 26 °C under shaking (230 rpm) to an absorbance of ~1.0 at 600 nm, harvested by centrifugation (3,000g) for 20 min at 4 °C, washed three times with water, resuspended in a small volume of ethanol, and stirred at room temperature overnight. The suspension was filtrated and collected cells were washed with ethanol, acetone (twice), and diethyl ether, and dried (recovery, 11.7 g). The pellet was resuspended in water (~15 mg/mL) with 0.02% NaN₃, sequentially treated overnight at room temperature with DNase/RNase and proteinase K (100 μ L of 10 mg/mL solutions per gram dry weight for each enzyme), then underwent dialysis (14-kDa cutoff) and lyophilization. For PW extraction (45), bacteria were resuspended in 45% aqueous phenol (10 mL per g bacteria) and stirred for 30 min at 68 °C. After centrifugation (5,600g) for 20 min at 4 °C, the upper water phase was collected. The extraction was repeated with the same volume of water as had been collected. Combined water phases and the phenolic phase were dialyzed against deionized water at room temperature (14-kDa cut-off) and lyophilized. Prior to lyophilization, the dialyzed phenolic phase (PP) was centrifuged (600g for 5 min at 20 °C) and divided into supernatant (sup) and sediment (sed). Lipopolysaccharide recovered from the water phase (77.4 mg; *Pst* Δ pagL WP) was used as such, whereas a phenol-chloroform-petroleum ether extraction (46) was performed with the material of the PP (4.06 g PP-sup; 1.42 g PP-sed). Each was resuspended in phenol (90%, w/v)-chloroform-petroleum ether (2:5:8 (vol/vol/vol); ~0.1 mg/mL), then the respective suspension was stirred for 30 min at room temperature, and centrifuged (6,000g) for 20 min at 20 °C. The supernatant was collected and the extraction was repeated once. Combined supernatants were

evaporated in vacuum until phenol crystallization began (at room temperature and normal pressure). Lipopolysaccharide from PP-sup was precipitated for 4 days at 4 °C with 1.37 mL water and collected by centrifugation (4,400g) for 20 min at 20 °C. Lipopolysaccharide from PP-sed was precipitated with 1.60 mL water as described for PP-sup. Precipitates were washed twice with 80% phenol (4,400g, 20 °C, 20 min) and three times with acetone (4,400g, 20 °C, 20 min) and dried. This resulted in the following yields: *Pst* Δ pagL PP-sup sed: 5.3 mg; *Pst* Δ pagL PP-sed sed: 58.9 mg.

Preparation and purification of lipopolysaccharide of P. cichorii ATCC10857/DSM50259.

Bacteria were grown in King's B medium at 26 °C under shaking (230 rpm) to an absorbance of ~1.0 at 600 nm, harvested by centrifugation (3,000g) for 20 min at 4 °C, resuspended in a small volume of water, boiled for 45 min, and lyophilized. The bacterial mass was washed with ethanol, acetone (twice), and diethyl ether, and afterwards dried (recovery, 13.5 g). For PW extraction (45), bacteria were resuspended in 45% aqueous phenol (10 mL per g bacteria) with an Ultra-Turrax and stirred for 20 min at 68 °C. After centrifugation (5,600g) for 20 min at 4 °C, the upper water phase was collected. The extraction was repeated with the same volume of water as had been collected. The separately treated water phases (WP) and the phenolic phase (PP) were extensively dialyzed against deionized water at 4 °C (14-kDa cut-off) and lyophilized. Before lyophilization, the dialyzed phenolic phase (PP) was centrifuged (600g for 5 min at 20 °C) and divided into supernatant (sup) and sediment (sed). All pellets were resuspended in water (10 mg/mL) and sequentially treated with DNase/RNase and proteinase K (100 μ L of 10 mg/mL solutions per gram dry weight for each enzyme), then underwent dialysis and lyophilization. This resulted in lyophilizates of 0.23 g and 0.38 g for the water phases, respectively, and 0.59 g for the supernatant of the PP (*Pci* lipopolysaccharide), which was the lipopolysaccharide containing fraction.

Selected lipopolysaccharide preparations were further purified by gel permeation chromatography (GPC) on Sephacryl S-400 HR (GE Healthcare) on a column (2.5 $\times$ 120 cm) as described (47). Purification of 80 mg of *Pci* lipopolysaccharide in four runs $\times$ 20 mg resulted in 31 mg of purified lipopolysaccharide (*Pci* S400). Purification of 41.8 mg of *Pst* WP in two runs (21.8/20.0 mg) resulted in 17 mg of purified lipopolysaccharide (*Pst* WP-S400), 19.9 mg of *Pst* PP-sup sed2 in 8.7 mg of purified lipopolysaccharide (*Pst* PP-sup sed2-S400), and 19.8 mg of *Pst* PP-sup sed3 in 9.4 mg of purified lipopolysaccharide (*Pst* PP-sup sed3-S400).

Preparation and purification of lipid A samples.

Starting from lipopolysaccharide preparations *Pst* WP (335 mg) and *Pci* (404 mg), lipid A samples were generated as described earlier (5) with three modifications: 1) heating for 3 h at 100 °C was done under reflux, 2) removal of SDS was achieved by six (*Pst*) or seven (*Pci*) washes with 120 mL 2 M HCl/ethanol (1:99 (vol/vol)), and 3) reextraction of the water phase was performed just twice with CHCl₃. Lipid A samples were further fractionated by reversed-phase HPLC (fig. S1,S2) essentially as described (5), but with some modifications. A semi-preparative Kromasil C18 column (5 μ m, 100 Å, 10 $\times$ 250 mm, MZ Analysentechnik) was used and the sample (10 mg/mL in CHCl₃/CH₃OH 4:1, v/v) was eluted using a gradient that consisted of methanol-chloroform-water (57:12:31, v/v/v) containing 10 mM NH₄OAc as mobile phase A and chloroform-methanol (70.2:29.8, v/v) with 50 mM NH₄OAc as mobile phase B. The initial solvent system consisted of 2% B and was maintained for 20 min, followed by a linear three step gradient raising from 2 to 17% B (20–50 min), 17 to 27% B (50–85 min), and 27 to 100% B (85–165 min). The solvent was held at 100% B for 10 min, the column re-equilibrated in 12 min to 2% B and held there for additional 10 min before the next injection. The flow rate was 2 mL/min using a splitter between the evaporative light-scattering detector (Sedex model 75C ELSD, S.E.D.E.R.E., France) equipped with a low-flow nebulizer recording the chromatogram and the fraction collector. Nitrogen (purity 99.996%) was used as gas to nebulize the post column flow stream at 3.5 bar into the detector at 50 °C setting the photomultiplier

gain to 10. The detector signal was transferred to the Gilson HPLC Chemstation (Trilution LC, version 2.1, Gilson) for detection and integration of the ELSD signal.

Repurification of lipopolysaccharide from P. cichorii ATCC10857/DSM50259 by DOC-GPC. The S400-purified material of *P. cichorii* (Pci S400) was further fractionated on Sephacryl S-200 HR (GE Healthcare) on a column (1.5 × 120 cm) using a desoxycholate (DOC) containing buffer as described (48). Approximately 5 mg lipopolysaccharide were resuspended in 1.5 mL eluting buffer and applied to the column. A representative chromatogram is depicted in figure S11A,B. Material of two runs was combined in pools as indicated and lyophilized. Resulting sediments were washed four times with 30 mL ethanol (3,363g (swing out rotor) for 10 min at 4 °C) and supernatants were discarded. Sediments were dried under a stream of nitrogen, stepwise dissolved in 9 mL water, transferred into dialysis tubes (12- to 16-kDa cut-off), and 1 mL 40 mM MgSO₄-solution was added. Dialysis against water was performed for three days at 4 °C with twelve water exchanges in total. Final yields starting from 10.4 mg Pci S400 were: Pci S400/S200 pool 1, 0.184 mg; Pci S400/S200 pool 2, 0.609 mg; Pci S400/S200 pool 3, 0.827 mg; Pci S400/S200 pool 4, 3.46 mg. SDS-PAGE analysis of the collected pools (fig. S11B) is shown in figure S11C. A 10% Bis-Tris NuPAGE gel (Thermo Fisher Scientific) was used according to manufacturer's instruction. For silver staining, the gel was incubated for 2 h in fixing solution (30% (v/v) ethanol, 10% (v/v) acetic acid in water), oxidized for 10 min in fixing solution containing 0.7% (w/v) periodic acid, and washed three times with water. The gel was stained for 30 min in 0.1% (w/v) aqueous silver nitrate solution (in the dark) and after transferring the gel for 10 seconds in water it was developed in a 3% (w/v) sodium carbonate/0.02% (v/v) formaldehyde solution for appr. 10 min (in the dark). The reaction was stopped with 1% (v/v) acetic acid and the gel was washed three times for 10 min with water.

Heat-detergent-promoted repurification of lipopolysaccharide from P. aeruginosa H4.

The heat-detergent-promoted repurification procedure was adapted from (49). Dried lipopolysaccharide preparations were resuspended in aqueous 1% (w/v) SDS solution (20 mg/mL lipopolysaccharide), incubated for 10 min at 100 °C, and dispersed in an ultrasonic bath for 10 min. 12 mL CHCl₃ and 8 mL MeOH per mL SDS-lipopolysaccharide suspension were added. Suspensions were sonicated for 10 min and incubated overnight on a rotator at 4 °C. Samples were centrifuged (10 min, 2000g, 4 °C), the supernatant was discarded, and the pellet dried under a stream of nitrogen. This procedure was repeated four times using 1% (w/v) SDS solution and two times using water to remove residual SDS. Finally, samples were washed three times with water and suspended in water. Aliquots of lipopolysaccharide samples were analysed on NuPAGE gels (Thermo Fisher Scientific) according to manufacturer's instruction. For silver staining, gels were incubated 2 h in fixing solution (25% (v/v) isopropanol, 7% (v/v) acetic acid in water), oxidized for 10 min in fixing solution containing 0.7% (w/v) periodic acid, and washed three times with water. Gels were stained for 10 min in 0.1% (w/v) aqueous silver nitrate solution, the reaction was stopped with 6% (w/v) sodium carbonate, 0.04% (v/v) formaldehyde solution, and gels were washed with 1% (v/v) acetic acid and water. SDS-PAGE analysis of the original and repurified lipopolysaccharide sample is shown in figure S11D.

Determination of absolute configurations of hydroxy fatty acids in lipopolysaccharide preparations

The absolute configuration of hydroxy fatty acids in *P. syringae* pv. *tomato* DC3000 and *P. cichorii* ATCC10857/DSM50259 lipopolysaccharide was determined by generating their respective L-phenylethylamides (50, 51) in comparison to authentic standards. The gas-liquid chromatography-mass spectrometry (GLC-MS) analyses were performed on an Agilent Technologies 6890N gas chromatograph coupled to a 5975 inert XL Mass Selective Detector (Agilent Technologies). A 30-m HP-5MS column (Hewlett-Packard) was used and a temperature gradient of 150 °C (kept for 3 min) to 320 °C at 5 °C/min and finally maintaining this temperature for 10 min was applied.

Quantification of 3-OH-C10:0 (1) by stable isotope dilution analysis (SIDA-UHPLC-MS/MS). 3-OH-C10:0 (1) was analysed by means of a newly developed SIDA-UHPLC-MS/MS method (fig. S10). Therefore, its deuterium-labelled twin molecule 3-OH-C10:0-d₂ (1-d₂) was synthesized as reported below.

Internal Standard (IS).

A stock solution (20 mL) of the 3-OH-C10:0-d₂ (1-d₂) (5 mmol/L, 3.8 mg/20 mL) was prepared in MeOD, its exact concentration was verified by means of quantitative NMR (qNMR) and it was stored at -20 °C until use.

Sample preparation.

Samples were solved in water, acetonitrile or a mixture of acetonitrile/water. After adding 2 µL of the IS and the use of a Vortexer (2 min, 250 rpm, VWR, Darmstadt, Germany), samples were equilibrated for one hour and were shaken again (2 min, 250 rpm).

Ultra High Performance Liquid Chromatography-Mass Spectrometry (UHPLC-MS/MS).

A QTRAP 6500 mass spectrometer (Sciex, Darmstadt, Germany) was used and operated in the full-scan mode (ion spray voltage, -4500 V): curtain gas, 35 V; temperature, 500 °C; gas 1, 55 V; gas 2, 65 V; collision-activated dissociation, -3 V; and entrance potential, -10 V. For tuning direct flow injection with a syringe pump (10 µL/min) and compound solutions in acetonitrile/water were used. The samples were separated by means of a Nexera X2 UHPLC (Shimadzu Europa GmbH, Duisburg, Germany) consisting of two LC pump systems 30AD, a DGU-20A5 degasser, a SIL-30AC autosampler, a CTO-30A column oven, and a CBM-20A controller and equipped with a 100 × 2.1 mm, 100 Å, Kinetex 1.7 µm C18 column (Phenomenex). Chromatography was performed with an injection volume of 2 µL and a flow rate of 0.4 mL/min. The solvent system consisted of A: acetonitril (0.1% formic acid) and B: formic acid (0.1% in water, pH 3.5). The following gradient was used: 0 min, 30% A; 1 min, 30% A; 5 min, 100% A; 6 min, 100% A; 8 min, 30% A; 10 min, 30% A. Data acquisition and instrumental control was performed with Analyst 1.6.2 software (Sciex, Darmstadt, Germany). After optimizing instrument settings, analytes and the internal standard were analysed using the MRM transition Q1/Q3 of m/z 187.1/58.3 as qualifier (DP = -80 V, CE = -16 V, CXP = -7 V) and Q1/Q3 of m/z 187.1/187.1 as quantifier (DP = -80 V, CE = -8 V, CXP = -19 V) for 3-OH-C10:0 (1) and Q1/Q3 of m/z 189.0/58.9 as qualifier (DP = -55 V, CE = -16 V, CXP = -9 V) and Q1/Q3 of m/z 189.0/189.1 as quantifier (DP = -55 V, CE = -8 V, CXP = -13 V) for 3-OH-C10:0-d₂ (1-d₂).

Calibration Curve and Linear Range.

A stock solution of purified 3-OH-C10:0-d₂ (IS, 1-d₂) and the analyte 3-OH-C10:0 (1) was prepared in MeOD, and its exact concentration was verified by means of quantitative NMR (qNMR). Thereafter, the IS (1-d₂) and the analyte (1) were mixed in 10 micromolar ratios from 0.05 to 50 keeping a constant concentration of the internal standard. Triplicate UHPLC-MS/MS analysis calibration curves were prepared by plotting peak area ratios of each analyte to the internal standard against concentration ratios of each analyte to the IS using linear regression, showing linear responses with correlation coefficients of >0.99 each. The response was linear for chosen molar ratios and the contents of 3-OH-C10:0 (1) in the samples was calculated using the respective calibration function. Determination of the limit of detection (LOD) at a signal-to-noise ratio of 3 and the limit of quantitation (LOQ) at a signal-to-noise ratio of 10 revealed the following values: LOD: ≤0.002 µM; LOQ ≤0.01 µM.

High-Performance Liquid Chromatography (HPLC)

For preparative chromatography an HPLC apparatus (Jasco, Groß-Umstadt, Germany) consisting of two PU-2087 Plus pumps, an AS-2055 Plus autosampler, a Sedex LT-ELSD detector Model 85 (Sedere, Alfortville, France), a MD-2010 Plus diode array detector and a Rh 7725i type Rheodyne injection valve (Rheodyne, Bensheim, Germany) was used.

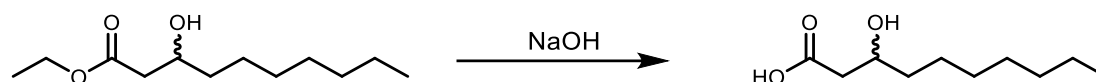
Nuclear Magnetic Resonance Spectroscopy (NMR)

One- and two-dimensional ^1H and ^{13}C NMR spectra were acquired on a 500 MHz Bruker Avance III equipped with a Triple Res. Cryo-TCI probe and a 400 MHz Avance III spectrometer equipped with a Double Resonance Broadband probe (Bruker, Rheinstetten, Germany). Chemical shifts are reported in parts per million, relative to solvent signals: ^1H NMR: 7.26 ppm (CDCl_3), 3.31 ppm (MeOD); ^{13}C NMR: 77.16 ppm (CDCl_3), 49.0 ppm (MeOD). All pulse sequences were taken from Bruker software library. For data processing Topspin NMR software (version 3.1 and 3.2; Bruker, Rheinstetten, Germany) and for data interpretation MestReNova 9.1.0-14011 (Mestrelab Research S.L., Santiago de Compostela, Spain) were used. Quantitative NMR (qNMR) experiments were carried out on a Bruker Avance III 400 MHz spectrometer as reported by (52).

Synthetic compounds

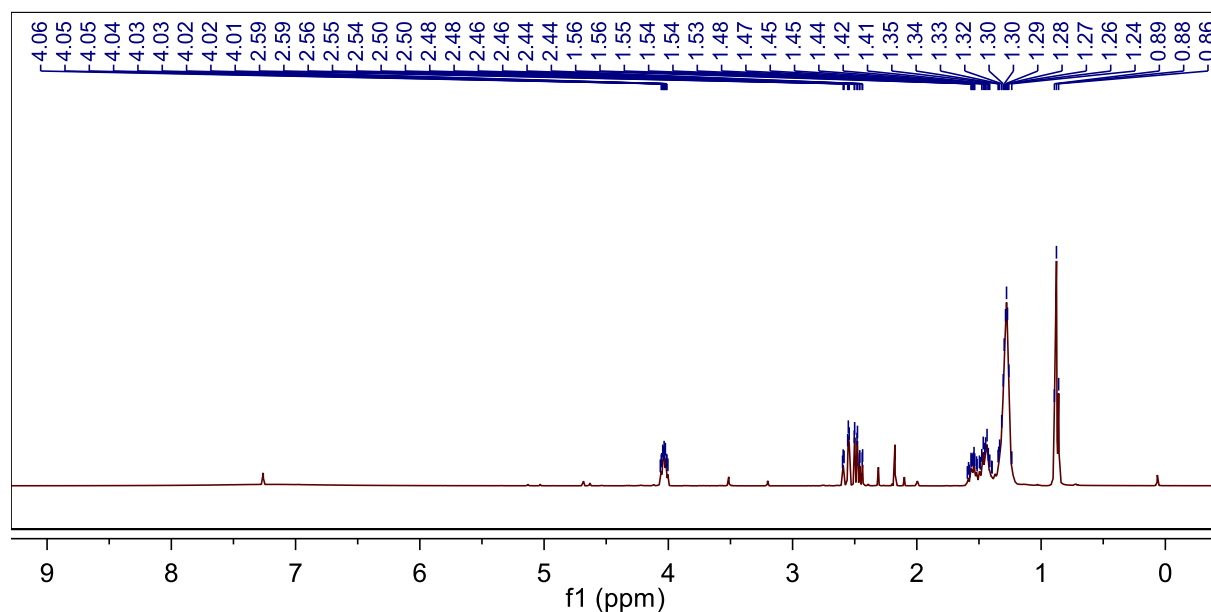
Synthetic compounds were obtained from commercial suppliers or generated in this study as indicated in table S2. Synthesis, purification, and analysis of compounds generated in this study are described in detail below. For dose-response assays with 3-OH-C10:0 (**1**) (fig. 1B) and the comparison of (*R*)-3-OH-C10:0 (**28**) and (*S*)-3-OH-C10:0 (**29**) (fig. 2F), the exact concentrations of the stock solutions in MeOH were verified by means of qNMR.

Synthesis of 3-hydroxydecanoic acid, 3-OH-C10:0 (**1**)

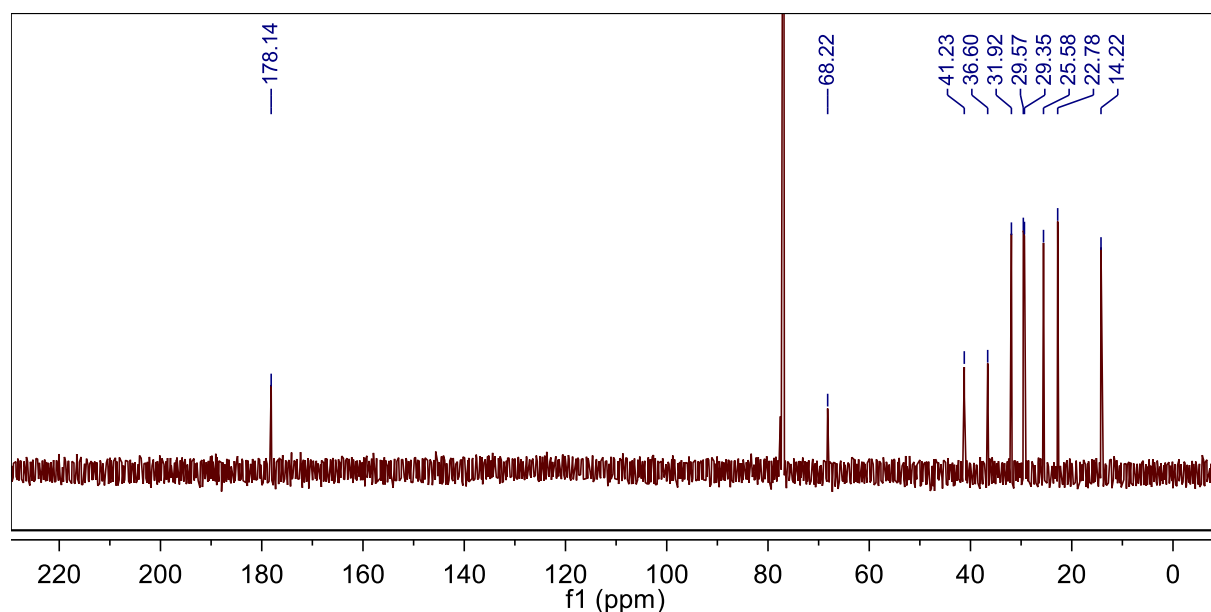


According to (53) ethyl 3-hydroxydecanoate (1.2 g, 5.5 mmol) was stirred over night at room temperature with an excess of NaOH (1 N, 11 mL, 11 mmol). The reaction mixture was washed with ether (2 × 20 mL), the aqueous phase was acidified with 1 N HCl and extracted with ether (3 × 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to afford 3-hydroxydecanoic acid (755 mg, 73%) as a white solid. ^1H NMR (400 MHz, CDCl_3) δ 0.88 (t, J = 7.1 Hz, 3H, H-C(10)), 1.36 – 1.22 (m, 10H), 1.62 – 1.38 (m, 2H, H-C(4)), 2.47 (ddd, J = 16.6, 9.0, 1.3 Hz, 1H, H_a -C(2)), 2.57 (dt, J = 16.5, 2.4 Hz, 1H, H_b -C(2)), 4.03 (tdd, J = 8.1, 4.6, 3.1 Hz, 1H, H-C(3)). ^{13}C NMR (101 MHz, CDCl_3) δ 14.2 (C(10)), 22.8 (C(9)), 25.6 (C(5)), 29.4 (C(7)), 29.6 (C(6)), 31.9 (C(8)), 36.6 (C(4)), 41.2 (C(2)), 68.2 (C(3)), 178.1 (C(1)).

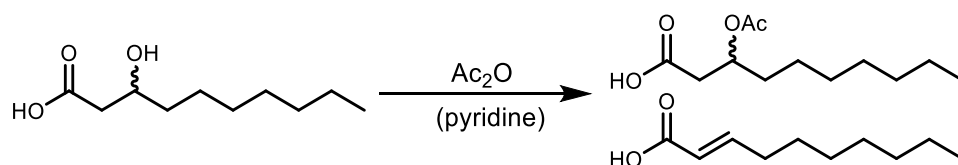
^1H -NMR spectra of 3-OH-C10:0 (**1**)



^{13}C -NMR spectra of 3-OH-C10:0 (**1**)



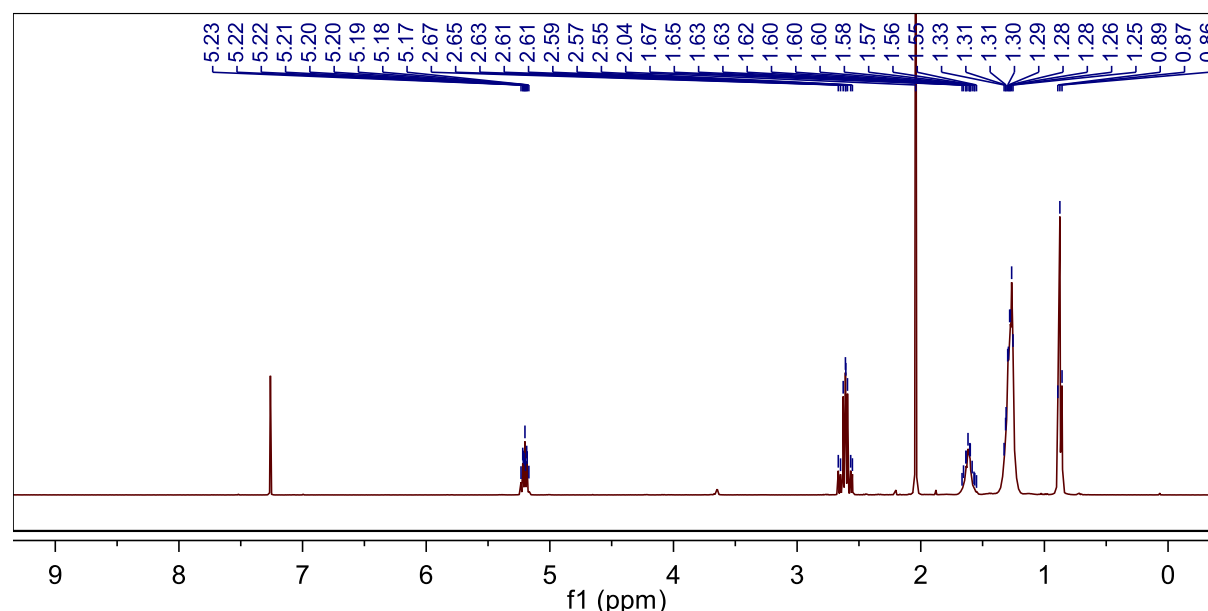
Synthesis of 3-acetoxydecanoic acid, 3-AcO-C10:0 (**25**)/(*E*)-Dec-2-enoic acid, *trans*- Δ^2 -C10:1 (**20**)



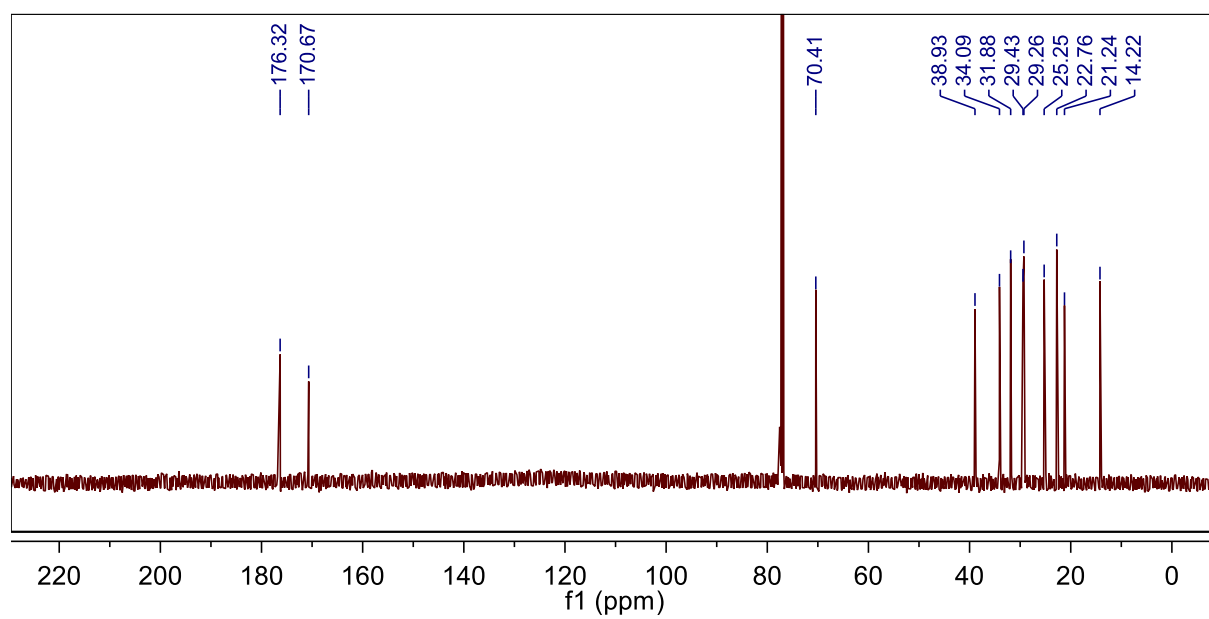
According to a procedure previously reported by (54) with slight modifications 3-acetoxydecanoic acid was synthesized. After 3-hydroxydecanoic acid (220 mg, 1.16 mmol) was dissolved in anhydrous pyridine (5 mL) acetic anhydride (131 μL , 1.4 mmol) was added under

ice cooling and the mixture was stirred at room temperature overnight. Afterwards, 5 mL distilled water and 10 mL 1 N hydrochloric acid were added, and the mixture was extracted with diethylether (3×30 mL). The combined ether layers were washed with brine, dried over Na_2SO_4 and concentrated in vacuum. The crude product was purified by means of silica gel column chromatography (hexane/EtOAc, 50/50, v/v) to obtain a mixture of 3-acetoxy-decanoic acid and (*E/Z*)-dec-2-enoic acid. The product mix was separated by RP-HPLC using a Hyperclone ODS (C18) 120 A (250×21.2 mm, 5 μm , Phenomenex, Aschaffenburg, Germany) column, 0.1% formic acid as solvent A and methanol/2-propanol (80/20, v/v) as solvent B. The column was operated with a flow rate of 18 mL/min and the following gradient: 0 min, 70% B; 2 min 70% B; 10 min, 100% B; 13 min, 100% B; 14 min, 70% B; 20 min, 70% B. The effluent was monitored by means of ELSD and UV detection at 210 nm. The fractions were collected individually in several runs. After evaporation of the solvent, extraction of the aqueous phase with EtOAc (3×10 mL) and subsequent drying over anhydrous Na_2SO_4 , 3-acetoxydecanoic acid (2.8 mg, 0.012 mmol, 1%) and (*E*)-dec-2-enoic acid (1.6 mg, 0.01 mmol, 0.9%) acid was afforded as white solid. 3-acetoxydecanoic acid: ^1H NMR (400 MHz, CDCl_3) δ 0.87 (d, $J = 7.0$ Hz, 3H, H-C(10)), 1.39 – 1.19 (m, 10H), 1.70 – 1.51 (m, 2H, H-C(4)), 2.04 (s, 3H, -OAc), 2.58 (dd, $J = 15.9, 5.5$ Hz, 1H, $\text{H}_a\text{-C}(2)$), 2.64 (dd, $J = 15.9, 7.2$ Hz, 1H, $\text{H}_b\text{-C}(2)$), 5.20 (tt, $J = 7.1, 5.5$ Hz, 1H, H-C(3)); ^{13}C NMR (101 MHz, CDCl_3) δ 14.2 (C(10)), 21.2 (C(2')), 22.8 (C(9)), 25.3 (C(5)), 29.3 (C(7)), 29.4 (C(6)), 31.9 (C(8)), 34.1 (C(4)), 38.9 (C(2)), 70.4 (C(3)), 170.7 (C(1')), 176.3 (C(1')). (*E*)-dec-2-enoic acid: ^1H NMR (400 MHz, CDCl_3) δ 0.88 (t, $J = 7.1$ Hz, 3H, H-C(10)), 1.37 – 1.19 (m, 8H), 1.47 (dq, $J = 12.1, 7.0$ Hz, 2H, H-C(5)), 2.23 (qd, $J = 7.1, 1.6$ Hz, 2H, H-C(4)), 5.82 (dt, $J = 15.6, 1.6$ Hz, 1H, H-C(2)), 7.08 (dt, $J = 15.6, 7.0$ Hz, 1H, H-C(3)); ^{13}C NMR (101 MHz, CDCl_3) δ 14.2 (C(10)), 22.8 (C(9)), 28.0 (C(7)), 29.2 (C(5)), 29.2 (C(6)), 31.9 (C(8)), 32.5 (C(4)), 120.5 (C(2)), 152.7 (C(3)), 171.4 (C(1)).

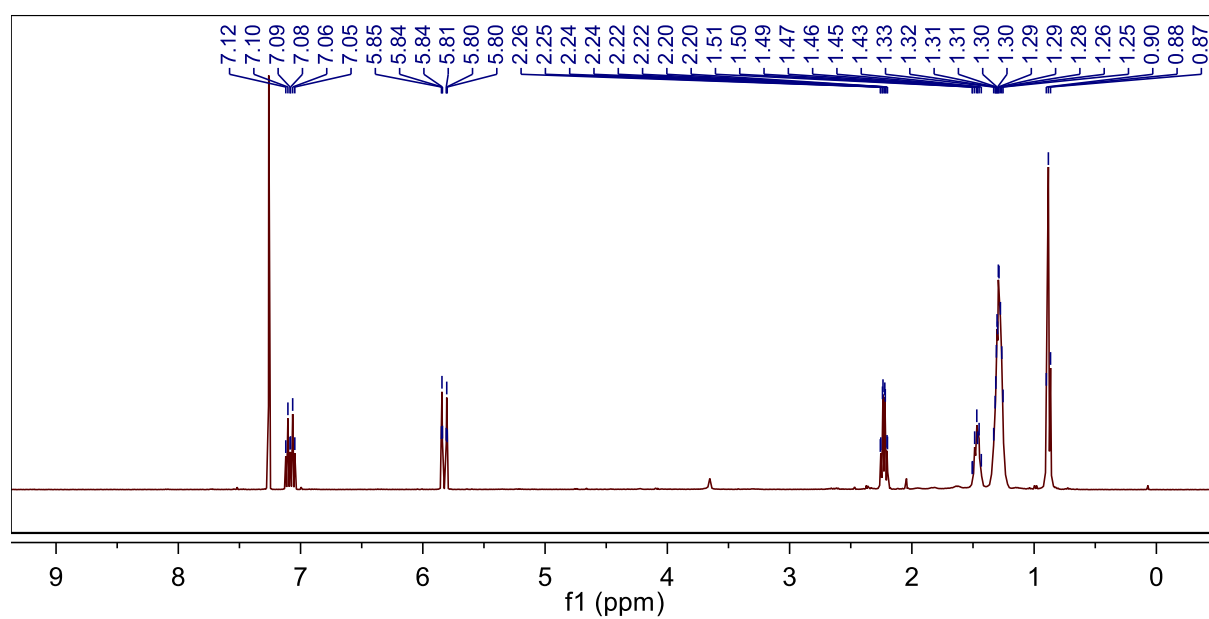
^1H -NMR spectra of 3-AcO-C10:0 (25)



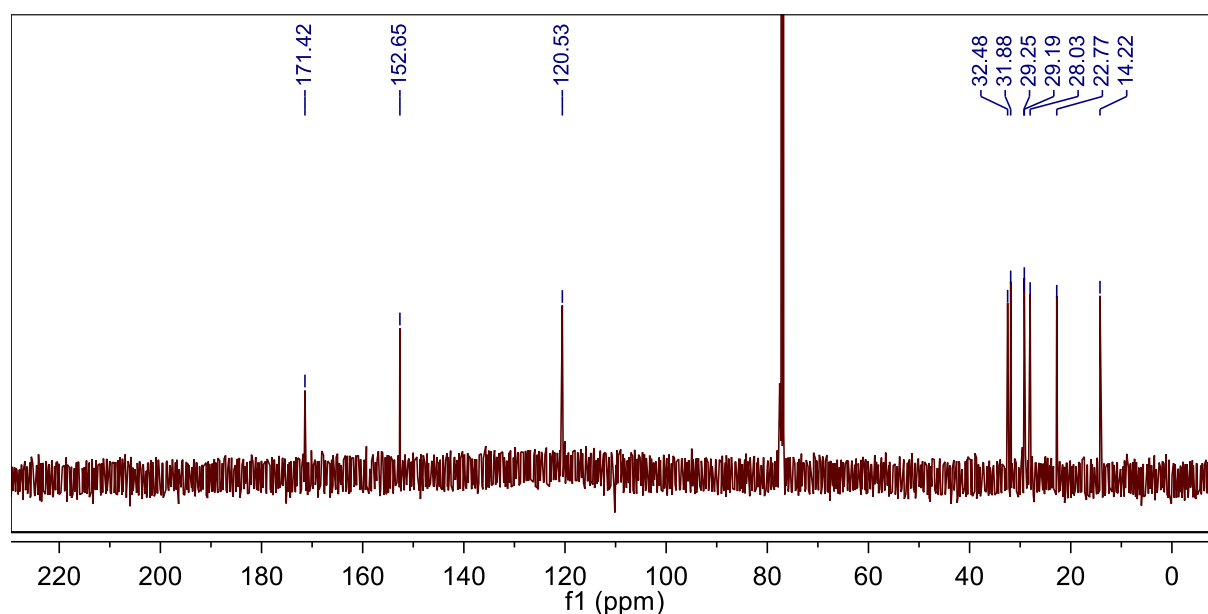
^{13}C -NMR spectra of 3-AcO-C10:0 (25)



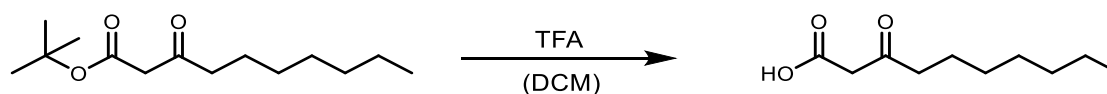
^1H -NMR spectra of *trans*- Δ^2 -C10:1 (20)



^{13}C -NMR spectra of *trans*- Δ^2 -C10:1 (**20**)

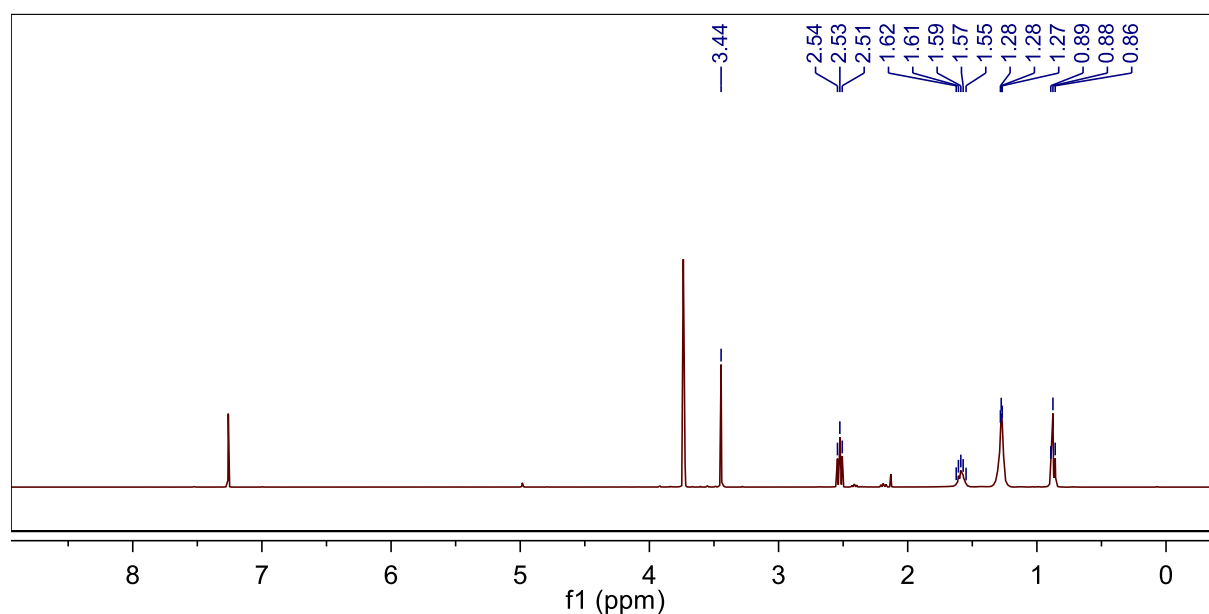


Synthesis of 3-oxodecanoic acid, 3-oxo-C10:0 (**23**)

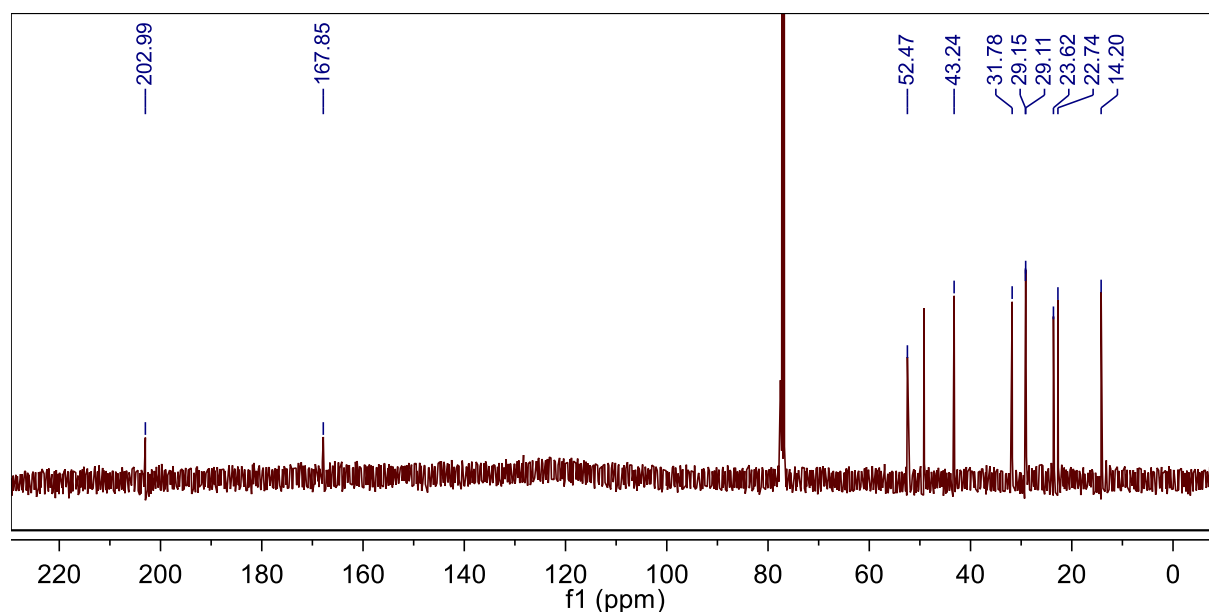


Using a standard reaction described by (53) *tert*-butyl 3-oxo-decanoate was deprotected by dissolving in TFA/DCM (1/3, v/v) (15 mL) and stirring for 12 h. Afterwards the solution was concentrated to dryness under a flow of nitrogen to give 3-oxodecanoic acid in a high purity ($\geq 98\%$) without further purification. ^1H NMR (400 MHz, CDCl_3) δ 0.88 (t, $J = 7.3$ Hz, 3H, H-C(10)), 1.34 – 1.21 (m, 8H), 1.59 (p, $J = 9.0, 7.9$ Hz, 2H, H-C(5)), 2.53 (t, $J = 7.4$ Hz, 2H, H-C(4)), 3.44 (s, 2H, H-C(2)). ^{13}C NMR (101 MHz, CDCl_3) δ 14.2 (C(10)), 22.7 (C(9)), 23.6 (C(5)), 29.1 (C(7)), 29.2 (C(6)), 31.8 (C(8)), 43.2 (C(4)), 52.5 (C(2)), 167.9 (C(1)), 203.0 (C(3)).

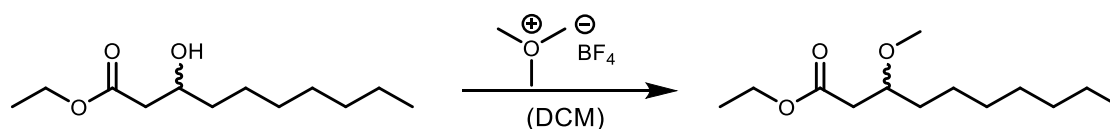
¹H-NMR spectra of 3-oxo-C10:0 (23)



¹³C-NMR spectra of 3-oxo-C10:0 (23)



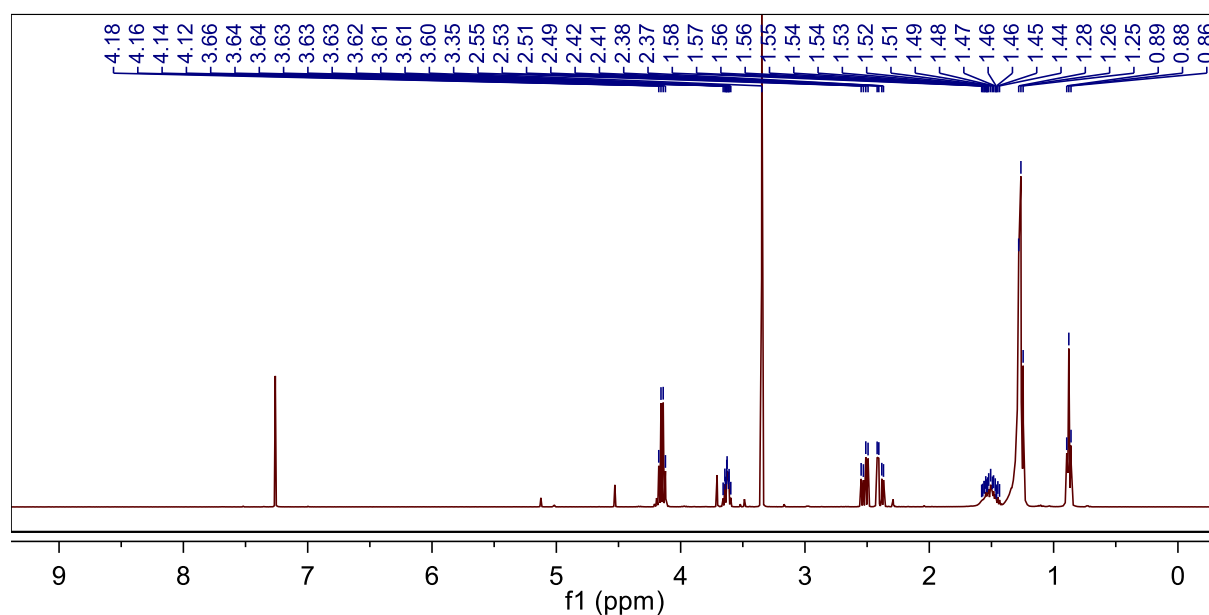
Synthesis of ethyl 3-methoxydecanoate (24a)



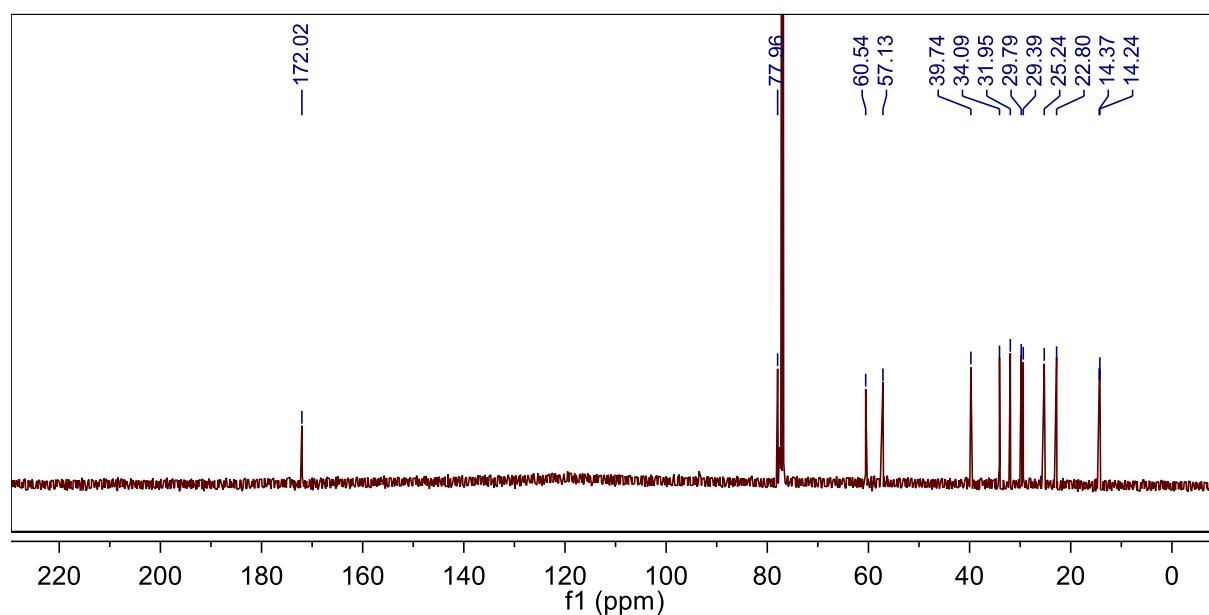
Following a literature procedure (55) with some modifications, ethyl 3-methoxy-decanoate was synthesized. To a solution of ethyl 3-hydroxy-decanoate (0.2 g, 0.92 mmol) in anhydrous DCM (30 mL) at room temperature activated mol. sieves (4Å, 1.2 g), proton sponge (1.68 g, 7.82 mmol) and trimethyloxonium tetrafluoroborate (0.98 g, 6.44 mmol) were added. The reaction mixture was stirred overnight, diluted with diethylether (80 mL), and then filtered to remove

the solids. The filtrate was washed with HCl (1 N, 2 × 30 mL), dried over Na₂SO₄ and concentrated in vacuum. The crude product was purified by means of silica gel chromatography (hexane/EtOAc, 95/5, v/v) to yield ethyl 3-methoxydecanoate (176 mg, 0.76 mmol, 83%). ¹H NMR (400 MHz, CDCl₃) δ 0.87 (t, *J* = 6.8 Hz, 3H, H-C(10)), 1.26 (t, *J* = 7.1 Hz, 3H, H-C(2')), 1.37 – 1.17 (m, 10H) 1.61 – 1.42 (m, 2H, H-C(4)), 2.39 (dd, *J* = 15.1, 5.4 Hz, 1H, H_a-C(2)), 2.52 (dd, *J* = 15.0, 7.3 Hz, 1H, H_b-C(2)), 3.35 (s, 3H, O-Me), 3.67 – 3.58 (m, 1H, H-C(3)), 4.15 (q, *J* = 7.1 Hz, 2H, H-C(1')). ¹³C NMR (101 MHz, CDCl₃) δ 14.2 (C(2')), 14.4 (C(10)), 22.8 (C(9)), 25.2 (C(5)), 29.4 (C(7)), 29.8 (C(6)), 32.0 (C(8)), 34.1 (C(4)), 39.7 (C(2)), 57.1 (O-Me), 60.5 (C(1')), 78.0 (C(3)), 172.0 (C(1)).

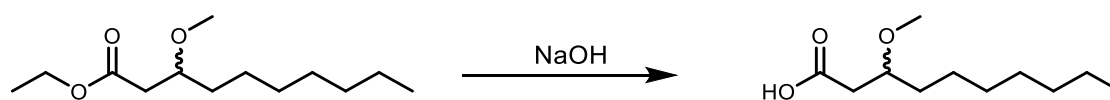
¹H-NMR spectra of ethyl 3-methoxydecanoate (**24a**)



¹³C-NMR spectra ethyl 3-methoxydecanoate (**24a**)

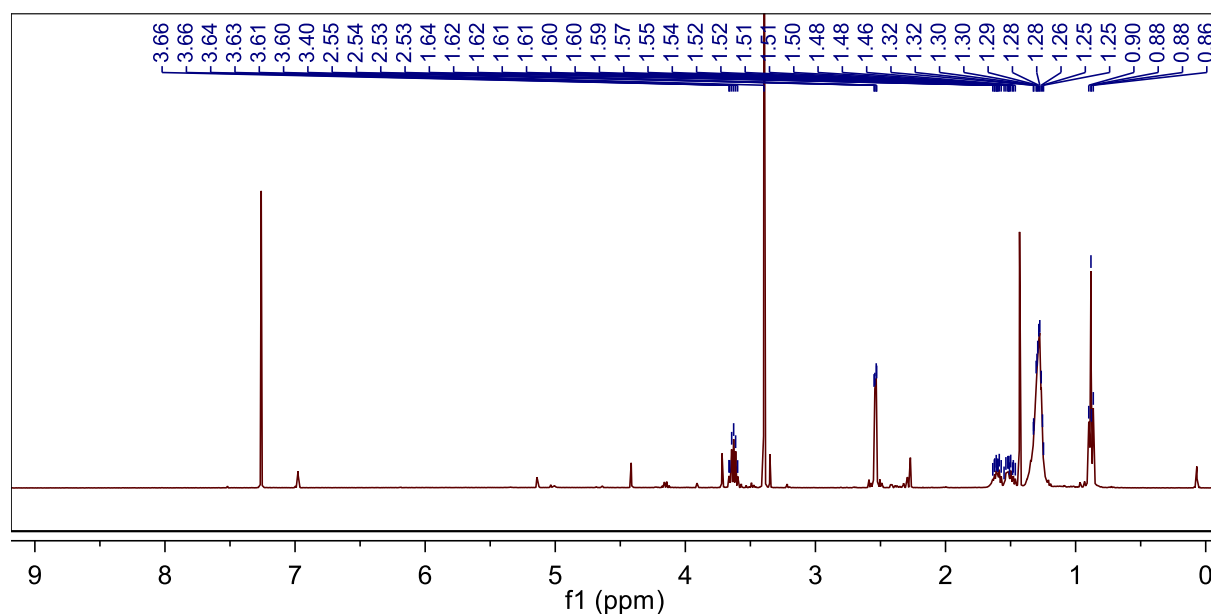


Synthesis of 3-methoxydecanoic acid, 3-MeO-C10:0 (**24**)

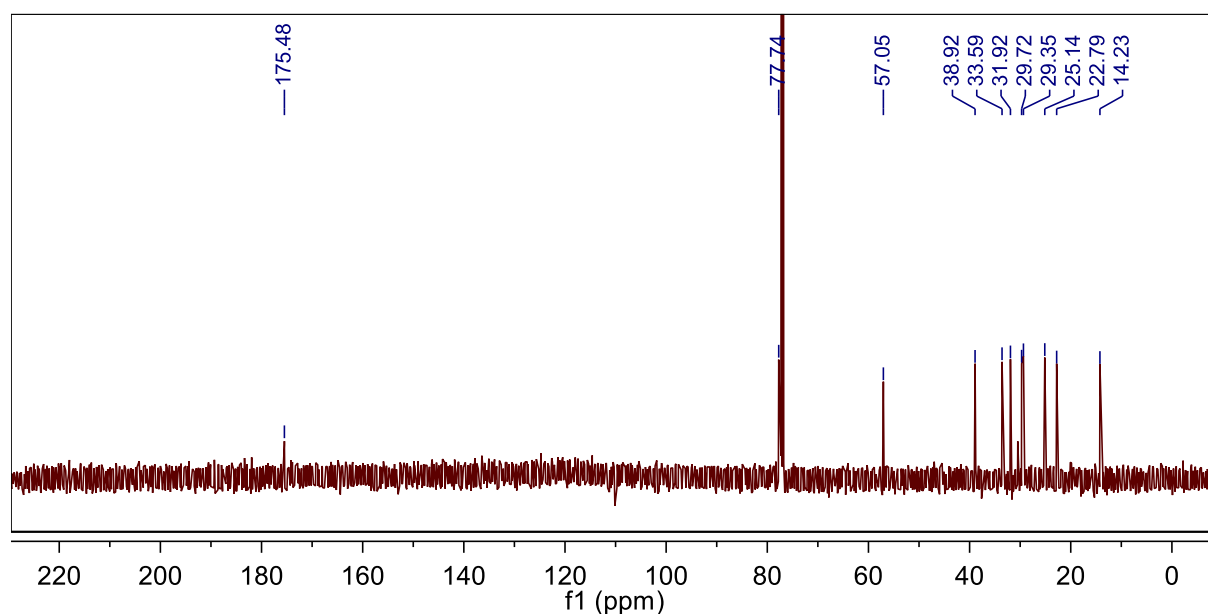


According to the procedure already described for the hydrolysis of ethyl 3-hydroxydecanoate to 3-hydroxydecanoic acid, 3-methoxydecanoic acid was synthesized from ethyl 3-methoxydecanoate (50 mg, 0.22 mmol). Thereby, 3-methoxydecanoic acid was afforded as a viscous oil (6.8 mg, 0.033 mmol, 15%). ^1H NMR (400 MHz, CDCl_3) δ 0.92 – 0.84 (t, 3H, J = 6.5 Hz, H-C(10)), 1.39 – 1.22 (m, 10H), 1.50 (ddd, J = 14.2, 8.7, 6.0 Hz, 1H), 1.67 – 1.56 (m, 1H), 2.54 (dd, J = 6.0, 1.9 Hz, 2H), 3.40 (s, 3H), 3.69 – 3.57 (m, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ 14.2 (C(10)), 22.8 (C(9)), 25.1 (C(5)), 29.4 (C(7)), 29.7 (C(6)), 31.9 (C(8)), 33.6 (C(4)), 39.0 (C(2)), 57.1 (O-Me), 175.5 (C(1)).

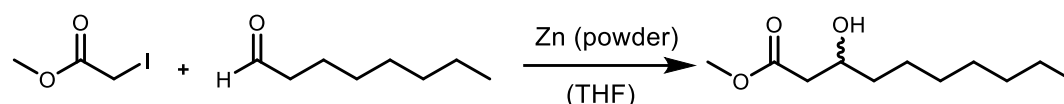
^1H -NMR spectra of 3-MeO-C10:0 (**24**)



¹³C-NMR spectra of 3-MeO-C10:0 (24)

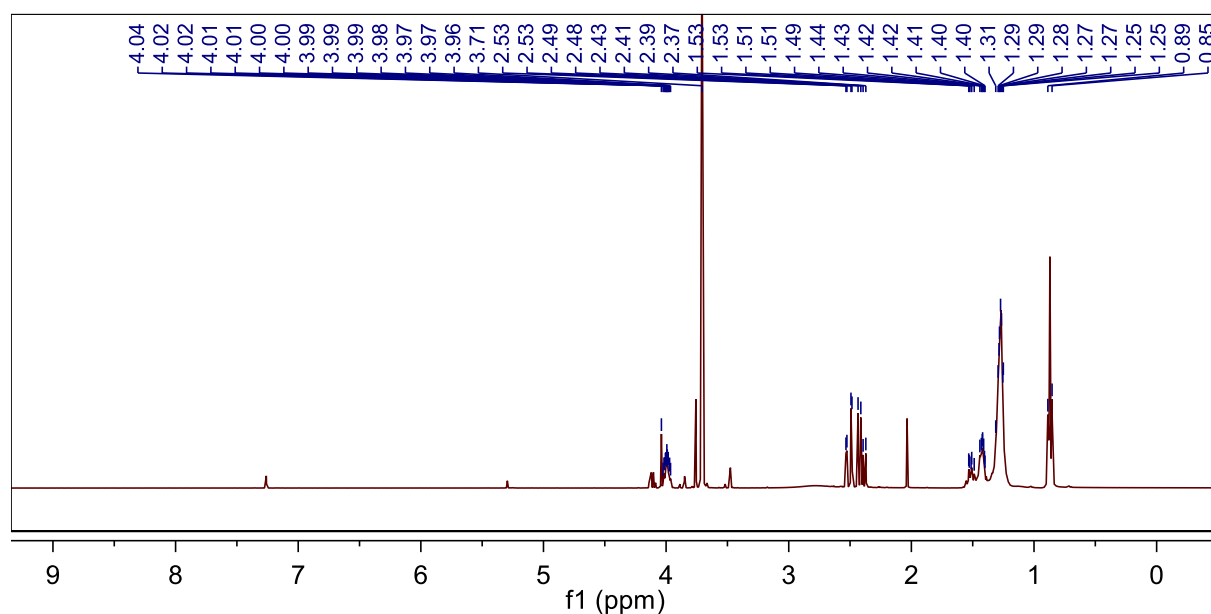


Synthesis of methyl 3-hydroxydecanoate, Me-3-OH-C10:0 (26)

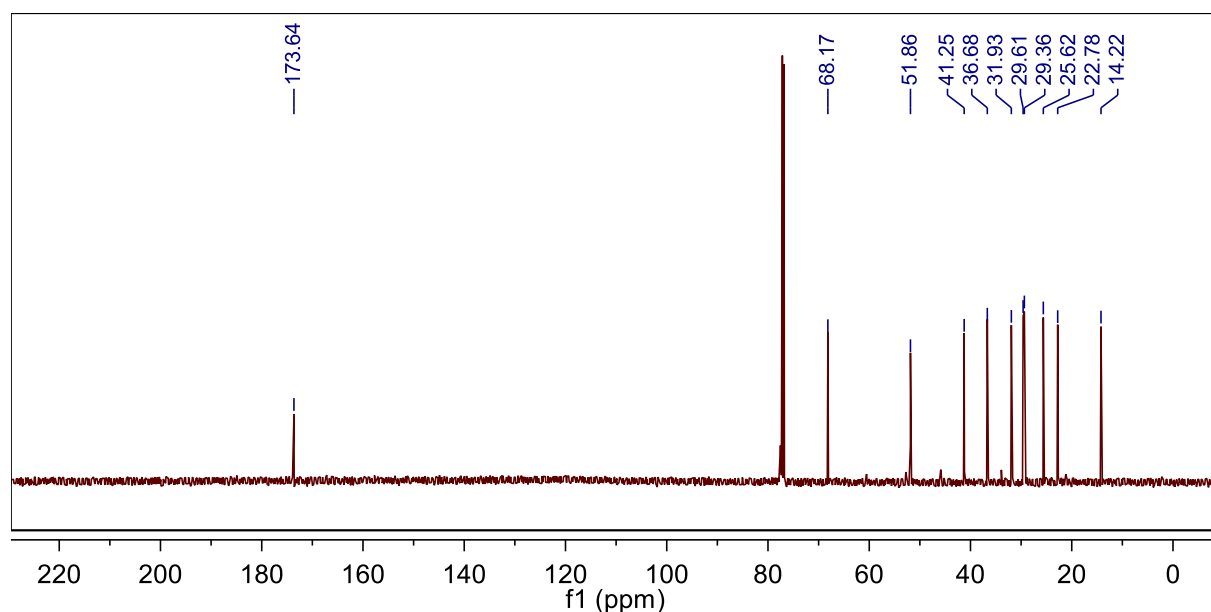


Methyl 3-hydroxy-decanoate was synthesized according to a modified procedure reported by (56). Methyl bromoacetate (3.7 mL, 39 mmol) and zinc powder (2.55 g, 39 mmol) were added to a solution of octanal (1.22 mL, 7.8 mmol) in toluene (10.0 mL). The mixture was refluxed for 20 min and then cooled to room temperature. The reaction mixture was diluted with ether (50 mL) and HCl (1 N, 50 mL). The separated organic layer was washed with saturated aqueous NaHCO₃-solution and brine, dried over Na₂SO₄ and concentrated under reduced pressure. Column chromatography of the crude product was achieved by hexane/EtOAc (85/15, v/v) resulting in Methyl 3-hydroxydecanoate (1.1 g, 39%) as colorless oil. ¹H NMR (400 MHz, CDCl₃) δ 0.87 (t, *J* = 13.8 Hz, 3H, H-C(10)), 1.37 – 1.20 (m, 10H), 1.61 – 1.37 (m, 2H, H-C(4)), 2.40 (dd, *J* = 16.4, 9.0 Hz, 1H, H_a-C(2)), 2.51 (dd, *J* = 16.4, 3.2 Hz, 1H, H_b-C(2)), 3.71 (s, 3H, H-C(1')), 3.99 (dddd, *J* = 8.8, 7.7, 4.5, 3.1 Hz, 1H, H-C(3)). ¹³C NMR (101 MHz, CDCl₃) δ 14.2 (C(10)), 22.8 (C(9)), 25.6 (C(5)), 29.4 (C(7)), 29.6 (C(6)), 31.9 (C(8)), 36.7 (C(4)), 41.3 (C(2)), 51.9 (C(1')), 68.2 (C(3)), 173.6 (C(1)).

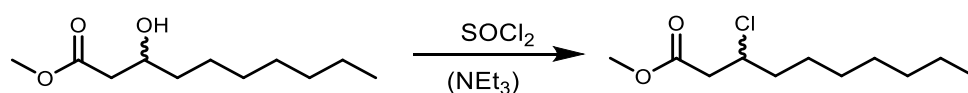
¹H-NMR spectra of Me-3-OH-C10:0 (26)



¹³C-NMR spectra of Me-3-OH-C10:0 (26)



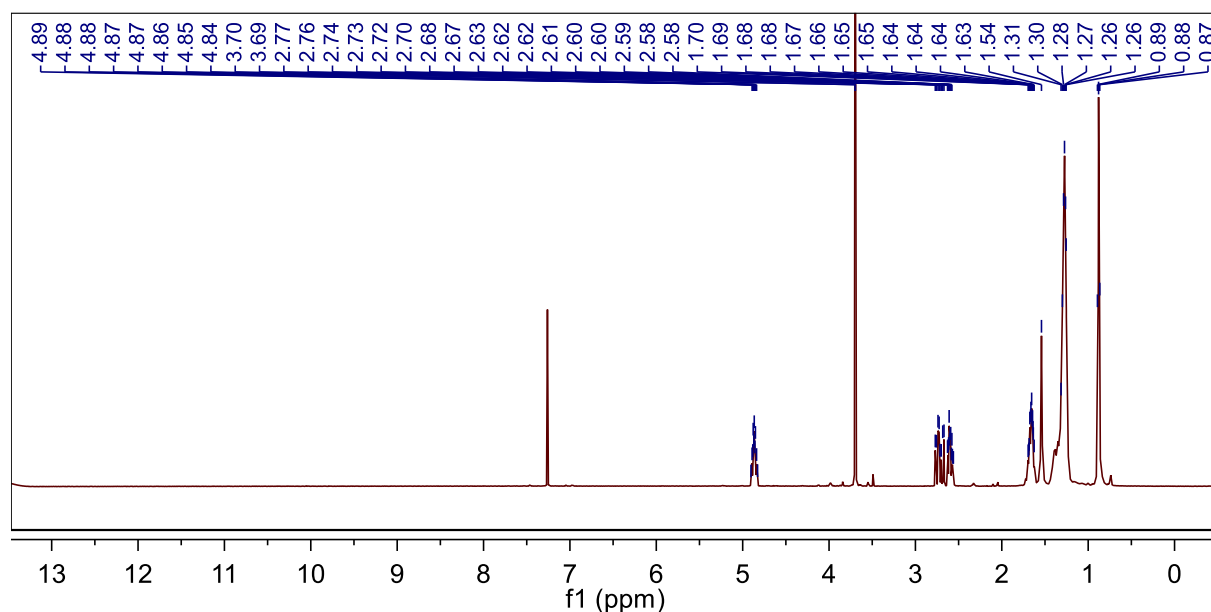
Synthesis of methyl 3-chlorodecanoate, Me-3-Cl-C10:0 (27)



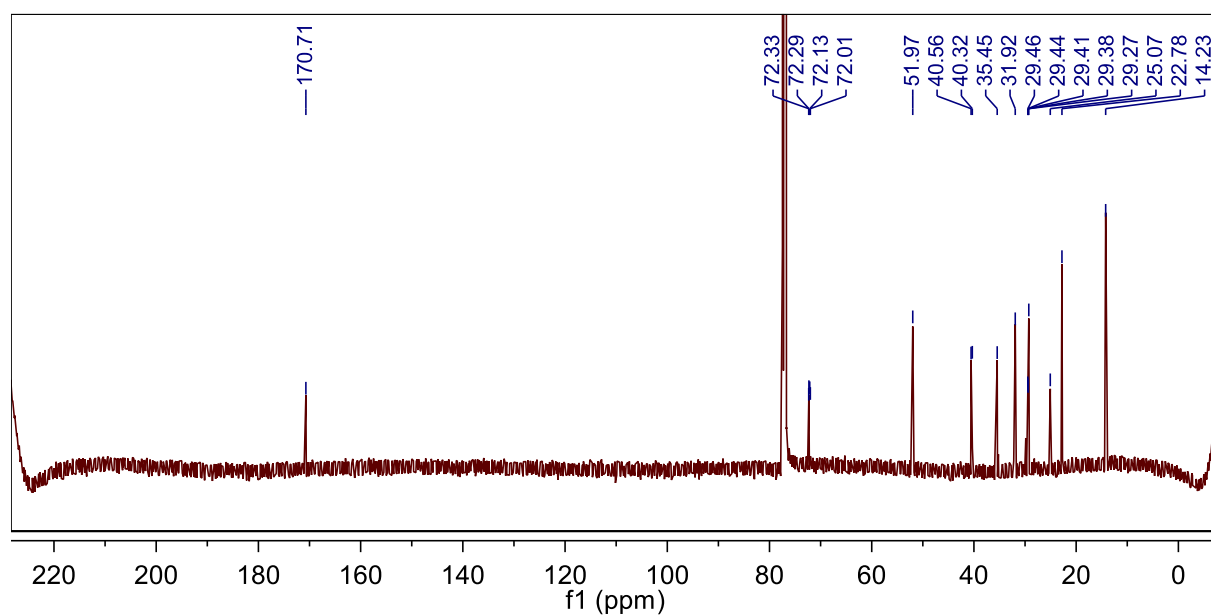
According to (57) a solution of methyl 3-hydroxydecanoate (100 mg, 0.494 mmol) and trimethylamine (7.5 mg, 0.074 mmol) in dry diisopropyl ether (5 mL) one half of SOCl₂ (69 mg, 0.581 mmol) in dry diisopropyl ether (5 mL) was added dropwise at -10°C. The reaction mixture was stirred for 10 min at -10 °C and after that allowed to warm to room temperature. Subsequently the second half of SOCl₂ solution was added dropwise and the mixture was heated to 75 °C for 3 h. After cooling to room temperature, the reaction mixture was neutralized by

the addition of saturated aqueous NaHCO₃ solution. The mixture was extracted with CHCl₃ (3 × 30 mL), the combined organic layers were washed with brine and dried over Na₂SO₄. The solvent was removed under reduced pressure and the crude product was subjected to a column filled with conditioned silica gel. Chromatography was performed using hexane/EtOAc (95/5) to yield methyl 3-chlorodecanoate (15 mg, 14%). ¹H NMR (500 MHz, CDCl₃) δ 0.88 (t, *J* = 6.8 Hz, 3H, H-C(10)), 1.27 (dt, *J* = 9.4, 5.1 Hz, 10H), 1.73 – 1.60 (m, 2H, H-C(4)), 2.59 (dddd, *J* = 15.6, 8.6, 6.0, 3.1 Hz, 1H, H_a-C(2)), 2.72 (ddd, *J* = 28.2, 16.0, 6.8 Hz, 1H, H_b-C(2)), 3.70 (d, *J* = 1.8 Hz, 3H, H-C(1')), 4.87 (ddt, *J* = 13.5, 9.6, 5.4 Hz, 1H, H-C(3)). ¹³C NMR (126 MHz, CDCl₃) δ 14.2 (C(10)), 22.8 (C(9)), 25.1 (C(5)), 29.3 (C(6)), 29.4 (C(7)), 31.9 (C(8)), 40.3 (C(4)), 40.6 (C(2)), 52.0 (C(1')), 73.1 – 70.9 (C(3)), 170.7 (C(1)).

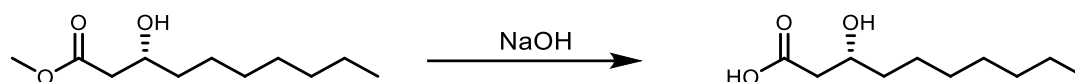
¹H-NMR spectra of Me-3-Cl-C10:0 (27)



¹³C-NMR spectra of Me-3-Cl-C10:0 (27)

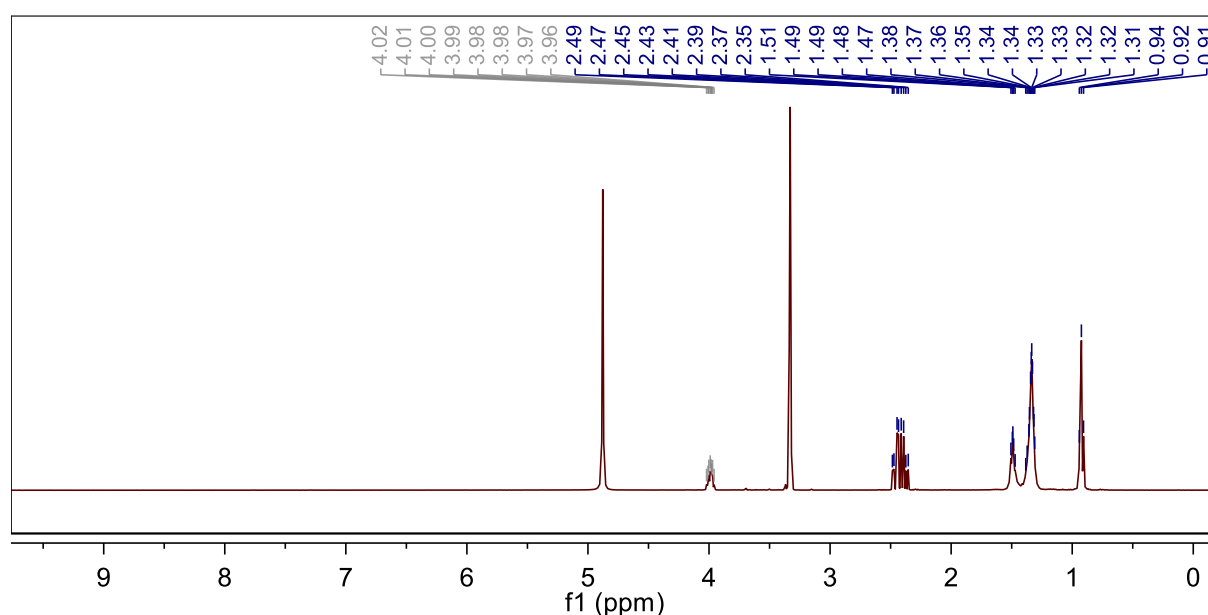


Synthesis of (*R*)-3-hydroxydecanoic acid, (*R*)-3-OH-C10:0 (**28**)

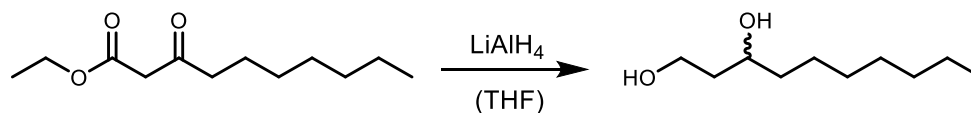


According to the procedure already described for the hydrolysis of ethyl 3-hydroxydecanoate to 3-hydroxydecanoic acid, (*R*)-3-hydroxydecanoic acid was synthesized from methyl (*R*)-3-hydroxydecanoate (50 mg, 0.25 mmol). Thereby, (*R*)-3-hydroxydecanoic acid was afforded as a white solid (37 mg, 0.2 mmol, 79%). ¹H NMR (400 MHz, MeOD) δ 0.92 (d, J = 7.0 Hz, 3H, H-C(10)), 1.45 – 1.22 (m, 10H), 1.57 – 1.41 (m, 2H, H-C(4)), 2.38 (dd, J = 15.2, 8.1 Hz, 1H, H_a-C(2)), 2.46 (dd, J = 15.2, 4.9 Hz, 1H, H_b-C(2)), 4.05 – 3.93 (m, 1H, H-C(3)).

¹H-NMR spectra of (*R*)-3-OH-C10:0 (**28**)



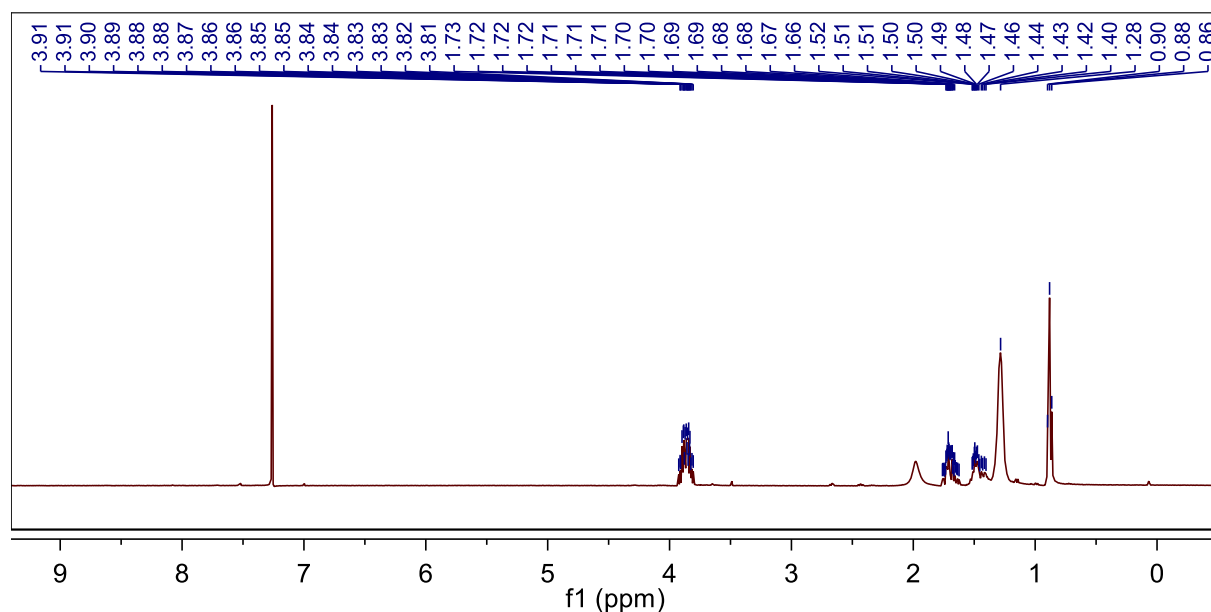
Synthesis of decan-1,3-diol, 1,3-decandiol (**32**)



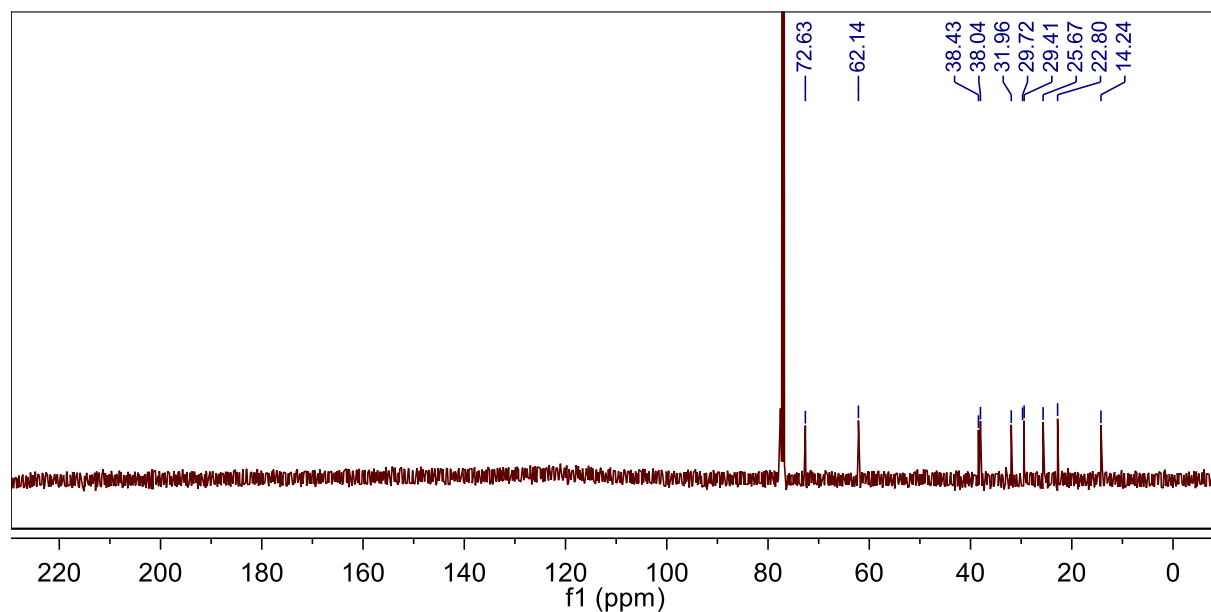
Decan-1,3-diol was synthesized according to a modified procedure described by (58) previously. To a vigorously stirred suspension of LiAlH₄ (35 mg, 0.93 mmol) in dry THF (7 mL) a solution of ethyl 3-oxodecanoate (50 mg, 0.23 mmol) in dry THF (3 mL) was added dropwise at 0°C under argon atmosphere. The solution was stirred 1 h at 0 °C, warmed to room temperature and was further stirred overnight. The reaction mixture was quenched by slow addition of HCl (1 N) at 0 °C and afterwards extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with saturated aqueous NaHCO₃ solution and brine, dried over Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by means of silica gel using hexane/EtOAc (75/25, v/v) yielding in decan-1,3-diol (15 mg, 37%). ¹H NMR (400 MHz, CDCl₃) δ 0.88 (t, J = 7.2 Hz, 3H, H-C(10)), 1.36 – 1.22 (m, 10H), 1.55 – 1.38 (m, 2H, H-C(4)), 1.80 – 1.61 (m, 2H, H-C(2)), 3.95 – 3.78 (m, 3H, H-C(1), H-C(3)). ¹³C NMR (101

MHz, CDCl₃) δ 14.2 (C(10)), 22.8 (C(9)), 25.7 (C(5)), 29.4 (C(7)), 29.7 (C(6)), 32.0 (C(8)), 38.0 (C(4)), 38.4 (C(2)), 62.1 (C(1)), 72.6 (C(3)).

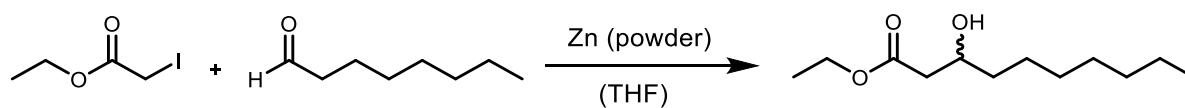
¹H-NMR spectra of 1,3-decandiol (**32**)



¹³C-NMR spectra of 1,3-decandiol (**32**)



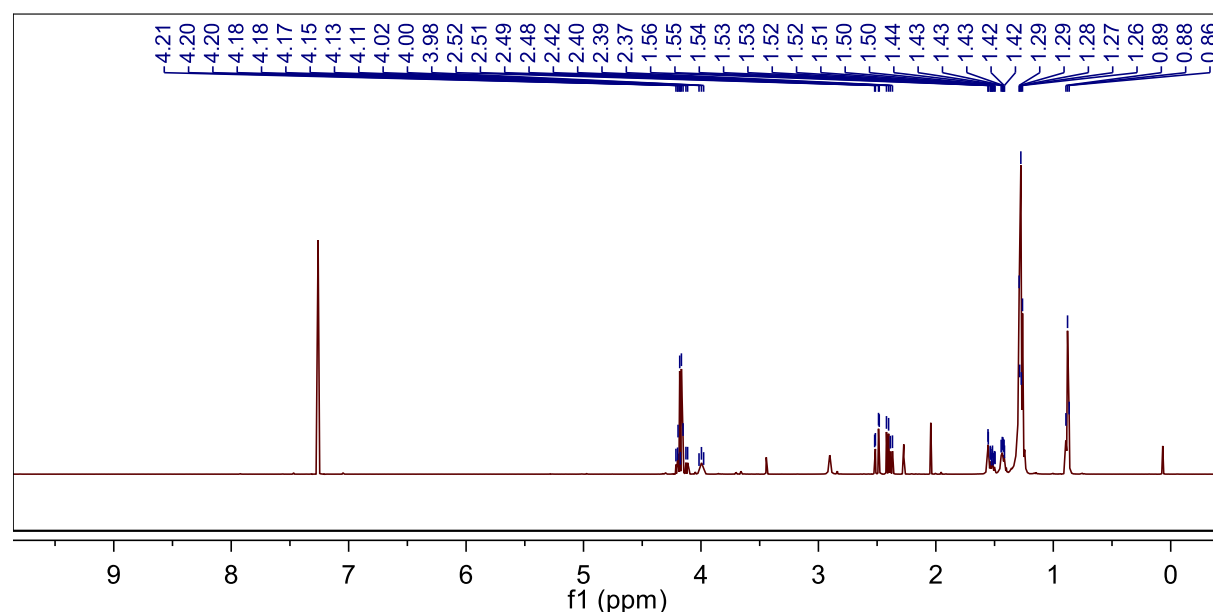
Synthesis of ethyl 3-hydroxydecanoate, Et-3-OH-C10:0 (**33**)



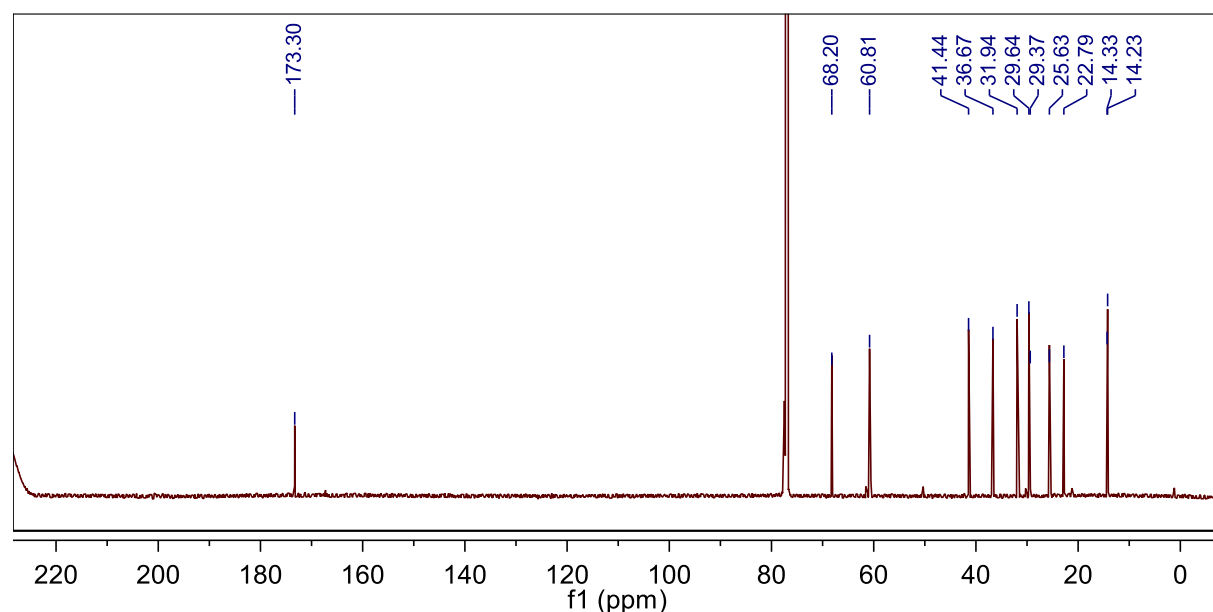
Following a literature procedure (56) with some modifications, ethyl 3-hydroxydecanoate was synthesized. Therefore, ethyl bromoacetate (5.55 mL, 50 mmol) and zinc powder (3.27 g, 50

mmol) were added to a solution of octanal (1.56 mL, 10 mmol) in toluene (15.0 mL). After refluxing for 20 min and cooling to room temperature the mixture was diluted with ether (50 mL) and HCl (1 N, 50 mL). The separated organic layer was washed with saturated aqueous NaHCO₃-solution and brine, dried over Na₂SO₄ and concentrated under reduced pressure. Column chromatography (silica column) of the crude product was achieved by a mixture of hexane/EtOAc (90/10, v/v) resulting in ethyl 3-hydroxydecanoate (1.2 g, 56%) as colourless oil: ¹H NMR (500 MHz, CDCl₃) δ 0.88 (t, *J* = 7.0 Hz, 3H, H-C(10)), 1.20 – 1.37 (m, 10H), 1.37 – 1.59 (m, 2H, H-C(4)), 2.39 (dd, *J* = 16.4, 9.1 Hz, 1H, H_a-C(2)), 2.50 (dd, *J* = 16.4, 3.1 Hz, 1H, H_b-C(2)), 4.00 (t, *J* = 9.3 Hz, 1H, H-C(3)), 4.17 (q, *J* = 7.1 Hz, 2H, H-C(1')). ¹³C NMR (126 MHz, CDCl₃) δ 14.2 (C(2')), 14.3 (C(10)), 22.8 (C(9)), 25.6 (C(5)), 29.4 (C(7)), 29.6 (C(6)), 31.9 (C(8)), 36.7 (C(4)), 41.4 (C(2)), 60.8 (C(1')), 68.2 (C(3)), 173.3 (C(1)).

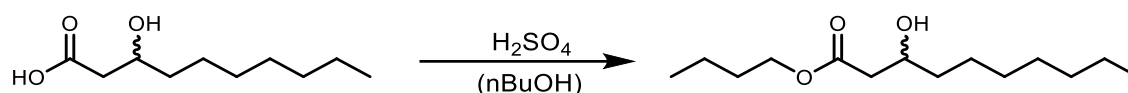
¹H-NMR spectra of Et-3-OH-C10:0 (**33**)



¹³C-NMR spectra of Et-3-OH-C10:0 (**33**)

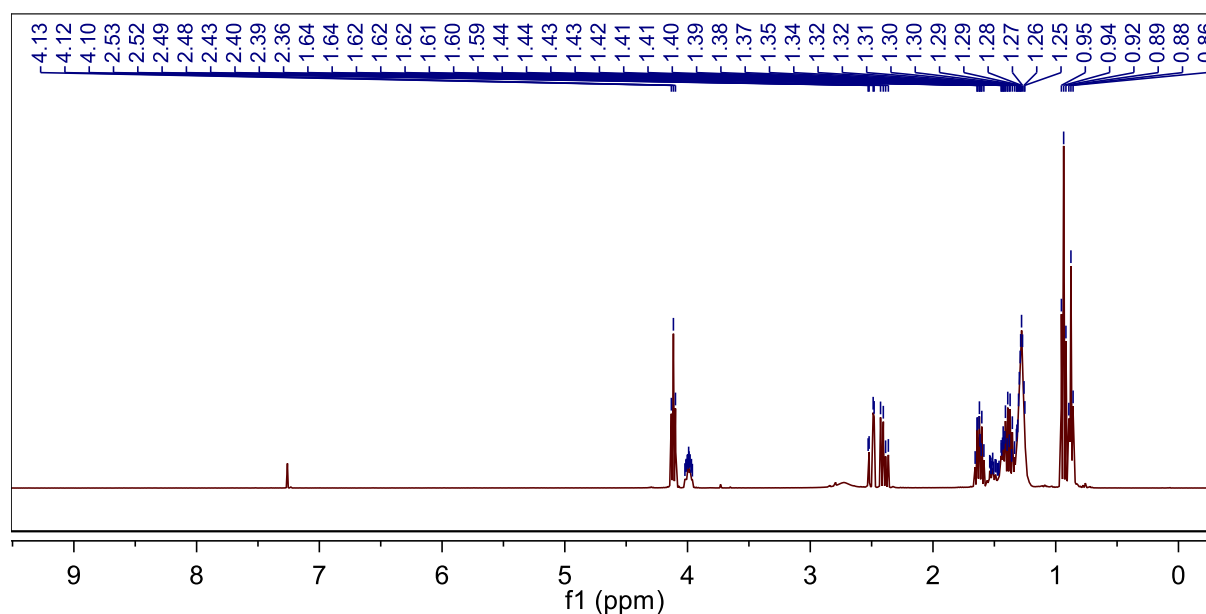


Synthesis of butyl 3-hydroxydecanoate, *n*But-3-OH-C10:0 (**34**)

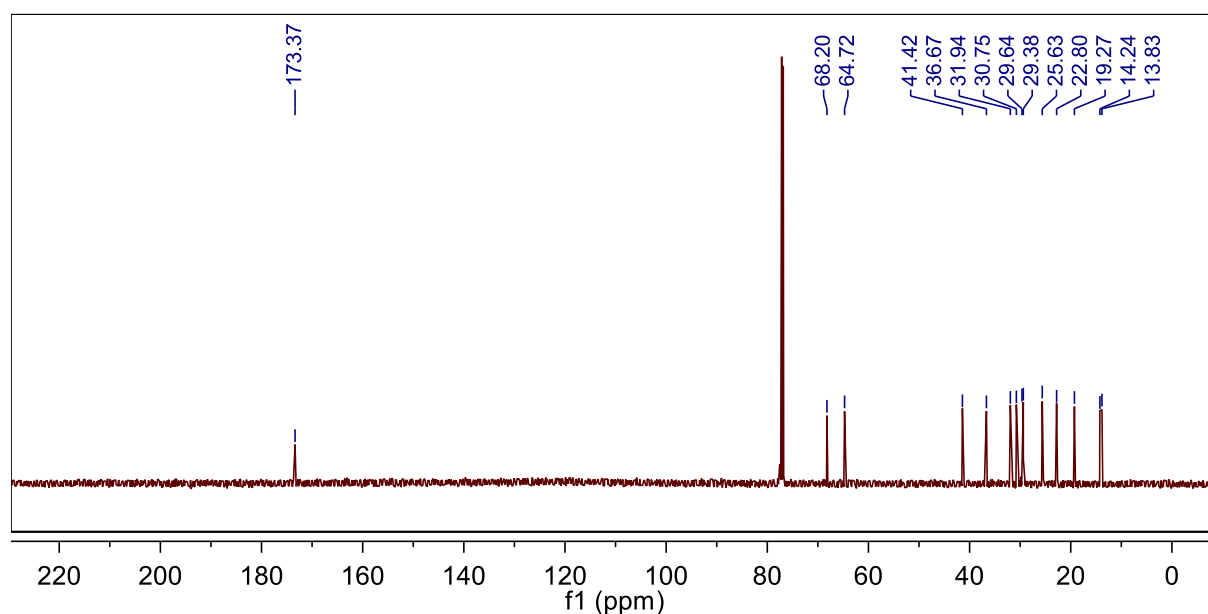


Butyl 3-hydroxydecanoate was synthesized according to (59). Thereby, sulfuric acid (2 drops) was added to a solution of 3-hydroxydecanoic acid (41 mg, 0.22 mmol) in *n*-BuOH (10 mL) at 0°C. After stirring overnight a solution of NaHCO₃ (10%, 20 mL) was added to end the reaction. The resulting mixture was extracted three times with EtOAc (30 mL), and subsequently, the combined organic layers were washed with brine (30 mL), dried over Na₂SO₄ and concentrated in vacuum. The crude product was purified by means of silica gel column chromatography (hexane/EtOAc, 90/10, v/v) to provide butyl 3-hydroxydecanoate (46 mg, 83%). ¹H NMR (400 MHz, CDCl₃) δ 0.88 (t, *J* = 6.8 Hz, 3H, H-C(10)), 0.94 (t, *J* = 7.4 Hz, 3H, H-C(4')), 1.23 – 1.31 (m, 10H), 1.57 – 1.33 (m, 4H), 1.62 (tt, *J* = 8.6, 6.6 Hz, 2H, H-C(2')), 2.40 (dd, *J* = 16.4, 9.0 Hz, 1H, H_a-C(2)), 2.50 (dd, *J* = 16.4, 3.1 Hz, 1H, H_b-C(2)), 3.99 (dddd, *J* = 8.9, 7.7, 4.5, 3.1 Hz, 1H, H-C(3)), 4.12 (t, *J* = 6.7 Hz, 2H, H-C(1')). ¹³C NMR (101 MHz, CDCl₃) δ 13.8 (C(4')), 14.2 (C(10)), 19.3 (C(3')), 22.8 (C(9')), 25.6 (C(5)), 29.4 (C(7)), 29.6 (C(6)), 30.8 (C(3')), 31.9 (C(8)), 36.7 (C(4)), 41.4 (C(2)), 64.7 (C(1')), 68.2 (C(3)), 173.4 (C(1)).

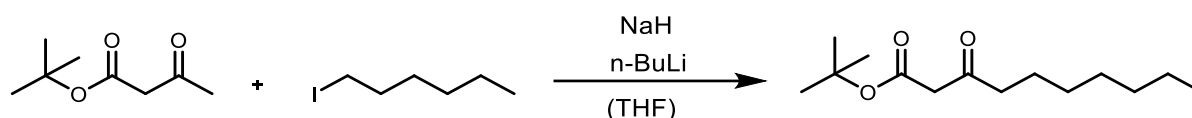
¹H-NMR spectra of *n*But-3-OH-C10:0 (**34**)



^{13}C -NMR spectra of *n*But-3-OH-C10:0 (**34**)

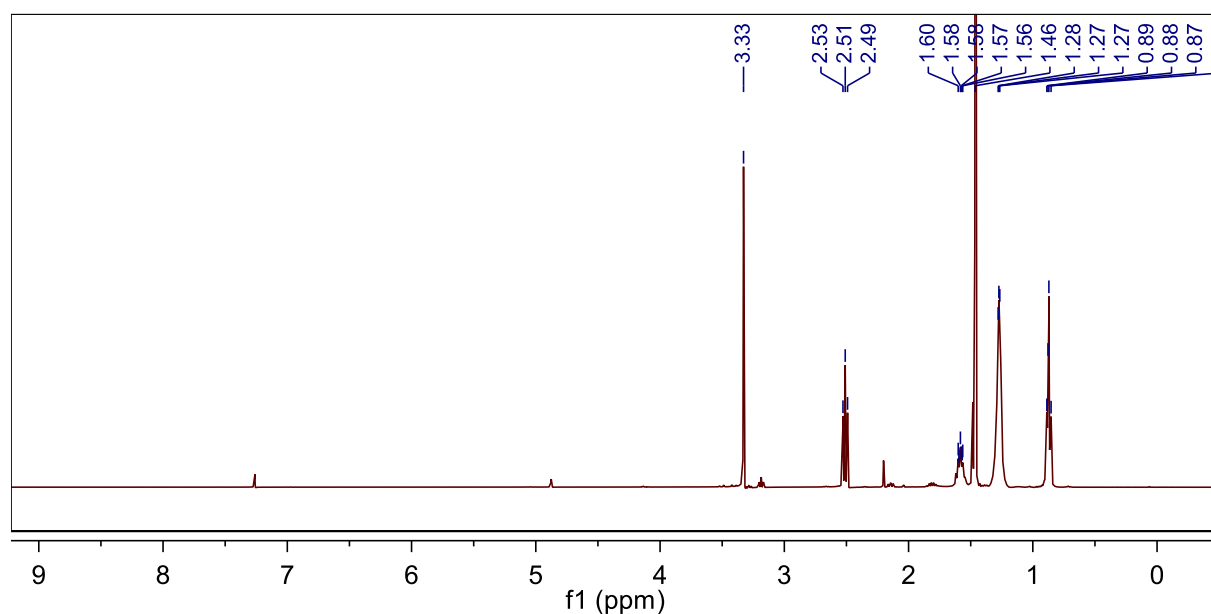


Synthesis of *tert*-butyl 3-oxodecanoate (**35a**)

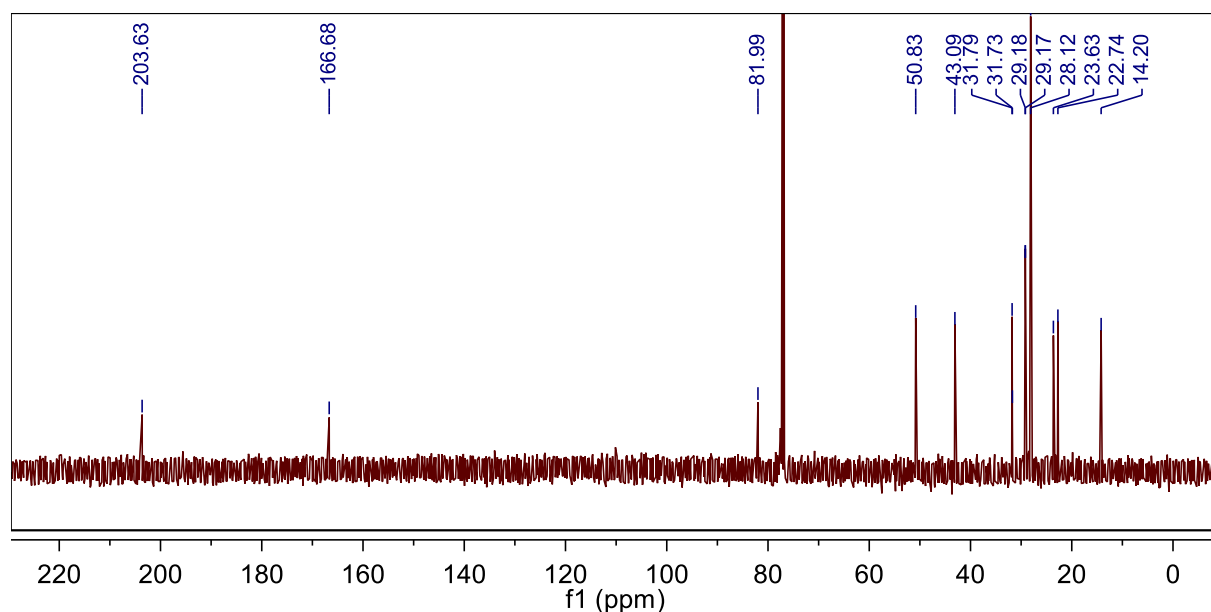


tert-Butyl 3-oxodecanoate was synthesized according to a modified procedure previously described by (60). *tert*-Butyl acetoacetate (1.5 mL, 10 mmol) was added at 0 °C to a stirred suspension of NaH (0.48 g, 12 mmol) in THF (80 mL) dropwise and was stirred for 10 min. Thereafter, *n*-BuLi (1.6 M hexane solution, 2.3 mL, 11 mmol) was added slowly and the mixture was stirred at 0 °C for 30 min. To this mixture 1-iodohexane (3.15 g, 5 mmol) was added dropwise, and the resulting reaction was stirred for additional 30 min at 0 °C. The reaction was ended by addition of 1 N HCl (20 mL). After extraction of the solution with ether (three times, 50 mL), the combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was subjected to column filled with activated silica gel. Chromatography was performed using hexane/EtOAc (95/5, v/v) to give *tert*-butyl 3-oxodecanoate (0.85 g, 70%) as a colorless oil. ^1H NMR (400 MHz, CDCl_3) δ 0.93 – 0.82 (m, 3H, H-C(10)), 1.27 (d, J = 5.2 Hz, 10H), 3.33 (s, 2H, H-C(2)), 1.46 (s, 9H, H-C(2')), 1.63 – 1.55 (m, 1H, H-C(5)), 2.51 (t, J = 7.4 Hz, 2H, H-C(4)). ^{13}C NMR (101 MHz, CDCl_3) δ 14.2 (C(10)), 22.7 (C(9)), 23.6 (C(5)), 28.1 (C(2')), 29.2 (C(7)), 29.2 (C(6)), 31.8 (C(8)), 43.1 (C(4)), 50.8 (C(2)), 82.0 (C(1')), 166.7 (C(1)), 203.6 (C(3)).

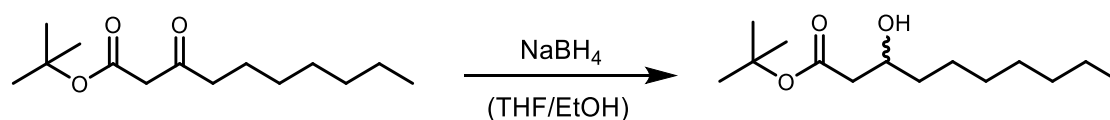
¹H-NMR spectra of *tert*-butyl 3-oxo-decanoate (**35a**)



¹³C-NMR spectra of *tert*-butyl 3-oxo-decanoate (**35a**)



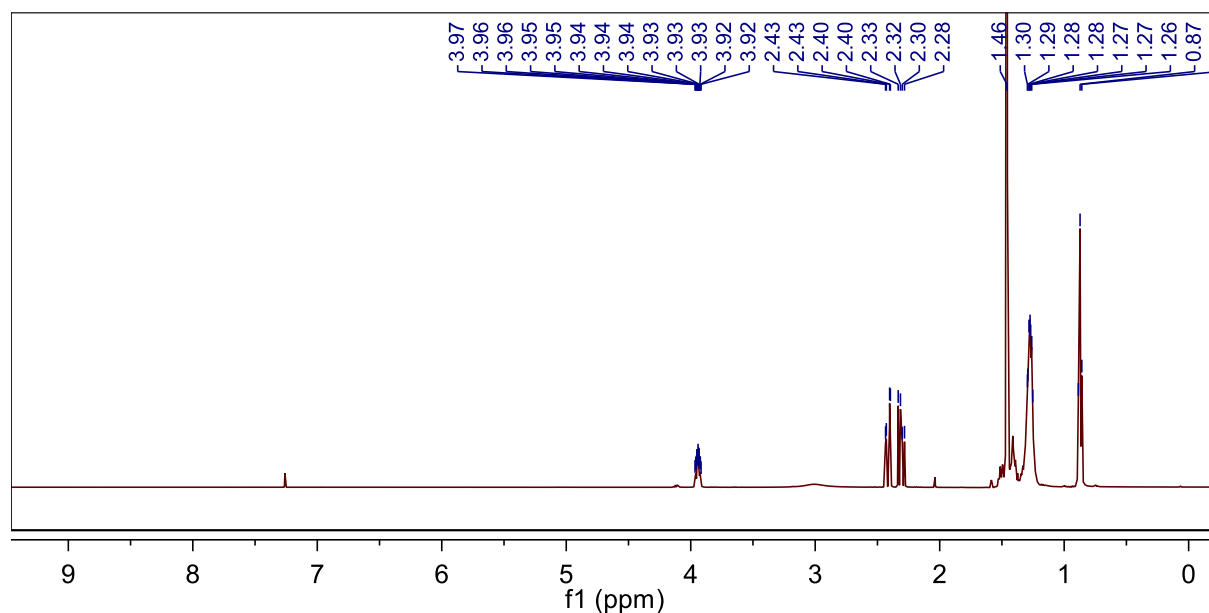
Synthesis of *tert*-butyl 3-hydroxydecanoate, tBut-3-OH-C10:0 (**35**)



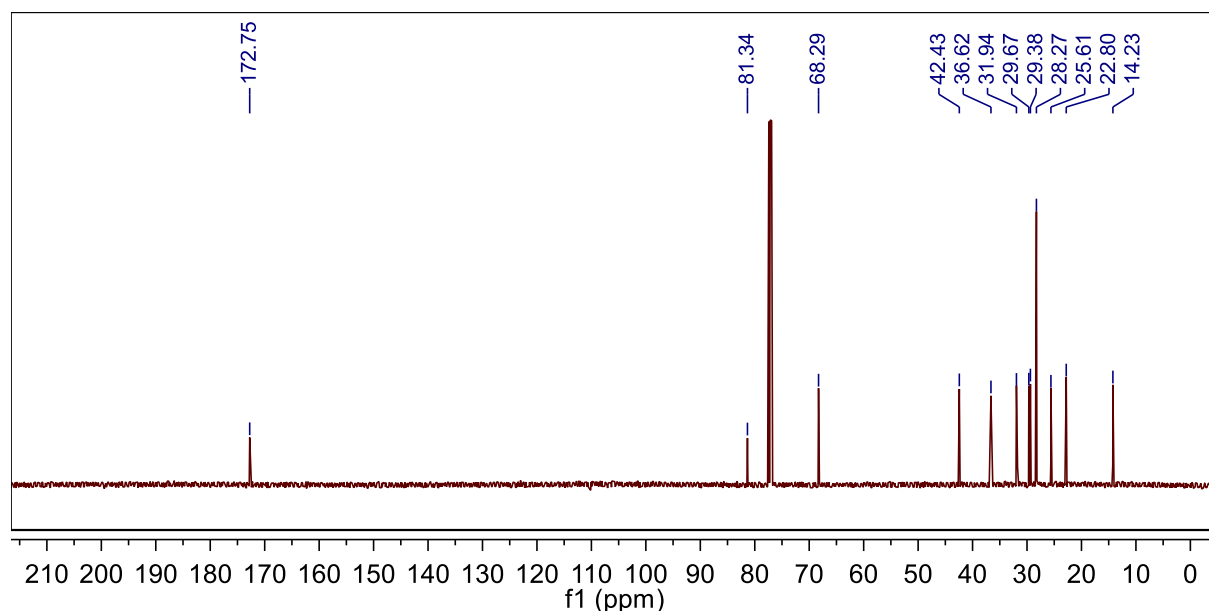
According to a synthesis reported by (61) *tert*-Butyl 3-hydroxydecanoate was generated. To an ice-cold solution (0 °C) of *tert*-Butyl 3-oxodecanoate (0.31 g, 1 mmol) in ethanol (0.25 mL) and THF (2.25 mL) sodium borohydride (0.3 g, 8 mmol) was added. The resulting slurry was stirred vigorously for 1 h and afterwards was quenched by the slow addition of HCl (1 N) until pH 5 was reached. Subsequent the mixture was extracted with EtOAc (3 × 30 mL). The combined

organic layers were washed with 20 mL of brine solution, dried over Na₂SO₄ and concentrated under reduced pressure to yield *tert*-Butyl 3-hydroxydecanoate (0.15 g, 61%). ¹H NMR (500 MHz, CDCl₃) δ 0.87 (m, t, *J* = 7.3 Hz, 3H, H-C(10)) 1.33 – 1.16 (m, 10H), 1.46 (s, 9H, H-C(2')), 1.54-1.33 (m, 2H, H-C(4)), 2.31 (dd, *J* = 16.3, 9.0 Hz, 1H, H_a-C(2)), 2.42 (dd, *J* = 16.3, 3.1 Hz, 1H, H_b-C(2)), 3.94 (dddd, *J* = 8.9, 7.6, 4.6, 3.0 Hz, 1H, H-C(3)). ¹³C NMR (126 MHz, CDCl₃) δ 14.2 (C(10)), 22.8 (C(9)), 25.6 (C(5)), 28.3 (C(2')), 29.4 (C(7)), 29.7 (C(6)), 31.9 (C(8)), 36.6 (C(4)), 42.4 (C(2)), 68.3 (C(3)), 81.3 (C(1')), 172.8 (C(1)).

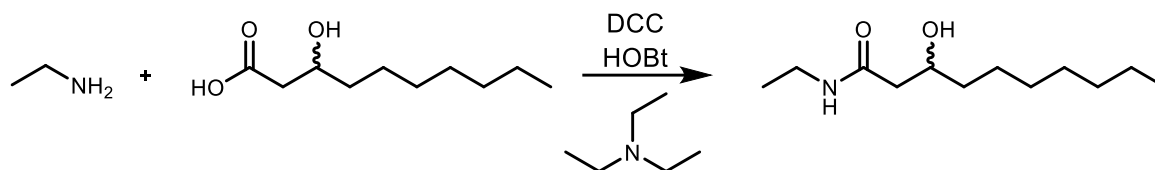
¹H-NMR spectra of *t*But-3-OH-C10:0 (35)



¹³C-NMR spectra of *t*But-3-OH-C10:0 (35)

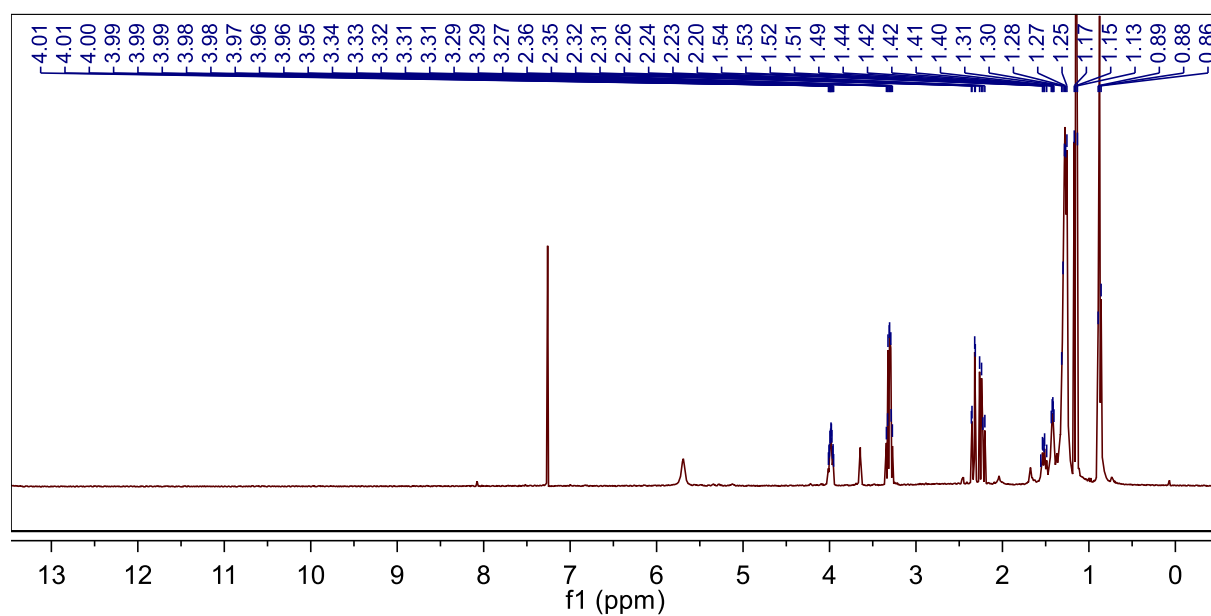


Synthesis of *N*-ethyl-3-hydroxydecanamide, Et-*N*-3-OH-C10:0 (**36**)

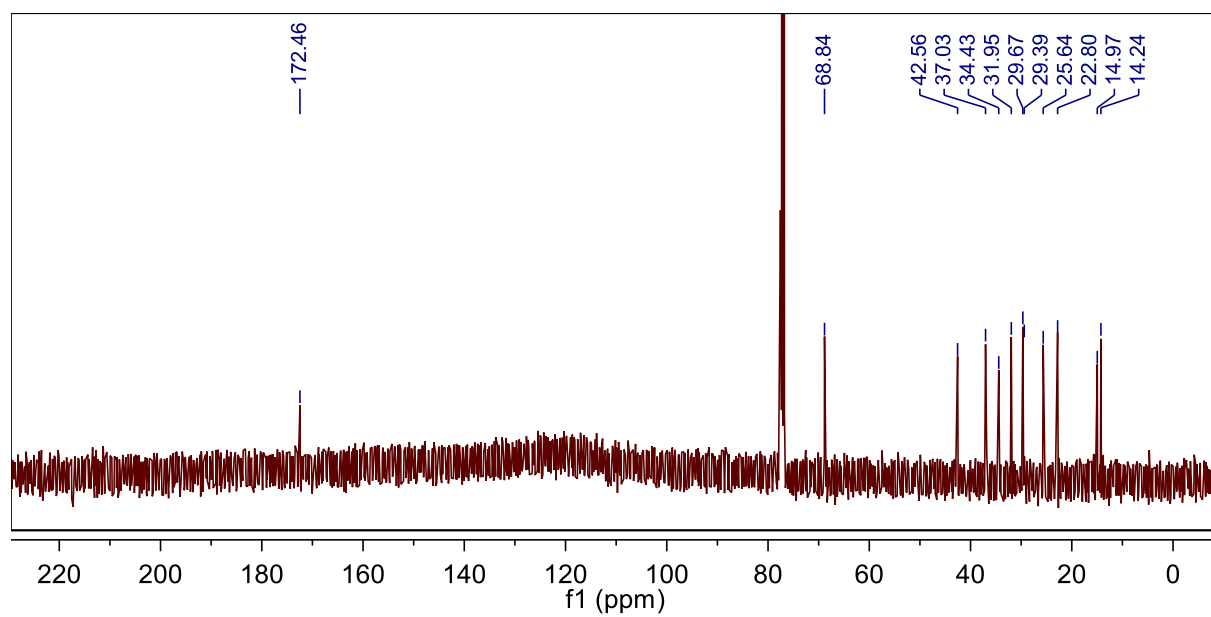


N-Ethyl-3-hydroxydecanamide was synthesized according to (62) with slight modifications. To a solution of 3-hydroxydecanoic acid (140 mg, 0.75 mmol) in dry DCM (20 mL) 1-hydroxybenzotriazole (112 mg, 0.83 mmol) was added at 0 °C, followed by dicyclohexylcarbodiimide (186 mg, 0.9 mmol). The mixture was stirred for 1 h at 0°C and afterwards was allowed to warm to room temperature. Thereafter, ethylamine (18 µL, 0.83 mmol) dissolved in DMF (10 mL) was added. Subsequently, triethylamine (125 µL, 0.9 mmol) was added and the reaction mixture was stirred for 24 h. The precipitate was filtered off and the filtrate was evaporated under reduced pressure. The reaction product was acidified with HCl (1 N) and extracted with CHCl₃ (3 × 30 mL). The combined organic layers were washed with a solution of NaHCO₃ (10%, 30 mL) and saturated brine (30 mL), followed by drying over anhydrous Na₂SO₄. The crude product was further purified by means of RP-HPLC chromatography using a Hyperclone ODS (C18) 120 A (250 × 21.2 mm, 5 µm, Phenomenex, Aschaffenburg, Germany) column with 0.1% formic acid as solvent A, acetonitrile as solvent B. chromatography was performed with a flow rate of 21 mL/min and the following gradient was used: 0 min, 30% B; 3 min 30% B; 20 min, 100% B; 24 min, 100% B; 25 min, 30% B; 30 min, 30% B. The effluent was monitored by UV detection at 200 nm and the fractions were collected individually in several runs. After evaporation of the solvent, extraction of the aqueous phase with EtOAc (3 × 10 mL) and subsequent drying over anhydrous Na₂SO₄, *N*-ethyl-3-hydroxydecanamide (24 mg, 12%) was afforded as white solid. ¹H NMR (400 MHz, CDCl₃) δ 0.88 (t, *J* = 7.1 Hz, 2H, H-C(10)), 1.15 (t, *J* = 7.3 Hz, 3H, H-C(2')), 1.25 – 1.38 (m, 10H), 1.46 – 1.38 (m, 2H, H-C(4)), 2.23 (dd, *J* = 15.3, 9.0 Hz, 1H, H_a-C(2)), 2.34 (dd, *J* = 15.3, 2.8 Hz, 1H, H_b-C(2)), 3.31 (qd, *J* = 7.3, 5.6 Hz, 2H, H-C(1')), 3.98 (dddd, *J* = 11.9, 7.8, 4.5, 2.7 Hz, 1H, H-C(3)). ¹³C NMR (101 MHz, CDCl₃) δ 14.2 (C(10)), 15.0 (C(2')), 22.8 (C(9)), 25.6 (C(5)), 29.4 (C(7)), 29.7 (C(6)), 32.0 (C(8)), 34.4 (C(1')), 37.0 (C(4)), 42.6 (C(2)), 68.8 (C(3)), 172.5 (C(1)).

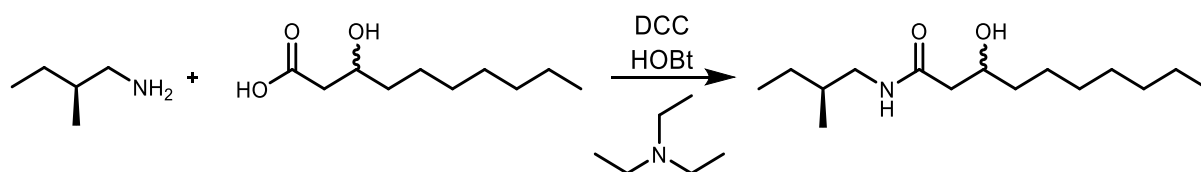
^1H -NMR spectra of Et-*N*-3-OH-C10:0 (**36**)



^{13}C -NMR spectra of Et-*N*-3-OH-C10:0 (**36**)

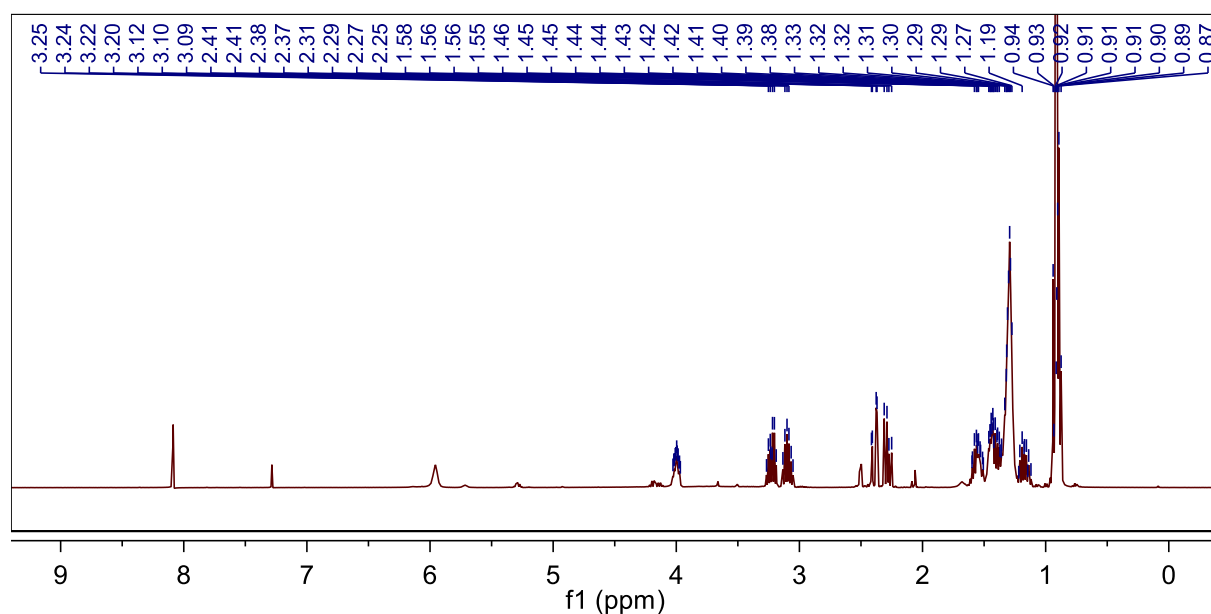


Synthesis of 3-hydroxy-*N*-((*S*)-2-methylbutyl)decanamide, 2-Me-But-*N*-3-OH-C10:0 (37)

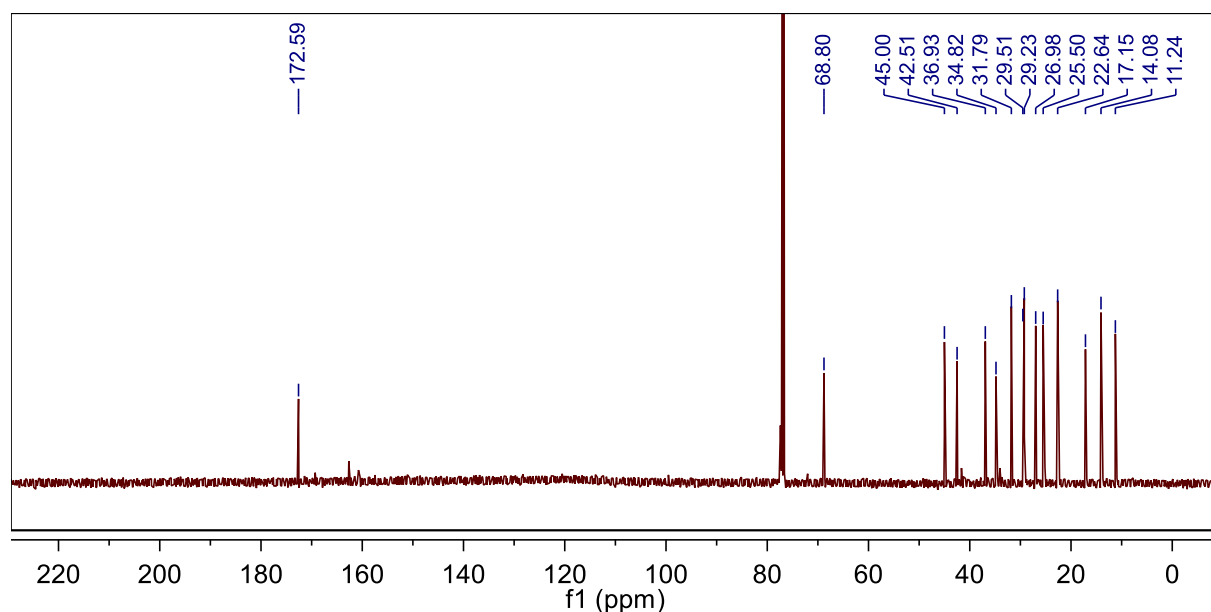


With slight modifications 3-hydroxy-*N*-((*S*)-2-methylbutyl)decanamide was synthesized according to (62). To a solution of 3-hydroxy-decanoic acid (140 mg, 0.75 mmol) in dry DCM (20 mL) 1-hydroxybenzotriazole (112 mg, 0.83 mmol) was added at 0°C, followed by dicyclohexylcarbodiimide (186 mg, 0.9 mmol). The mixture was stirred for 1 h at 0°C and afterwards was allowed to warm to room temperature. Thereafter, 2(*S*)-methylbutylamine (72 mg, 0.83 mmol) dissolved in DMF (10 mL) was added to the reaction mixture. Subsequently, triethylamine (125 μ L, 0.9 mmol) was added and the reaction mixture was stirred for 24 h. The precipitate was filtered off and the filtrate was evaporated under reduced pressure. The reaction product was acidified with HCl (1 N) and extracted with CHCl_3 (3 $\times$ 30 mL). The combined organic layers were washed with a solution of NaHCO_3 (10%, 30 mL) and saturated brine solution (30 mL), followed by drying over anhydrous Na_2SO_4 . The crude product was further purified by RP-HPLC using a Hyperclone ODS (C18) 120 A (250 $\times$ 21.2 mm, 5 μ m, Phenomenex, Aschaffenburg, Germany) column with 0.1% formic acid as solvent A, acetonitrile as solvent B. The column was operated with a flow rate of 21 mL/min and the following gradient: 0 min, 30% B; 3 min 30% B; 20 min, 100% B; 24 min, 100% B; 25 min, 30% B; 30 min, 30% B. The effluent was monitored by UV detection at 200 nm and the fractions were collected individually in several runs. After evaporation of the solvent, extraction of the aqueous phase with EtOAc (3 $\times$ 10 mL) and subsequent drying over anhydrous Na_2SO_4 , 3-hydroxy-*N*-((*S*)-2-methylbutyl)decanamide (16 mg, 9%) was afforded as white solid. ^1H NMR (400 MHz, CDCl_3) δ 0.95 – 0.86 (m, 9H, H-C(10), H-C(4'), H-C(5')), 1.17 (dt, J = 13.4, 7.4 Hz, 1H), 1.35 – 1.26 (m, 10H), 1.48 – 1.35 (m, 2H, H-C(4)), 1.55 (ddd, J = 13.2, 10.5, 6.4 Hz, 1H, H-C(2')), 2.28 (dd, J = 15.2, 9.0 Hz, 1H, H_a -C(2)), 2.39 (dd, J = 15.2, 2.8 Hz, 1H, H_b -C(2)), 3.09 (dq, J = 13.3, 6.3 Hz, 1H, H-C(1')), 3.23 (dq, J = 13.4, 6.0 Hz, 1H, H-C(1')), 4.00 (ddt, J = 8.8, 7.5, 2.8 Hz, 1H, H-C(3)). ^{13}C NMR (101 MHz, CDCl_3) δ 11.2 (C(4')), 14.1 (C(10)), 17.2 (C(5')), 22.6 (C(9)), 25.5 (C(5)), 27.0 (C(3')), 29.2 (C(7)), 29.5 (C(6)), 31.8 (C(8)), 34.8 (C(2')), 36.9 (C(4)), 42.5 (C(2)), 45.0 (C(1')), 68.8 (C(3)), 172.6 (C(1)).

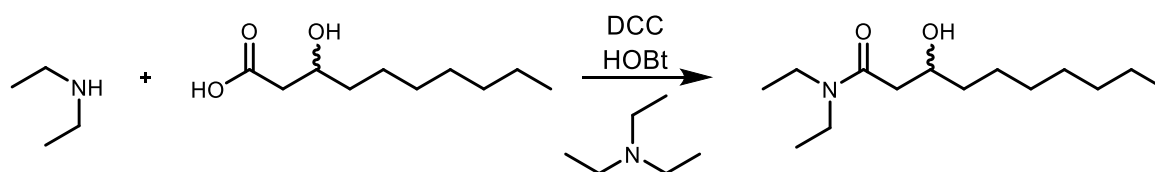
^1H -NMR spectra of 2-Me-But-*N*-3-OH-C10:0 (**37**)



^{13}C -NMR spectra of 2-Me-But-*N*-3-OH-C10:0 (**37**)



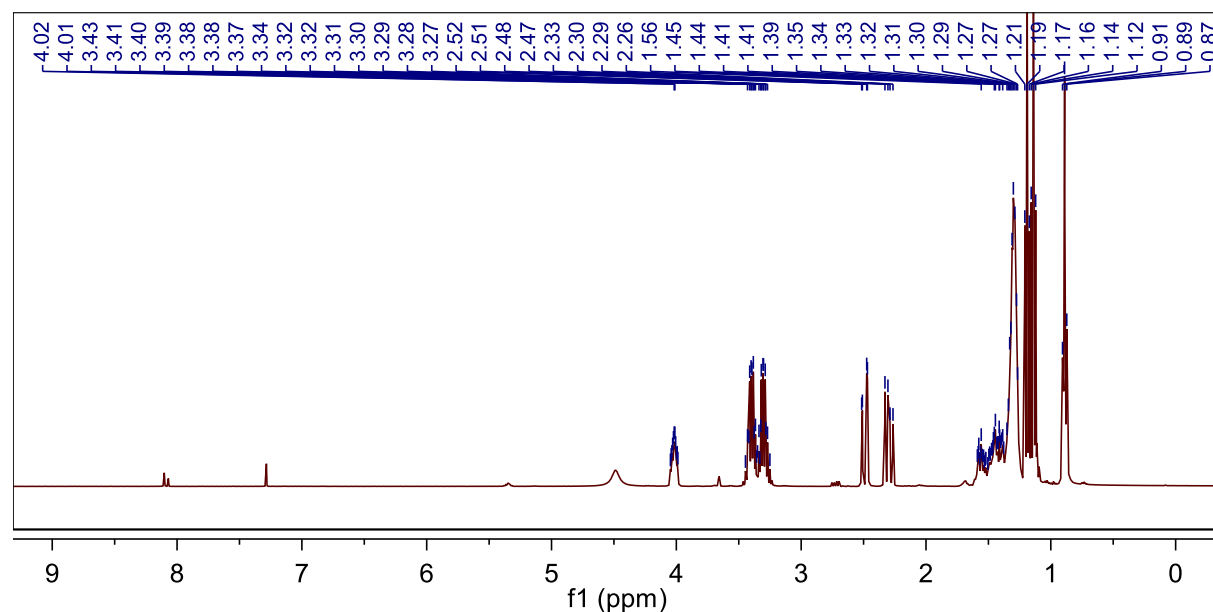
Synthesis of *N,N*-diethyl-3-hydroxydecanamide, $\text{Et}_2\text{-N-3-OH-C10:0}$ (**38**)



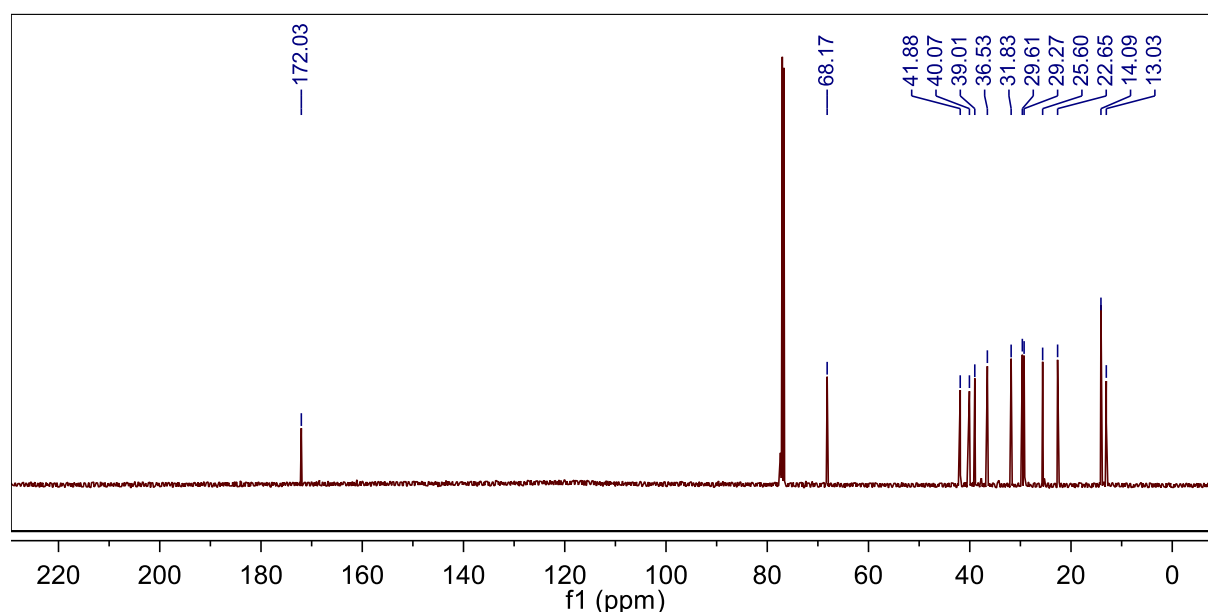
N,N-Diethyl-3-hydroxydecanamide was synthesized according to a modified procedure described by (62). To a solution of 3-hydroxydecanoic acid (140 mg, 0.75 mmol) in dry DCM (20 mL) 1-hydroxybenzotriazole (112 mg, 0.83 mmol) was added at 0 °C, followed by

dicyclohexylcarbodiimide (186 mg, 0.9 mmol). The mixture was stirred for 1 h at 0°C and afterwards was allowed to warm to room temperature, thereafter, diethyl amine (90.7 mg, 0.83 mmol) dissolved in DMF (10 mL) was added to the reaction mixture. Subsequently, triethylamine (125 μ L, 0.9 mmol) was added and the reaction mixture was stirred for 24 h. The precipitate was filtered off and the filtrate was evaporated under reduced pressure. The reaction product was acidified with HCl (1 N) and extracted with CHCl₃ (3 $\times$ 30 mL). The combined organic layers were washed with a solution of NaHCO₃ (10%, 30 mL) and saturated brine (30 mL), followed by drying over anhydrous Na₂SO₄. The crude product was further purified by means of RP-HPLC using a Hyperclone ODS (C18) 120 A (250 $\times$ 21.2 mm, 5 μ m, Phenomenex, Aschaffenburg, Germany) column. Chromatography was performed by using 0.1% formic acid as solvent A, acetonitrile as solvent B, a flow rate of 21 mL/min and following gradient: 0 min, 30% B; 3 min 30% B; 20 min, 100% B; 24 min, 100% B; 25 min, 30% B; 30 min, 30% B. The effluent was monitored by UV detection at 200 nm and the fractions were collected individually in several runs. After evaporation of the solvent, extraction of the aqueous phase with EtOAc (3 $\times$ 10 mL) and subsequent drying over anhydrous Na₂SO₄, *N,N*-diethyl-3-hydroxydecanamide (20 mg, 10%) was afforded as white solid. ¹H NMR (400 MHz, CDCl₃) δ 0.89 (t, *J* = 7.1 Hz, 3H, H-C(10)), 1.14 (t, *J* = 7.1 Hz, 3H, H-C(2')), 1.19 (t, *J* = 7.2 Hz, 3H, H-C(2')), 1.30 (dq, *J* = 10.2, 5.1, 4.4 Hz, 10H), 1.50 – 1.37 (m, 1H, H-C(4)), 1.62 – 1.50 (m, 1H, H-C(4)), 2.30 (dd, *J* = 16.2, 9.6 Hz, 1H, H_a-C(2)), 2.49 (dd, *J* = 16.2, 2.3 Hz, 1H, H_b-C(2)), 3.30 (qd, *J* = 7.3, 5.6 Hz, 1H, H-C(1')), 3.40 (qd, *J* = 6.9, 4.2 Hz, 1H, H-C(1')), 4.02 (dddd, *J* = 9.7, 7.2, 4.6, 2.3 Hz, 1H, H-C(3)). ¹³C NMR (101 MHz, CDCl₃) δ 13.0 (C(2')), 14.1 (C(10)), 22.7 (C(9)), 25.6 (C(5)), 29.3 (C(7)), 29.6 (C(6)), 31.8 (C(8)), 36.5 (C(4)), 39.0 (C(2)), 40.1 (C(1')), 41.9 (C(1')), 68.2 (C(3)), 172.0 (C(1)).

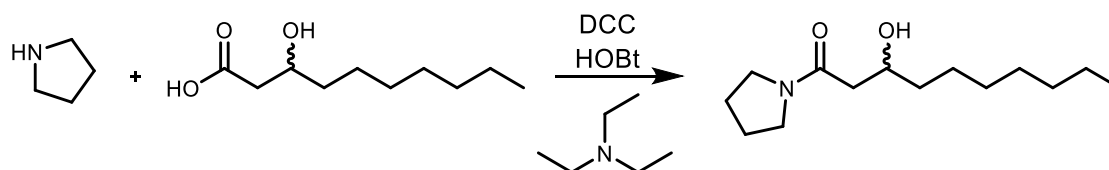
¹H-NMR spectra of Et₂-*N*-3-OH-C10:0 (**38**)



¹³C-NMR spectra of Et₂-N-3-OH-C10:0 (**38**)



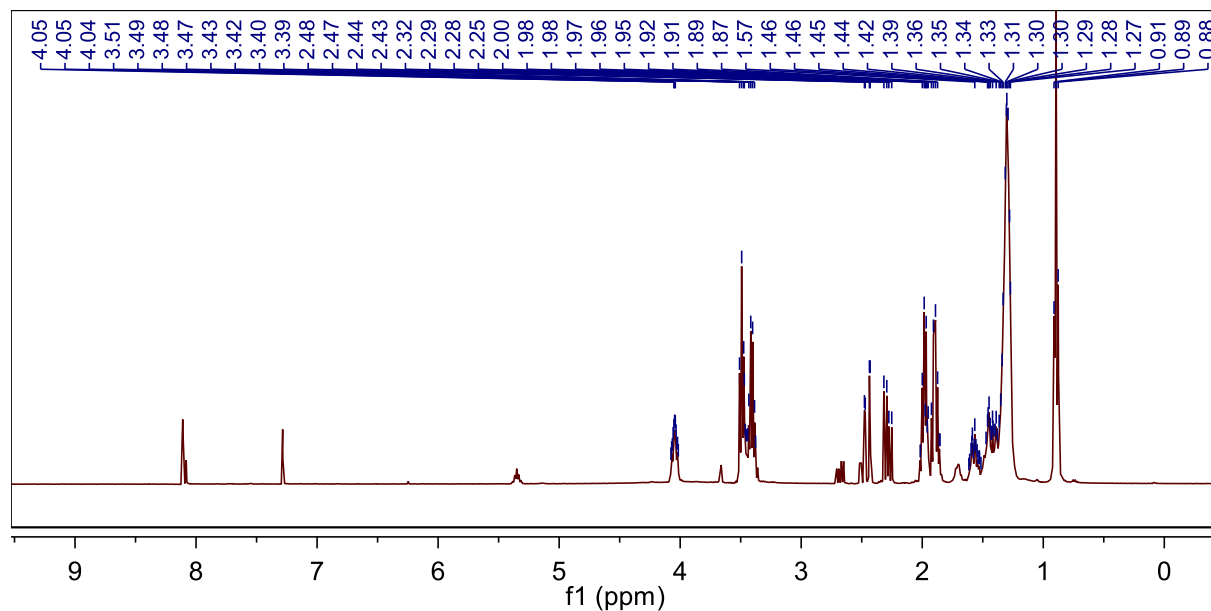
Synthesis of 3-hydroxy-1-(pyrrolidin-1-yl)decan-1-one, Pyr-N-3-OH-C10:0 (**39**)



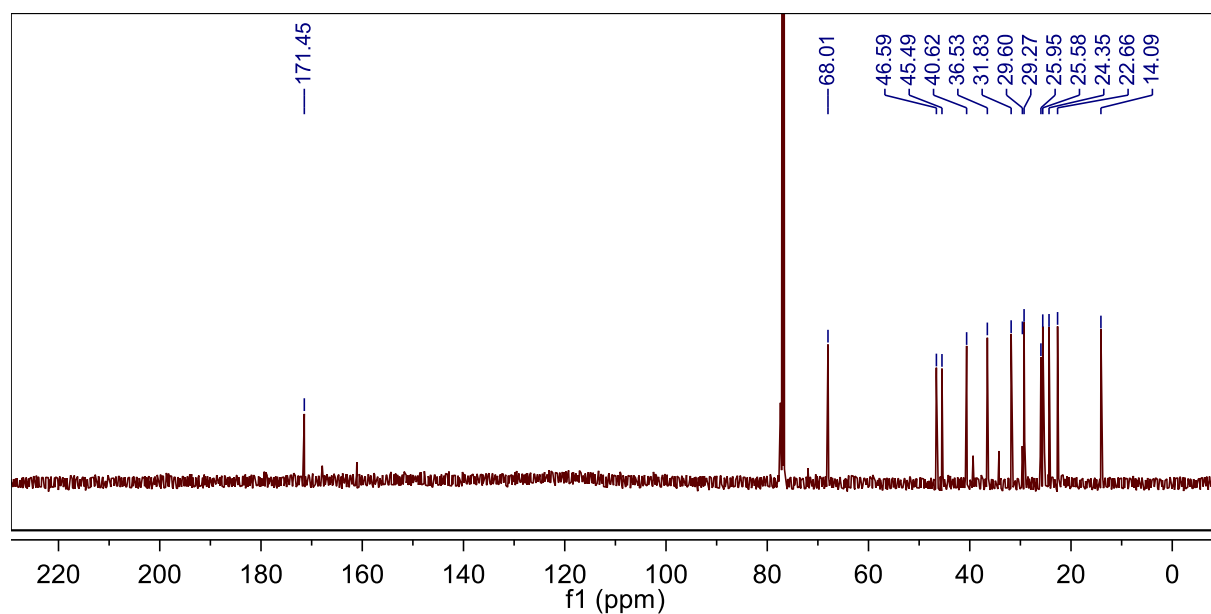
33-Hydroxy-1-(pyrrolidin-1-yl)decan-1-one was synthesized according to a modified procedure reported by (62). To a solution of 3-hydroxydecanoic acid (140 mg, 0.75 mmol) in dry DCM (20 mL) 1-hydroxybenzotriazole (112 mg, 0.83 mmol) was added at 0°C, followed by dicyclohexylcarbodiimide (186 mg, 0.9 mmol). The mixture was stirred for 1 h at 0°C and afterwards was allowed to warm to room temperature and pyrrolidine (59 mg, 0.83 mmol) dissolved in DMF (10 mL) was added. Subsequently triethylamine (125 µL, 0.9 mmol) was added and the reaction mixture was stirred for 24 h. The precipitate was filtered off and the filtrate was evaporated under reduced pressure. The reaction product was acidified with HCl (1 N) and extracted with CHCl₃ (3 × 30 mL). The combined organic layers were washed with a solution of NaHCO₃ (10%, 30 mL) and saturated brine (30 mL), followed by drying over anhydrous Na₂SO₄. The crude product was further purified by means of RP-HPLC using a Hyperclone ODS (C18) 120 A (250 × 21.2 mm, 5 µm, Phenomenex, Aschaffenburg, Germany) column and 0.1% formic acid as solvent A, acetonitrile as solvent B. Chromatography was performed with a flow rate of 21 mL/min and the following gradient: 0 min, 30% B; 3 min 30% B; 20 min, 100% B; 24 min, 100% B; 25 min, 30% B; 30 min, 30% B. The effluent was monitored by UV detection at 200 nm and the fractions were collected individually in several runs. After evaporation of the solvent, extraction of the aqueous phase with EtOAc (3 × 10 mL) and subsequent drying over anhydrous Na₂SO₄, 3-Hydroxy-1-(pyrrolidin-1-yl)decan-1-one (18 mg, 9%) was afforded as white solid. ¹H NMR (400 MHz, CDCl₃) δ 0.89 (t, 3H, *J* = 7.1 Hz, H-C(10)), 1.37 – 1.21 (m, 10H), 1.50 – 1.38 (m, 1H, H-C(4)), 1.64 – 1.51 (m, 1H, H-C(4)), 1.89 (p, *J* = 7.8, 7.2 Hz, 2H, H-C(2')), 1.98 (q, *J* = 6.4 Hz, 2H, H-C(2')), 2.28 (dd, *J* = 16.4, 9.6 Hz, 1H, H_a-C(2)), 2.45 (dd, *J* = 16.4, 2.5 Hz, 1H, H_b-C(2)), 3.41 (q, *J* = 6.3 Hz, 2H, H-C(1')), 3.49 (t, *J* = 6.9 Hz, 2H, H-C(1')), 4.05 (dddd, *J* = 9.8, 7.3, 4.6, 2.4 Hz, 1H, H-C(3)). ¹³C NMR

(101 MHz, CDCl₃) δ 14.1 (C(10)), 22.7 (C(9)), 24.4 (C(5)), 25.6 (C(2')), 25.9 (C(2')), 29.3 (C(7)), 29.6 (C(6)), 31.8 (C(8)), 36.5 (C(4)), 40.6 (C(2)), 45.5 (C(1')), 46.6 (C(1')), 68.0 (C(3)), 171.5 (C(1)).

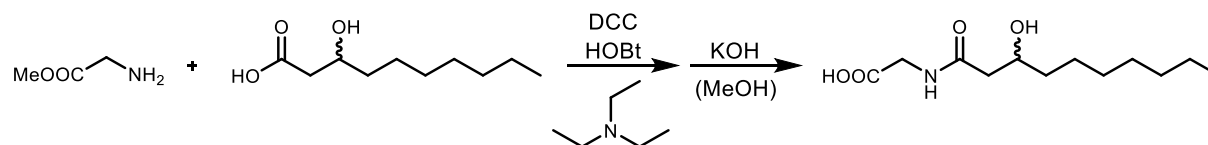
¹H-NMR spectra of Pyr-*N*-3-OH-C10:0 (**39**)



¹³C-NMR spectra of Pyr-*N*-3-OH-C10:0 (**39**)

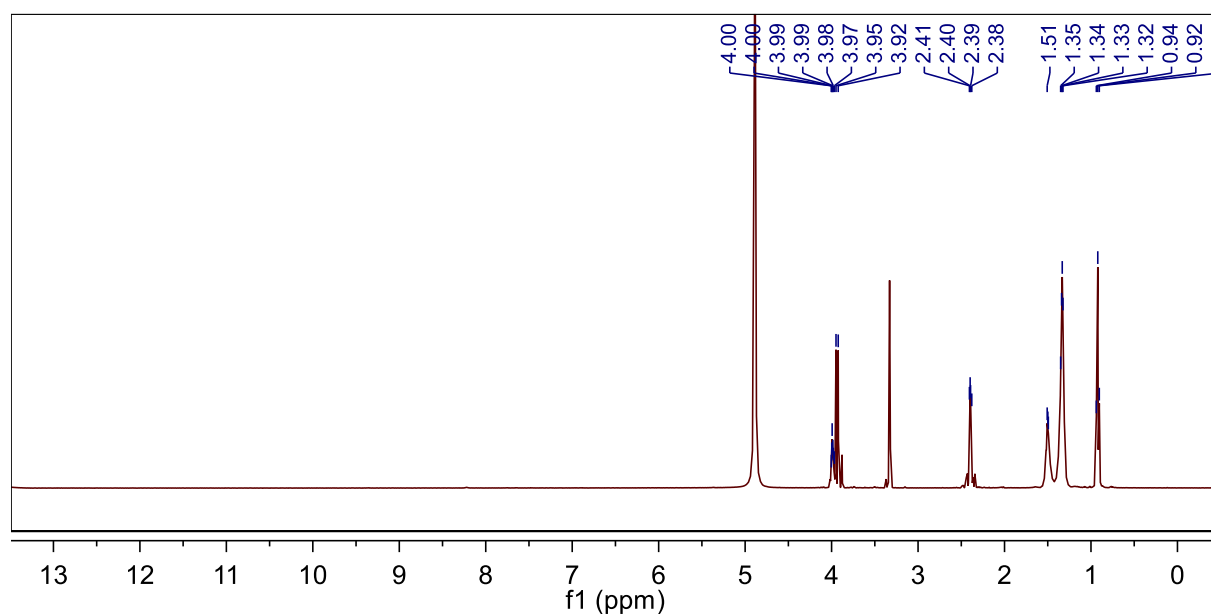


Synthesis of (3-hydroxydecanoyl)glycine, Gly-*N*-3-OH-C10:0 (**40**)

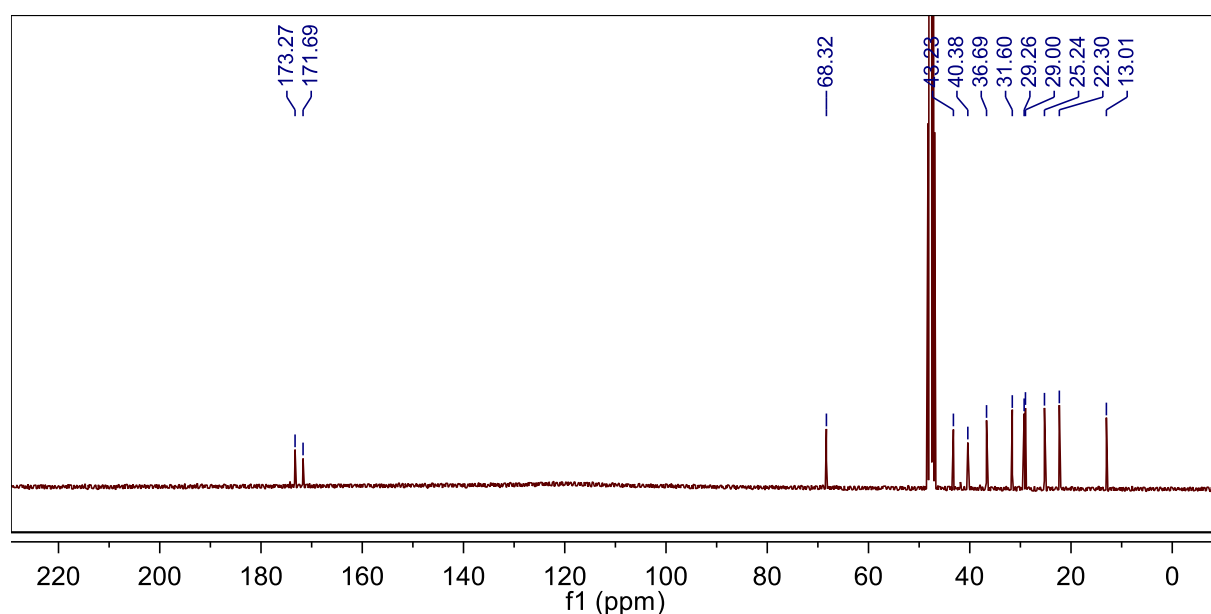


(3-Hydroxydecanoyl)glycine was synthesized according to a modified procedure described by (62). To a solution of 3-hydroxydecanoic acid (36 mg, 0.19 mmol) in dry DCM (10 mL) 1-hydroxybenzotriazole (27 mg, 0.2 mmol) was added at 0°C, followed by dicyclohexylcarbodiimide (41.3 mg, 0.2 mmol). The mixture was stirred for 1 h at 0°C and afterwards was allowed to warm to room temperature. Thereafter, glycine methyl ester hydrochloride (120.5 mg, 0.96 mmol) dissolved in DMF (2 mL) was added. Subsequently triethylamine (130 μ L, 0.96 mmol) was added and the reaction mixture was stirred for 12 h. The precipitate was filtered off and the filtrate was evaporated under reduced pressure. The reaction product was acidified with HCl (1 N, 10 mL) and extracted with DCM (3 $\times$ 30 mL). The combined organic layers were washed with a solution of NaHCO₃ (10%, 30 mL) and saturated brine solution (30 mL), followed by drying over anhydrous Na₂SO₄. The crude product was further purified by means of RP-HPLC chromatography using a Hyperclone ODS (C18) 120 A (250 $\times$ 21.2 mm, 5 μ m, Phenomenex, Aschaffenburg, Germany) column with 0.1% formic acid as solvent A, acetonitrile as solvent B, operated with a flow rate of 21 mL/min and following gradient: 0 min, 50% B; 3 min 50% B; 13 min, 100% B; 18 min, 100% B; 20 min, 50% B; 25 min, 50% B. The effluent was monitored by UV detection at 200 nm and the fractions were collected individually in several runs. After evaporation of the solvent and lyophilization, (3-hydroxydecanoyl)glycine-methylester was deprotected by stirring with NaOH (1 N, 5 mL, 5 mmol) in MeOH (20 mL) at room temperature for 14 h. The reaction was neutralized with HCl (1 N) and afterwards extracted with EtOAc (3 $\times$ 30 mL). The combined organic layers were washed with saturated brine solution, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to yield (3-hydroxydecanoyl)glycine (18 mg, 39 %) as white powder. ¹H NMR (400 MHz, MeOD) δ 0.92 (d, J = 6.9 Hz, 3H, H-C(10)), 1.44 – 1.26 (m, 10H), 1.63 – 1.42 (m, 2H, H-C(4)), 2.39 (dd, J = 6.4, 4.4 Hz, 2H, H-C(2)), 3.92 (s, 1H, H-C(2')), 3.95 (s, 1H, H-C(2')), 3.99 (p, J = 2.2 Hz, 1H, H-C(3)). ¹³C NMR (101 MHz, MeOD) δ 13.0 (C(10)), 22.3 (C(9)), 25.2 (C(5)), 29.0 (C(7)), 29.3 (C(6)), 31.6 (C(8)), 36.7 (C(4)), 40.4 (C(2')), 43.2 (C(2)), 68.3 (C(3)), 171.7 (C(1)), 173.3 (C(1')).

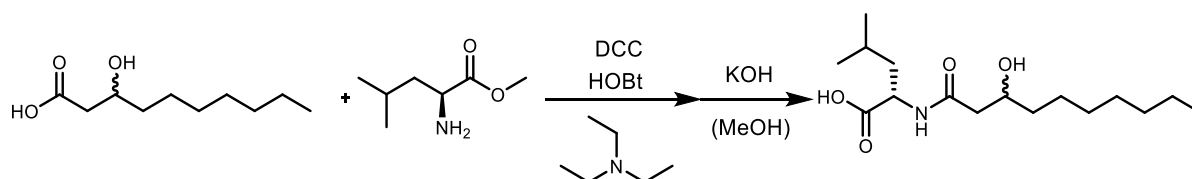
¹H-NMR spectra of Gly-*N*-3-OH-C10:0 (**40**)



¹³C-NMR spectra of Gly-*N*-3-OH-C10:0 (**40**)



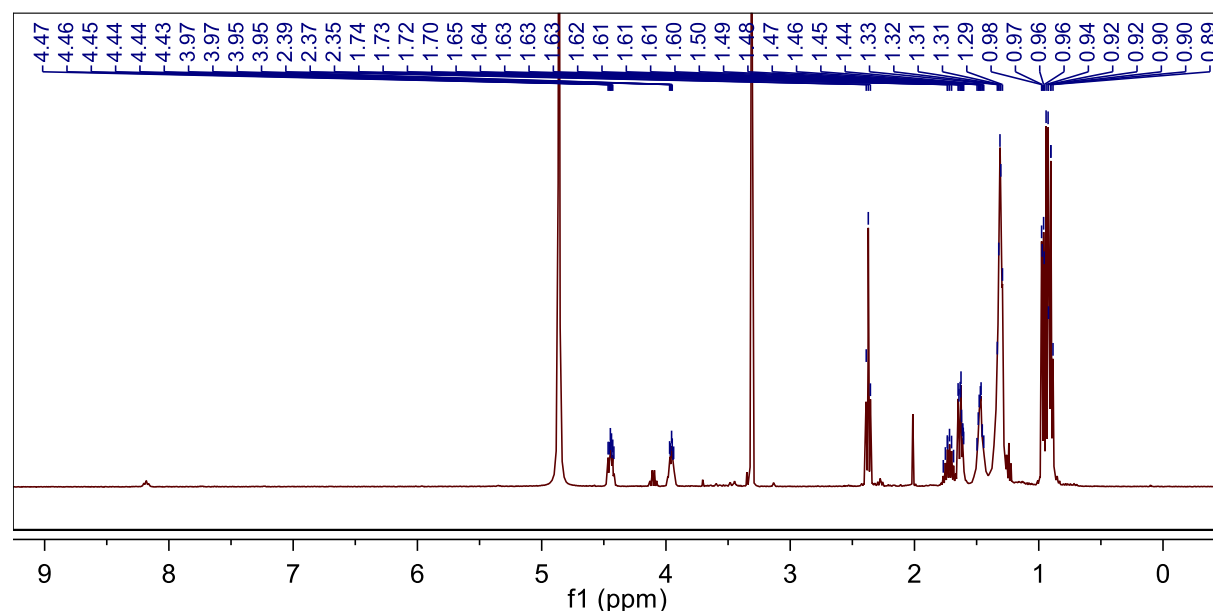
Synthesis of (3-hydroxydecanoyl)-L-leucine, Leu-*N*-3-OH-C10:0 (**41**)



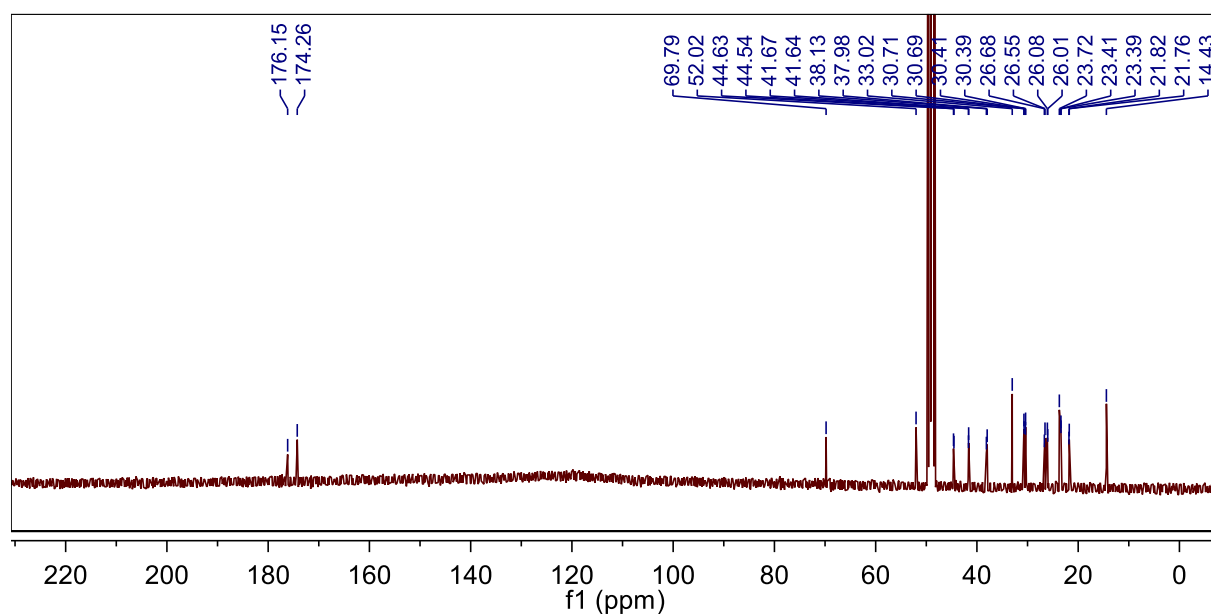
According to a procedure reported by (62), with some modifications, (3-hydroxydecanoyl)-L-leucine was synthesized. Thereby, to a solution of 3-hydroxydecanoic acid (470 mg, 2.5 mmol) in dry DCM (10 mL) 1-hydroxybenzotriazole (371 mg, 2.75 mmol) was added at 0 °C, followed

by dicyclohexylcarbodiimide (619 mg, 3 mmol). The mixture was stirred for 1 h at 0 °C and afterwards was allowed to warm to room temperature. Thereafter, leucinemethylester hydrochloride (499.7 mg, 2.75 mmol) dissolved in DMF (5 mL) was added. Subsequently triethylamine (420 μ L, 3 mmol) was added and the reaction mixture was stirred for 12 h. The precipitate was filtered off and the filtrate was evaporated under reduced pressure. The reaction product was acidified with HCl (1 N, 10 mL) and extracted with DCM (3 $\times$ 30 mL). The combined organic layers were washed with a solution of NaHCO₃ (10%, 30 mL) and saturated brine (30 mL), followed by drying over anhydrous Na₂SO₄. The crude product was further purified by RP-HPLC chromatography using a Hyperclone ODS (C18) 120 A (250 $\times$ 21.2 mm, 5 μ m, Phenomenex, Aschaffenburg, Germany) column with 0.1% formic acid as solvent A, acetonitrile as solvent B, operated with a flow rate of 21 mL/min and following gradient: 0 min, 50% B; 3 min 50% B; 13 min, 100% B; 18 min, 100% B; 20 min, 50% B; 25 min, 50% B. The effluent was monitored by UV detection at 200 nm and the fractions were collected individually in several runs. After evaporation of the solvent and lyophilization, (3-hydroxydecanoyl)-L-leucine-methylester was deprotected by stirring with NaOH (1 N, 20 mL, 20 mmol) in MeOH (40 mL) at room temperature for 14 h. The reaction was neutralized with 1 N HCl and afterwards was extracted with EtOAc (3 $\times$ 50 mL). The combined organic layers were washed with saturated brine solution, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to yield (3-hydroxydecanoyl)-L-leucine (45 mg, 6 %) as white powder. ¹H NMR (400 MHz, MeOD) δ 0.90 (t, J = 7.2 Hz, 3H, H-C(10)), 0.93 (d, J = 6.4 Hz, 2H, H-C(5')), 0.97 (dd, J = 6.5, 1.9 Hz, 3H, H-C(5')), 1.53 – 1.42 (m, 2H, H-C(4)), 1.31 (m, 10H), 1.67 – 1.58 (m, 2H, H-C(4')), 1.72 (dq, J = 13.0, 6.3 Hz, 1H, H-C(3')), 2.37 (t, J = 6.7 Hz, 2H, H-C(2)), 3.95 (td, J = 5.4, 4.5, 2.0 Hz, 1H, H-(3)), 4.44 (td, J = 7.4, 3.6 Hz, 1H, H-C(2')). ¹³C NMR (101 MHz, MeOD) δ 14.4 (C(10)), 21.8 (C(9)), 23.4 (C(5')), 26.1 (C(4')), 26.6 (C(5)), 30.4 (C(7)), 30.7 (C(6)), 33.0 (C(8)), 38.1 (C(4)), 41.7 (C(3')), 44.6 (C(2)), 52.0 (C(2')), 69.8 (C(3)), 174.3 (C(1)), 176.2 (C(1')).

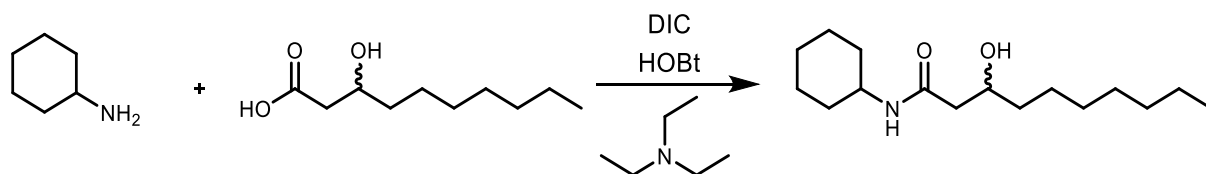
¹H-NMR spectra of Leu-*N*-3-OH-C10:0 (**41**)



¹³C-NMR spectra of Leu-*N*-3-OH-C10:0 (**41**)

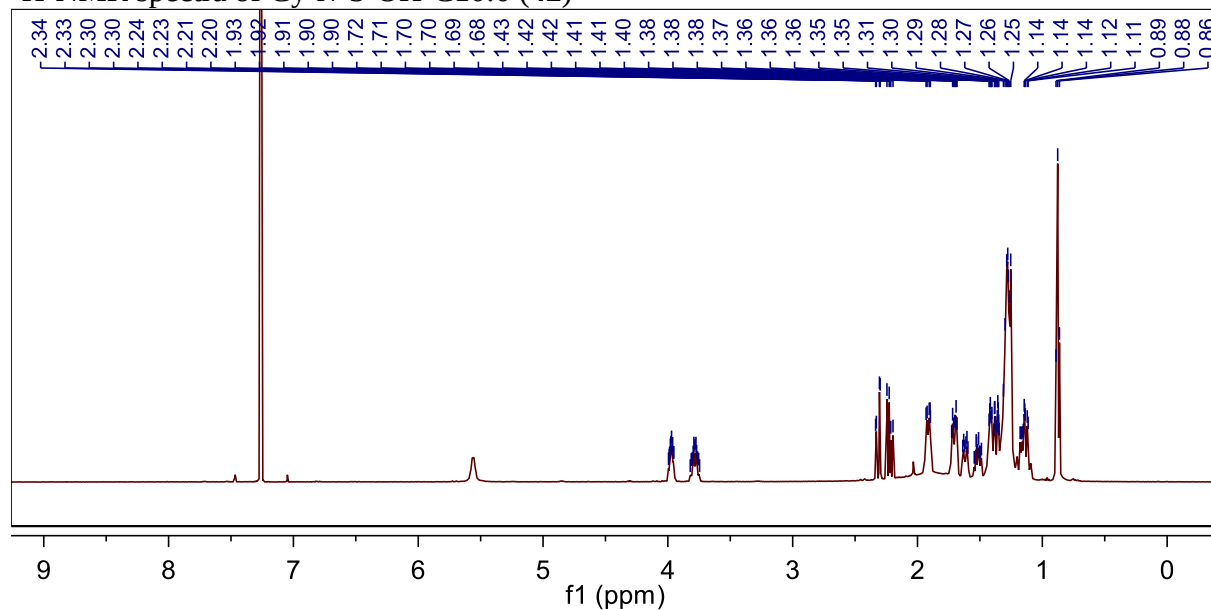


Synthesis of *N*-cyclohexyl-3-hydroxydecanamide, Cy-*N*-3-OH-C10:0 (**42**)

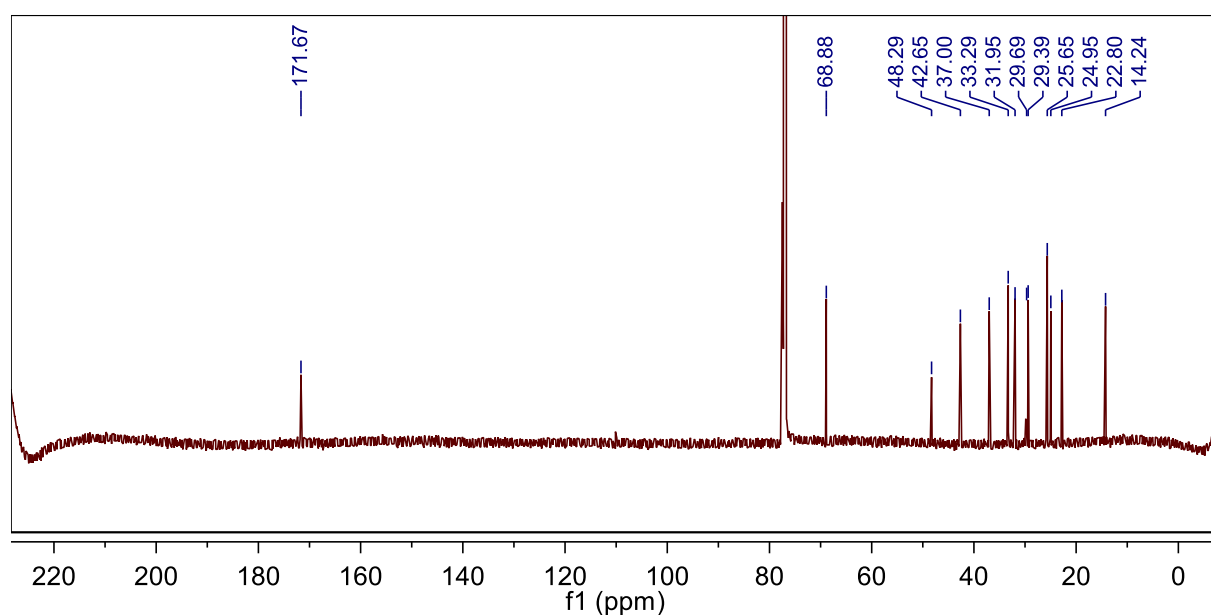


According to (63) *N*-cyclohexyl-3-hydroxydecanamide was generated. Triethylamine (28 μ L, 0.21 mmol), diisopropylcarbodiimide (32.5 μ L, 0.21 mmol) and 1-hydroxybenzotriazole (28.4 mg, 0.21 mmol) were added to a solution of 3-hydroxydecanoic acid (40 mg, 0.21 mg) at 0°C in DCM (15 mL). Afterwards cyclohexylamine (24.2 μ L, 0.21 mmol) was added at 0°C, the reaction mixture was allowed to warm to room temperature and stirred for 14 h. After evaporation of the solvent the residue was solved in acetonitrile/water (50/50, v/v) and purified by means of RP-HPLC using a Hyperclone ODS (C18) 120 A (250 $\times$ 21.2 mm, 5 μ m, Phenomenex, Aschaffenburg, Germany) column with 0.1% formic acid as solvent A, acetonitrile as solvent B, operated with a flow rate of 21 mL/min and following gradient: 0 min, 55% B; 15 min, 100% B; 20 min, 100% B; 22 min, 55% B; 30 min, 55% B. The effluent was monitored by UV detection at 200 nm and the fractions were collected individually in several runs. After evaporation of the solvent and lyophilization, *N*-cyclohexyl-3-hydroxydecanamide (23 mg, 41%) was yielded as white powder. ¹H NMR (500 MHz, CDCl₃) δ 0.88 (t, J = 6.8 Hz, 3H, H-C(10)), 1.19 – 1.08 (m, 3H, H-C(2'), H-C(4')), 1.33 – 1.23 (m, 10H), 1.44 – 1.34 (m, 3H, H-C(4), H-C(3')), 1.51 (ddd, J = 11.5, 8.4, 5.6 Hz, 1H, H-C(4)), 1.62 (dt, J = 12.8, 3.8 Hz, 1H, H-C(4')), 1.70 (dt, J = 13.6, 3.9 Hz, 2H, H-C(3')), 1.91 (dd, J = 12.3, 3.7 Hz, 2H, H-C(2')), 2.22 (dd, J = 15.2, 9.0 Hz, 1H, H_a-C(2)), 2.32 (dd, J = 15.2, 2.7 Hz, 1H, H_b-C(2)), 3.78 (tdt, J = 11.9, 8.0, 3.9 Hz, 1H, H-C(1')), 3.97 (dtd, J = 9.1, 4.6, 2.3 Hz, 1H, H-C(3)). ¹³C NMR (126 MHz, CDCl₃) δ 14.2 (C(10)), 22.8 (C(9)), 24.9 (C(3')), 25.7 (C(4')), 29.4 (C(7)), 29.7 (C(6)), 31.9 (C(8)), 33.3 (C(2')), 37.0 (C(4)), 42.7 (C(2)), 48.3 (C(1')), 68.9 (C(3)), 171.7 (C(1)).

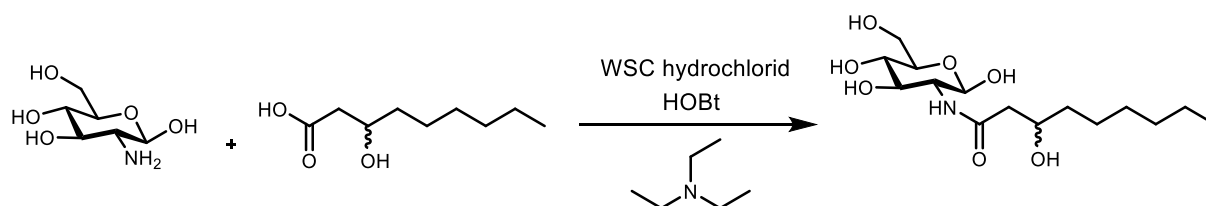
¹H-NMR spectra of Cy-*N*-3-OH-C10:0 (**42**)



¹³C-NMR spectra of Cy-*N*-3-OH-C10:0 (**42**)



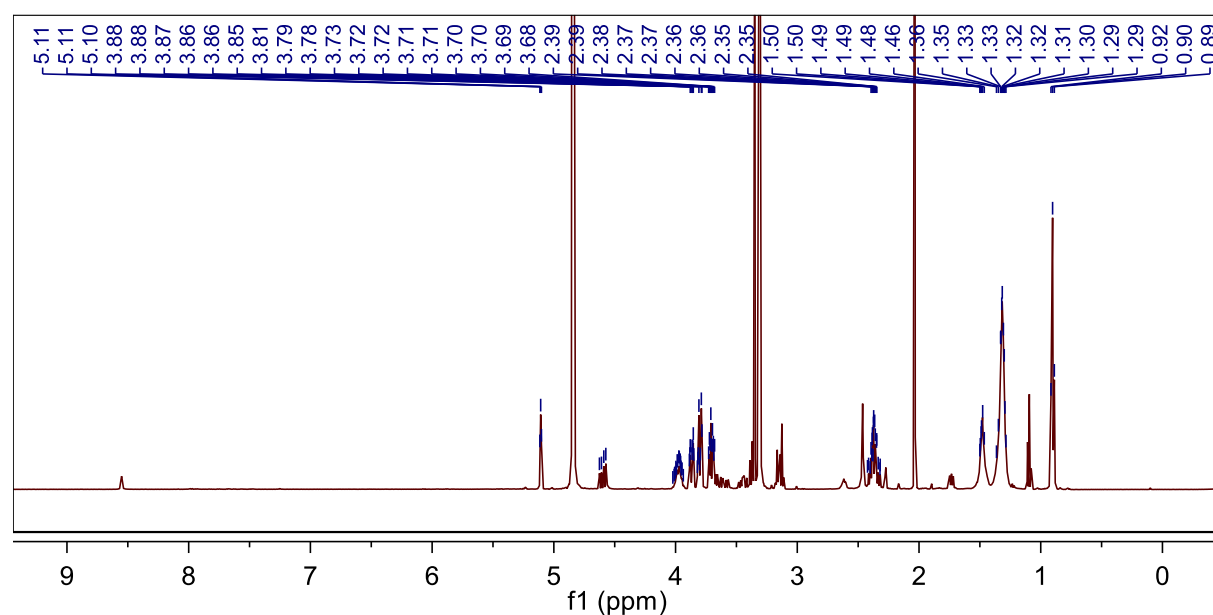
Synthesis of 3-hydroxy-*N*-((2*S*,3*R*,4*R*,5*S*,6*R*)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)decanamide, Glc-*N*-3-OH-C10:0 (**43**)



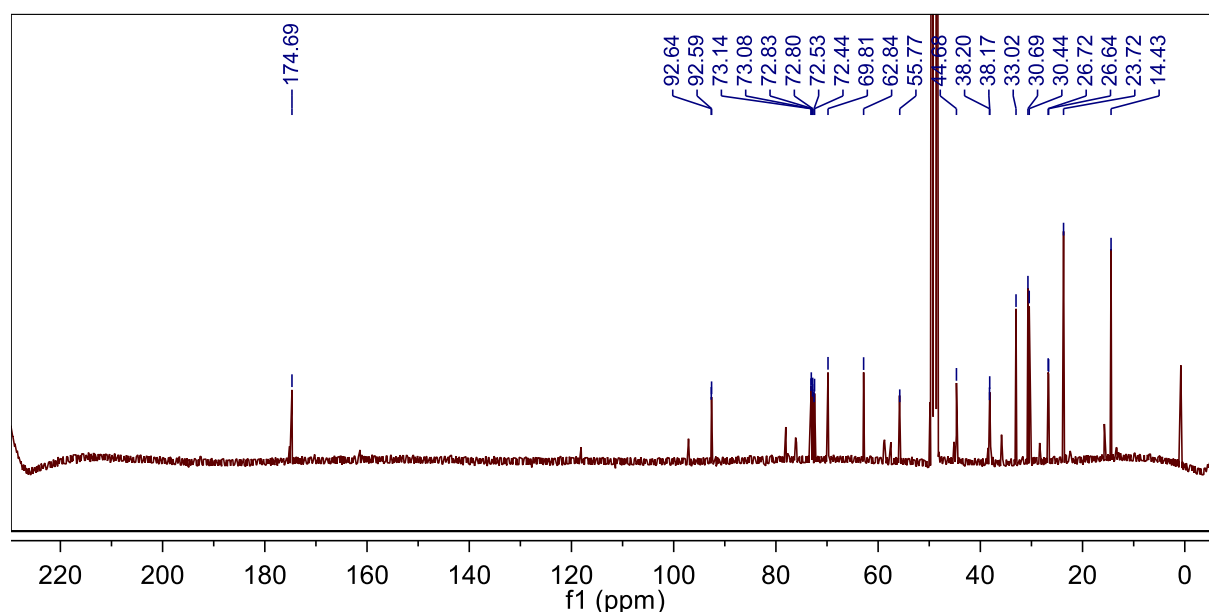
According to procedure reported (64) with slight modifications 3-hydroxy-*N*-((2*S*,3*R*,4*R*,5*S*,6*R*)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)decanamide

was synthesized. To a solution of 3-hydroxydecanoic acid (49 mg, 0.26 mmol), glucosamine hydrochloride (65 mg, 0.3 mmol) and triethyl amine (55 μ L, 0.4 mmol) in MeOH/water (10 mL, 50/50, v/v), WSC hydrochloride (77 mg, 0.4 mmol) and 1-hydroxybenzotriazole (35 mg, 0.26 mmol) were added. The solution was stirred at room temperature overnight and after evaporation of the organic solvent, the residue was chromatographed by means of RP-HPLC using a Hyperclone ODS (C18) 120 A (250 $\times$ 21.2 mm, 5 μ m, Phenomenex, Aschaffenburg, Germany) column with 0.1% formic acid as solvent A, acetonitrile as solvent B. Chromatography was performed by using a flow rate of 21 mL/min and the following gradient: 0 min, 10% B; 15 min, 70% B; 20 min, 70% B; 22 min, 10% B; 30 min, 10% B. The effluent was monitored by UV detection at 200 nm and the fractions were collected individually in several runs. After evaporation of the organic solvent and lyophilization 3-hydroxy-*N*-((2*S*,3*R*,4*R*,5*S*,6*R*)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)decanamide (12 mg, 13%, mixture of anomers) was afforded as white powder. ^1H NMR (500 MHz, MeOD) δ 0.93 – 0.85 (m, 3H, H-C(10)), 1.40 – 1.25 (m, 10H), 1.48 (q, J = 6.7 Hz, 2H, H-C(4)), 2.49 – 2.22 (m, 2H, H-C(2)), 3.35 – 3.39 (m, 1H, H-C(5')), 3.74 – 3.67 (m, 2H, H-C(5'), H-C(6')), 3.83 – 3.77 (m, 2H, H-C(3'), H-C(6')), 3.90 – 3.85 (m, 1H, H-C(2')), 3.98 (tt, J = 7.6, 5.5 Hz, 1H, H-C(3)), 5.11 (t, J = 3.2 Hz, 1H, H-C(1')). ^{13}C NMR (126 MHz, MeOD) δ 14.4 (C(10)), 23.7 (C(9)), 26.7 (C(5)), 30.4 (C(7)), 30.7 (C(6)), 33.0 (C(8)), 38.2 (C(4)), 44.7 (C(2)), 55.8 (C(2')), 62.8 (C(6')), 69.8 (C(3)), 72.5 (C(5')), 72.8 (C(3')), 73.1 (C(4')), 92.6 (C(1')), 174.7 (C(1)).

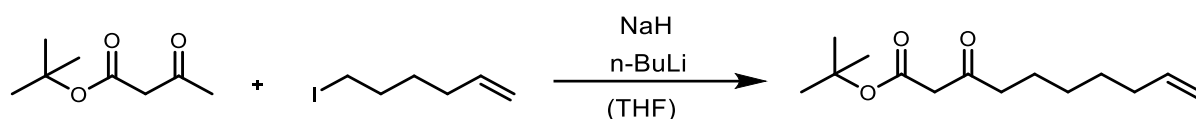
^1H -NMR spectra of Glc-*N*-3-OH-C10:0 (**43**)



¹³C-NMR spectra of Glc-*N*-3-OH-C10:0 (**43**)

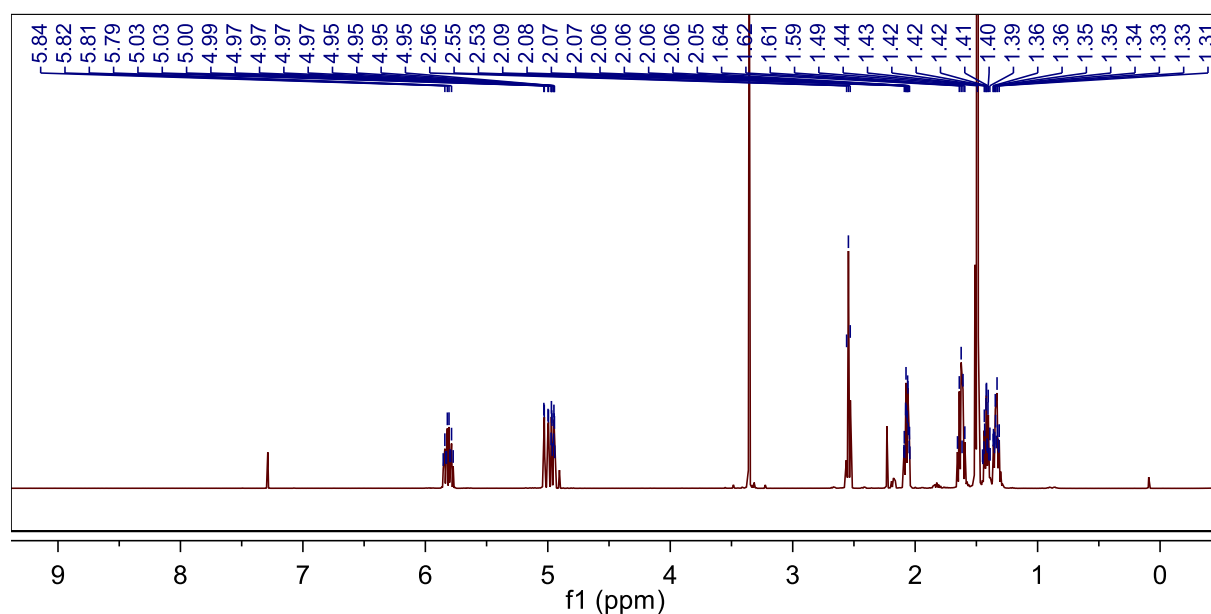


Synthesis of *tert*-butyl 3-oxodecen-9-oate (**1-d₂-a**)

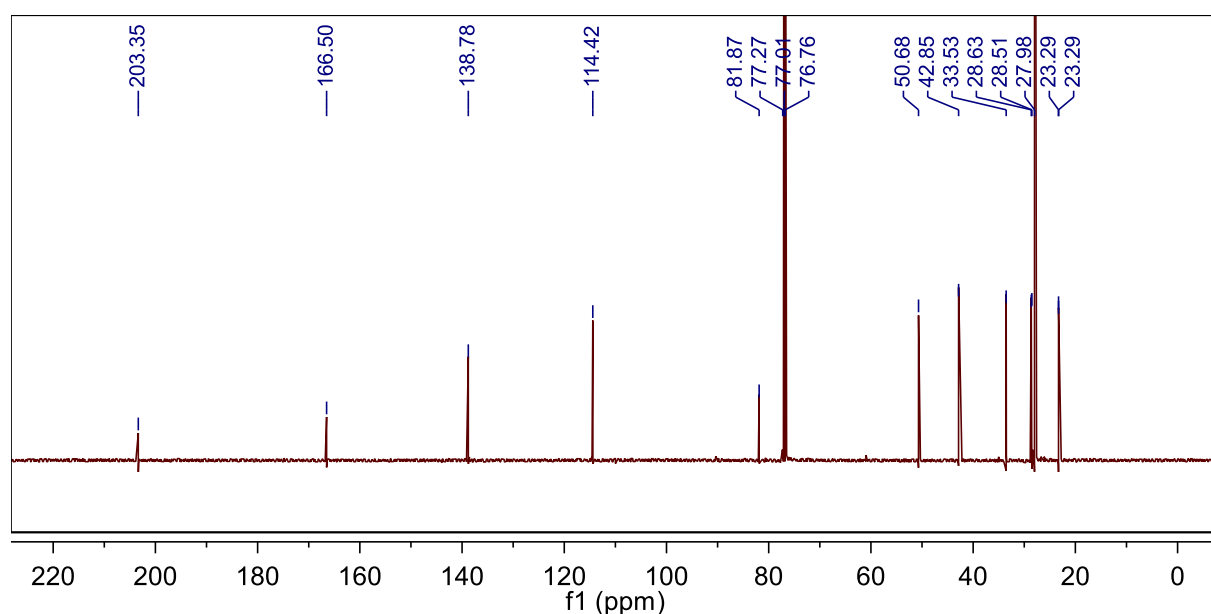


According to a modified procedure reported by (60) *tert*-butyl acetoacetate (0.53 g, 3.3 mmol) was added dropwise to a stirred suspension of NaH (0.16 g, 4 mmol) in THF (10 mL) at 0 °C and was stirred for 10 min. After that *n*-BuLi (1.6 M hexane solution, 0.8 mL, 3.7 mmol) was added slowly and the mixture was stirred at 0 °C for 30 min. To this mixture 6-iodo-1-hexene (1.05 g, 5 mmol) was added dropwise, and the resulting reaction was stirred for additional 30 min at 0 °C. The reaction was ended by addition of HCl (1 N, 20 mL). After extraction of the solution ether (three times with 50 mL), the combined organic layers were washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure. The crude product was subjected to a column filled with silica gel. Chromatography was performed by using hexane/EtOAc (95/5, v/v) to give *tert*-butyl 3-oxodecen-9-oate (0.51 g, 64%) as a colourless oil. ¹H NMR (500 MHz, CDCl₃) δ 1.37 – 1.29 (m, 2H, H-C(6)), 1.46 – 1.38 (m, 2H, H-C(7)), 1.49 (s, 9H, H-C(2')), 1.62 (p, *J* = 7.4 Hz, 2H, H-C(5)), 2.07 (dtd, *J* = 7.7, 6.7, 1.4 Hz, 2H, H-C(8)), 2.55 (t, *J* = 7.4 Hz, 2H, H-C(4)), 3.36 (s, 2H, H-C(2)), 4.96 (ddt, *J* = 10.2, 2.3, 1.2 Hz, 1H, Hb-C(10)), 5.01 (dq, *J* = 17.1, 1.7 Hz, 1H, Ha-C(10)), 5.81 (ddt, *J* = 17.0, 10.2, 6.6 Hz, 1H, H-C(9)). ¹³C NMR (126 MHz, CDCl₃) δ 23.3 (C(5)), 28.0 (C(2')), 28.5 (C(7)), 28.6 (C(6)), 33.5 (C(8)), 42.9 (C(4)), 50.7 (C(2)), 81.9 (C(1')), 114.4 (C(10)), 138.8 (C(9)), 166.5 (C(1)), 203.3 (C(3)).

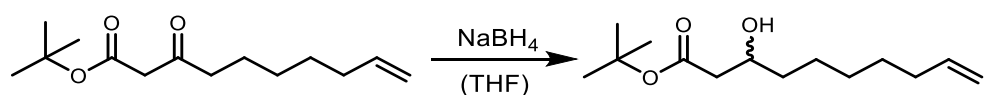
¹H-NMR spectra of *tert*-butyl 3-oxodecen-9-oate (**1-d₂-a**)



¹³C-NMR spectra of *tert*-butyl 3-oxodecen-9-oate (**1-d₂-a**)



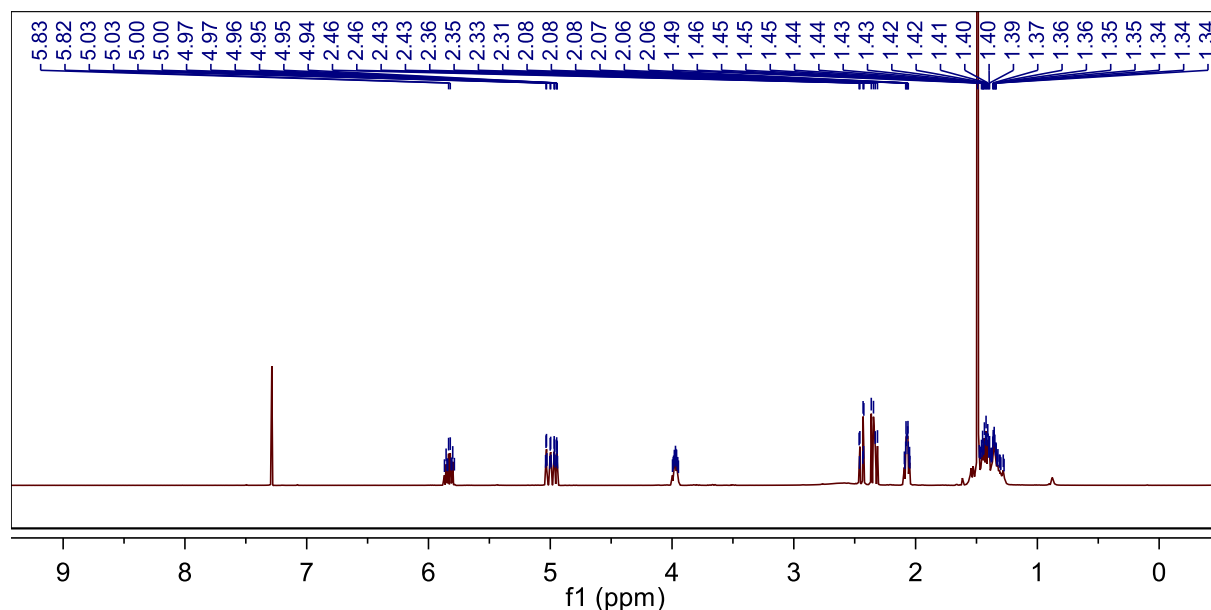
Synthesis of *tert*-butyl 3-hydroxydecen-9-oate (**1-d₂-b**)



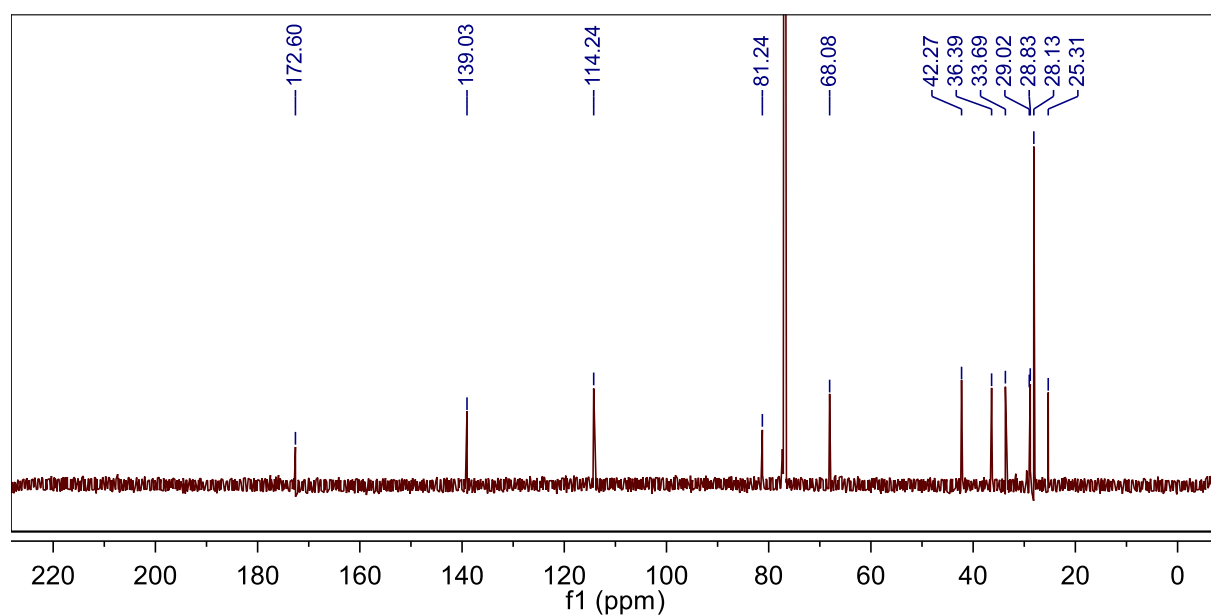
tert-Butyl 3-hydroxydecen-9-oate was synthesized according to a modified procedure described by (61). To an ice-cold solution (0 °C) of *tert*-butyl 3-oxo-decen-9-oate (0.48 g, 2 mmol) solved in ethanol (0.5 mL) and THF (4.5 mL) sodium borohydride (60.2 mg, 16 mmol) was added. The resulting slurry was stirred vigorously for 1 h and afterwards was quenched by the slow addition of HCl (1 N) until pH 5 was reached. Subsequent the mixture was extracted with EtOAc (3 × 40 mL). The combined organic layers were washed with a solution of brine

(20 mL), dried over Na₂SO₄ and concentrated under reduced pressure to yield *tert*-butyl 3-hydroxydecen-9-oate (0.26 g, 54%). ¹H NMR (400 MHz, CDCl₃) δ 1.62 – 1.22 (m, 8H), 1.49 (s, 9H, H-C(2')), 2.13 – 1.99 (m, 2H, H-C(8)), 2.33 (dd, *J* = 16.4, 9.0 Hz, 1H, H_a-C(2)), 2.44 (dd, *J* = 16.4, 3.1 Hz, 1H, H_b-C(2)), 4.01 – 3.93 (m, 1H, H-C(3)), 4.95 (dq, *J* = 10.2, 1.2 Hz, 1H, H-C(10)), 5.01 (dq, *J* = 17.2, 1.7 Hz, 1H, H-C(10)), 5.83 (ddt, *J* = 16.9, 10.2, 6.7 Hz, 1H, H-C(9)). ¹³C NMR (101 MHz, CDCl₃) δ 25.3 (C(5)), 28.1 (C(2')), 28.8 (C(7)), 29.0 (C(6)), 33.7 (C(8)), 36.4 (C(4)), 42.3 (C(2)), 68.1 (C(3)), 81.2 (C(1')), 114.2 (C(10)), 139.0 (C(9)), 172.6 (C(1)).

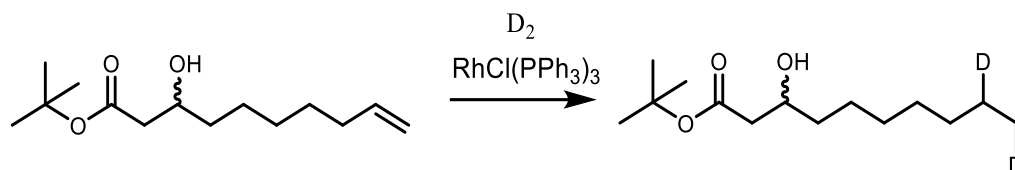
¹H-NMR spectra of *tert*-butyl 3-hydroxydecen-9-oate (**1-d₂-b**)



¹³C-NMR spectra of *tert*-butyl 3-hydroxydecen-9-oate (**1-d₂-b**)

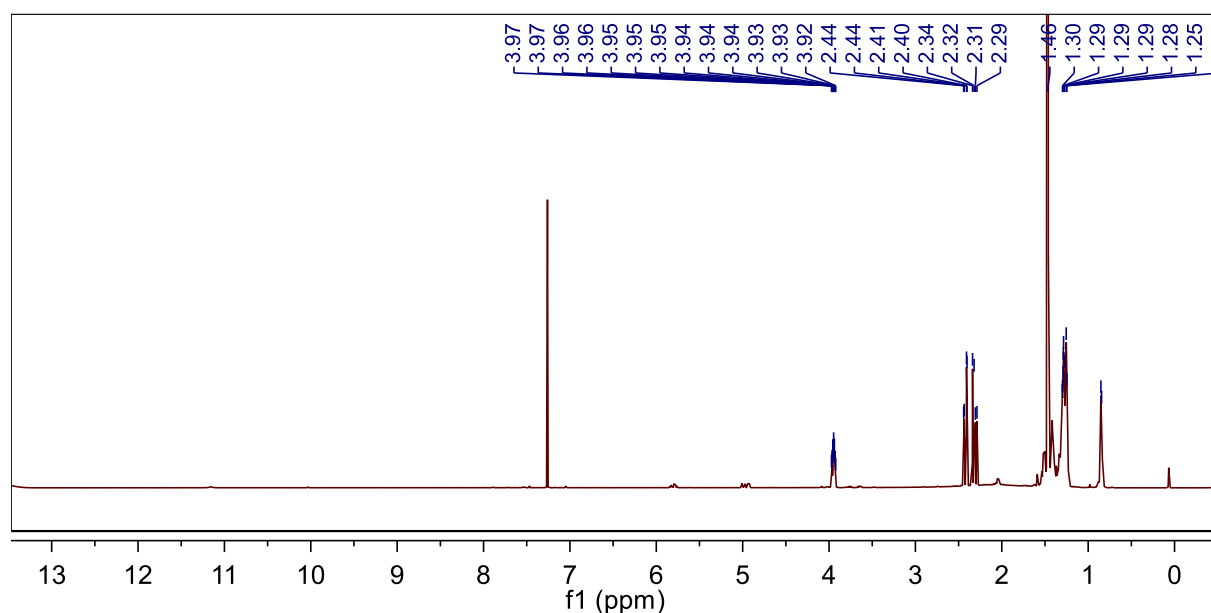


Synthesis of *tert*-butyl 3-hydroxydecanoate-d₂ (**1-d₂-c**)

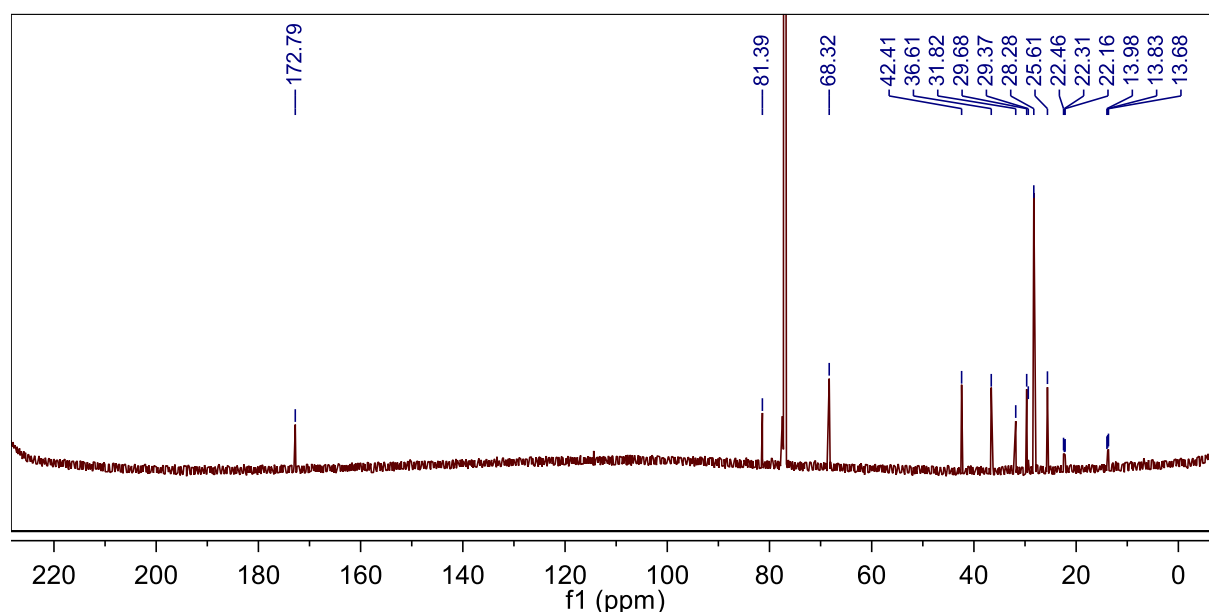


According to a modified procedure published (65) Wilkinson's catalyst [tris(triphenylphosphine)rhodium(I) chloride] (3.1 g, 3.3 mmol) was added to a solution of *tert*-butyl 3-hydroxydecen-9-oate (200 mg, 0.83 mmol) in toluene (10 mL) and was deuterated in an autoclave at 5 bar for 3 h. Afterwards the solvent was removed under reduced pressure and the crude product was purified by means of silica gel column chromatography using hexane/EtOAc (95/5, v/v) to yield *tert*-butyl 3-hydroxydecanoate-d₂ (110 mg, 54%) as colourless oil. ¹H NMR (500 MHz, CDCl₃) δ 0.88 (d, *J* = 4.5 Hz, 2H, H-C(10)), 1.47 – 1.25 (m, 8H), 1.49 (s, 9H, H-C(2')), 1.58 – 1.52 (m, 2H, H-C(4)), 2.34 (dd, *J* = 16.3, 9.1 Hz, 1H, H_a-C(2)), 2.45 (dd, *J* = 16.3, 3.0 Hz, 1H, H_b-C(2)), 3.97 (dddd, *J* = 8.9, 7.6, 4.7, 3.0 Hz, 1H, H-C(3)). ¹³C NMR (126 MHz, CDCl₃) δ 13.7 (t, *J* = 19.0 Hz, C(10)), 22.2 (t, *J* = 19.0 Hz, C(9)), 25.5 (C(5)), 28.1 (C(2')), 29.2 (C(7)), 29.5 (C(6)), 31.7 (C(8)), 36.5 (C(4)), 42.3 (C(2)), 68.2 (C(3)), 81.2 (C(1')), 172.6 (C(1)).

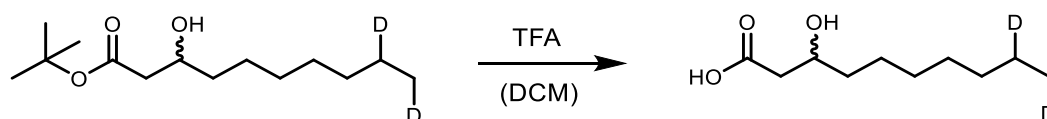
¹H-NMR spectra of *tert*-butyl 3-hydroxydecanoate-d₂ (**1-d₂-c**)



^{13}C -NMR spectra of *tert*-butyl 3-hydroxydecanoate- d_2 (**1-d₂-c**)

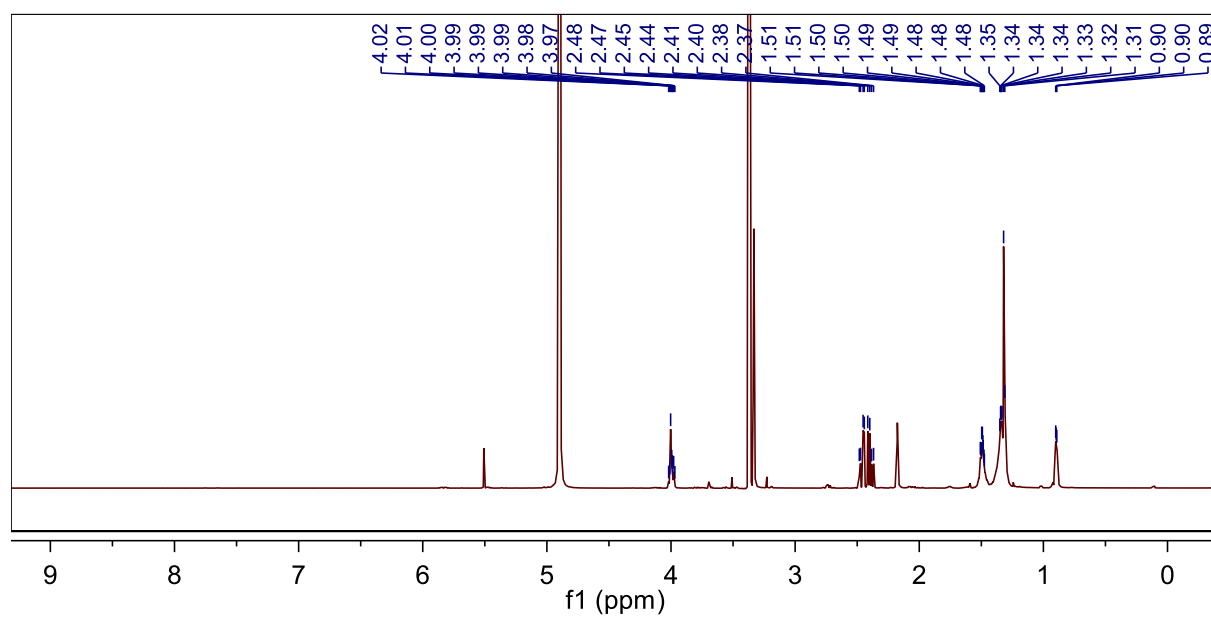


Synthesis of 3-hydroxydecanoic acid- d_2 , 3-OH-C10:0- d_2 (**1-d₂**)

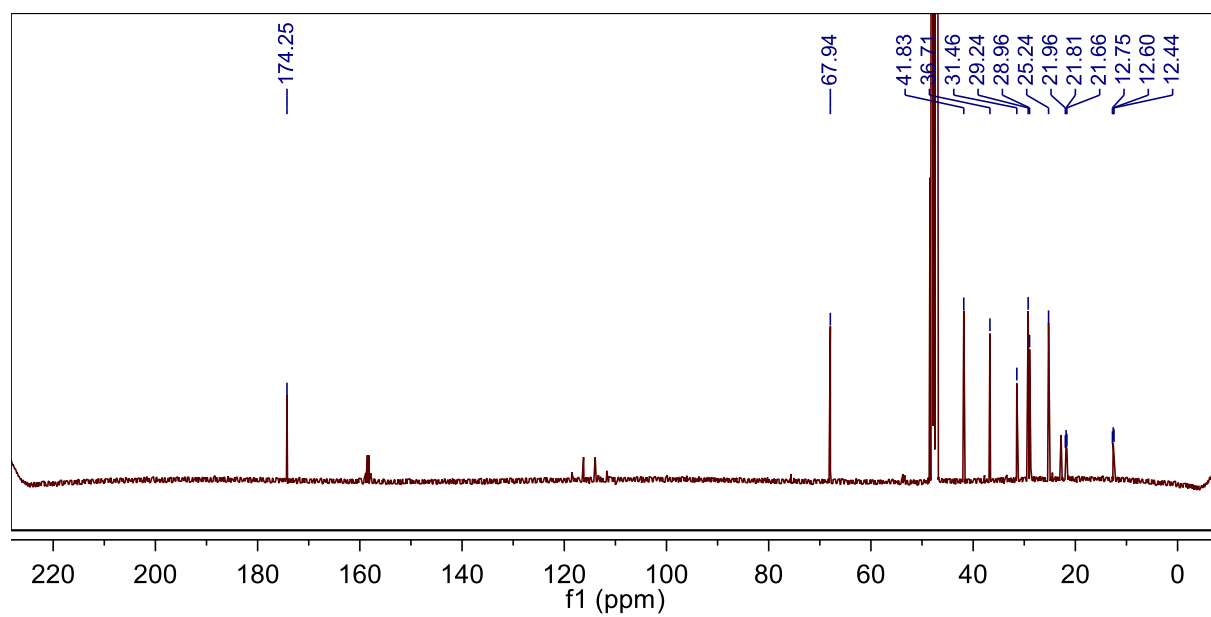


A standard deprotection reaction previously reported by (53) was used. Thereby, *tert*-butyl 3-hydroxydecanoate- d_2 was dissolved in TFA/DCM (1/3, v/v) (15 mL) and stirring for 12 h. Afterwards the solution was concentrated until dryness under a flow of nitrogen to give 3-hydroxydecanoic acid- d_2 in high purity ($\geq 98\%$) without further purification. ^1H NMR (500 MHz, MeOD) δ 0.90 (d, $J = 4.2$ Hz, 2H, H-C(10)), 1.39 – 1.27 (m, 8H), 1.49 (ddd, $J = 6.5, 4.9, 2.1$ Hz, 2H, H-C(4)), 2.39 (dd, $J = 15.2, 8.1$ Hz, 1H, H_a -C(2)), 2.46 (dd, $J = 15.2, 4.8$ Hz, 1H, H_b -C(2)), 4.03 – 3.96 (m, 1H, H-C(3)). ^{13}C NMR (126 MHz, MeOD) δ 12.6 (t, $J = 19.1$ Hz, C(10)), 21.8 (t, $J = 19.3$ Hz, C(9)), 25.2 (C(5)), 29.0 (C(7)), 29.2 (C(6)), 31.5 (C(8)), 36.7 (C(4)), 41.8 (C(2)), 67.9 (C(3)), 174.3 (C(1)).

^1H -NMR spectra of 3-OH-C10:0-d₂ (**1-d₂**)



^{13}C -NMR spectra of 3-OH-C10:0-d₂ (**1-d₂**)



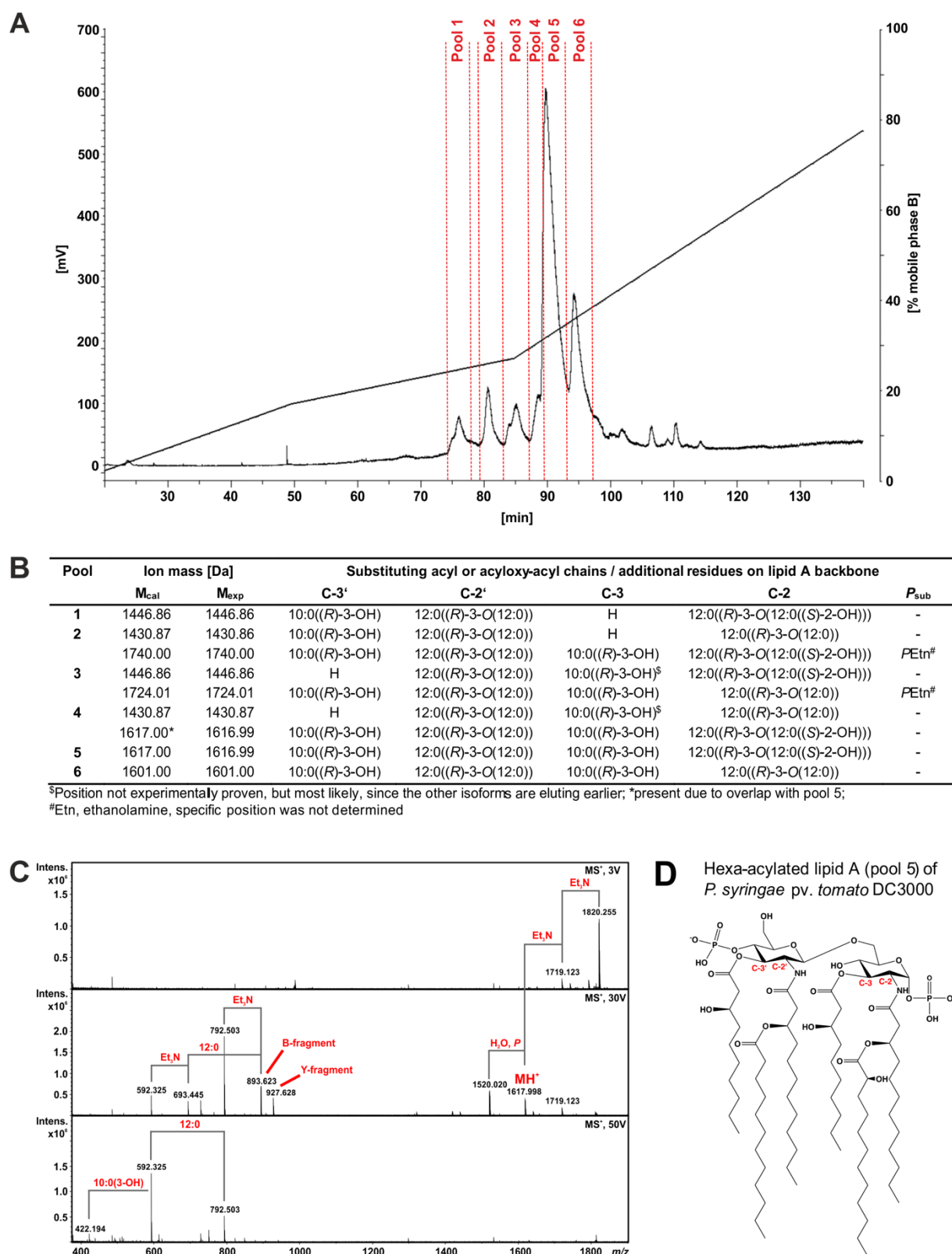


Fig. S1. HPLC purification and mass spectrometric analysis of lipid A of *P. syringae* pv. *tomato* DC3000. (A) Reversed-phase HPLC profile obtained for the lipid A of *P. syringae* pv. *tomato* DC3000 isolated after mild acetate buffer hydrolysis of the respective lipopolysaccharide. For further details see materials & methods. (B) Electrospray Ionization Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry (ESI-FT-ICR-MS) was performed on a 7 Tesla APEX Qe instrument (Bruker Daltonics, USA). For initial molecule

identification, samples were dissolved in a water/propan-2-ol/7 M triethylamine/acetic acid mixture (50:50:0.06:0.02, v/v/v/v) and spectra were acquired in the negative ion mode with nano-ESI using the Triversa Nanomate (Advion, USA) as ion source with a spray voltage set to -1.1 kV. Mass spectra were recorded in broad band mode and the mass scale was externally calibrated with glycolipids of known structure. **(C)** Representative example for the MS analysis, the fragmentation of the lipid A of pool 5 ($M_{\text{exp}} = 1616.99$ Da). To determine the acyl chain pattern, selected samples were dissolved in chloroform/methanol 4:1, v/v ($0.5 \mu\text{g}/\mu\text{L}$). Shortly before mass spectrometric analysis, $20 \mu\text{L}$ of this solution were mixed with $150 \mu\text{L}$ water/propan-2-ol/30 mM ammonium acetate/acetic acid mixture (15:15:1:0.04, v/v/v/v) and afterwards $0.5 \mu\text{L}$ triethylamine and $25 \mu\text{L}$ methanol were added. Spectra were recorded in positive ion mode at collision energies of 3 V, 15 V, 30 V, and 50 V using the Triversa Nanomate (Advion, USA) as nano-ESI ion source with a spray voltage set to 1.1 kV. Under this ionization condition, intensive Et_3N adduct ions are formed. Applying increased collision energies via CID leads to the formation of the $[\text{M}+\text{H}]^+$ ion as well as the abundant B-fragment (according to the nomenclature of (66)), in this case GlcN II carrying three fatty acids and the phosphate group in position 4; m/z 893.623 (67). At a collision energy of 30 V, the corresponding Y-fragment ion (here: GlcN I carrying three fatty acids and the phosphate group in position 1; m/z 927.628) can also be detected. At a collision energy of 50 V further loss of ester bond fatty acids can be observed. The general distribution of 3-OH-acyl chains in *Pseudomonas* lipid A molecules is assigned according to earlier published structural analysis (68). **(D)** Chemical structure of the hexa-acylated lipid A of *P. syringae* pv. *tomato* DC 3000 present in pool 5.

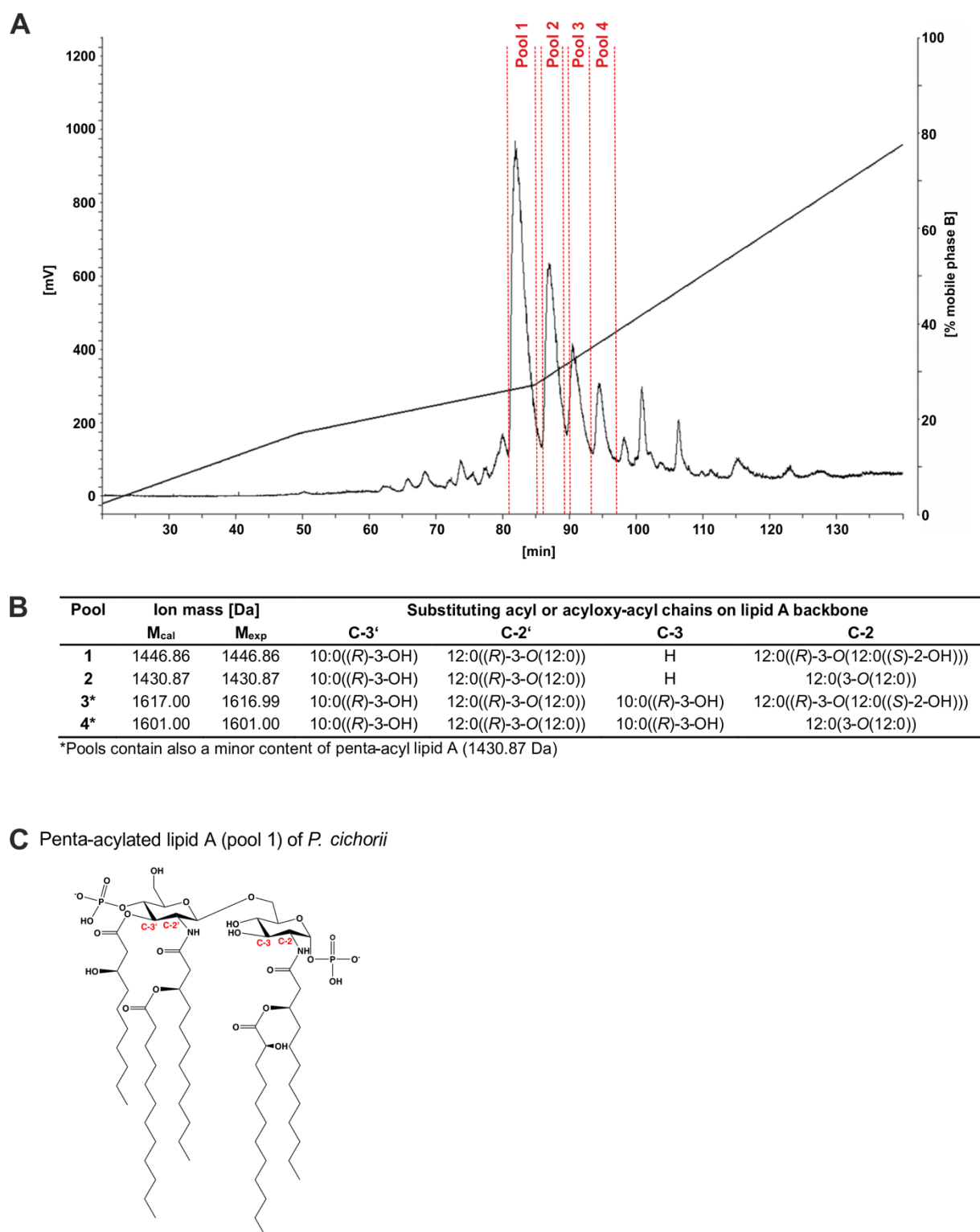


Fig. S2. HPLC purification and mass spectrometric analysis of lipid A of *P. cichorii* ATCC10857/DSM50259. (A) Reversed-phase HPLC profile obtained for the lipid A of *P. cichorii* after mild acetate buffer hydrolysis of the respective lipopolysaccharide. For further details see materials & methods. (B) Electrospray Ionization Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry (ESI-FT-ICR-MS) was performed as described in fig. S1. (C) Chemical structure of the penta-acylated lipid A of *P. cichorii* ATCC10857/DSM50259 present in pool 1.

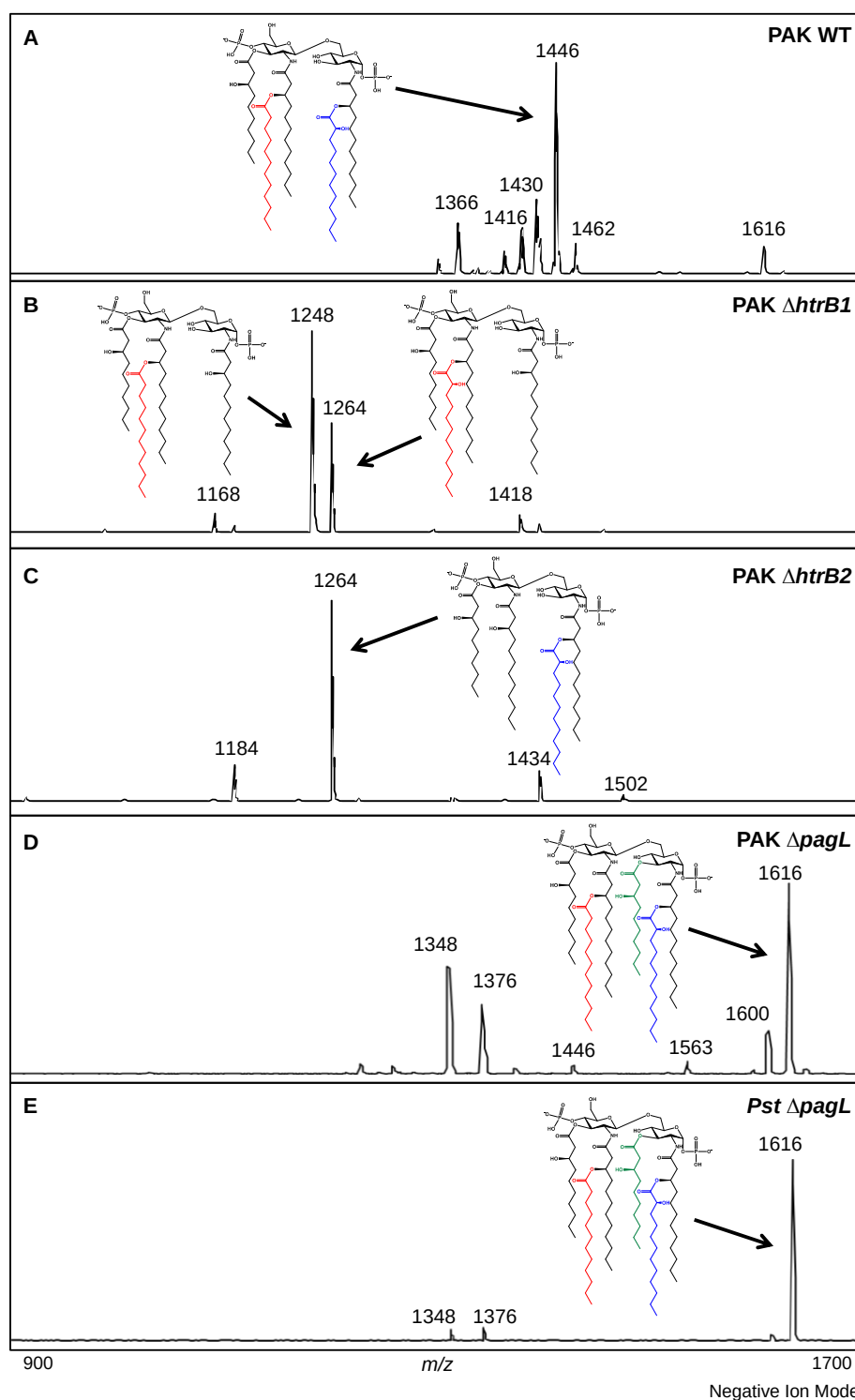


Fig. S3. Mass spectrometric analysis of lipid A of *P. aeruginosa* PAK wild type, $\Delta htrB1$, $\Delta htrB2$, and $\Delta pagL$ mutant, and *P. syringae* $\Delta pagL$ mutant. MALDI-TOF MS of (A) *P. aeruginosa* PAK wild type (WT) major ion peak m/z 1446.8; (B) *P. aeruginosa* PAK $\Delta htrB1$ major peaks m/z 1248 and 1264 corresponding to a loss of the secondary 2-OH-dodecanoyl chain at position C-2; (C) *P. aeruginosa* PAK $\Delta htrB2$ major peak at m/z 1264 resulting from a loss of the secondary dodecanoyl chain at position C-2'; (D,E) *P. aeruginosa* PAK $\Delta pagL$ and *P. syringae* pv. *tomato* DC3000 (*Pst*) $\Delta pagL$ major peak at m/z 1616 of hexa-acylated lipid A. (A–E) Lipid A structures corresponding to the major peaks are illustrated. Bacterial culture conditions, lipopolysaccharide/lipid A extraction (table S3), and MALDI-TOF MS analysis were performed as described (69).

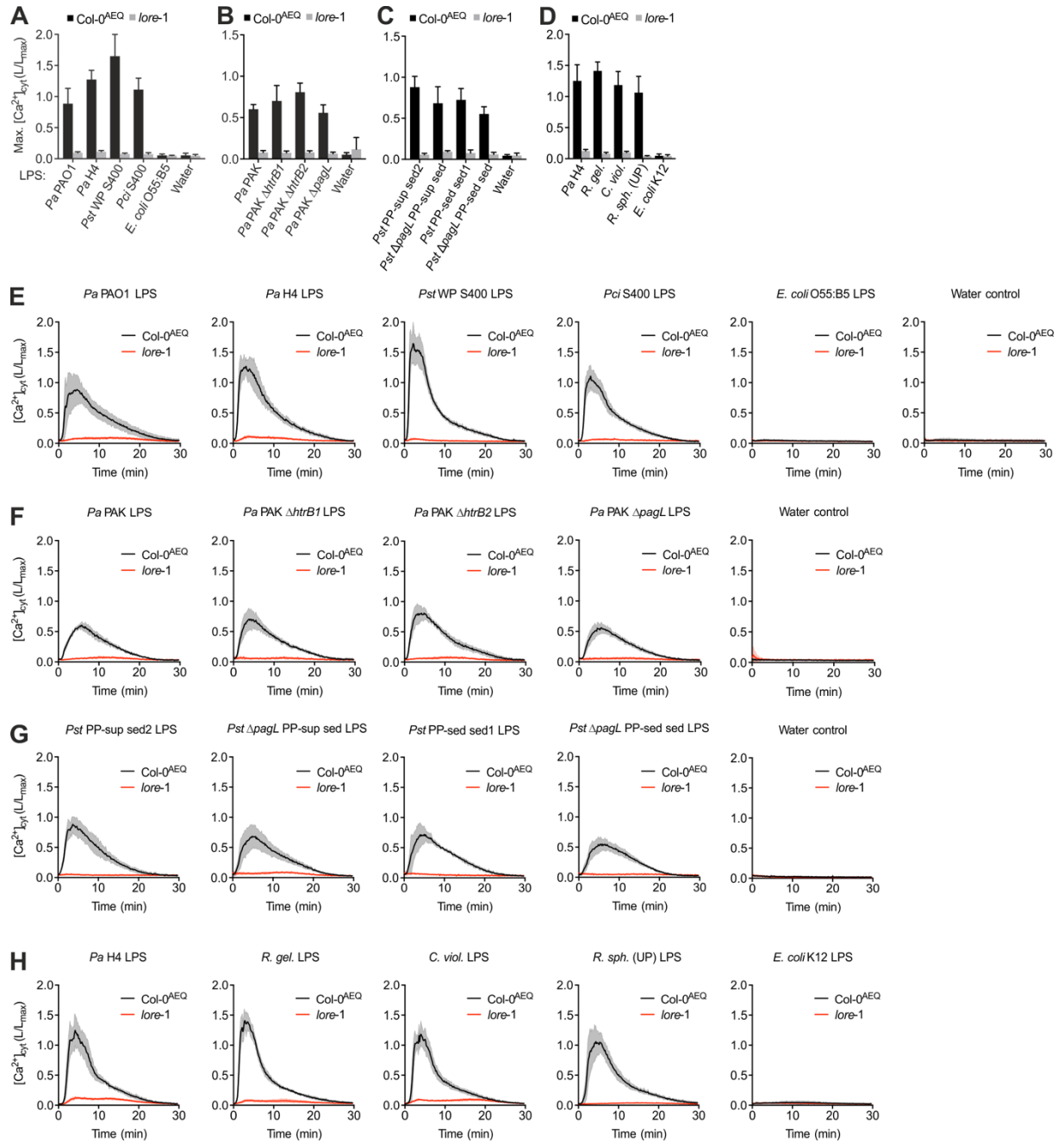


Fig. S4. LORE-dependent immune sensing of lipopolysaccharide preparations correlates with the presence of medium-chain 3-OH-acyl chains in the lipid A moiety. (A–D) Maximum $[Ca^{2+}]_{cyt}$ elevations in Arabidopsis seedlings treated with the indicated lipopolysaccharide (LPS) preparations (25 μ g/mL; tables S1 and S3) or water (mean $\pm$ SD, $n=6$). (E–H) Kinetics of $[Ca^{2+}]_{cyt}$ elevations shown in (A–C). Experiments were repeated three (A,C,D) or four times (B) with similar results. *Pa*, *P. aeruginosa*; *Pst*, *P. syringae* pv. *tomato* DC3000; *Pci*, *P. cichorii*; *E. coli*, *Escherichia coli*; *R. gel.*, *Rubrivivax gelatinosus* Dr2; *C. viol.*, *Chromobacterium violaceum*; *R. sph.* (UP), *Rhodobacter sphaeroides* (Ultrapure).

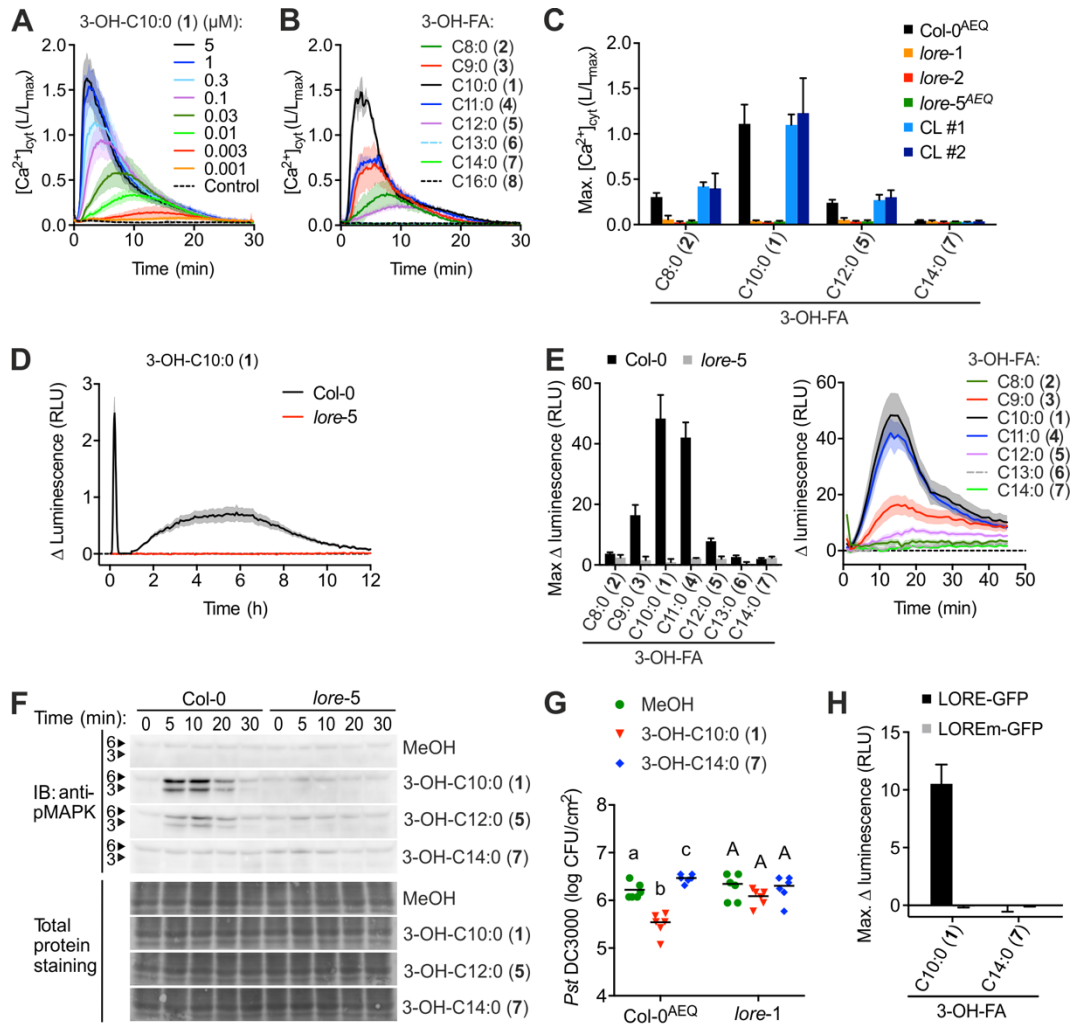


Fig. S5. Synthetic 3-OH-FAs trigger LORE-dependent immune responses in a chain length-specific manner. (A) Kinetics of $[\text{Ca}^{2+}]_{\text{cyt}}$ elevations shown in fig. 1B. Arabidopsis Col-0^{AEQ} seedlings were treated with different concentrations of 3-OH-10:0 (1) or water (mean $\pm$ SD, $n=12$). Concentration of the 3-OH-C10:0 (1) stock solution was determined by quantitative NMR. (B) Kinetics of $[\text{Ca}^{2+}]_{\text{cyt}}$ elevations shown in fig. 1C. Col-0^{AEQ} seedlings were treated with 3-OH-FAs of the indicated chain length (1 μM ; mean $\pm$ SD, $n=3$). (C) Maximum $[\text{Ca}^{2+}]_{\text{cyt}}$ elevations of Arabidopsis Col-0^{AEQ}, *lore-1*, *lore-2*, and *lore-5*^{AEQ} mutants, and two complementation lines (CL #1 and #2) treated with 3-OH-FAs of the indicated chain length (1 μM ; mean $\pm$ SD, $n=4$). (D) Biphasic kinetics of ROS production of Arabidopsis Col-0 leaf discs treated with 1 μM 3-OH-C10:0 (1) measured over 12 hours. (E) Maximum ROS accumulation (left) of Arabidopsis Col-0 and *lore-5* leaf discs treated with 3-OH-FAs of the indicated chain length (1 μM ; mean $\pm$ SE, $n=6$). Kinetics of ROS production (right) of Arabidopsis Col-0 leaf discs shown in the left panel. (F) Activation of AtMPK3 and AtMPK6 upon treatment of Arabidopsis seedlings with 3-OH-FAs (5 μM) or MeOH was analysed by immunoblot (IB). Total protein staining shows equal loading. (G) *Pst* DC3000 titre four days post infection in Arabidopsis plants pre-treated with 10 μM 3-OH-FAs or MeOH for two days before infection. Individual data (symbols) and means (lines) of two experiments are shown ($n=6$). Different letters indicate significant differences (one-way ANOVA with Tukey's post-hoc test applied separately to Col-0^{AEQ} (small letters) and *lore-1* (capitals), $p<0.01$). (H) Maximum ROS accumulation in leaf discs from *N. benthamiana* transiently expressing LORE-GFP or kinase-inactive LOREm-GFP elicited with 1 μM 3-OH-FAs of the indicated chain length (mean $\pm$ SE, $n=8$). Experiments were repeated two (C,H), three (A,D,E,F), or four times (B) with similar results.

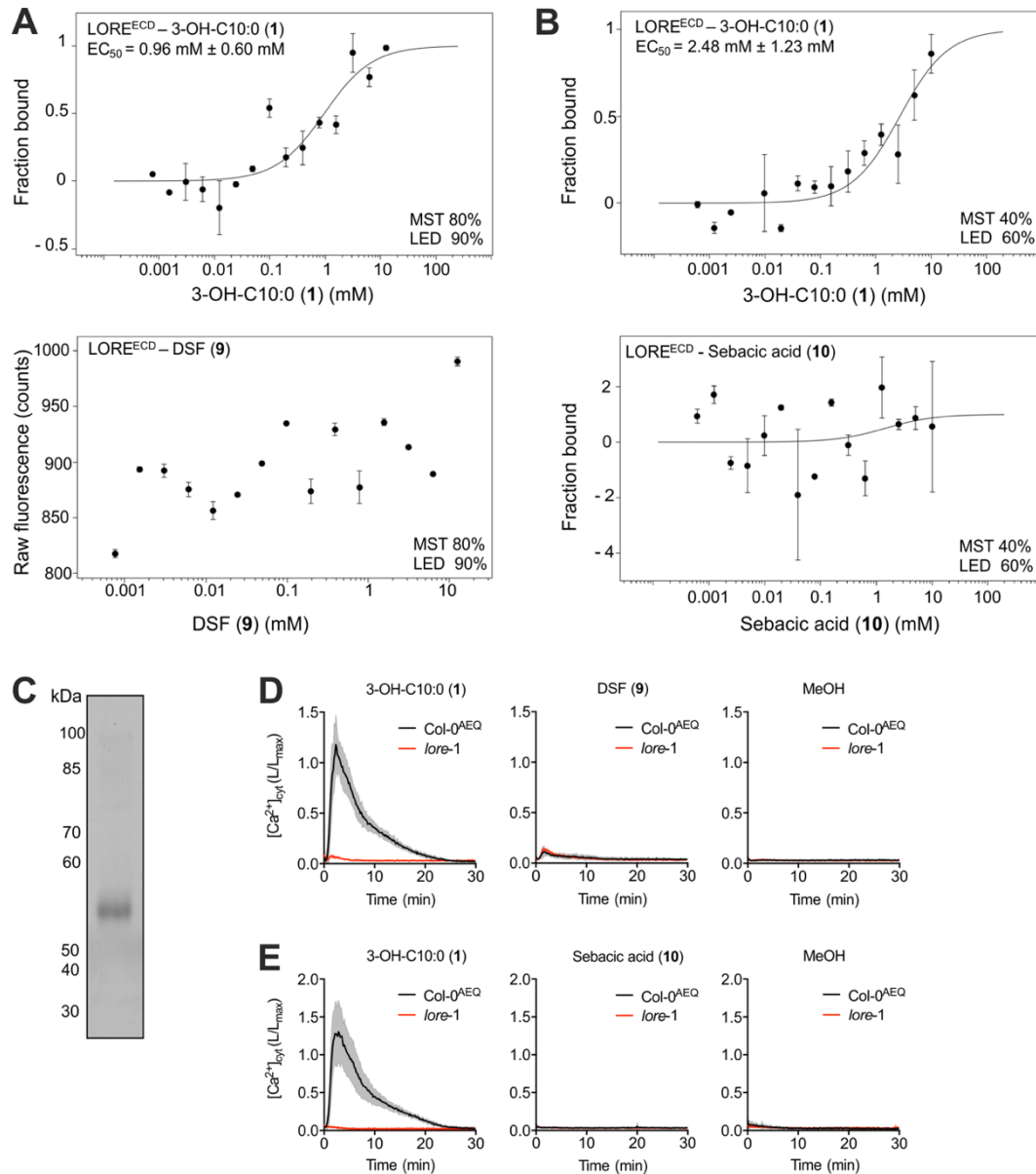


Fig. S6. LORE directly binds synthetic 3-OH-C10:0 (1) fatty acid. (A,B) Quantification of binding between 3-OH-C10:0 (1) or the elicitor-inactive control compounds (see D,E) diffusible signalling factor (DSF, 9) and sebacic acid (10) and the LORE ectodomain (LORE^{ECD}) by microscale thermophoresis (MST). Data points indicate the fraction of fluorescently labelled LORE^{ECD} bound to the compound (fraction bound) or the raw fluorescence (mean ± SE, $n=4$ (A(1)), $n=2$ (A(9)), $n=2$ (B(1)), $n=3$ (B(10))). LORE^{ECD} was fluorescently labelled using the His-Tag labelling kit (A) or the reactive amine NHS labelling kit (B). Measurements were performed at room temperature at an LED power of 90% and MST power of 80% (A) or an LED power of 60% and MST power of 40% (B). The standard error of the regression fit is $X^2=24.93$ for (A(1)), $X^2=18.49$ for (B(1)), and $X^2=214.40$ for (B(10)). (A) is the full figure of fig. 1G. The low affinity of the LORE ectodomain for its ligand 3-OH-C10:0 (1) *in vitro* likely reflects the high stringency conditions of the binding buffer (containing high salt concentrations) that were required to purify and to maintain solubility of the LORE^{ECD}. As this prevented obtaining a high-confidence K_d value, EC_{50} values are presented. (C) Coomassie stained gel of purified LORE^{ECD} used in MST assays (A,B). (D,E) [Ca²⁺]_{cyt} elevations of Arabidopsis seedlings treated with the indicated compounds (5 μM; mean ± SD, $n=6$). Experiments were repeated one (A(9)), two (A(1),B(10)), or three times (B(1),D,E) with similar results.

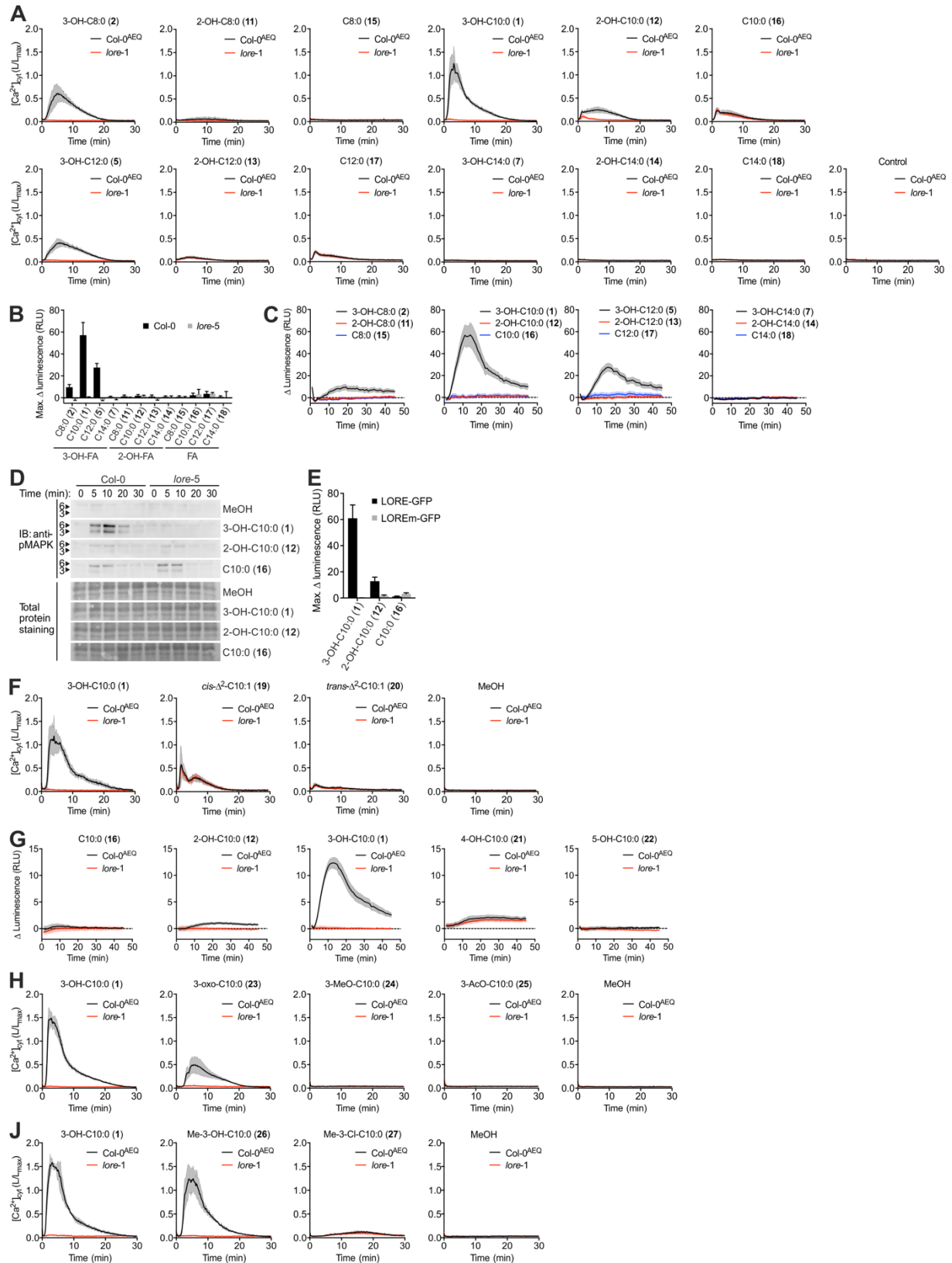


Fig. S7. The 3-hydroxyl group is critical for LORE-mediated immune sensing of mc-3-OH-FAs. (A) Kinetics of $[Ca^{2+}]_{cyt}$ elevations shown in fig. 2A. Arabidopsis seedlings were treated with the indicated FAs (5 μ M) or MeOH (mean $\pm$ SD, $n=6$). (B) Maximum ROS accumulation in Arabidopsis leaf discs treated with different FAs (5 μ M; mean $\pm$ SE, $n=6$). (C) Kinetics of ROS production in Col-0 leaf discs shown in (B). For better visibility *lore-5* is omitted. (D) Activation of AtMPK3 and AtMPK6 in Arabidopsis seedlings treated with FAs (5

μM) or MeOH was analysed by immunoblot (IB). Total protein staining shows equal loading. **(E)** Maximum ROS accumulation in leaf discs from *N. benthamiana* transiently expressing LORE-GFP or kinase-inactive LOREm-GFP elicited with 5 μM FAs (mean $\pm$ SE, $n=8$). **(F)** $[\text{Ca}^{2+}]_{\text{cyt}}$ elevations of Arabidopsis seedlings treated with different FAs (5 μM ; mean $\pm$ SD, $n=6$). **(G)** Kinetics of ROS accumulation shown in fig. 2C. Arabidopsis leaf discs were treated with the indicated FAs (5 μM ; mean $\pm$ SE, $n=8$). **(H,J)** Kinetics of $[\text{Ca}^{2+}]_{\text{cyt}}$ elevations shown in fig. 2D,E. Arabidopsis seedlings were treated with the indicated compounds (5 μM) or MeOH (mean $\pm$ SD, $n=6$ **(H)**, $n=3$ **(J)**). Experiments were repeated two **(A,B,F,G)** or three times **(D,E,H,J)** with similar results.

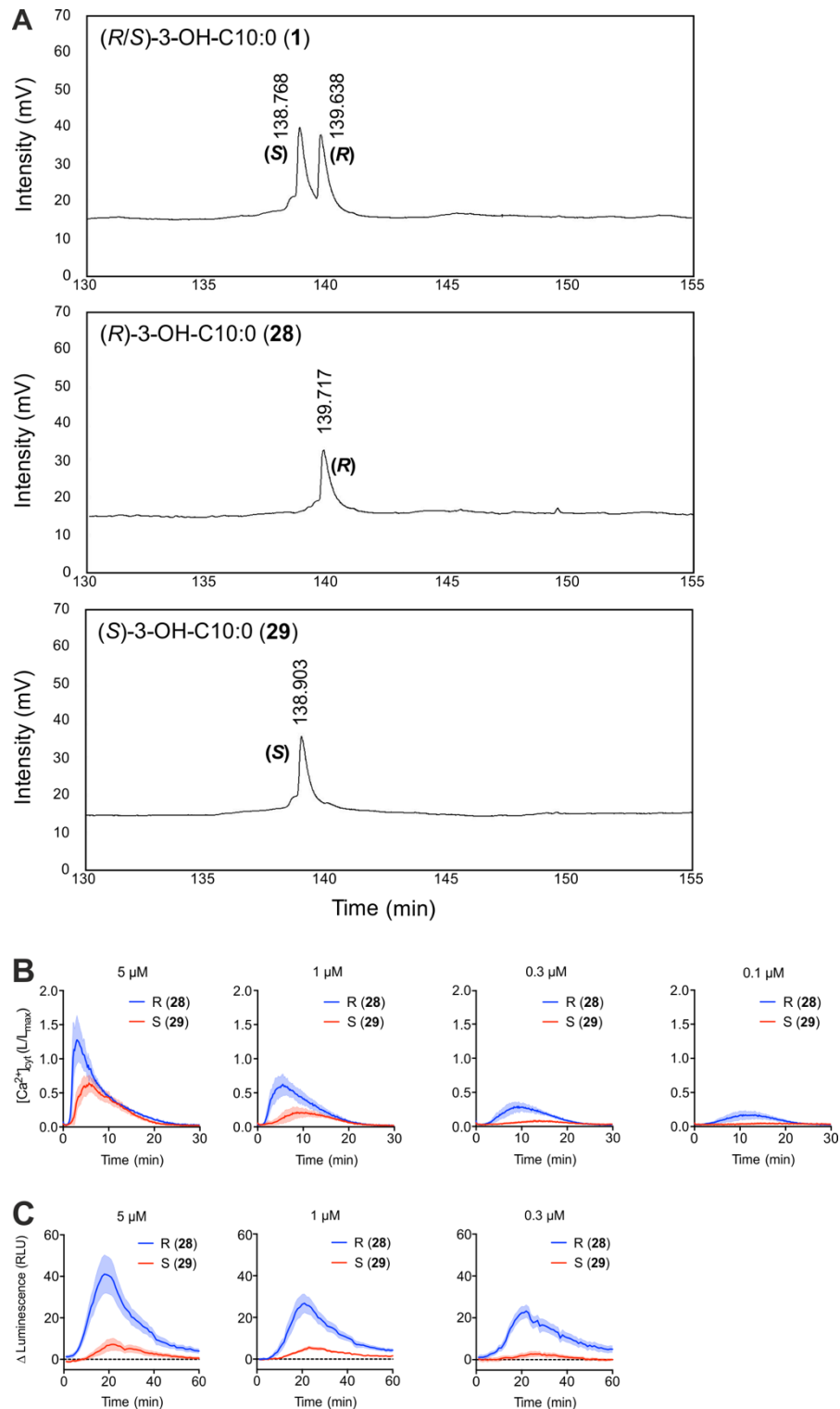


Fig. S8. (R)-3-OH-C10:0 (28) is a stronger elicitor of LORE-mediated immunity than (S)-3-OH-C10:0 (29). (A) Gas chromatographic separation and purity check of racemic 3-OH-C10:0 (1), (R)-3-OH-C10:0 (28), and (S)-3-OH-C10:0 (29). (B) Kinetics of [Ca²⁺]_{cyt} elevations shown in fig. 2F. Arabidopsis seedlings were treated with different concentrations of (R)-3-OH-C10:0 (28) or (S)-3-OH-C10:0 (29) (mean $\pm$ SD, $n=6$). (C) ROS accumulation in Arabidopsis leaf discs treated with different concentrations of (R)-3-OH-C10:0 (28) or (S)-3-OH-C10:0 (29) (mean $\pm$ SE, $n=6$). Concentrations of the stock solutions used in (B,C) were determined by quantitative NMR. Experiments were repeated two (C) or four times (B) with similar results.

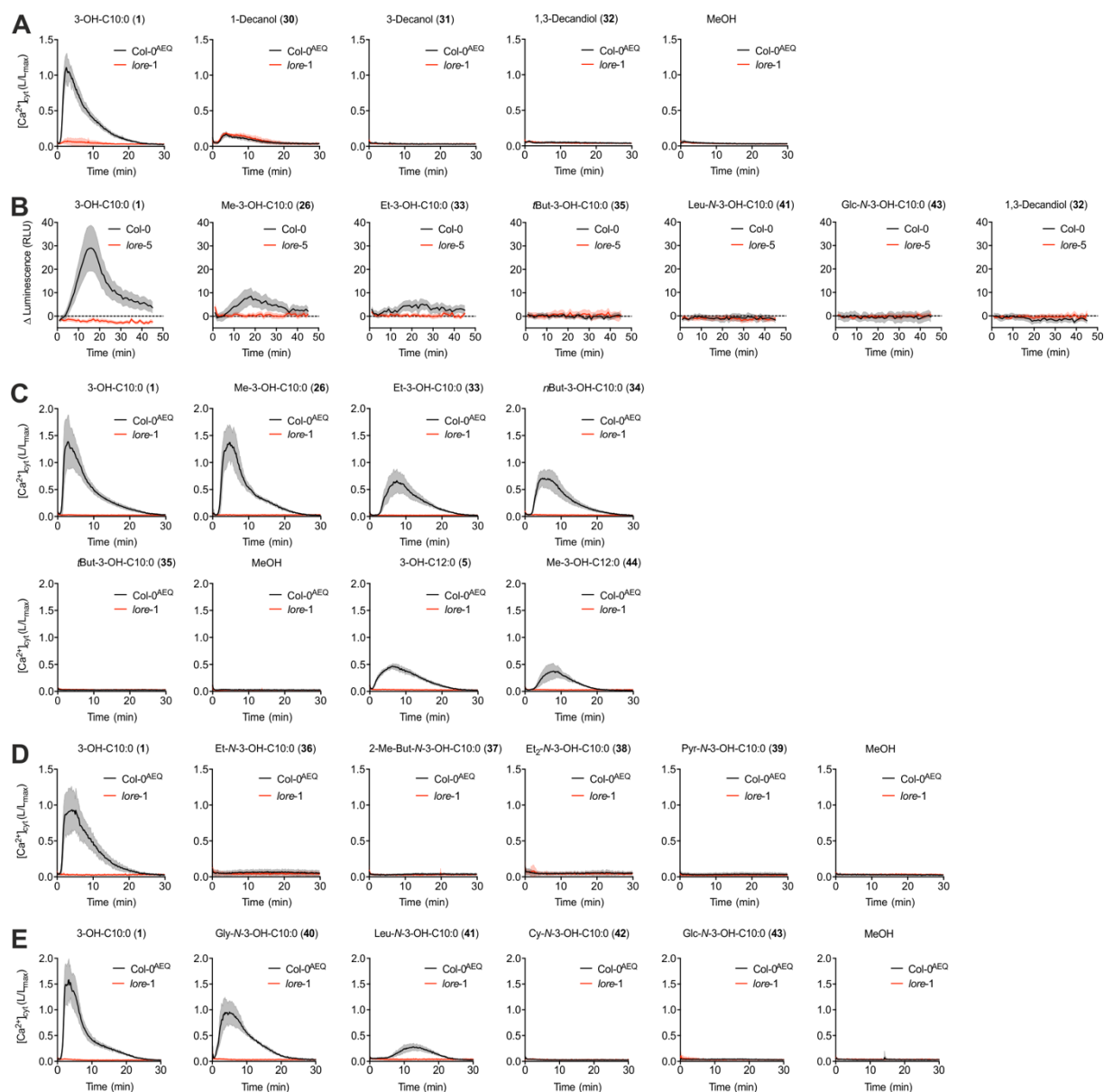


Fig. S9. Mc-3-OH-FAs in their free form show the strongest elicitor activity in Arabidopsis. (A) Kinetics of $[Ca^{2+}]_{cyt}$ elevations shown in fig. 2G. Arabidopsis seedlings were treated with the indicated compounds (5 μ M) or MeOH (mean $\pm$ SD, $n=6$). (B) ROS production in Arabidopsis leaf discs treated with the indicated compounds (5 μ M; mean $\pm$ SE, $n=6$). (C–E) Kinetics of $[Ca^{2+}]_{cyt}$ elevations shown in fig. 2H–K. Arabidopsis seedlings were treated with the indicated compounds (5 μ M) or MeOH (mean $\pm$ SD, $n=6$). (A–E) Experiments were repeated twice with similar results.

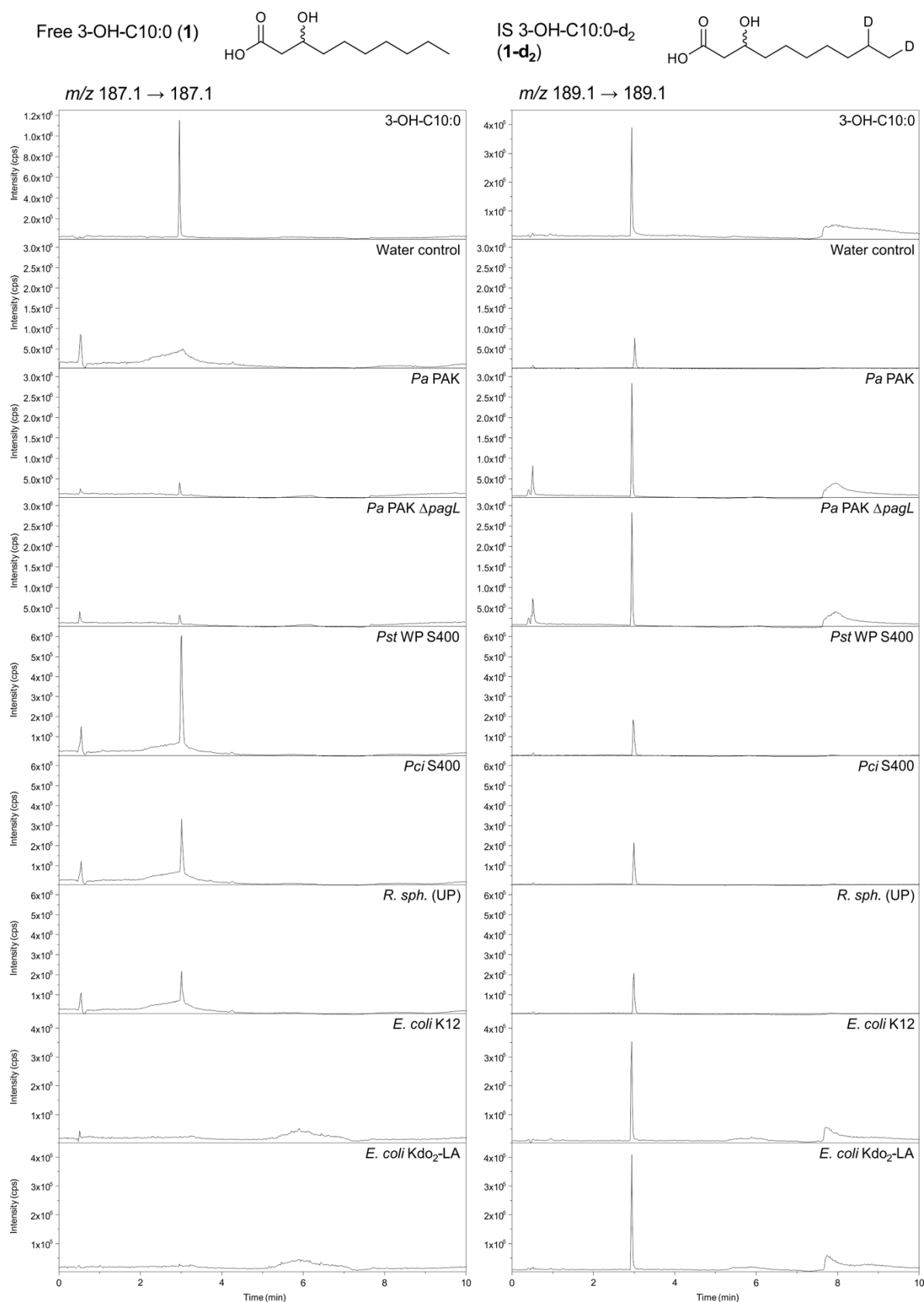


Fig. S10. Quantification of free 3-OH-C10:0 (1) by SIDA-UHPLC-MS/MS_{MRM}. HPLC-MS/MS (MRM) chromatograms (ESI[−]) of 3-OH-C10:0 (1) and its deuterium-labelled twin molecule 3-OH-C10:0-d₂ (1-d₂) applied as internal standard (IS) measured in the following samples (table S3): Controls 3-OH-C10:0 and water; lipopolysaccharide preparations *Pa* PAK, *Pa* PAK Δ *pagL*, *Pst* WP S400, *Pci* S400, *R. sph.* (UP), *E. coli* K12, *E. coli* Kdo₂-LA, and

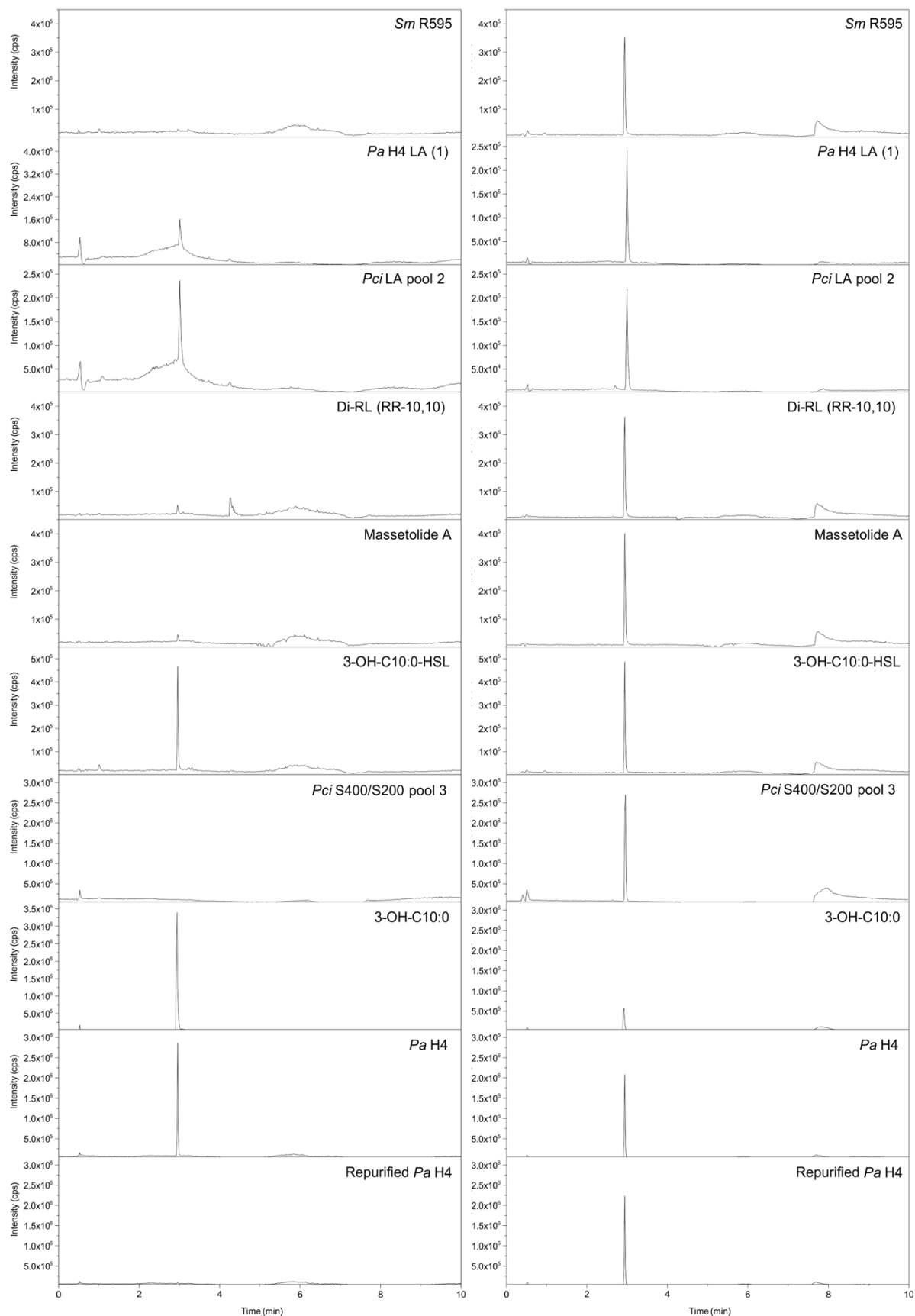


Fig. S10 (continued).

Sm R595; lipid A samples *Pa* H4 LA (1) and *Pci* LA pool 2; rhamnolipid Di-RL (RR-10,10); lipopeptide massetolide A; acyl-homoserine-lactone 3-OH-C10:0-HSL; DOC-GPC repurified lipopolysaccharide *Pci* S400/S200 pool 3; heat-detergent-promoted lipopolysaccharide repurification samples 3-OH-C10:0 control, *Pa* H4, and repurified *Pa* H4.

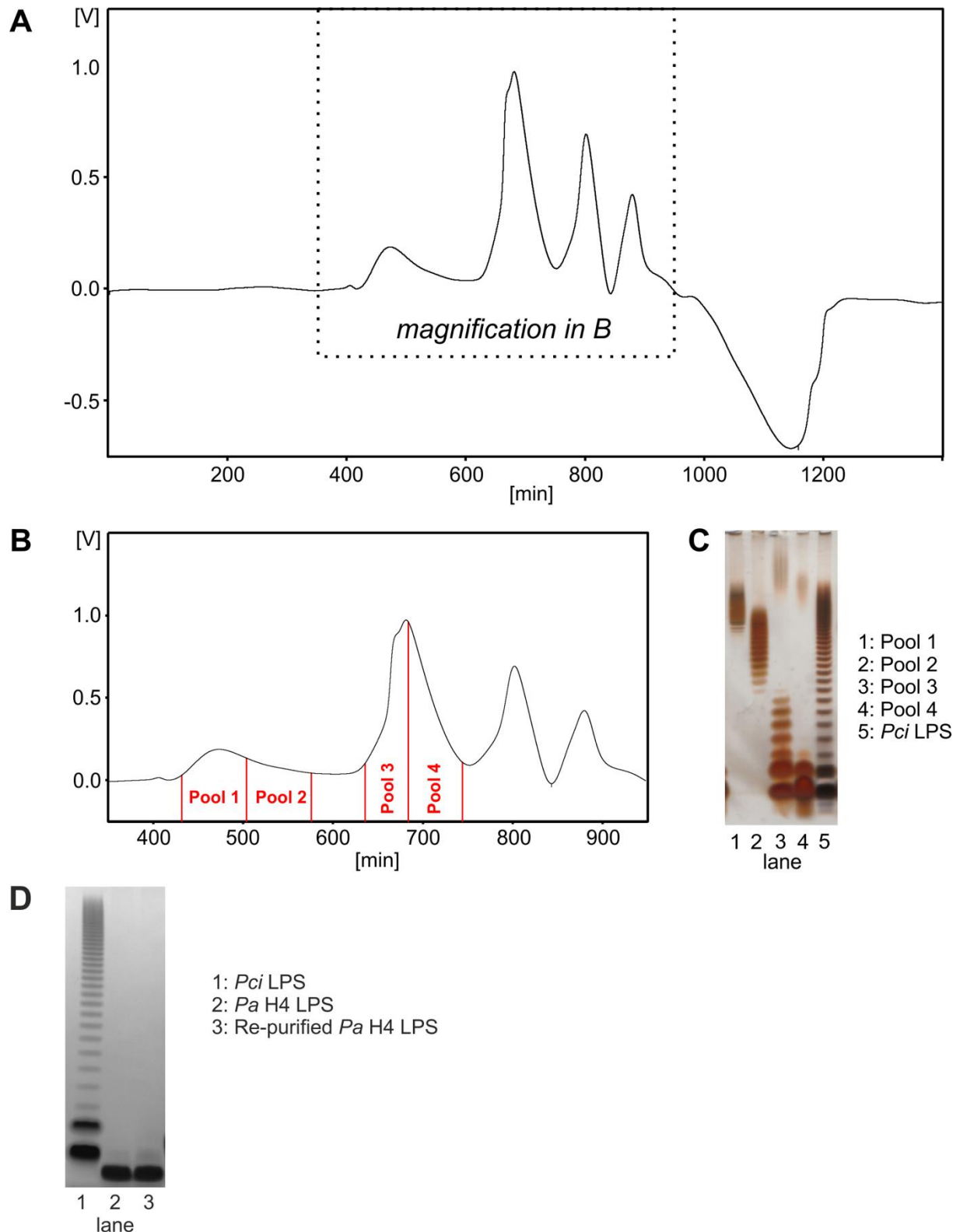


Fig. S11. DOC-GPC- and heat-detergent-promoted lipopolysaccharide repurification. (A–C) Fractionation of S400-purified lipopolysaccharide from *P. cichorii* (*Pci* S400) on Sephacryl S-200 HR using a desoxycholate (DOC)-containing buffer. The complete GPC chromatogram is depicted in (A), the indicated region is magnified in (B) with the chosen fractionation indicated. For further assays material of two comparable GPC runs was combined. (C) Silver-stained 10% Bis-Tris NuPAGE gel of these combined column pools. (D) Silver-stained 10% Bis-Tris NuPAGE gel of *P. aeruginosa* (*Pa*) H4 lipopolysaccharide before and after heat-detergent-repurification.

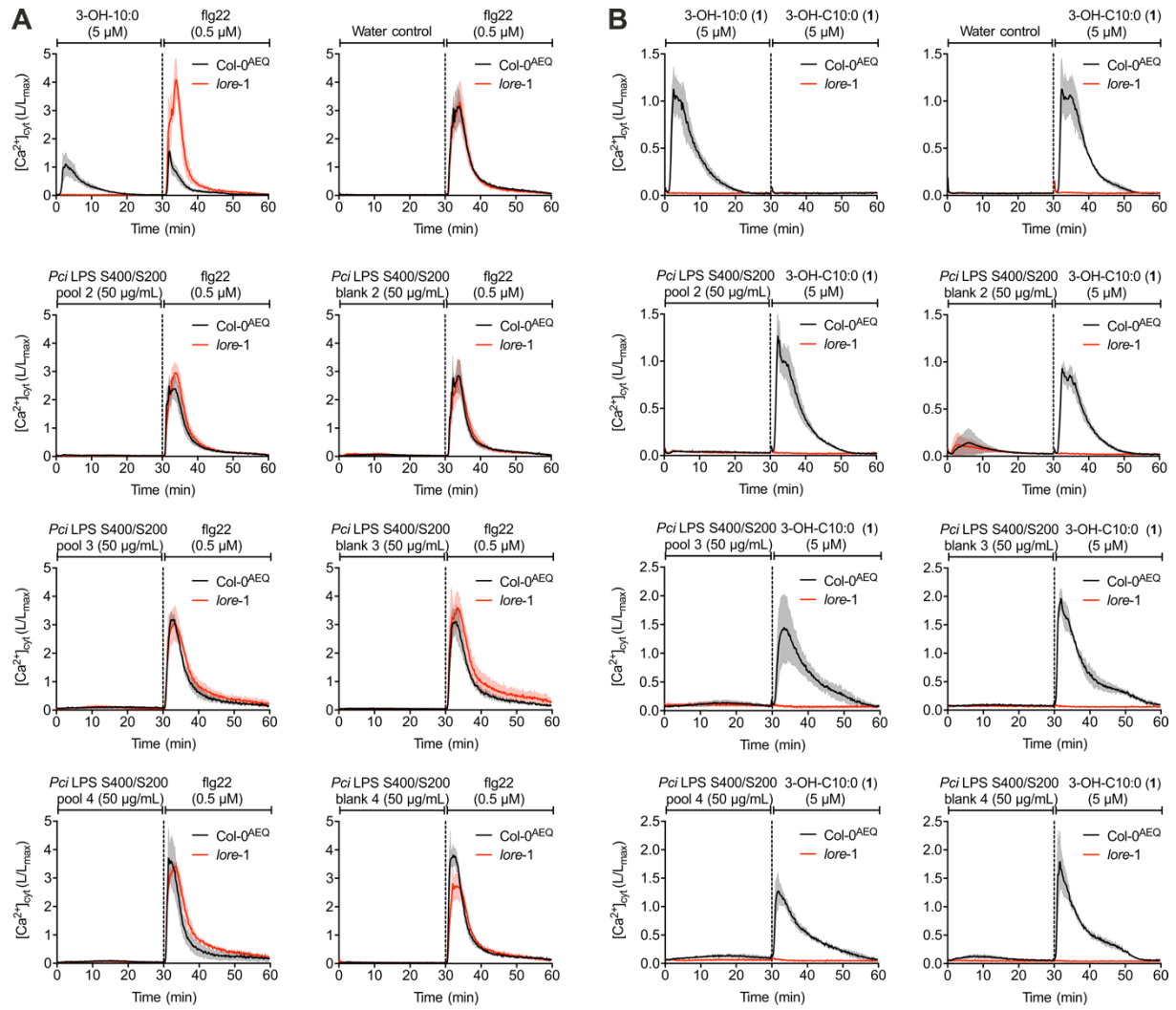


Fig. S12. DOC-GPC-repurified lipopolysaccharide does not activate LORE-mediated immunity. (A,B) $[Ca^{2+}]_{cyt}$ was measured over time in Arabidopsis seedlings treated with *P. cichorii* (*Pci*) S400/S200 lipopolysaccharide (repurified by DOC-GPC; 50 μ g/mL final concentration; table S3). After 30 min, (A) flg22 or (B) synthetic 3-OH-C10:0 (**1**) was added and the measurement continued for 30 min (mean $\pm$ SD, $n=3$ each). Full figure of fig. 3A. Note that the very weak $[Ca^{2+}]_{cyt}$ elevation observed in pool 4 correlates with the amount of free 3-OH-C10:0 (**1**) detected in that sample aliquot (table S3). The experiment was repeated once with all lipopolysaccharide pools at 25 μ g/mL and three times with pools 3 and 4 at 50 μ g/mL with essentially the same result.

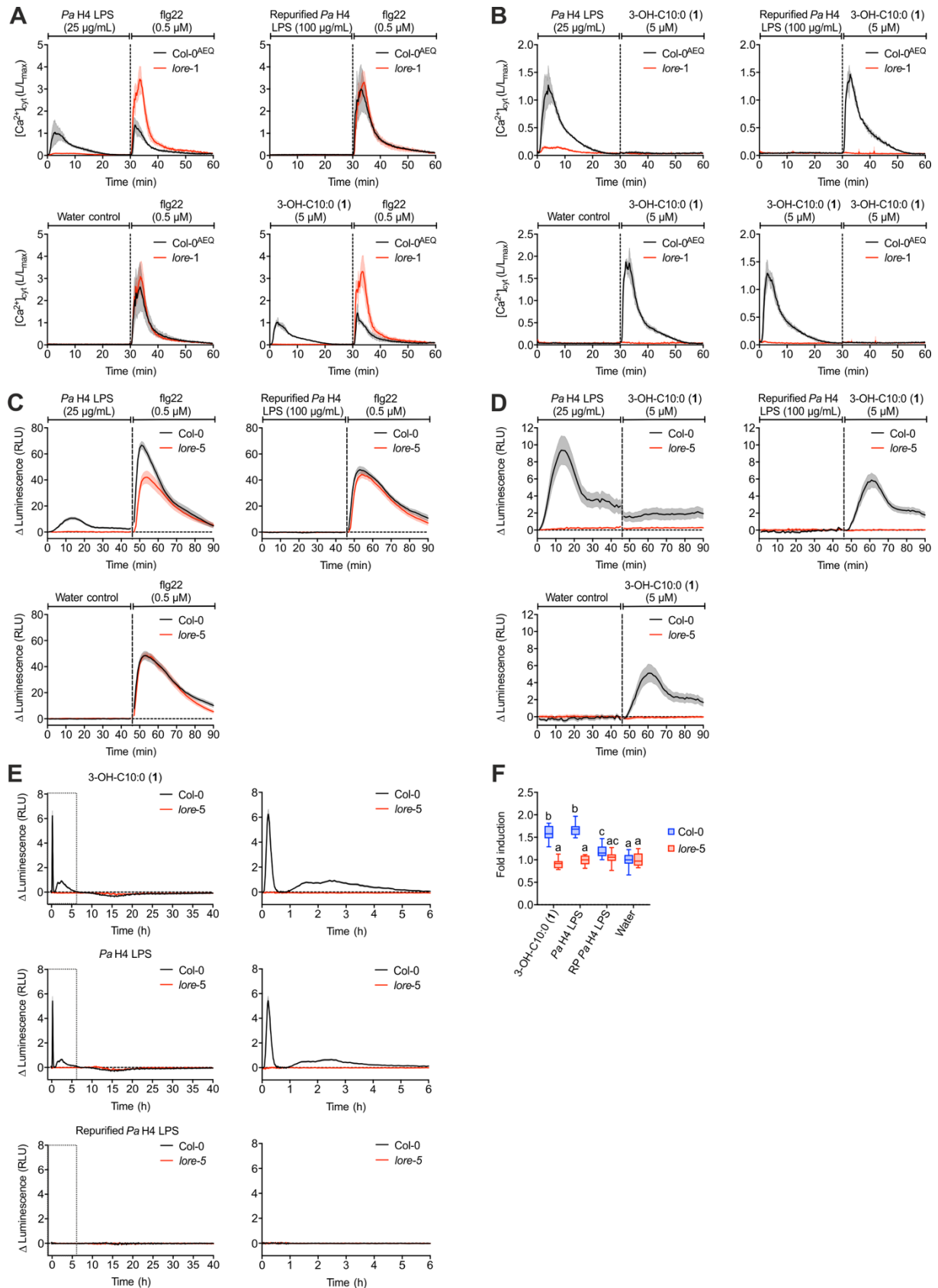


Fig. S13. Heat-detergent-repurified lipopolysaccharide does not activate LORE-mediated immunity. (A,B) $[Ca^{2+}]_{cyt}$ was measured over time in Arabidopsis seedlings treated with heat-detergent-repurified lipopolysaccharide from *P. aeruginosa* (*Pa*) H4 (100 µg/mL final concentration; table S3). After 30 min, (A) flg22 or (B) synthetic 3-OH-C10:0 (1) was added and the measurement continued for 30 min (mean $\pm$ SD, $n=3$ each). Full figure of fig. 3B. (C,D)

ROS production was measured over time in *Arabidopsis* leaf discs treated with heat-detergent-repurified lipopolysaccharide from *P. aeruginosa* (*Pa*) H4 (100 µg/mL final concentration; table S3). After 45 min, (C) flg22 or (D) synthetic 3-OH-C10:0 (**1**) was added and the measurement continued for 45 min (mean ± SE, *n*=6 each). (E) ROS production was measured over 40 h (left) in leaf discs treated with heat-detergent-repurified lipopolysaccharide from *P. aeruginosa* (*Pa*) H4 (25 µg/mL final concentration; table S3) (mean ± SE, *n*=6). For better visibility, the first 6 h (dashed lines) are enlarged on the right. (F) Induction of peroxidase activity in *Arabidopsis* leaf discs (*n*=12) 24 h after treatment with heat-detergent-repurified (RP) lipopolysaccharide from *P. aeruginosa* (*Pa*) H4 (25 µg/mL final concentration; table S3). Different letters indicate significant differences (one-way ANOVA with Tukey's post-hoc test, *p*<0.05). Experiments were repeated two (C,D,E), three (A,B), or four times (F) with similar results.

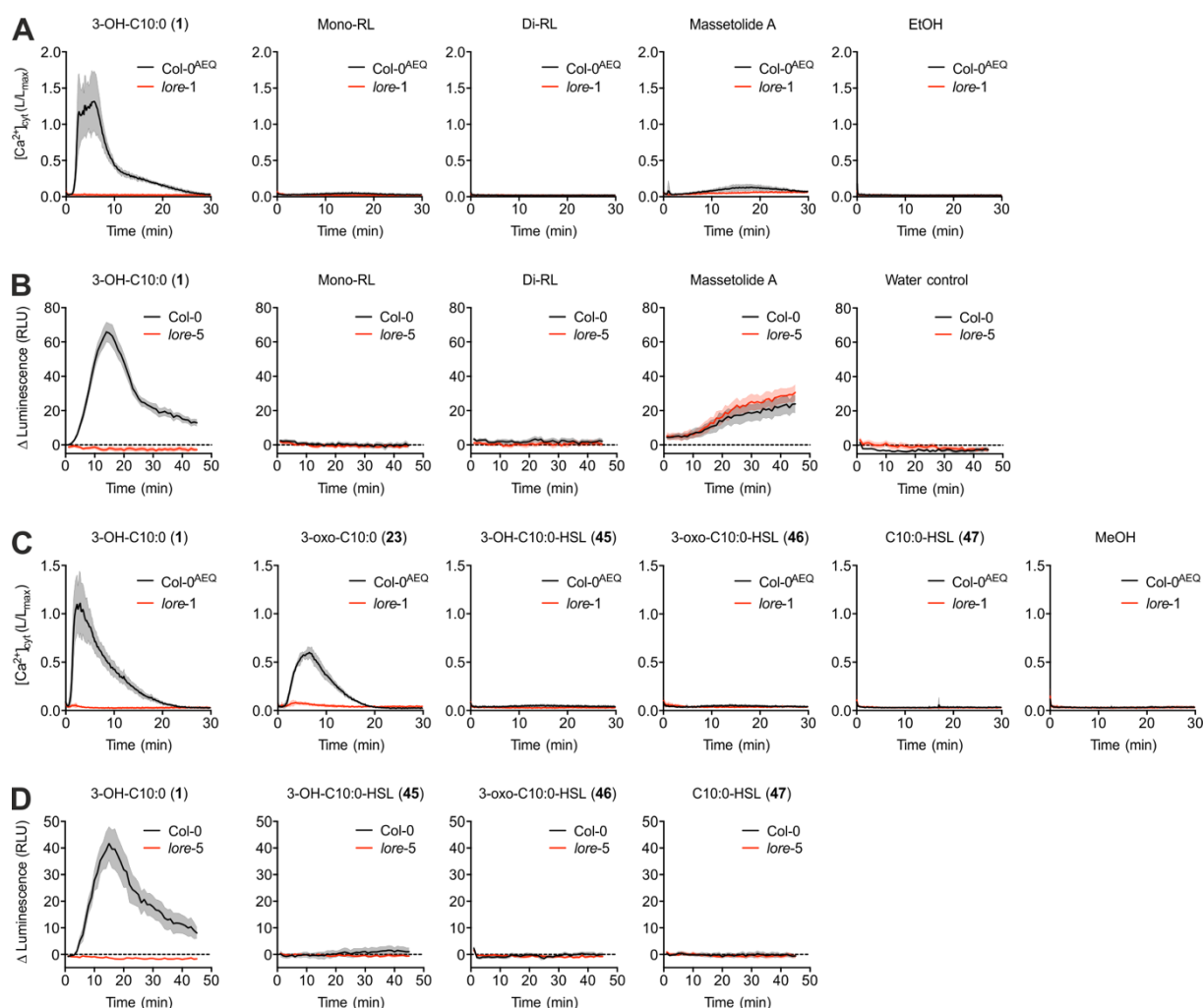


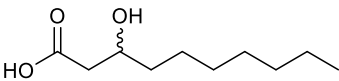
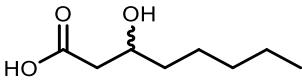
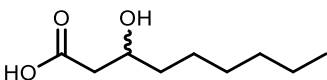
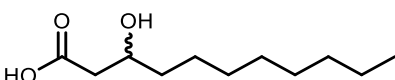
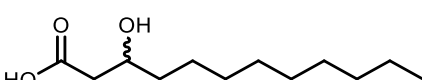
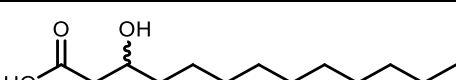
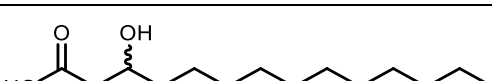
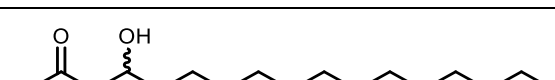
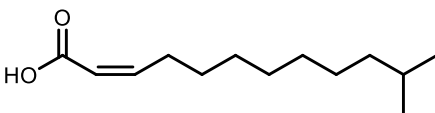
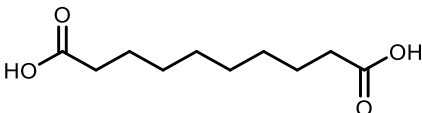
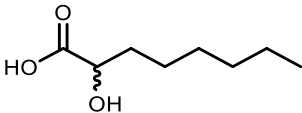
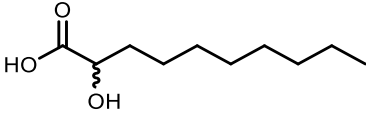
Fig. S14. Rhamnolipid, lipopeptide, and homoserine-lactone comprising 3-OH-decanoyl building blocks, but devoid of free 3-OH-C10:0 (1), do not activate LORE-mediated immune signalling. (A) Kinetics of $[Ca^{2+}]_{cyt}$ elevations shown in fig. 3D. Arabidopsis seedlings were treated with the indicated compounds (5 μ M) or the respective amount of EtOH as control (mean $\pm$ SD, $n=6$). (B) ROS production in Arabidopsis leaf discs were treated with the indicated compounds (5 μ M; mean $\pm$ SE, $n=6$). (C) Kinetics of $[Ca^{2+}]_{cyt}$ elevations shown in fig. 3E. Arabidopsis seedlings were treated with the indicated compounds (5 μ M) or the respective amount of MeOH as control (mean $\pm$ SD, $n=6$). (D) ROS production in Arabidopsis leaf discs treated with the indicated compounds (5 μ M; mean $\pm$ SE, $n=6$). (A–D) Experiments were repeated twice with similar results. RL, rhamnolipid; HSL, homoserine-lactone.

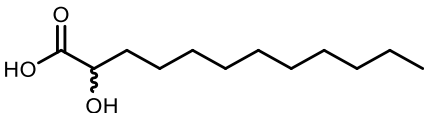
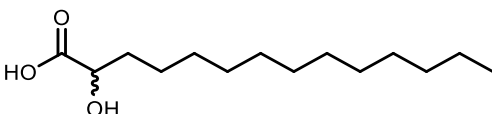
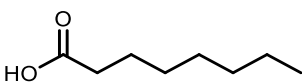
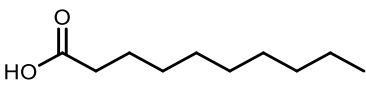
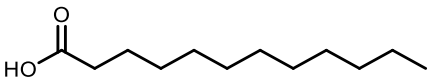
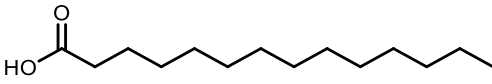
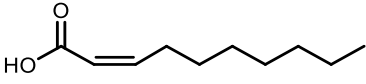
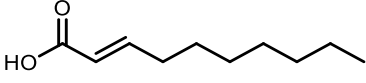
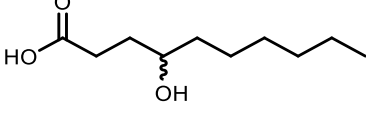
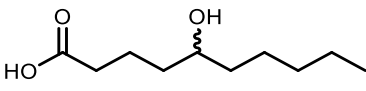
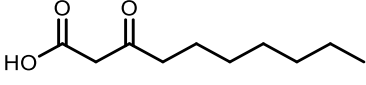
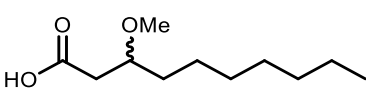
Table S1. Lipid A acylation patterns of selected bacterial species used to assess LORE-dependent immune sensing in Arabidopsis.

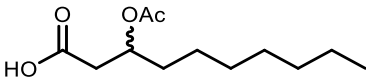
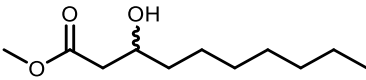
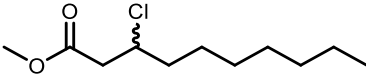
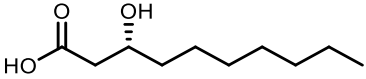
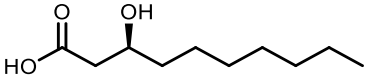
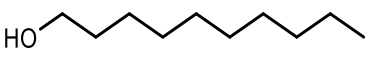
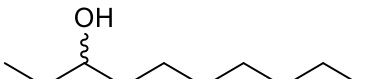
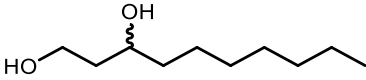
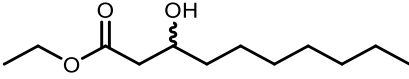
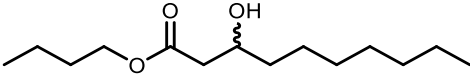
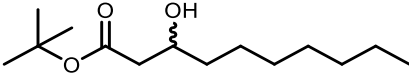
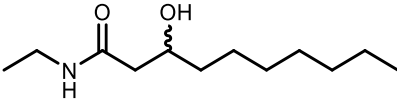
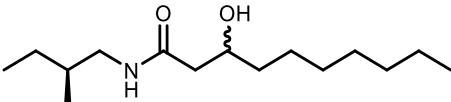
Bacterial species	Immune response in Arabidopsis	LORE dependent	Substituting acyl chains on lipid A backbone								Reference for lipid A structure
			C-3'		C-2'		C-3		C-2		
			primary ^s	secondary	primary ^s	secondary	primary ^s	secondary	primary ^s	secondary ^s	
<i>Escherichia coli</i>	No	—	(R)-3-OH-C14:0	C14:0	(R)-3-OH-C14:0	C12:0	(R)-3-OH-C14:0	—	(R)-3-OH-C14:0	—	(70)
<i>Salmonella enterica</i>	No	—	(R)-3-OH-C14:0	C14:0	(R)-3-OH-C14:0	C12:0	(R)-3-OH-C14:0	—	(R)-3-OH-C14:0	—	(70)
<i>Burkholderia cepacia</i>	No	—	(R)-3-OH-C14:0	—	(R)-3-OH-C16:0	C14:0	(R)-3-OH-C14:0	—	(R)-3-OH-C16:0	—	(71)
<i>Pseudomonas aeruginosa</i> H4	Yes	Yes	(R)-3-OH-C10:0	—	(R)-3-OH-C12:0	C12:0	((R)-3-OH-C10:0)*	—	(R)-3-OH-C12:0	(S)-2-OH-C12:0	(5)
<i>Pseudomonas syringae</i> pv. tomato DC3000	Yes	Yes	(R)-3-OH-C10:0	—	(R)-3-OH-C12:0	C12:0	((R)-3-OH-C10:0)**	—	(R)-3-OH-C12:0	(S)-2-OH-C12:0	Fig. S1
<i>Pseudomonas syringae</i> pv. tomato DC3000 Δ pagL	Yes	Yes	(R)-3-OH-C10:0	—	(R)-3-OH-C12:0	C12:0	(R)-3-OH-C10:0	—	(R)-3-OH-C12:0	(S)-2-OH-C12:0	Fig. S3
<i>Pseudomonas cichorii</i> ATCC10857/DSM50259	Yes	Yes	(R)-3-OH-C10:0	—	(R)-3-OH-C12:0	C12:0	((R)-3-OH-C10:0)*	—	(R)-3-OH-C12:0	(S)-2-OH-C12:0	Fig. S2
<i>Pseudomonas aeruginosa</i> PAK	Yes	Yes	(R)-3-OH-C10:0	—	(R)-3-OH-C12:0	C12:0	((R)-3-OH-C10:0)*	—	(R)-3-OH-C12:0	(S)-2-OH-C12:0	Fig. S3, (69)
<i>Pseudomonas aeruginosa</i> PAK Δ htrB1	Yes	Yes	(R)-3-OH-C10:0	—	(R)-3-OH-C12:0	C12:0	((R)-3-OH-C10:0)	—	(R)-3-OH-C12:0	—	Fig. S3, (69)
<i>Pseudomonas aeruginosa</i> PAK Δ htrB2	Yes	Yes	(R)-3-OH-C10:0	—	(R)-3-OH-C12:0	—	((R)-3-OH-C10:0)	—	(R)-3-OH-C12:0	(S)-2-OH-C12:0	Fig. S3, (69)
<i>Pseudomonas aeruginosa</i> PAK Δ pagL	Yes	Yes	(R)-3-OH-C10:0	—	(R)-3-OH-C12:0	C12:0	(R)-3-OH-C10:0	—	(R)-3-OH-C12:0	(S)-2-OH-C12:0	Fig. S3, (15)
<i>Xanthomonas campestris</i>	Yes	Yes	(R)-3-OH-C10:0 to (R)-3-OH-C13:0 [#]	C10:0 to C13:0 [#]	(R)-3-OH-C10:0 to (R)-3-OH-C13:0 [#]	—	(R)-3-OH-C10:0 to (R)-3-OH-C13:0 [#]	C10:0 to C13:0 [#]	(R)-3-OH-C10:0 to (R)-3-OH-C13:0 [#]	—	(72)
<i>Rhodobacter sphaeroides</i>	Yes	Yes	(R)-3-OH-C10:0	—	(R)-3-OH-C14:0	cis- Δ^7 -C14:1	(R)-3-OH-C10:0	—	3-oxo-C14:0	—	(73)
<i>Rubrivivax gelatinosus</i> Dr2 (former <i>Rhodocyclus gelatinosus</i> Dr2)	Yes	Yes	(R)-3-OH-C10:0	—	(R)-3-OH-C10:0	C12:0	(R)-3-OH-C10:0	—	(R)-3-OH-C10:0	C12:0/C14:0	(74, 75)
<i>Chromobacterium violaceum</i>	Yes	Yes	(R)-3-OH-C10:0	—	(R)-3-OH-C12:0	C12:0	(R)-3-OH-C10:0	—	(R)-3-OH-C12:0	C12:0	(75, 76)

[§]Primary 3-hydroxy acyl chains are generally in the (R)-configuration and secondary 2-hydroxy acyl chains in the (S)-configuration (7). *Mainly penta-acylated. **Mainly hexa-acylated. [#]Length heterogeneity ranging from 10 to 13 carbon atoms.

Table S2. List of synthetic compounds 1–47 tested in this study.

Compound acronym and (no.)	IUPAC name	Structural formula	Source/Reference
3-OH-C10:0 (1)	3-hydroxydecanoic acid		Matreya LLC, M-1727 & synthesized in this study
3-OH-C8:0 (2)	3-hydroxyoctanoic acid		Matreya LLC, M-1745
3-OH-C9:0 (3)	3-hydroxynonanoic acid		Matreya LLC, M-1725
3-OH-C11:0 (4)	3-hydroxyundecanoic acid		Matreya LLC, M-1729
3-OH-C12:0 (5)	3-hydroxydodecanoic acid		Matreya LLC, M-1731
3-OH-C13:0 (6)	3-hydroxytridecanoic acid		Matreya LLC, M-1733
3-OH-C14:0 (7)	3-hydroxytetradecanoic acid		Matreya LLC, M-1735
3-OH-C16:0 (8)	3-hydroxyhexadecanoic acid		Matreya LLC, M-1739
DSF (9)	<i>cis</i> -11-methyl-2-dodecenoic acid (diffusible signal factor)		Sigma-Aldrich, Taufkirchen, Germany, 42052
Sebacic acid (10)	decanedioic acid		Sigma-Aldrich, Taufkirchen, Germany, 283258
2-OH-C8:0 (11)	2-hydroxyoctanoic acid		Sigma-Aldrich, Taufkirchen, Germany, H7396
2-OH-C10:0 (12)	2-hydroxydecanoic acid		Matreya LLC, M-1758

2-OH-C12:0 (13)	2-hydroxydodecanoic acid		Matreya LLC, M-1701
2-OH-C14:0 (14)	2-hydroxytetradecanoic acid		Matreya LLC, M-1703
C8:0 (15)	octanoic acid (caprylic acid)		Sigma- Aldrich, Taufkirchen, Germany, W279900
C10:0 (16)	decanoic acid (capric acid)		Sigma- Aldrich, Taufkirchen, Germany, 21409
C12:0 (17)	dodecanoic acid (lauric acid)		Sigma- Aldrich, Taufkirchen, Germany, L4250
C14:0 (18)	tetradecanoic acid (myristic acid)		Sigma- Aldrich, Taufkirchen, Germany, M3128
<i>cis</i> -Δ ² -C10:1 (19)	(<i>Z</i>)-dec-2-enoic acid		Sigma- Aldrich, Taufkirchen, Germany, 19699
<i>trans</i> -Δ ² - C10:1 (20)	(<i>E</i>)-dec-2-enoic acid		synthesized in this study
4-OH-C10:0 (21)	4-hydroxydecanoic acid		Sigma- Aldrich, Taufkirchen, Germany, D804
5-OH-C10:0 (22)	5-hydroxydecanoic acid		Sigma- Aldrich, Taufkirchen, Germany, H135
3-oxo-C10:0 (23)	3-oxodecanoic acid		synthesized in this study
3-MeO-C10:0 (24)	3-methoxydecanoic acid		synthesized in this study

3-AcO-C10:0 (25)	3-acetoxydecanoic acid		synthesized in this study
Me-3-OH-C10:0 (26)	methyl 3-hydroxydecanoate		Matreya LLC, M-1728 & synthesized in this study
Me-3-Cl-C10:0 (27)	methyl 3-chlorodecanoate		synthesized in this study
(R)-3-OH-C10:0 (28)	(R)-3-hydroxydecanoic acid		synthesized in this study from Me-(R)-3-OH-C10:0 Carbosynth, Compton Berkshire, United Kingdom, FH24326
(S)-3-OH-C10:0 (29)	(S)-3-hydroxydecanoic acid		Chemspace, Riga, Latvia, FCH1217832
1-Decanol (30)	decan-1-ol		Sigma-Aldrich, Taufkirchen, Germany, 239763
3-Decanol (31)	decan-3-ol		Sigma-Aldrich, Taufkirchen, Germany, CDS001495
1,3-Decandiol (32)	decane-1,3-diol		synthesized in this study
Et-3-OH-C10:0 (33)	ethyl 3-hydroxydecanoate		synthesized in this study
nBut-3-OH-C10:0 (34)	butyl 3-hydroxydecanoate		synthesized in this study
tBut-3-OH-C10:0 (35)	tert-butyl 3-hydroxydecanoate		synthesized in this study
Et-N-3-OH-C10:0 (36)	N-ethyl-3-hydroxydecanamide		synthesized in this study
2-Me-But-N-3-OH-C10:0 (37)	3-hydroxy-N-((S)-2-methylbutyl)decanamide		synthesized in this study

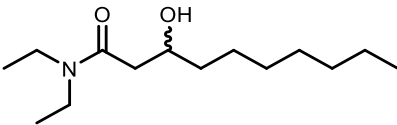
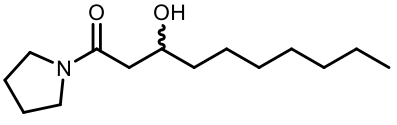
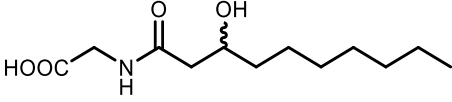
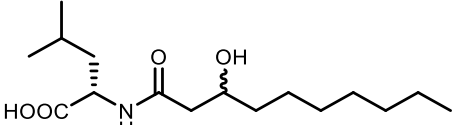
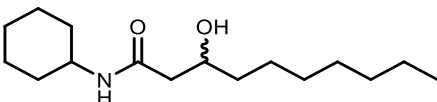
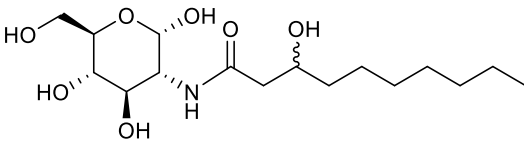
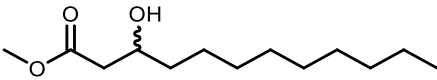
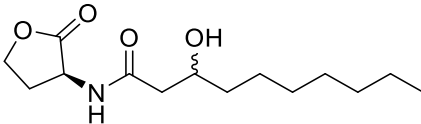
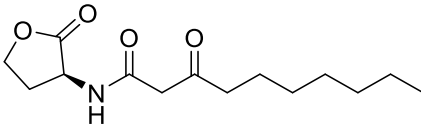
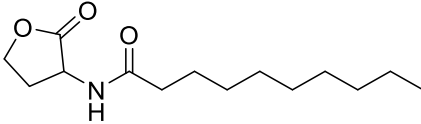
Et ₂ -N-3-OH-C10:0 (38)	<i>N,N</i> -diethyl-3-hydroxydecanamide		synthesized in this study
Pyr-N-3-OH-C10:0 (39)	3-hydroxy-1-(pyrrolidin-1-yl)decan-1-one		synthesized in this study
Gly-N-3-OH-C10:0 (40)	(3-hydroxydecanoyl)glycine		synthesized in this study
Leu-N-3-OH-C10:0 (41)	(3-hydroxydecanoyl)-L-leucine		synthesized in this study
Cy-N-3-OH-C10:0 (42)	<i>N</i> -cyclohexyl-3-hydroxydecanamide		synthesized in this study
Glc-N-3-OH-C10:0 (43)	3-hydroxy- <i>N</i> -((2 <i>S</i> ,3 <i>R</i> ,4 <i>R</i> ,5 <i>S</i> ,6 <i>R</i>)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2 <i>H</i> -pyran-3-yl)decanamide		synthesized in this study
Me-3-OH-C12:0 (44)	methyl 3-hydroxydodecanoate		Matreya LLC, M-1732
3-OH-C10:0-HSL (45)	<i>N</i> -(3-hydroxydecanoyl)-L-homoserine lactone		Univ. of Nottingham HC10
3-oxo-C10:0-HSL (46)	<i>N</i> -(3-oxodecanoyl)-L-homoserine lactone		Sigma-Aldrich, Taufkirchen, Germany, O9014
C10:0-HSL (47)	<i>N</i> -decanoyl-DL-homoserine lactone		Sigma-Aldrich, Taufkirchen, Germany, 17248

Table S3. Quantification of free 3-OH-C10:0 (1) in lipopolysaccharide, lipid A, lipopeptide, rhamnolipid, and acyl-homoserine-lactone samples.

Sample	Bacterial strain	Isolation procedure [#]	Further purification	Source/Reference	Sample conc. (for quantification)	Free 3-OH-C10 (μM)	Sample conc. (for biological assays)	Immune response in Arabidopsis	LORE-dependent
Controls									
Water control	–	–	–	–	–	<LOQ	–	No	–
3-OH-C10:0	–	–	–	–	4 μM	2.49	1 – 5 μM (mostly)	Yes	Yes
Lipopolysaccharide									
<i>Pa</i> PAO1	<i>Pseudomonas aeruginosa</i> PAO1	Phenol/water	–	(5, 77)	100 μg/mL	0.50 ^{\$}	20 – 25 μg/mL	Yes	Yes
<i>Pa</i> H4	<i>Pseudomonas aeruginosa</i> H4	PCP	–	(5, 78)	100 μg/mL	0.95 ^{\$}	20 – 25 μg/mL	Yes	Yes
<i>Pa</i> PAK	<i>Pseudomonas aeruginosa</i> PAK	Phenol/water	–	Robert Ernst, (69)	100 μg/mL	0.42 ^{\$}	20 – 25 μg/mL	Yes	Yes
<i>Pa</i> PAK Δ <i>htrB1</i>	<i>Pseudomonas aeruginosa</i> PAK Δ <i>htrB1</i>	Phenol/water	–	Robert Ernst, (69)	100 μg/mL	0.49 ^{\$}	20 – 25 μg/mL	Yes	Yes
<i>Pa</i> PAK Δ <i>htrB2</i>	<i>Pseudomonas aeruginosa</i> PAK Δ <i>htrB2</i>	Phenol/water	–	Robert Ernst, (69)	100 μg/mL	0.35 ^{\$}	20 – 25 μg/mL	Yes	Yes
<i>Pa</i> PAK Δ <i>pagL</i>	<i>Pseudomonas aeruginosa</i> PAK Δ <i>pagL</i>	Phenol/water	–	Robert Ernst, (15)	100 μg/mL	0.34 ^{\$}	20 – 25 μg/mL	Yes	Yes
<i>Pst</i> WP	<i>Pseudomonas syringae</i> pv. <i>tomato</i> DC3000	Phenol/water	–	Ranf/Gisch, this study	100 μg/mL	0.14 ^{\$}	20 – 25 μg/mL	Yes	Yes
<i>Pst</i> WP S400	<i>Pseudomonas syringae</i> pv. <i>tomato</i> DC3000	Phenol/water	GPC S400	Ranf/Gisch, this study	100 μg/mL	2.71 ^{\$}	20 – 25 μg/mL	Yes	Yes
<i>Pst</i> PP-sup sed2	<i>Pseudomonas syringae</i> pv. <i>tomato</i> DC3000	1) Phenol/water 2) PCP	–	Ranf/Gisch, this study	100 μg/mL	0.30 ^{\$}	20 – 25 μg/mL	Yes	Yes
<i>Pst</i> PP-sup sed2 S400	<i>Pseudomonas syringae</i> pv. <i>tomato</i> DC3000	1) Phenol/water 2) PCP	GPC S400	Ranf/Gisch, this study	100 μg/mL	1.64 ^{\$}	20 – 25 μg/mL	Yes	Yes
<i>Pst</i> PP-sup sed3	<i>Pseudomonas syringae</i> pv. <i>tomato</i> DC3000	1) Phenol/water 2) PCP	–	Ranf/Gisch, this study	100 μg/mL	0.30 ^{\$}	20 – 25 μg/mL	Yes	Yes
<i>Pst</i> PP-sup sed3 S400	<i>Pseudomonas syringae</i> pv. <i>tomato</i> DC3000	1) Phenol/water 2) PCP	GPC S400	Ranf/Gisch, this study	100 μg/mL	1.95 ^{\$}	20 – 25 μg/mL	Yes	Yes

Sample	Bacterial strain	Isolation procedure [#]	Further purification	Source/Reference	Sample conc. (for quantification)	Free 3-OH-C10 (μM)	Sample conc. (for biological assays)	Immune response in Arabidopsis	LORE-dependent
<i>Pst</i> PP-sed sed1	<i>Pseudomonas syringae</i> pv. <i>tomato</i> DC3000	1) Phenol/water 2) PCP	–	Ranf/Gisch, this study	100 μg/mL	0.31 ^{\$}	20 – 25 μg/mL	Yes	Yes
<i>Pst</i> PP-sed sed2	<i>Pseudomonas syringae</i> pv. <i>tomato</i> DC3000	1) Phenol/water 2) PCP	–	Ranf/Gisch, this study	100 μg/mL	0.23 ^{\$}	20 – 25 μg/mL	Yes	Yes
<i>Pst</i> PCP	<i>Pseudomonas syringae</i> pv. <i>tomato</i> DC3000	1) Phenol/water 2) PCP	–	Ranf/Gisch, (5)	100 μg/mL	0.38 ^{\$}	20 – 50 μg/mL	Yes	Yes
<i>Pst</i> Δ pagL PP-sup sed	<i>Pseudomonas syringae</i> pv. <i>tomato</i> DC3000 Δ pagL	1) Phenol/water 2) PCP		Ranf, this study	100 μg/mL	0.41 ^{\$}	20 – 50 μg/mL	Yes	Yes
<i>Pst</i> Δ pagL PP-sed sed	<i>Pseudomonas syringae</i> pv. <i>tomato</i> DC3000 Δ pagL	1) Phenol/water 2) PCP		Ranf, this study	100 μg/mL	0.33 ^{\$}	20 – 50 μg/mL	Yes	Yes
<i>Pci</i>	<i>Pseudomonas cichorii</i> ATCC10857/DSM50259	Phenol/water	–	Ranf/Gisch, this study	100 μg/mL	0.16 ^{\$}	20 – 25 μg/mL	Yes	Partially
<i>Pci</i> S400	<i>Pseudomonas cichorii</i> ATCC10857/DSM50259	Phenol/water	GPC S400	Ranf/Gisch, this study	100 μg/mL	1.12 ^{\$}	20 – 25 μg/mL	Yes	Yes
<i>R. sph.</i>	<i>Rhodobacter sphaeroides</i>	–	–	InvivoGen	100 μg/mL	1.97 ^{\$}	20 – 25 μg/mL	Yes	Yes
<i>R. sph.</i> (UP)	<i>Rhodobacter sphaeroides</i>	–	Ultrapure	InvivoGen	100 μg/mL	0.62 ^{\$}	20 – 25 μg/mL	Yes	Yes
<i>R. gel.</i>	<i>Rubrivivax gelatinosus</i> Dr2	PCP	–	(74)	100 μg/mL	2.15 ^{\$}	20 – 25 μg/mL	Yes	Yes
<i>C. viol.</i>	<i>Chromobacterium violaceum</i>	Phenol/water	–	Chris Galanos, (79)	100 μg/mL	0.64 ^{\$}	20 – 25 μg/mL	Yes	Yes
<i>E. coli</i> O55:B5	<i>Escherichia coli</i> O55:B5	–	Ultrapure	InvivoGen	1 mg/mL	<LOQ	20 – 50 μg/mL	No	–
<i>E. coli</i> K12	<i>Escherichia coli</i> K12	–	Ultrapure	InvivoGen	1 mg/mL	<LOQ	20 – 50 μg/mL	No	–
<i>Pa</i> F1	<i>P. aeruginosa</i> Fisher-type 1 (F1) (Habs 6)	Phenol/water	–	(5, 80, 81)	100 μg/mL	0.57 ^{\$}	20 – 50 μg/mL	Yes	Yes
<i>Pa</i> PAO1 Δ algC	<i>Pseudomonas aeruginosa</i> PAO1 Δ algC	PCP	–	(5, 77)	100 μg/mL	0.28 ^{\$}	20 – 50 μg/mL	Yes	Yes
<i>Pa</i> R5	<i>Pseudomonas aeruginosa</i> R5	PCP	–	(5, 82)	100 μg/mL	0.76 ^{\$}	20 – 50 μg/mL	Yes	Yes
<i>Pa</i> PAN1	<i>Pseudomonas aeruginosa</i> PAN1	PCP	–	(5, 83)	100 μg/mL	0.37 ^{\$}	20 – 50 μg/mL	Yes	Yes
<i>Pa</i> PAC 1R Δ algC	<i>Pseudomonas aeruginosa</i> PAC 1R Δ algC	PCP	–	(5, 77)	100 μg/mL	1.06 ^{\$}	20 – 50 μg/mL	Yes	Yes
<i>Psa</i>	<i>Pseudomonas syringae</i> pv. <i>apii</i>	Phenol/water	–	(5)	100 μg/mL	0.43 ^{\$}	20 – 50 μg/mL	Yes	Yes

Sample	Bacterial strain	Isolation procedure [#]	Further purification	Source/Reference	Sample conc. (for quantification)	Free 3-OH-C10 (μM)	Sample conc. (for biological assays)	Immune response in Arabidopsis	LORE-dependent
<i>Palc</i>	<i>Pseudomonas alcaligenes</i> 537	Phenol/water	–	(5)	100 μg/mL	0.26 ^{\$}	20 – 50 μg/mL	Yes	Yes
<i>Pfluor</i>	<i>Pseudomonas fluorescens</i> ATCC49271	PCP	–	(5, 84)	100 μg/mL	0.28 ^{\$}	20 – 50 μg/mL	Yes	Yes
<i>Xcm</i> (1)	<i>Xanthomonas campestris</i> pv. <i>malvacearum</i> GSPB1386	Phenol/water	–	(5, 85)	100 μg/mL	0.09 ^{\$}	20 – 50 μg/mL	Yes	Yes
<i>Xcm</i> (2)	<i>Xanthomonas campestris</i> pv. <i>malvacearum</i> GSPB2388	Phenol/water	–	(5, 85)	100 μg/mL	0.11 ^{\$}	20 – 50 μg/mL	Yes	Yes
<i>Xcph</i>	<i>Xanthomonas campestris</i> pv. <i>phaseoli</i> var. <i>fuscans</i> GSPB271	Phenol/water	–	(5, 85)	100 μg/mL	0.03 ^{\$}	20 – 50 μg/mL	Yes	Yes
<i>Xcb</i>	<i>Xanthomonas campestris</i> pv. <i>begoniae</i> GSPB525	Phenol/water	–	(5, 86)	100 μg/mL	0.17 ^{\$}	20 – 50 μg/mL	Yes	Yes
<i>Bcp</i>	<i>Burkholderia cepacia</i> GIFU645/ATCC25416	Phenol/water	–	(5, 87)	1 mg/mL	<LOQ	20 – 50 μg/mL	No	–
<i>Bpm</i>	<i>Burkholderia pseudomallei</i> GIFU12046/3P-62	Phenol/water	–	(5, 88)	1 mg/mL	<LOQ	20 – 50 μg/mL	No	–
<i>E. coli</i> O111:B4	<i>Escherichia coli</i> O111:B4	–	Ultrapure	InvivoGen	1 mg/mL	<LOQ	20 – 50 μg/mL	No	–
<i>E. coli</i> Kdo ₂ -LA	<i>Escherichia coli</i> KPM 53	PCP	HPLC	(5, 89)	1 mg/mL	<LOQ	20 – 50 μg/mL	No	–
<i>Sm</i> Typh	<i>Salmonella enterica</i> sv. Typhimurium	–	TLRgrade	Enzo Life Sciences	1 mg/mL	<LOQ	20 – 50 μg/mL	No	–
<i>Sm</i> Minnesota	<i>Salmonella enterica</i> Minnesota	–	TLRgrade	Enzo Life Sciences	1 mg/mL	<LOQ	20 – 50 μg/mL	No	–
<i>Sm</i> R595	<i>Salmonella enterica</i> sv. Minnesota R595	–	Ultrapure	InvivoGen	1 mg/mL	<LOQ	20 – 50 μg/mL	No	–
<i>Pa</i> H4 LPS-OH	<i>P. aeruginosa</i> H4	PCP	Hydrazinolysis	Ranf/Gisch, (5)	1 mg/mL	<LOQ	20 – 50 μg/mL	No	–
<i>Pa</i> H4 core OS	<i>P. aeruginosa</i> H4	PCP	1) NaAc hydrolysis 2) GPC of core oligo-saccharides	Ranf/Gisch, (5)	1 mg/mL	<LOQ	20 – 50 μg/mL	No	–
Lipid A									
<i>Pa</i> H4 LA (1)	<i>P. aeruginosa</i> H4	1) Phenol/water 2) PCP	1) NaAc hydrolysis 2) HPLC	Ranf/Gisch, (5)	100 μg/mL	0.34 ^{\$}	25 μg/mL	Yes	Yes

Sample	Bacterial strain	Isolation procedure [#]	Further purification	Source/Reference	Sample conc. (for quantification)	Free 3-OH-C10 (µM)	Sample conc. (for biological assays)	Immune response in Arabidopsis	LORE-dependent
<i>Pa</i> H4 LA (2)	<i>P. aeruginosa</i> H4	1) Phenol/water 2) PCP	1) NaAc hydrolysis 2) HPLC	Ranf/Gisch, (5)	100 µg/mL	0.13 ^{\$}	25 µg/mL	Yes	Yes
<i>Pst</i> WP LA pool 1	<i>Pseudomonas syringae</i> pv. <i>tomato</i> DC3000	Phenol/water	1) NaAc hydrolysis 2) HPLC	Ranf/Gisch, this study	100 µg/mL	0.05 ^{\$}	25 µg/mL	Very weak	Yes
<i>Pst</i> WP LA pool 2	<i>Pseudomonas syringae</i> pv. <i>tomato</i> DC3000	Phenol/water	1) NaAc hydrolysis 2) HPLC	Ranf/Gisch, this study	100 µg/mL	0.05 ^{\$}	25 µg/mL	Weak	Yes
<i>Pst</i> WP LA pool 3	<i>Pseudomonas syringae</i> pv. <i>tomato</i> DC3000	Phenol/water	1) NaAc hydrolysis 2) HPLC	Ranf/Gisch, this study	100 µg/mL	0.07 ^{\$}	25 µg/mL	Weak	Yes
<i>Pst</i> WP LA pool 4	<i>Pseudomonas syringae</i> pv. <i>tomato</i> DC3000	Phenol/water	1) NaAc hydrolysis 2) HPLC	Ranf/Gisch, this study	100 µg/mL	0.14 ^{\$}	25 µg/mL	Yes	Yes
<i>Pst</i> WP LA pool 5	<i>Pseudomonas syringae</i> pv. <i>tomato</i> DC3000	Phenol/water	1) NaAc hydrolysis 2) HPLC	Ranf/Gisch, this study	100 µg/mL	0.23 ^{\$}	25 µg/mL	Yes	Yes
<i>Pst</i> WP LA pool 6	<i>Pseudomonas syringae</i> pv. <i>tomato</i> DC3000	Phenol/water	1) NaAc hydrolysis 2) HPLC	Ranf/Gisch, this study	100 µg/mL	0.16 ^{\$}	25 µg/mL	Yes	Yes
<i>Pci</i> LA pool 1	<i>Pseudomonas cichorii</i> ATCC10857/DSM50259	Phenol/water	1) NaAc hydrolysis 2) HPLC	Ranf/Gisch, this study	100 µg/mL	0.35 ^{\$}	25 µg/mL	Yes	Yes
<i>Pci</i> LA pool 2	<i>Pseudomonas cichorii</i> ATCC10857/DSM50259	Phenol/water	1) NaAc hydrolysis 2) HPLC	Ranf/Gisch, this study	100 µg/mL	0.76 ^{\$}	25 µg/mL	Yes	Yes
Rhamnolipid & Lipopeptide									
EtOH control	–	–	–	–	–	<LOQ	–	No	–
Mono-RL (R-10,10)	<i>P. aeruginosa</i> PA14	Centrifugal partition chromatography	–	Dorey/Hubert, (13, 44)	5 mM	0.28	5 µM	No	–
Di-RL (RR-10,10)	<i>P. aeruginosa</i> PA14	Centrifugal partition chromatography	–	Dorey/Hubert, (13, 44)	5 mM	0.05	5 µM	No	–
Massetolide A	<i>P. fluorescens</i> strain SS101	–	–	Dorey/Ongena/Raaijmakers, (12)	1 mM	0.030	5 µM	Weak	Partially
Acyl-homoserine-lactone									
MeOH control	–	–	–	–	–	<LOQ	–	No	–
C10:0-HSL	synthetic	–	–	Sigma-Aldrich	1 mg/mL (3.92 mM)	<LOQ	5 µM	No	–

Sample	Bacterial strain	Isolation procedure [#]	Further purification	Source/Reference	Sample conc. (for quantification)	Free 3-OH-C10 (μM)	Sample conc. (for biological assays)	Immune response in Arabidopsis	LORE-dependent
3-OH-C10:0-HSL	synthetic	–	–	University of Nottingham	1 mg/mL (3.69 mM)	0.80	5 μM	No	–
3-Oxo-C10:0-HSL	synthetic	–	–	Sigma-Aldrich	1 mg/mL (3.71 mM)	<LOQ	5 μM	No	–
DOC-GPC lipopolysaccharide repurification									
Water control	–	–	–	–	–	<LOQ		No	–
3-OH-C10:0	–	–	–	–	20 μM	24.89	5 μM	Yes	Yes
<i>Pci</i> S400/S200 pool 2	<i>Pseudomonas cichorii</i> ATCC10857/DSM50259	Phenol/water	GPC S400 & DOC-GPC S200	Ranf/Gisch, this study	200 μg/mL	0.07	50 μg/mL	No	–
<i>Pci</i> S400/S200 pool 3	<i>Pseudomonas cichorii</i> ATCC10857/DSM50259	Phenol/water	GPC S400 & DOC-GPC S200	Ranf/Gisch, this study	200 μg/mL	<LOQ	50 μg/mL	No	–
<i>Pci</i> S400/S200 pool 4	<i>Pseudomonas cichorii</i> ATCC10857/DSM50259	Phenol/water	GPC S400 & DOC-GPC S200	Ranf/Gisch, this study	200 μg/mL	0.13	50 μg/mL	Very weak	Yes
Heat-detergent-promoted lipopolysaccharide repurification									
Water control	–	–	–	–	–	<LOQ		No	–
3-OH-C10:0	–	–	–	–	20 μM	22.41	5 μM	Yes	Yes
<i>Pa</i> H4	<i>Pseudomonas aeruginosa</i> H4	PCP	–	(5, 78)	100 μg/mL	5.38 ^{\$}	25 μg/mL	Yes	Yes
Repurified <i>Pa</i> H4	<i>Pseudomonas aeruginosa</i> H4	PCP	Heat-detergent repurification	Ranf/Gisch, this study	400 μg/mL	<LOQ	100 μg/mL	No	–

[#]LPS preparations were generated using phenol/water extraction (45), phenol-chloroform-petroleum ether (PCP) extraction (46) or a combination of the two protocols in the indicated order. ^{\$}Note that the total content of 3-OH-C10:0 in LPS samples is presumably significantly higher because only free 3-OH-C10:0 molecules (dissolved from LPS aggregates/micelles) but not the fraction still aggregated with LPS is considered under the assay conditions.

Author contributions

SR initiated and managed the project and designed plant experiments. CD, CS, and TH designed chemical syntheses and analytics. CD and CS performed chemical syntheses and analytics. AK, NG, TG, CEC, RE, and SR prepared lipopolysaccharide. NG, DS, CEC, and RE performed structural analysis of lipopolysaccharide/lipid A samples. NG, AK, and SR designed lipopolysaccharide repurification approaches. RS and SD initiated rhamnolipid and lipopeptide experiments and prepared rhamnolipid samples. ACV, MS, AK, and SR designed infection experiments. ACV, AK, and MS performed infection experiments. AK performed and analysed calcium, ROS, and peroxidase assays. LR performed and analysed MAPK activation and gene expression experiments. ESL, YB, and SR designed binding assays. ESL performed protein expression and binding assays. AK, CD, CS, NG, LR, ACV, ESL, YB, and SR analysed the data. SR drafted the manuscript. AK, CD, NG, CS, LR, TG, ESL, and ACV contributed methods sections. AK, CD, NG, SD, YB, RH, TH, and SR contributed ideas, interpreted results, and critically revised the manuscript. All authors discussed the results and approved the manuscript.