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Supporting Information

for

New Structural Variants of Homoserine Lactones in Bacteria

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Determination of the absolute configuration of *N*-(3-hydroxydecanoyl)homoserine lactones

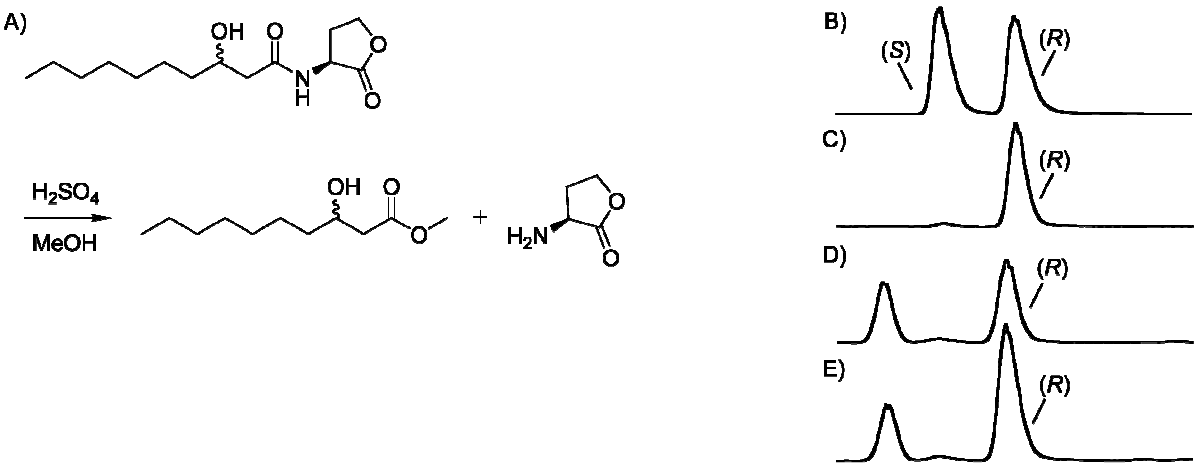

Figure S1. Enantiomer analysis of 3OH-C_{10}-HSL from *Phaeobacter gallaeciensis* T5. A) Derivatization procedure; B) Synthetic racemic mixture of (*S*)-**19** and (*R*)-**19**; C) Synthetic (*R*)-**19**; D) Extract of *Phaeobacter gallaeciensis* T5 after treatment with H_2SO_4/MeOH; E) Co-injection of C and D;

Synthesis (general methods). Chemicals were purchased from Fluka or Sigma-Aldrich and used without further purification. NMR-spectra were obtained using a Bruker AMX400 (^{1}H-NMR: 400 MHz, ^{13}C-NMR: 100 MHz) spectrometer with TMS as an internal standard. Optical rotations were determined on a Dr. Kernchen Propol Digital Automatic Polarimeter. Column chromatography was carried out using Merck

Kieselgel 60. Thin layer chromatography was carried out using 0.2 mm precoated plastic sheets Polygram Sil G/UV_{254} (Marcherey-Nagel, Düren, Germany). Solvents were purified by distillation and dried according to standard methods.

7-Methyloctanoic acid (**2**): Similar to the method of Klimentová et al.,[24] 6-bromohexanoic acid (0.5 g, 2.56 mmol, 1.0 equiv.) was converted into the sodium salt by stirring the acid in a solution of sodium methoxide prepared from Na (0.06 g, 2.56 mmol, 1.0 equiv.) in anhydrous methanol (20 mL) for 0.5 h. Methanol was evaporated in vacuo and the white precipitate was dried. The salt was suspended in dry THF (20 mL), cooled to 0℃, and the catalyst Li_2CuCl_4 in THF (0.52 mL, 0.1 M, 0.52 mmol, 0.2 equiv.) was added, followed by slow addition of isopropylmagnesium chloride (0.79 mL, 6.4 mmol, 2.5 equiv.). The reaction mixture was stirred for 2 h at 0℃ and was allowed to warm up to room temperature. The reaction was stopped by adding conc. HCl, the layers were separated and the aqueous phase was extracted with diethyl ether (3×). The combined organic extracts were dried ($MgSO_4$) and evaporated in vacuo. Purification by flash column chromatography (pentane/diethyl ether 5:1) yielded the desired acid **2** (0.36 g, 2.28 mmol, 89%) as colorless oil. TLC: R_F = 0.20 (pentane/diethyl ether 5:1); ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 10.18 (s, 1H, COOH), 2.34 (t, ^3J = 7.5 Hz, 2H, CH_2COO), 1.64 (quint, ^3J = 7.4 Hz, 2H, CH_2CH_2COO), 1.52 (sept, ^3J = 6.6 Hz, 1H, CH), 1.36-1.28 (m, 4H, CH_2CH_2CH_2CH), 1.19-1.14 (m, 2H, CH_2CH), 0.86 (d, ^3J = 6.6 Hz, 6H, CH(CH_3)$_2$); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 180.2 (COO), 38.7 (*C*H_2CH), 34.1 (*C*H_2COO), 29.3 (CH_2), 27.8 (CH), 26.9 (CH_2), 24.7 (CH_2), 22.5 (CH(*C*H_3)$_2$); EI-MS (70 eV): *m*/*z* (%): 158 (<1) [*M*]$^+$, 143 (10), 115 (25), 97 (29), 83 (21), 73 (100), 69 (28), 57 (67), 41 (76).

***N*-(7-Methyloctanoyl)-L-homoserine lactone (3)**: According to a method of Chhabra et al.,[25] L-homoserine lactone hydrobromide (0.18 g, 1.0 mmol, 1.0 equiv.) was dissolved in H_2O. Then triethylamine (0.13 mL, 1.0 mmol, 1.0 equiv.), the acid **3** (0.16 g, 1.0 mmol, 1.0 equiv.), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 0.18 g, 1.0 mmol, 1.0 equiv.) were added and the reaction mixture was stirred for 3 h at room temperature. The solvent was evaporated in vacuo, the residue was extracted with EtOAc (5×), and the combined organic extracts were washed successively with H_2O, saturated $NaHCO_3$ solution, and brine. After drying ($MgSO_4$), the solvent was removed, and the crude product was purified by flash column chromatography (CH_2Cl_2/MeOH 9:1) to yield the desired *iso*C_9-HSL **3** (0.104 g, 0.43

mmol, 43%) as a white solid. TLC: R_F = 0.82 (dichloromethane/methanol 5:1); $[\alpha]_D^{23}$: +14.9 (*c* = 11.2 in dichloromethane); m.p. 127°C (from dichloromethane/methanol 5:1); ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 6.31 (br s, 1H, NH), 4.59 (ddd, ^2J = 11.4 Hz, ^3J = 8.4 Hz, ^3J = 6.4 Hz, 1H, CHN), 4.46 (t, ^3J = 9.0 Hz, 1H, OCH_2), 4.32-4.25 (m, 1H, OCH_2), 2.88-2.78 (m, 1H, NCHCH_2), 2.25 (t, ^3J = 7.6 Hz, 2H, CH_2CON), 2.15 (ddd, ^2J = 23 Hz, ^3J = 11.6 Hz, ^3J = 8.9 Hz, 1H, NCHCH_2), 1.68-1.61 (m, 2H, CH_2CH_2CON), 1.51 (sept, ^3J = 6.6 Hz, 1H, C$H(CH_3)_2$), 1.34-1.26 (m, 4H, CH_2CH_2), 1.19-1.13 (m, 2H, CH_2CH), 0.86 (d, ^3J = 6.6 Hz, 6H, CH(C$H_3)_2$). ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 175.7 (CON), 173.9 (CO_2), 66.1 (CH_2O), 49.1 (CHN), 38.7 (CH_2), 36.1 (CH_2CON), 30.4 (CH_2), 29.4 (CH_2), 27.9 ($CH(CH_3)_2$), 27.0 (CH_2), 25.4 (CH_2), 22.6 (CH($CH_3)_2$); EI-MS (70 eV): *m*/*z* (%): 241 (3) $[M]^+$, 198 (6), 156 (20), 143 (100), 125 (23), 102 (27), 83 (21), 69 (15), 57 (84), 43 (58).

5-Methyl-1-hexanol (**5**): According to the method of Fürstner et al.[i] 1-bromopropanol (2.8 mL, 32.0 mmol, 1.0 equiv.) and Li_2CuCl_4 in THF (6.0 mL, 0.1 M, 6.0 mmol, 0.2 equiv.) were added to an ice-cooled solution of isobutylmagnesium bromide in dry THF (25 mL), prepared from Mg (1.94 g, 80.0 mmol, 2.5 equiv.) and isobutyl bromide (8.74 mL, 80.0 mmol, 2.5 equiv.). The reaction mixture was stirred for 1.5 h at 0°C, allowed to warm up to room temperature and then quenched by the addition of conc. hydrochloric acid. The aqueous layer was separated and extracted with diethyl ether (3×). The combined organic extracts were successively washed with saturated $NaHCO_3$ solution and brine, dried ($MgSO_4$), and the solvent was removed. The crude product was purified by flash column chromatography (pentane/diethyl ether 5:1) to obtain the desired alcohol **5** (2.74 g, 23.6 mmol, 74%) as a colorless liquid. TLC: R_F = 0.15 (pentane/diethyl ether 5:1); ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 3.61 (t, ^3J = 6.7 Hz, 2H, CH_2OH), 2.68 (m, 2H, CH, OH), 1.54 (sept, ^3J = 6.6 Hz, 2H, CH_2CH_2OH), 1.39-1.29 (m, 2H, CH_2CH), 1.24-1.15 (m, 2H, CH_2CH_2CH), 0.88 (d, ^3J = 6.7 Hz, 6H, 2×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 62.7 (CH_2OH), 38.7 (CH_2), 32.9 (CH_2), 27.9 (CH), 23.5 (CH_2), 22.5 (CH($CH_3)_2$). EI-MS (70 eV): *m*/*z* (%): 98 (2) ($[M]^+$-H_2O), 83 (26), 73 (16), 69 (47), 55 (100), 41 (72).

5-Methylhexanal (6): This aldehyde was synthesized by the standard procedure of Corey and Suggs.[ii] The aldehyde **6** (0.95 g, 8.3 mmol, 38%) was obtained as a colorless liquid. TLC: R_F = 0.55 (pentane/diethyl ether 5:1); ^{1}H NMR (400 MHz, $CDCl_3$,

TMS): δ = 9.76 (t, ^3J = 1.9 Hz, 1H, CHO), 2.41 (td, ^2J = 7.5 Hz, ^3J = 1.9 Hz, 2H, CH_2CHO), 1.68-1.51 (m, 3H, CH(CH_3)$_2$, CH_2CH_2CHO), 1.25-1.18 (m, 2H, CH_2CH), 0.89 (d, ^3J = 6.7 Hz, 6H, 2×CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 202.9 (CHO), 44.0 (CH_2CHO), 38.3 (CH_2CH), 27.7 (CH), 22.3 (CH(CH_3)$_2$), 19.8 (CH_2CH_2CH); EI-MS (70 eV): *m*/*z* (%): 96 (52), 86 (12), 81 (57), 71 (68), 55 (93), 43 (100).

Ethyl 7-methyl-3-oxooctanoate (7): Similar to a method of Holmquist and Roskamp,[26] tin(II) chloride (0.104 g, 0.58 mmol, 10 mol%) was added to a solution of ethyl diazoacetate (0.62 mL, 5.8 mmol, 1.0 equiv.) in dry CH_2Cl_2 (10 mL) under an nitrogen atmosphere. A solution of the aldehyde **6** (0.66 g, 5.8 mmol, 1.0 equiv.) in dry CH_2Cl_2 (10 mL) was added dropwise to this suspension at room temperature. After evolution of nitrogen had stopped (~ 2.5 h), the reaction mixture was transferred to a separatory funnel, quenched with brine (40 mL), and extracted with CH_2Cl_2 (3×). The organic layers were collected, dried ($MgSO_4$), and the solvent was removed under reduced pressure. Purification by flash column chromatography (pentane/diethyl ether 9:1) afforded the β-keto ester **7** (0.48 g, 2.4 mmol, 41%) as yellow oil. TLC: R_F = 0.39 (pentane/diethyl ether 5:1); ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ = 4.20 (q, ^3J = 7.1 Hz, 2H, OCH_2), 3.49 (s, 2H, COCH_2CO), 2.52 (t, ^3J = 7.4 Hz, 2H, CH_2CH_2CO), 1.65-1.48 (m, 3H, CH, CH_2CH_2CO), 1.28 (t, ^3J = 7.1 Hz, 3H, CH_2CH_3), 1.23-1.13 (m, 2H, CHCH_2), 0.88 (d, ^3J = 6.6 Hz, 6H, CH(CH_3)$_2$); ^{13}C NMR (75 MHz, $CDCl_3$, TMS): δ = 202.9 (CO), 167.2 (COO), 61.2 (OC$H_2$$CH_3$), 49.3 (COC$H_2$COO), 43.2 ($CH_2CH_2$CO), 38.2 ($CH_2$), 27.8 (CH), 22.4 (CH(C$H_3$)$_2$), 21.3 ($CH_2$), 14.0 ($CH_2CH_3$); EI-MS (70 eV): *m*/*z* (%): 200 (>1) $[M]^+$, 182 (6), 157 (8), 143 (12), 130 (73), 113 n(27), 95 (85), 88 (41), 84 (48), 69 (58), 55 (26), 43 (100).

Synthesis of the (*R*)- or (*S*)-Ru-[Cl_2BINAP·NEt_3]-catalysts. As described by Taber and Silverberg,[27] under anhydrous nitrogen Ru(1,5-cyclooctadiene)Cl_2 (0.39 g, 0.14 mmol) and (*R*)-(-)- or (*S*)-(+)-2,2'-tris(diphenylphosphino)-1,1'-binaphthyl were dissolved in dry xylene (4 mL) and triethylamine (0.3 mL) was added. The reaction mixture was stirred under reflux for 2.5 h, concentrated under an inert atmosphere, and the catalyst was obtained as a red residue, which was finally suspended in dry tetrahydrofuran (10 mL).

Ethyl (*R*)-3-hydroxy-7-methyloctanoate (8): Similar to a method described by Taber and Silverberg,[27] the ester **7** (0.48 g, 2.4 mmol) was dissolved in dry ethanol (10 mL) and the freshly synthesized (*R*)-Ru-[Cl_2BINAP·NEt_3] catalyst (1.5 mL) as well as

concentrated hydrochloric acid (0.2 mL) were added. The reaction mixture was stirred under a H_2 atmosphere (20 bar) for 20 h at 80°C. After removal of the solvent, the crude product was purified by flash column chromatography (pentane/diethyl ether 5:1) to give the β-hydroxy ethyl ester **8** (0.39 g, 1.5 mmol, 64%) as a colorless liquid. TLC: R_F = 0.28 (pentane/diethyl ether 5:1); $[\alpha]_D^{23}$: -24.0 (*c* = 2.25 in ethyl acetate); ^{1}H NMR (300 MHz, $CDCl_3$, TMS): δ = 4.17 (q, ^3J = 7.1 Hz, 2H, OCH_2), 4.02-4.00 (m, 1H, C*H*OH), 3.05 (br s, 1H, OH), 2.50 (dd, ^2J = 16.3 Hz, ^3J = 3.5 Hz, 1H, $COCH_2CO$), 2.40 (dd, ^2J = 16.3 Hz, ^3J = 8.7 Hz, 1H, $COCH_2CO$), 1.61-1.33 (m, 5H, C*H*$(CH_3)_2$, C*H*$_2$C*H*$_2$CHOH), 1.27 (t, ^3J = 7.1 Hz, 3H, CH_2CH_3), 1.23-1.15 (m, 2H, C*H*$_2$CH$(CH_3)_2$), 0.88 (d, ^3J = 6.6 Hz, CH(C*H*$_3$)$_2$); ^{13}C NMR (75 Hz, $CDCl_3$, TMS): δ = 173.0 (COO), 68.0 (CHOH), 60.5 (OCH_2), 41.3 (CH_2), 38.8 (CH_2), 35.7 (CH_2), 27.8 (*C*H$(CH_3)_2$), 23.2 (CH_2), 22.5 (CH(*C*H$_3$)$_2$), 14.1 (CH_2CH_3); EI-MS (70 eV): *m*/*z* (%): 157 (2), 138 (9). 117 (100), 95 (16), 89 (29), 71 (52), 55 (31), 43 (64).

(*R*)-3-Hydroxy-7-methyloctanoic acid (9): The ester **8** was saponified according to the method of Hsiao et al.[iii] The acid **9** (0.07 g, 0.41 mmol, 98%) was obtained as colorless liquid. TLC: R_F = 0.17 (pentane/diethyl ether 2:1); $[\alpha]_D^{23}$: -24.7 (*c* = 1.10 in ethyl acetate); ^{1}H NMR (200 MHz, $CDCl_3$, TMS): δ = 7.08 (br s, 1H, COOH), 4.05 (br s, 1H, OH), 3.51 (q, ^3J = 7.0 Hz, 1H, C*H*OH), 2.57 (dd, ^2J = 16.9 Hz, ^3J = 3.8 Hz, 1H, CH_2COO), 2.46 (dd, ^2J = 16.9 Hz, ^3J = 8.6 Hz, 1H, CH_2COO), 1.61-1.37 (m, 5H, C*H*$(CH_3)_2$, C*H*$_2$C*H*$_2$CHOH), 1.25-1.18 (m, 2H, C*H*$_2$CH$(CH_3)_2$), 0.87 (d, ^3J = 6.6 Hz, 6H, CH(C*H*$_3$)$_2$); ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ = 170.8 (COO), 68.1 (CHOH), 38.7 (*C*H$_2$COO), 36.7 (CH_2), 27.8 (CH_2), 23.2 (*C*H$(CH_3)_2$), 22.5 (CH(*C*H$_3$)$_2$), 15.1 (CH_2CH_3); EI-MS (70 eV): *m*/*z* (%): 157 (2) $[M]^+$-OC_2H_5), 139 (17), 117 (100), 95 (20), 89 (35), 71 (57), 55 (27), 43 (47).

(*R*)-*N*-(3-Hydroxy-7-methyloctanoyl)-L-homoserine lactone (10): The preparation was performed as described for **3**. The homoserine lactone **10** (0.036 g, 0.14 mmol, 47%) was obtained as white solid. TLC: R_F = 0.91 (acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 6.81 (d, ^3J = 6.2 Hz, 1H, NH), 4.57 (ddd, ^2J = 11.7 Hz, ^3J = 5.3 Hz, ^3J = 2.9 Hz, 1H, CHN), 4.48 (td, ^2J = 9.1 Hz, ^3J = 1.4 Hz, 1H, OCH_2), 4.29 (ddd, ^2J = 11.1 Hz, ^3J = 9.2 Hz, ^3J = 6.1 Hz, 1H, OCH_2), 4.09-3.99 (m, 1H, C*H*OH), 2.83-2.74 (m, 2H, NCHC*H*$_2$), 2.47 (dd, ^2J = 15.3 Hz, ^3J = 2.8 Hz, 1H, CH_2CON), 2.35 (dd, ^2J = 15.4 Hz, ^3J = 9.0 Hz, 1H, CH_2CON), 2.24-2.13 (m, 1H, NCHC*H*$_2$), 1.54 (sept, ^3J = 6.6 Hz, 1H, C*H*$(CH_3)_2$), 1.47-1.12 (m, 6H, C*H*$_2$C*H*$_2$C*H*$_2$), 0.87 (d, ^3J = 6.6 Hz, CH(C*H*$_3$)$_2$); ^{13}C

NMR (100 MHz, $CDCl_3$, TMS): δ = 175.5 (CON), 173.0 (COO), 68.6 (CHOH), 66.1 (CH_2O), 49.1 (CHN), 42.5 (CH_2CO), 38.7 (CH_2), 37.2 (CH_2CHOH), 30.0 (CH_2CHN), 27.9 ($CH(CH_3)_2$), 23.3 (CH_2), 22.5 ($CH(CH_3)_2$); EI-MS (70 eV): *m*/*z* (%): 257 (2) $[M]^+$, 239 (2), 214 (2), 196 (4), 172 (80), 155 (11), 143 (29), 125 (11), 102 (100), 97 (18), 83 (11), 74 (19), 69 (22), 57 (73), 43 (78).

7-(Tetrahydro-2*H*-pyran-2-yloxy)-1-heptanol: As described by Parham and Anderson,[31] a solution of 1,7-heptanediol (6.17 mL, 50.0 mmol, 1.0 equiv.), *p*-toluenesulfonic acid (0.48 g, 2.5 mmol, 0.5 equiv.) and 3,4-dihydro-2*H*-pyran (3.62 mL, 40.0 mmol, 0.8 equiv.) in dry CH_2Cl_2 (75 mL) was stirred at room temperature for 1.5 h. The organic solvent was removed under reduced pressure, and the product was separated from unprotected and biprotected alcohol by column chromatography (pentane/diethyl ether 2:1). The THP-ether (4.38 g, 20.3 mmol, 41%) was obtained as colorless liquid. TLC: R_F = 0.10 (pentane/diethyl ether 2:1); ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 4.57 (t, ^3J = 3.5 Hz, 1H, CH), 3.89-3.83 (m, 1H, OCH_2 THP-ether), 3.72 (dt, ^2J = 9.6 Hz, ^3J = 6.8 Hz, 1H, OCH_2 chain), 3.58 (t, ^3J = 6.7 Hz, 2H, CH_2OH), 3.52-3.47 (m, 1H, OCH_2 THP-ether), 3.38 (dt, ^2J = 9.6 Hz, ^3J = 6.6 Hz, 1H, OCH_2 chain), 2.93 (s, 1H, OH), 1.86-1.80 (m, 1H, $CHCH_2$), 1.74-1.68 (m, 1H, $CHCH_2$), 1.59-1.50 (m, 8H, $CHCH_2CH_2CH_2$, OCH_2CH_2 chain, CH_2CH_2OH), 1.39-1.35 (m, 6H, $CH_2CH_2CH_2CH_2CH_2OH$); ^{13}C-NMR (100 MHz, $CDCl_3$, TMS): δ = 98.6 (CH), 67.4 (CH_2), 62.5 (CH_2), 62.1 (CH_2), 32.5 (CH_2), 30.6 (CH_2), 29.5 (CH_2), 29.1 (CH_2), 26.0 (CH_2), 25.5 (CH_2), 25.3 (CH_2), 19.4 (CH_2); EI-MS (70 eV): *m*/*z* (%): 215 (1) $[M]^+$, 143 (2), 131 (1), 115 (4), 101 (19), 97 (27), 85 (100), 67 (12), 55 (53), 41 (30).

7-(Tetrahydro-2*H*-pyran-2-yloxy)-heptanal (**14**): This aldehyde was synthesized by oxidation of 7-(Tetrahydro-2*H*-pyran-2-yloxy)-1-heptanol as described for **6** (2.89 g, 13.5 mmol, 67% yield). TLC: R_F = 0.65 (pentane/diethyl ether 1:1); ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 9.76 (t, ^3J = 1.8 Hz, 1H, CHO), 4.59 (t, ^3J = 3.5 Hz, 1H, CH), 3.89-3.83 (m, 1H, OCH_2 THP-ether), 3.73 (dt, ^2J = 9.5 Hz, ^3J = 6,8 Hz, 1H, OCH_2 chain), 3.53-3.49 (m, 1H, OCH_2THP-ether), 3.39 (dt, ^2J = 9.6 Hz, ^3J = 6.5 Hz, 1H, OCH_2 chain), 2.43 (dt, ^2J = 7.5 Hz, ^3J = 1.8 Hz, 1H, CH_2CHO), 2.33 (t, ^3J = 7.5 Hz, 1H, CH_2CHO), 1.86-1.78 (m, 1H, $CHCH_2$), 1.74-1.48 (m, 9H, $CHCH_2$, $OCH_2CH_2CH_2$ THP-ether, CH_2CH_2CHO OCH_2CH_2 chain), 1.43-1.33 (m, 4H, $CH_2CH_2CH_2CH_2CHO$); EI-MS (70 eV): *m*/*z* (%): 213 (3) $[M]^+$, 141 (2), 113 (12), 101 (52), 95 (69), 85 (100), 69 (40), 67 (34), 55 (49), 41 (81).

2-(Tetradec-7-enyloxy)tetrahydropyran (**15**): As described by Martinez and Ruiz,[32] heptyltriphenylphosphonium bromide (7.2 g, 16.3 mmol, 1.2 equiv.) was dissolved in dry THF (60 mL), cooled to –30℃ and deprotonated b y addition of *n*-BuLi in hexane (10.4 mL, 1.6 M, 16.3 mmol, 1.2 equiv.). After stirring for 45 min, the aldehyde **14** (2.76 g, 12.9 mmol, 1.0 equiv.) in dry THF (5 mL) was added dropwise. The reaction mixture was stirred for 2 h at room temperature and hydrolyzed by addition of diluted hydrochloric acid. The aqueous layer was separated, extracted with diethyl ether (3×), the combined organic extracts were dried ($MgSO_4$), and concentrated under reduced pressure. Purification by column chromatography (pentane/diethyl ether 9:1) yielded **15** (1.74 g, 5.8 mmol, 45%) as a colorless liquid with only minor amounts of the *E* isomer (<3% detected by GC-MS). TLC: R_F = 0.65 (pentane/diethyl ether 9:1); ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 5.34 (t, ^3J = 4.2 Hz, 2H, CHCH), 4.58-4.57 (m, 1H, OCHO), 3.88-3.84 (m, 1H, OCH_2 THP-ether), 3.75-3.70 (m, 1H, OCH_2 chain), 3.50-3.47 (m, 1H, OCH_2 THP-ether), 3.40-3.35 (m, 1H, OCH_2 chain), 2.01 (s, 4H, CH_2CH-CHCH_2), 1.86-1.80 (m, 1H, OCHCH_2), 1.73-1.67 (m, 1H, OCHCH_2), 1.61-1.49 (m, 6H, 3×CH_2), 1.34-1.28 (m, 14H, 7×CH_2), 0.88 (t, ^3J = 6.1 Hz, 3H, CH_3); ^{13}C NMR (200 MHz, $CDCl_3$, TMS): δ = 129.9 (CH), 129.7 (CH), 98.7 (OCH), 67.5 (OCH_2), 62.1 (OCH_2), 31.7 (CH_2), 30.7 (CH_2), 29.7 (2×CH_2), 29.6 (CH_2), 29.1 (CH_2), 28.9 (CH_2), 27.2 (CH_2), 27.1 (CH_2), 26.1 (CH_2), 25.5 (CH_2), 22.6 (CH_2), 19.6 (CH_2), 14.0 (CH_3); EI-MS (70 eV): *m*/*z* (%): 296 (1) [*M*]$^+$, 223 (6), 205 (2), 192 (2), 138 (2), 124 (3), 101 (11), 96 (20), 85 (100), 67 (50), 55 (59), 41 (68).

(*Z*)-7-Tetradecen-1-ol: According to standard procedures, a solution of **15** (2.50 g, 8.4 mmol, 1.0 equiv.) and *p*-toluenesulfonic acid (0.18 g, 0.84 mmol, 0.1 equiv.) in dry methanol (50 mL) was stirred for 1.5 h at room temperature. Then the solvent was removed under reduced pressure and the crude product was purified by column chro-matography (pentane/diethyl ether 5:1) to obtain (*Z*)-7-tetradecen-1-ol (1.09 g, 5.15 mmol, 61%) as colorless oil. TLC: R_F = 0.40 (pentane/diethyl ether 2:1); ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 5.40-5.33 (m, 2H, CHCH), 3.62 (t, ^3J = 6.7 Hz, 2H, CH_2OH), 2.00-1.98 (m, 4H, CH_2CHCHCH_2), 1.60-1.51 (m, 2H, CH_2CH$_2$CHOH), 1.40-1.20 (m, 12H, CH_2-chain), 0.88 (t, ^3J = 6.7 Hz, 3H, CH_3); ^{13}C NMR (200 MHz, $CDCl_3$, TMS): δ = 130.0 (CH), 129.6 (CH), 62.8 (CH_2OH), 32.6 (CH_2), 31.7 (CH_2), 29.7 (2×CH_2), 29.0 (CH_2), 28.9 (CH_2), 27.2 (CH_2), 27.1 (CH_2), 25.6 (CH_2), 22.6 (CH_2), 14.0 (CH_3); EI-MS (70 eV): *m*/*z* (%): 194 (10) [*M*-18]$^+$, 166 (3), 151 (2), 138 (7), 123 (9), 109 (22), 96 (52), 82 (73), 67 (80), 55 (83), 44 (100).

(*Z*)-7-Tetradecenal (**16**): This aldehyde was synthesized from (*Z*)-7-Tetradecen-1-ol: as described for **6** (0.805 g, 3.4 mmol, 65%). TLC: R_F = 0.45 (pentane/diethyl ether 2:1); ^{1}H-NMR (400 MHz, $CDCl_3$, TMS): δ = 9.76 (t, ^3J = 1.8 Hz, 1H, CHO), 5.40-5.29 (m, 2H, CHCH), 2.42 (dt, ^2J = 7.4 Hz, ^3J = 2.2 Hz, 2H, CH_2CHO), 2.06-1.94 (m, 4H, CH_2CHCHCH_2), 1.67-1.59 (m, 2H, CH_2CH_2CHO), 1.37-1.27 (m, 12H, 6×CH_2), 0.88 (t, ^3J = 6.4 Hz, 3H, CH_3); ^{13}C-NMR (200 MHz, $CDCl_3$, TMS): δ = 202.7 (CHO), 130.2 (CH), 129.3 (CH), 43.8 (CH_2), 31.7 (CH_2), 29.6 (CH_2), 29.4 (CH_2), 28.9 (CH_2), 28.7 (CH_2), 27.2 (CH_2), 26.9 (CH_2), 22.6 (CH_2), 21.9 (CH_2), 13.5 (CH_3); EI-MS (70 eV): *m*/*z* (%): 210 (1) $[M]^+$, 192 (8), 163 (2), 149 (4), 135 (12), 121 (25), 111 (21), 98 (37), 81 (45), 67 (64), 55 (100), 41 (89).

Ethyl (2*E*,9*Z*)-2,9-hexadecadienoate (**17**): Sodium hydride (0.25 g, 10.4 mmol, 4.5 equiv.) was suspended in dry dimethoxy ethane (5 mL), and diethylphosphonoacetate (1.4 g, 6.54 mmol, 3.0 equiv.) was added at 0°C. The mixture was allowed to stir for 15 min at room temperature, then the aldehyde **16** (0.46 g, 2.18 mmol, 1.0 equiv.) in dry dimethoxy ethane (2 mL) was added, and the reaction mixture was stirred for 12 h under reflux. The organic phase was extracted successively with H_2O and saturated NaCl-solution, dried ($MgSO_4$), and concentrated under reduced pressure. The crude product was purified by column chromatography (pentane/diethyl ether 15:1), and the desired ester **17** (0.3 g, 1.1 mmol, 50%) was obtained as yellow oil. The NMR analysis showed the product to be the almost pure 2*E*,9*Z*-diastereomer. TLC: R_F = 0.45 (pentane/diethyl ether 5:1); ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 6.96 (dt, ^2J = 15.6 Hz, ^3J = 7.0 Hz, 1H, COOCHC*H*), 5.81 (dt, ^2J = 15.6 Hz, ^3J = 1.5 Hz, 1H, COOCH), 5.40-5.30 (m, 2H, CH_2C*H*C*H*CH_2), 4.18 (q, ^3J = 7.1 Hz, 2H, CH_2COO), 2.19 (m, 2H, COOCHCHCH_2), 1.38-1.22 (m, 15H, 6×CH_2, COOCH_2CH_3), 0.90-0.84 (m, 3H, CH_3); ^{13}C NMR (200 MHz, $CDCl_3$, TMS): δ = 166.8 (COO), 149.4 (COOCH-*C*H), 130.2 (CH), 129.5 (CH), 121.3 (COO*C*H), 60.1 (COO*C*H_2), 32.2 (CH_2), 31.8 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.0 (CH_2), 28.8 (CH_2), 27.9 (CH_2), 27.5 (CH_2), 27.0 (CH_2), 22.7 (CH_2), 14.3 (CH_3), 14.1 (CH_3); EI-MS (70 eV): *m*/*z* (%): 280 (4) $[M]^+$, 251 (11), 198 (16), 192 (25), 184 (9), 171 (8), 150 (20), 135 (27), 147 (49), 121 (26), 109 (34), 99 (40), 95 (61), 81 (87), 67 (70), 55 (100), 41 (80).

(2*E*,9*Z*)-*N*-(2,9-Hexadecadienoyl)-L-homoserine lactone (**11**): The ester **17** was saponified and directly used as crude material in the preparation of the AHL **11** according to the procedure described for **3**. Yield: 31%. TLC: R_F = 0.43 (dichlorometh-

ane/methanol 19:1); ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 6.87 (dt, ^{2}J = 15.3 Hz, ^{3}J = 7.0 Hz, 1H, C*H*CHCON), 6.26 (br s, 1H, NH), 5.84 (dt, ^{2}J = 15.3 Hz, ^{3}J = 1.5 Hz, 1H, CHCON), 5.40-5.32 (m, 2H, $CH_2CHCHCH_2$), 4.65 (ddd, ^{2}J = 11.5 Hz, ^{3}J = 8.6 Hz, ^{3}J = 6.1 Hz, 1H, CHN), 4.48 (dt, ^{2}J = 9.0 Hz, ^{3}J = 1.0 Hz, 1H, OCH_2), 4.30 (ddd, ^{2}J = 11.2 Hz, ^{3}J = 9.4 Hz, ^{3}J = 5.9 Hz, 1H, OCH_2), 3.04 (dd, ^{2}J = 29.4 Hz, ^{3}J = 6.6 Hz, 1H, NCHCH_2), 2.89-2.79 (m, 1H, NCHCH_2), 2.22-2.16 (m, 2H, CH_2CHCHCO), 2.05-1.96 (m, 4H, CH_2(CH)$_2$CH_2), 1.47-1.42 (m, 2H, CH_2CH$_2$(CH)$_2$CO), 1.39-1.23 (m, 12H, 6×CH_2), 0.89 (t, ^{3}J = 6.9 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 175.7 (COO), 166.4 (CON), 146.6 (*C*HCHCO), 130.2 (CH), 129.5 (CH), 122.4 (*C*HCO), 66.2 (CH_2O), 49.2 (NCH), 32.1 (CH_2), 31.7 (CH_2), 30.5 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.0 (CH_2), 28.8 (CH_2), 28.1 (CH_2), 27.2 (CH_2), 27.0 (CH_2), 22.6 (CH_2), 14.1 (CH_3). EI-MS (70 eV): *m*/*z* (%): 335 (7) $[M]^+$, 234 (16), 192 (9), 177 (11), 164 (15), 149 (20), 143 (23), 135 (25), 121 (23), 109 (20), 102 (13), 95 (39), 81 (100), 67 (58), 55 (91), 41 (68).

Methyl 3-hydroxyoctanoate (19): According to Katzenellenbogen et al., [S1] ethyl 3-oxooctanoate (0.186 g, 1.0 mmol, 1.0 equiv.) in dry methanol (2 mL) was added slowly to a solution of sodium borohydride (0.045 g, 1.2 mmol, 1.2 equiv.) in dry methanol (10 mL) and the reaction mixture was stirred for 2 h at room temperature. After hydrolysis by addition of diluted hydrochloric acid, the organic phase was diluted with diethyl ether. The aqueous phase was separated, extracted with diethyl ether (3×), the combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure. Purification by column chromatography (pentane/diethyl ether 5:1) yielded ethyl 3-hydroxyoctanoate (0.156 g, 0.83 mmol, 83%), which was directly transformed into the methyl ester. Therefore, concentrated hydrochloric acid (0.2 mL) was added to a solution of ethyl 3-hydroxyoctanoate (0.156 g, 0.83 mmol) in dry methanol (3 mL), and the reaction mixture was stirred for 18 h under reflux. The organic phase was extracted with saturated $NaHCO_3$ solution, dried ($MgSO_4$), and the solvent was removed in vacuo to give the desired methyl ester (0.141 g, 0.81 mmol, 98%) as colorless liquid. TLC: R_F = 0.11 (pentane/diethyl ether 5:1); ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 4.00 (bs, 1H, CH), 3.71 (s, 3H, OCH_3), 3.01 (bs, 1H, OH), 2.52 (dd, *J* = 16.3 Hz, *J* = 3.3 Hz, 1H, CH_2CO_2), 2.42 (dd, *J* = 16.3 Hz, *J* = 8.9 Hz, 1H, CH_2CO_2), 1.45-1.27 (m, 8H, 4×CH_2), 0.88 (t, *J* = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, 25℃, TMS): δ = 173.4 (CO_2), 68.0 (COH), 51.6 (OCH_3), 41.1 (CH_2CO_2), 36.5 (CH_2COH), 31.6 (CH_2), 25.1 (CH_2), 22.5 (CH_2), 13.9 (CH_3); EI-MS (70 eV): *m*/*z*

(%): 156 (1) $[M\text{-}H_2O]^+$, 125 (8), 103 (100), 83 (11), 74 (42), 71 (47), 61 (20), 55 (20), 43 (48).

Methyl (*S*)-3-Hydroxyoctanoate ((*S*-)19): Preparation as described for **8** with (*S*)-Ru-[Cl_2BINAP·NEt_3] catalyst. 91% yield.

(*S*)-3-Hyxdroxyoctanoic acid: Preparation as described for **9**: 98%. TLC: R_F = 0.17 (pentane/diethyl ether 2:1); ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 6.69 (s, OH), 4.04 (sept, ^3J = 4.1 Hz, 1H, C*H*OH), 2.57 (dd, ^2J = 16.6 Hz, ^3J = 3.2 Hz, 1H, C*H*H*COO), 2.47 (dd, ^2J = 16.6 Hz, ^3J = 8.9 Hz, 1H, CH_2COO), 1.50-1.40 (m, 2H, CH_2CHOH), 1.38-1.26 (m, 6H, 3×CH_2), 0.89 (t, ^3J = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 177.9 (COO), 68.1 (CH), 41.1 (*C*H_2COO), 36.4 (CH_2), 31.6 (CH_2), 25.1 (CH_2), 22.5 (CH_2), 14.0 (CH_3).

(*R*)-3-Hyxdroxyoctanoic acid: Preparation as described for **9**: 95%.

(*S*)-*N*-(3-Hydroxyoctanoyl)-L-homoserine lactone ((*S*)-18): Preparation as described for **3**: 54%. TLC: R_F = 0.47 (dichloromethane/methanol 9:1); ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 7.11 (d, ^3J = 7.9 Hz, 1H, NH), 4.67 (ddd, ^2J = 11.5 Hz, ^3J = 8.8 Hz, ^3J = 7.0 Hz, 1H, CHN), 4.47 (td, ^2J = 9.0 Hz, ^3J = 1.2 Hz, 1H, OCH_2), 4.29 (ddd, ^2J = 11.1 Hz, ^3J = 9.2 Hz, ^3J = 6.1 Hz, 1H, OCH_2), 4.03-3.99 (m, 1H, C*H*OH), 3.66 (s, 1H, OH), 2.76-2.69 (m, 2H, NCHCH_2), 2.44 (dd, ^2J = 15.1 Hz, ^3J = 2.9 Hz, 1H, CH_2CON), 2.35 (dd, ^2J = 15.1 Hz, ^3J = 9.0 Hz, 1H, CH_2CON), 2.30-2.19 (m, 1H, NCHCH_2), 1.50-1.41 (m, 2H, CH_2CH), 1.35-1.26 (m, 6H, CH_2CH_2CH_2), 0.89 (t, ^3J = 6.8 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 175.9 (CON), 173.0 (COO), 68.6 (CHOH), 66.1 (CH_2O), 48.9 (CHN), 44.5 (*C*H_2CO), 37.0 (CH_2), 31.6 (*C*H_2CH-OH), 29.7 (*C*H_2CHN), 25.1 (CH_2), 22.5 (CH_2), 14.0 (CH_3); EI-MS (70 eV): *m*/*z* (%): 243 (1) [*M*]$^+$, 225 (6), 172 (79), 158 (11), 143 (33), 125 (24), 102 (100), 83 (31), 74 (19), 57 (74), 43 (52).

(*R*)-*N*-(3-Hydroxyoctanoyl)-L-homoserine lactone ((*R*)-18): Preparation as described for **3**: 42%.

Ethyl 3-Oxodecanoate: Preparation as described for **7**: 87% yield. TLC: R_F = 0.13 (pentane/diethyl ether 19:1). ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ = 4.19 (q, J = 7.1 Hz, 2 H, OCH_2), 3.43 (s, 2 H, CH_2CO_2), 2.53 (t, J = 7.3 Hz, 2 H, CH_2(CH_2)$_2CO_2$), 1.59 (quint, J = 7.3 Hz, 2 H, CH_2(CH_2)$_3CO_2$), 1.34-1.22 (m, 11 H, 4 × CH_2, OCH_2CH_3), 0.88 (t, J = 6.8 Hz, 3 H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ = 202.8 (CO),

167.2 (CO_2), 61.2 (OCH_2CH_3), 49.2 (CH_2CO_2), 42.9 (CH_2CO), 31.5 (CH_2), 28.9 (2 × CH_2), 23.4 (CH_2), 22.5 (CH_2), 14.0 (CH_3), 13.9 (CH_3). EI-MS (70 eV): *m*/*z* (%): 214 (19 $[M]^+$, 196 (2), 172 (3), 143 (20), 131 (14), 130 (79), 127 (48), 115 (14), 109 (6), 102 (12), 98 (9), 97 (16), 88 (48), 87 (9), 83 (8), 70 (9), 69 (24), 67 (7), 58 (11), 57 (100), 56 (18), 55 (47), 53 (6), 45 (6), 43 (80), 42 (34), 41 (68), 39 (21).

Ethyl 3-hydroxydecanoate: This compound was prepared as described in the preparation of **19**. Yield 39% as colorless liquid. TLC: R_F = 0.32 (pentane/diethyl ether 2:1); ^{1}H NMR ($CDCl_3$, 400 MHz, TMS): δ = 4.17 (q, *J* = 7.1 Hz, 2H, OCH_2), 4.04-3.96 (m, 1H, C*H*OH), 2.97 (bs, 1H, OH), 2.50 (dd, *J* = 16.3 Hz, *J* = 3.2 Hz, 1H, CH_2CO_2), 2.40 (dd, *J* = 16.3 Hz, *J* = 8.9 Hz, 1H, CH_2CO_2), 1.56-1.24 (m, 15H, 6×CH_2, CH_3), 0.88 (t, *J* = 6.9 Hz, 3H, CH_3); ^{13}C NMR (100 Hz, $CDCl_3$, TMS): δ = 173.0 (s, CO_2), 68.0 (d, CH), 60.6 (t, OCH_2), 41.3 (t, *C*H_2CO_2), 36.5 (t, CH_2), 31.7 (t, CH_2), 29.4 (t, CH_2), 29.2 (t, CH_2), 25.4 (t, CH_2), 22.6 (t, CH_2), 14.1 (t, CH_2), 14.1 (q, CH_3), 14.0 (q, CH_3); EI-MS (70 eV): *m*/*z* (%): 153 (6), 127 (9), 117 (100), 89 (26), 88 (24), 71 (46), 69 (19), 61 (9), 60 (13), 57 (19), 55 (21), 43 (46), 41 (32).

Methyl 3-Hydroxydecanoate: Concentrated hydrochloric acid (0.2 mL) was added to a solution of ethyl 3-hydroxydecanoate (0.370 g, 1.71 mmol) in dry methanol (5 mL) and the reaction mixture was stirred for 18 h under reflux conditions. The organic phase was extracted with saturated $NaHCO_3$ solution, dried ($MgSO_4$), and the solvent was removed in vacuo to give the desired methyl ester (0.320 g, 1.58 mmol, 93%) as colorless liquid.

Methyl (*R*)-3-hydroxydecanoate: Preparation as described for **8**. 87% yield. TLC: R_F = 0.15 (pentane/diethyl ether 4:1); $[\alpha]_D^{27}$: +16.4 (*c* = 18.88 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, 25℃, TMS): δ = 4.05-3.97 (m, 1 H, CH), 3.71 (s, 3 H, OCH_3), 2.94 (bs, 1 H, OH), 2.52 (dd, ^2J = 16.3 Hz, *J* = 3.3 Hz, 1 H, CH_2CO_2), 2.41 (dd, ^2J = 16.3 Hz, *J* = 8.9 Hz, 1 H, CH_2CO_2), 1.56-1.24 (m, 12 H, 6 × CH_2), 0.88 (t, *J* = 6.8 Hz, 3 H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, 25℃, TMS): δ = 173.4 (CO_2), 68.0 (COH), 51.6 (OCH_3), 41.1 (*C*H_2CO_2), 36.5 (*C*H_2COH), 31.7 (CH_2), 29.4 (CH_2), 29.2 (CH_2), 25.4 (CH_2), 22.6 (CH_2), 14.0 (CH_3); EI-MS (70 eV): *m*/*z* (%): 184 (1) $[M\text{-}H_2O]^+$, 103 (100), 74 (32), 71 (37), 69 (17), 61 (18), 59 (11), 57 (15), 55 (19), 43 (53), 41 (29), 39 (9).

Results of Bioassays

Table S1. Relative fold induction of sensors pJBA132, most sensitive for *N*-(3-oxo-hexanoyl)homoserine lactone (3-oxo-C_6-HSL). Fold induction in the presence of 0.25 µg/mL 3-oxo-C_6-HSL was arbitrarily set to 100 %.

Conc µg/ml	3-oxo-C_6-HSL	C_8-HSL	(*R*)-3OH-C_8-HSL	(*S*)-3OH-C_8-HSL	isoC_9-HSL	3-OH-isoC_9-HSL	(*R*)-3OH-C_{10}-HSL	(2*E*,9*Z*)-$C_{16:2}$-HSL
0.025	88.6	0.3	0.1	0.2	-0.1	0.3	0.2	0.2
0.25	100.0	29.1	-0.1	0.2	-2.3	15.0	0.3	0.5
2.5	109.0	70.3	0.0	3.5	-0.1	40.0	0.1	28.0
25	105.3	88.5	2.8	43.3	0.3	63.0	0.4	58.6
250		83.0	49.8	61.7	4.7	38.2	0.6	45.5

Table S2. Relative fold induction of sensors pRK-C12, most sensitive for C_{12}-HSL. Fold induction in the presence of 0.25 µg/mL C_{12}-HSL was arbitrarily set to 100 %.

Conc µg/ml	3-oxo-C_6-HSL	C_8-HSL	(*R*)-3OH-C_8-HSL	(*S*)-3OH-C_8-HSL	isoC_9-HSL	3-OH-isoC_9-HSL	(*R*)-3OH-C_{10}-HSL	(2*E*,9*Z*)-$C_{16:2}$-HSL
0.025	10.5	-0.1	0.1	0.1	0.1	0.1	-0.1	0.3
0.25	100.0	0.1	-0.1	-0.1	0.1	0.8	0.1	9.8
2.5	105.0	0.8	0.1	0.3	2.6	9.0	9.8	118.8
25	87.1	34.1	2.8	9.1	52.4	114.0	71.3	139.4
250		145.2	89.1	180.0	149.6	133.7	65.8	0.1

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