

SUPPORTING INFORMATION

Synthesis and Characterization of Four Diastereomers of Monorhamnolipids

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Materials and Methods

Synthesis and Structural Characterization

^1H and ^{13}C -NMR spectra were recorded on a Bruker DRX-400 (400 MHz), DRX-500 (500 MHz), and DRX-600 (600 MHz) spectrometers using CDCl_3 and MeOD as solvents with TMS as the internal standard. All NMR spectra were analyzed and interpreted using MestReNova software.

All glassware was flame-dried previous to reactions and all reactions were achieved under argon gas. For flash chromatography, Silia Flash P60 Silica Gel, 40-63 μm , 60 Å (Silicycle, R1200308) was employed and a Biotage, Inc. Horizon HPFC system was utilized. Optical rotations measurements were performed on a Jasco P-1020[®] polarimeter, with a cell length of 50mm, and at a wavelength of 589 nm from a Na lamp, taking 100 readings per sample at concentrations 0.38-1.77 % w/v in dry CHCl_3 . Solvents used for experiments were dried and distilled according to literature procedures or purchased as anhydrous solvents from EMD Millipore. All chemicals were purchased from Aldrich Chemical Company, Acros Chemicals, Chem-Impex International, and EMD Chemicals.

Interfacial and Aggregation Behavior

Buffer solutions were prepared from sodium phosphate monobasic monohydrate (EMD, 98%), sodium dibasic anhydrous phosphate (EMD, 99%). Pyrene (Sigma Aldrich, $\geq 99.0\%$) was further purified by recrystallization from an ethanol (1-2 vol %) in water solution. Water was purified using a Milli-Q-UV system to a resistivity of 18.2 M Ω (Millipore Corp.) with a total organic carbon content <6 ppb.

Surfactant Stock Solution Preparation

All mRL solutions were prepared by dissolving purified mRL in 10 mM phosphate buffer at pH 8 unless otherwise specified, and stirred in a Teflon vial (VWR) with a Teflon coated stir bar for a minimum of 12 h. Phosphate buffer was prepared with appropriate amounts of sodium phosphate monobasic monohydrate and sodium dibasic anhydrous phosphate using purified water and then filtered sequentially through Acrodisc 25 mm syringe filters with 0.45 μm , 0.2 μm , and 0.1 μm Supor membranes.

Instrumentation and Measurement Procedures

Surface tension measurements were performed on a Fisher Scientific model 21 Surface Tensiometer using a Pt-Ir du Noüy ring (Fisher Scientific.) The tensiometer was calibrated with pure water at 72 mN/m before each experiment. Three independently-prepared samples were

tested starting with 5 mL of Milli-Q water adjusted to pH 4 or 8. Aliquots of mRL stock solution were then added to increase the mRL concentration.

A Photon Technologies, Inc. (PTI) Quanta Master 40 spectrofluorometer was used for steady-state fluorescence measurements. Samples were prepared by first preparing a rhamnolipid stock solution with 250 nM pyrene and transferred to a sealed 0.4 cm cuvette (Starna Cell) that had been previously silanized to prevent mRL adsorption (as described in the Supporting Information) and purged with N₂. Pyrene fluorescence was excited with 337 nm radiation and the emission was observed at 373 nm. All fluorescence experiments were performed at 25 °C. Three independently prepared surfactant/probe samples at each concentration were measured, and three instrumental replicates of data for each sample were acquired and averaged. Fluorescence intensities were corrected for the inner filter effect as described below.

Silanization Procedure for Fluorescence Cuvettes

To prevent mRL adsorption to the glass walls, fluorescence cuvettes were silanized with a fluoro-terminated silane. Fluorescence cuvettes were pretreated with a cleaning solution consisting of 4:1:1 (v/v) water, 30% (v/v) hydrogen peroxide and concentrated NH₄OH (14.5 M) by boil in the solution for at least 45 min, followed by thorough rinsing with Milli-Q water. They were then dried under vacuum for 2-3 h at 120 °C. Next, the silanizing solution was made in an environmental control box under constant Ar and N₂ flow to eliminate water contamination. This solution was made by mixing dry toluene 97.5% (v/v), dry (tridecafluoro-1,1,2,2-tetrahydrooctyl) triethoxysilane 2% (v/v), and dry pyridine 0.5% (v/v) for 10 min and immediately added to the cuvettes, which were filled for an even coating. The solution was allowed to sit in the cuvette for 4 h. The glassware was rinsed with several aliquots of dry toluene, followed by several aliquots of absolute ethanol. The cuvettes were placed under vacuum for 12 h at 100 °C to allow the coating to cure.

Inner Filter Effect Correction of Fluorescence Intensities

Gauthier et al. have provided a method for correcting for the inner filter effect to generate a corrected fluorescence intensity (F_{corr}) from the observed fluorescence intensity (F_{obsd}) using the following equation:

$$\frac{F_{corr}}{F_{obsd}} = \frac{2.3dA_{ex}}{1 - 10^{-dA_{ex}}} 10^{gA_{em}} \frac{2.3sA_{em}}{1 - 10^{-sA_{em}}}$$

here A_{ex} and A_{em} are the absorbance values at the excitation and emission wavelengths, respectively, d is the cuvette path length, s is the beam thickness, and g is the distance between the beam and the edge of the cuvette. A diagram describing these distances is shown in Figure S1. The inner filter effect correction measurements were done with the same excitation parameters used when measuring fluorescence. The values for s and g using a 0.4cm (d) cuvette s and g are 0.063 ± 0.008 and 0.169 ± 0.019 cm, respectively. F_{corr} values were used in place of F_{obsd} for all fluorescence intensity.

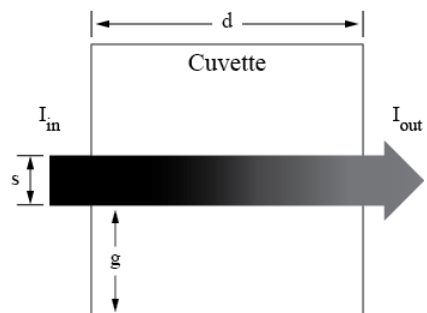


Figure S1. Diagram describing the cuvette path length values of g and s used to correct for the inner filter effect.

Synthesis of Monorhamnolipid Diastereomers

Synthesis of the Acceptor: Benzyl 3-hydroxydecanoate

Benzyl 3-oxodecanoate (5)

Meldrum's acid (19.5 g, 135 mmol) was dissolved in 200 mL dry THF at 0 °C in a flame-dried 500 mL round bottom flask. Dry pyridine (29.7 mL, 368 mmol) was added drop-wise to the reaction

mixture, and the reaction was allowed to stir for 1.5 h at 0 °C. Octanoyl chloride (21.0 mL, 123 mmol) was then added to the reaction mixture drop-wise over a 1 h period, and the reaction was allowed to stir for 1 h at 0 °C. The reaction mixture was then allowed to warm to room temperature for 1 h and was concentrated by rotoevaporation. The red syrup was redissolved in benzyl alcohol (38 mL, 368 mmol) and 240 mL benzene, and the reaction mixture was refluxed for 3 h. The reaction mixture was allowed to cool to room temperature, and was then concentrated by rotoevaporation. The crude, red oil was purified via flash chromatography (10% ethyl acetate/hexanes) to yield **5** as a pale yellow oil; yield 94%; TLC: R_f = 0.79 (30% ethyl acetate/hexanes); $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.37 – 7.29 (m, 5H), 5.16 (s, 2H), 3.46 (s, 2H), 2.49 (t, J = 7.4 Hz, 2H), 1.56 (p, J = 7.2 Hz, 2H), 1.32 – 1.20 (m, 8H), 0.90 – 0.84 (m, 3H); $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 202.7, 167.0, 135.3, 128.6, 128.5, 128.4, 128.3, 67.0, 49.2, 43.0, 31.6, 29.0, 28.9, 23.4, 22.5, 14.0.

Benzyl 3-hydroxydecanoate (6)

5 (32 g, 116 mmol) was dissolved in 200 mL dry THF at room temperature in a flame-dried 500 mL round bottom flask, and NaCNBH_3 (21.8 g, 348 mmol) was added to the reaction mixture. Then, 2 M HCl was slowly added to the cloudy reaction mixture over a 45 min period until the reaction mixture attains a pH \sim 3, and the solution becomes clear. The reaction was allowed to stir at room temperature for 30 min and was then quenched with saturated NaHCO_3 . After transfer to a separatory funnel, the solution was washed twice with CH_2Cl_2 . The organic layer was dried over MgSO_4 , and the solution was concentrated by rotoevaporation. Purification was achieved via flash chromatography (10% ethyl acetate/hexanes) to yield **6** as a colorless oil; yield 89%; TLC: R_f 0.67 (30% ethyl acetate/hexanes); $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.39 – 7.28 (m, 5H), 5.14 (s, 2H), 4.01 (tdd, J = 8.9, 4.7, 3.2 Hz, 1H), 2.77 (s, 1H), 2.50 (ddd, J = 25.4, 16.4, 6.1 Hz, 2H), 1.57 – 1.21 (m, 12H), 0.90 – 0.84 (m, 3H); $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 172.9, 135.6, 128.6, 128.4, 128.2, 68.0, 66.5, 41.3, 36.5, 31.8, 29.5, 29.2, 25.4, 22.6, 14.1.

Glycosylation of Benzyl 3-hydroxydecanoate by Peracetylated Rhamnose

Benzyl 3-*O*-(2,3,4-tri-*O*-acetyl- α -L-rhamnopyranosyl)decanoate (7a)

Rhamnose peracetate (18 g, 54.2 mmol) was dissolved in 200 mL dry CH₃CN at room temperature in a flame-dried 500 mL round bottom flask. The lipid **6** (6.85 g, 24.6 mmol) and Bi(OTf)₃ (1.61 g, 2.46 mmol) were added and the reaction mixture was refluxed for 45 min. The reaction mixture was allowed to cool to room temperature, and the black-brown solution was diluted with CH₂Cl₂. Celite was added, and the solution was filtered and concentrated to obtain a black-brown syrup. Purification was achieved by flash chromatography (10% ethyl acetate-hexanes) and the product was obtained as a colorless oil; yield 64%; TLC: R_f 0.55 (30% ethyl acetate-hexanes); ¹H-NMR (499 MHz, CDCl₃) δ 7.36 – 7.27 (m, 10H), 5.23 (ddd, *J* = 10.1, 3.4, 1.0 Hz, 2H), 5.14 (dt, *J* = 4.4, 2.2 Hz, 1H), 5.13 – 5.10 (m, 5H), 5.02 (td, *J* = 10.0, 5.9 Hz, 2H), 4.87 (d, *J* = 1.6 Hz, 1H), 4.83 (d, *J* = 1.6 Hz, 1H), 4.14 – 4.02 (m, 2H), 3.95 – 3.86 (m, 2H), 2.66 – 2.46 (m, 4H), 2.12 (d, *J* = 5.9 Hz, 6H), 2.04 – 1.99 (m, 6H), 1.96 (d, *J* = 1.5 Hz, 6H), 1.63 – 1.43 (m, 4H), 1.37 – 1.19 (m, 23H), 1.18 (d, *J* = 6.3 Hz, 3H), 1.15 (d, *J* = 6.3 Hz, 3H), 0.85 (td, *J* = 6.9, 3.7 Hz, 6H); ¹³C-NMR (126 MHz, CDCl₃) δ 171.1, 170.9, 170.1, 170.0, 169.9, 135.7, 135.7, 128.5, 128.5, 128.4, 128.2, 128.2, 97.4, 96.2, 76.1, 75.1, 71.1, 70.3, 70.2, 69.1, 69.1, 66.7, 66.7, 66.5, 66.4, 40.2, 39.5, 35.0, 33.4, 31.7, 29.5, 29.4, 29.1, 29.1, 25.1, 24.7, 22.6, 22.6, 20.9, 20.8, 20.7, 17.3, 14.1.

Benzyl 3-*O*-(2,3,4-tri-*O*-benzoyl- α -L-rhamnopyranosyl)decanoate (7b)

Rhamnose perbenzoate (2.2 equiv.) was dissolved in dry CH₃CN in a flame-dried round bottom flask. The lipid **6** (1 equiv.) and Bi(OTf)₃ (0.10 equiv.) were added to the reaction flask. The reaction was refluxed for 2.5 h and allowed to cool to room temperature. The yellow-brown reaction mixture was diluted with CH₂Cl₂ and Celite was added. The mixture was filtered and concentrated to a yellow-brown syrup. Purification was achieved by column chromatography (0-20% ethyl acetate-hexanes gradient). The mixture of diastereomers **7b** was obtained as clear oil; yield 33%; TLC: R_f 0.74 (30% ethyl acetate-hexanes); ¹H-NMR (499 MHz, CDCl₃) δ 8.11 – 8.06 (m, 4H), 7.98 – 7.90 (m, 4H), 7.84 – 7.78 (m, 4H), 7.61 – 7.55 (m, 2H), 7.51 – 7.43 (m, 6H), 7.41 – 7.19 (m, 21H), 5.79 (dd, *J* = 10.1, 3.3 Hz, 2H), 5.65 (td, *J* = 10.0, 1.6 Hz, 2H), 5.58 (dd, *J* = 3.3, 1.8

Hz, 1H), 5.56 (dd, $J = 3.3, 1.8$ Hz, 1H), 5.18 (t, $J = 3.8$ Hz, 5H), 5.14 (d, $J = 1.5$ Hz, 1H), 4.28 – 4.16 (m, 4H), 2.75 (dd, $J = 15.4, 7.9$ Hz, 1H), 2.71 (dd, $J = 15.5, 7.3$ Hz, 1H), 2.61 (t, $J = 15.5$ Hz, 1H), 2.60 (t, $J = 15.9$ Hz, 1H), 1.78 – 1.50 (m, 5H), 1.49 – 1.36 (m, 4H), 1.34 (d, $J = 6.3$ Hz, 3H), 1.31 (d, $J = 6.2$ Hz, 3H), 1.27 (dd, $J = 15.0, 9.0$ Hz, 18H), 0.86 (t, $J = 6.8$ Hz, 6H); ^{13}C -NMR (126 MHz, CDCl_3) δ 171.1, 170.9, 165.7, 165.7, 165.5, 165.4, 135.8, 135.7, 133.4, 133.3, 133.2, 133.2, 133.0, 129.8, 129.8, 129.7, 129.6, 129.5, 129.4, 129.3, 129.2, 128.5, 128.5, 128.5, 128.3, 128.2, 128.2, 97.5, 96.4, 76.4, 75.5, 71.8, 71.3, 71.2, 69.9, 67.1, 67.1, 66.6, 66.5, 40.3, 39.6, 35.1, 33.6, 31.7, 29.5, 29.4, 29.2, 29.1, 25.2, 24.8, 22.6, 17.6, 17.5, 14.0.

Debenzylation of Glycoside 7a and Separation of the Two Free Acid Diastereomers by Flash Column Chromatography

(R)-3-O-(2,3,4-tri-O-acetyl- α -L-rhamnopyranosyl)decanoic acid (8a)

7a (8.57 g, 15.6 mmol) was dissolved in 100 mL dry THF at room temperature in a flame-dried 250 mL round bottom flask, and a catalytic amount of 10 wt% Pd/C was added. The reaction mixture was placed under H_2 gas (1 atm) and stirred for 24 h at room temperature. The reaction mixture was diluted in CH_2Cl_2 , filtered through Celite and concentrated. Purification was achieved via flash chromatography (1:1 Et₂O:hexanes with 1% v/v AcOH). Product **8a** was obtained as a clear oil; yield 33%; $[\alpha]_{\text{D}} = -47.9^\circ$ ($c = 1.0$ in CHCl_3); TLC: R_f : 0.38 (1:1 Et₂O:hexanes with 1% v/v AcOH); ^1H -NMR (400 MHz, CDCl_3) δ 5.23 (dd, $J = 10.1, 3.4$ Hz, 1H), 5.14 (dd, $J = 3.4, 1.8$ Hz, 1H), 5.02 (t, $J = 9.9$ Hz, 1H), 4.82 (d, $J = 1.7$ Hz, 1H), 4.07 (dq, $J = 7.5, 5.9$ Hz, 1H), 3.93 (dq, $J = 9.9, 6.3$ Hz, 1H), 2.63 (dd, $J = 15.9, 7.6$ Hz, 1H), 2.52 (dd, $J = 15.9, 4.8$ Hz, 1H), 2.12 (s, 3H), 2.02 (s, 3H), 1.96 (s, 3H), 1.52 (ddd, $J = 23.0, 14.2, 5.3$ Hz, 2H), 1.32 – 1.21 (m, 10H), 1.15 (d, $J = 6.3$ Hz, 3H), 0.87 – 0.82 (m, 3H); ^{13}C -NMR (101 MHz, CDCl_3) δ 176.7, 170.2, 170.1, 170.0, 96.4, 75.1, 71.1, 70.3, 69.1, 66.8, 39.9, 33.6, 31.7, 29.5, 29.1, 24.8, 22.6, 20.9, 20.8, 20.7, 17.2, 14.0.

(S)-3-O-(2,3,4-Tri-O-acetyl- α -L-rhamnopyranosyl)decanoic acid (8b)

7a (8.57 g, 15.6 mmol) was dissolved in 100 mL dry THF at room temperature in a flame-dried 250 mL round bottom flask, and a catalytic amount of 10 wt% Pd/C was added. The reaction mixture was placed under H_2 gas (1 atm) and stirred for 24 h at room temperature. The reaction

mixture was diluted in CH₂Cl₂, filtered through Celite and concentrated. Purification was achieved via flash chromatography (1:1 Et₂O:hexanes with 1% v/v AcOH). Product **8b** was obtained as a colorless oil; yield 38%; [α]D = -30.6° (c = 1.0 in CHCl₃); TLC: R_f: 0.26 (1:1 Et₂O:hexanes with 1% v/v AcOH); ¹H-NMR (400 MHz, CDCl₃) δ 5.24 (dd, *J* = 10.1, 3.5 Hz, 1H), 5.12 (dd, *J* = 3.4, 1.8 Hz, 1H), 5.03 (t, *J* = 9.9 Hz, 1H), 4.89 (d, *J* = 1.8 Hz, 1H), 4.04 (dq, *J* = 11.7, 5.9 Hz, 1H), 3.93 (dq, *J* = 9.8, 6.3 Hz, 1H), 2.57 (dd, *J* = 15.8, 7.5 Hz, 1H), 2.49 (dd, *J* = 15.8, 5.3 Hz, 1H), 2.11 (s, 3H), 2.03 (s, 3H), 1.96 (s, 3H), 1.65 – 1.50 (m, 2H), 1.32 – 1.22 (m, 10H), 1.18 (d, *J* = 6.3 Hz, 3H), 0.89 – 0.84 (m, 3H); ¹³C-NMR (101 MHz, CDCl₃) δ 176.2, 170.2, 170.1, 170.1, 97.5, 76.2, 71.1, 70.3, 69.1, 66.8, 39.3, 35.0, 31.7, 29.4, 29.1, 25.1, 22.6, 20.9, 20.8, 20.7, 20.7, 17.2, 14.0.

Addition of the Second Lipid Tail

Benzyl (*R/S*)-3-*O*-[(*R*)-(3'-*O*-decyl)2,3,4-tri-*O*-acetyl- α -L-rhamnopyranosyl]decanoate (9a**)**

8a (545 mg, 1.18 mmol) was dissolved in 25 mL dry CH₂Cl₂ at room temperature in a flame-dried 100 mL round bottom flask. EDC (490 mg, 3.16 mmol) and DMAP (144 mg, 1.18 mmol) were added to the reaction mixture, and the reaction was allowed to stir for 10 min at room temperature. The lipid **6** (219 mg, 0.789 mmol) was dissolved in 5 mL dry CH₂Cl₂ and added to the reaction mixture, where it was stirred for 24 h at room temperature. The reaction was quenched with saturated NaHCO₃, and the product was extracted 3x with CH₂Cl₂. The combined organic layers were dried over MgSO₄, and the solution was filtered and concentrated. Purification was achieved via flash chromatography (10% ethyl acetate-hexanes), and the product **9a** was obtained as a colorless oil; yield 62%; TLC: R_f: 0.66 (30% ethyl acetate-hexanes); ¹H-NMR (400 MHz, CDCl₃) δ 7.35 – 7.27 (m, 10H), 5.26 – 5.17 (m, 4H), 5.14 (dd, *J* = 3.4, 1.8 Hz, 2H), 5.10 (s, 2H), 5.09 (d, *J* = 0.7 Hz, 2H), 5.03 (t, *J* = 9.9 Hz, 2H), 5.02 (t, *J* = 9.9 Hz, 2H), 4.81 (d, *J* = 1.7 Hz, 2H), 4.04 (dd, *J* = 12.5, 5.5 Hz, 2H), 3.92 (ddd, *J* = 10.0, 6.3, 3.8 Hz, 2H), 2.71 – 2.56 (m, 4H), 2.56 – 2.30 (m, 4H), 2.12 (d, *J* = 0.5 Hz, 6H), 2.01 (s, 3H), 1.97 (s, 3H), 1.96 (d, *J* = 0.6 Hz, 6H), 1.58 (s, 4H), 1.48 (s, 4H), 1.31 – 1.21 (m, 40H), 1.18 (d, *J* = 6.2 Hz, 6H), 0.89 – 0.83 (m, 12H); ¹³C-NMR (101 MHz, CDCl₃) δ 170.5, 170.5, 170.2, 170.2, 170.1, 170.1, 170.0, 128.6, 128.3, 77.2,

71.2, 70.9, 70.3, 69.1, 69.1, 66.7, 66.5, 66.4, 39.0, 33.9, 33.3, 33.2, 31.8, 29.6, 29.2, 25.2, 24.7, 22.6, 20.9, 20.8, 20.7, 17.3, 17.3, 14.1.

Benzyl (*R/S*)-3-*O*-[(*S*)-(3'-*O*-decyl)2,3,4-tri-*O*-acetyl- α -L-rhamnopyranosyl]decanoate (9b**)**

8b (331 mg, 0.719 mmol) was dissolved in 15 mL dry CH₂Cl₂ at room temperature in a flame-dried 100 mL round bottom flask. EDC (298 mg, 1.92 mmol) and DMAP (88 mg, 0.719 mmol) were added to the reaction mixture, and the reaction was allowed to stir for 10 min at room temperature. The lipid **6** (133 mg, 0.479 mmol) was dissolved in 5 mL dry CH₂Cl₂ and added to the reaction mixture, where it was stirred for 24 h at room temperature. The reaction was quenched with saturated NaHCO₃, and the product was extracted 3x with CH₂Cl₂. The combined organic layers were dried over MgSO₄, and the solution was filtered and concentrated. Purification was achieved via flash chromatography (10% ethyl acetate-hexanes), obtaining the product **9b** as a colorless oil; yield 67%; TLC: R_f: 0.53 (30% ethyl acetate-hexanes); ¹H-NMR (400 MHz, CDCl₃) δ 7.37 – 7.26 (m, 10H), 5.24 (dd, *J* = 3.4, 1.7 Hz, 1H), 5.23 – 5.17 (m, 3H), 5.15 (dd, *J* = 3.5, 1.8 Hz, 1H), 5.11 (dd, *J* = 3.4, 1.8 Hz, 1H), 5.09 (s, 2H), 5.08 (d, *J* = 0.8 Hz, 2H), 5.03 (t, *J* = 9.9 Hz, 1H), 5.03 (t, *J* = 9.9 Hz, 1H), 4.86 (d, *J* = 1.8 Hz, 1H), 4.85 (d, *J* = 1.8 Hz, 1H), 4.04 – 3.95 (m, 2H), 3.91 (dq, *J* = 12.5, 6.2 Hz, 2H), 2.68 – 2.53 (m, 4H), 2.39 (dddd, *J* = 23.0, 21.4, 11.3, 4.7 Hz, 4H), 2.11 (d, *J* = 1.1 Hz, 6H), 2.03 (s, 6H), 1.94 (d, *J* = 4.3 Hz, 6H), 1.55 (d, *J* = 13.8 Hz, 9H), 1.25 (d, *J* = 13.4 Hz, 40H), 1.19 (d, *J* = 6.3 Hz, 6H), 0.89 – 0.83 (m, 12H); ¹³C-NMR (101 MHz, CDCl₃) δ 170.4, 170.2, 170.2, 170.0, 169.9, 169.9, 128.5, 128.5, 128.4, 128.3, 128.3, 128.2, 97.2, 96.9, 75.7, 75.3, 71.2, 71.0, 70.2, 69.2, 66.7, 66.7, 66.4, 39.3, 39.2, 39.1, 39.0, 39.0, 34.9, 34.9, 33.9, 31.8, 31.7, 29.5, 29.3, 29.2, 29.1, 29.1, 25.2, 25.1, 22.6, 22.6, 20.9, 20.8, 20.7, 17.3, 17.3, 14.1.

Debenzylation of Glycosides **9a and **9b**, and Resolution of the Four Free Acid Diastereomers by Flash Column Chromatography**

(*R*)-3-(((*R*)-3-[2,3,4-Tri-*O*-acetyl- α -L-rhamnopyranosyl]decanoyl)oxy)decanoic acid (10a**)**

9a (158 mg, 0.219 mmol) was dissolved in 25 mL dry THF at room temperature in a flame-dried 100 mL round bottom flask, and a catalytic amount of 10 wt% Pd/C was added to the reaction

mixture. The reaction was then placed under H₂ gas (1 atm) at room temperature and stirred for 2.5 h. The mixture was diluted in CH₂Cl₂, filtered through Celite and concentrated. Purification was achieved via flash chromatography (1:1 Et₂O:hexanes with 1% v/v AcOH). Product **10a** was obtained as a clear oil; yield 37%; [α]_D = -53.1° (c = 1.0 in CHCl₃); TLC: R_f: 0.36 (60:40 Et₂O:hexanes with 1% v/v AcOH); ¹H-NMR (400 MHz, CDCl₃) δ 8.87 (s, 1H), 5.30 (m, 1H), 5.23 (dd, *J* = 10.1, 3.5 Hz, 1H), 5.10 (dd, *J* = 3.4, 1.7 Hz, 1H), 5.00 (t, *J* = 10.0 Hz, 1H), 4.83 (d, *J* = 1.6 Hz, 1H), 4.13 (qd, *J* = 8.7, 4.5 Hz, 1H), 3.99 (dq, *J* = 9.9, 6.2 Hz, 1H), 2.61 (dd, *J* = 15.6, 8.4 Hz, 1H), 2.54 (m, 2H), 2.44 (dd, *J* = 15.7, 4.1 Hz, 1H), 2.11 (s, 3H), 2.01 (s, 3H), 1.97 (s, 3H), 1.51 (ddd, *J* = 21.8, 13.5, 6.5 Hz, 4H), 1.23 (d, *J* = 3.0 Hz, 20H), 1.18 (d, *J* = 6.3 Hz, 3H), 0.84 (dd, *J* = 8.3, 5.5 Hz, 6H); ¹³C-NMR (101 MHz, CDCl₃) δ 173.6, 171.0, 170.9, 170.2, 170.2, 94.5, 72.7, 71.2, 70.8, 70.3, 69.6, 66.6, 39.5, 39.1, 34.2, 31.7, 31.7, 30.3, 29.5, 29.3, 29.1, 29.1, 25.1, 24.5, 22.6, 20.9, 20.8, 20.8, 17.2, 14.0.

(S)-3-(((R)-[3-2,3,4-Tri-O-acetyl- α -L-rhamnopyranosyl]decanoyl)oxy)decanoic acid (10b)

9a (158 mg, 0.219 mmol) was dissolved in 25 mL dry THF at room temperature in a flame-dried 100 mL round bottom flask, and a catalytic amount of 10 wt% Pd/C was added to the reaction mixture. The reaction was then placed under H₂ gas (1 atm) at room temperature and stirred for 2.5 h. The mixture was diluted in CH₂Cl₂, filtered through Celite and concentrated. Purification was achieved via flash chromatography (1:1 Et₂O: hexanes with 1% v/v AcOH). Product **10b** was obtained as a colorless oil; yield 35%; [α]_D = -40.9° (c = 1.0 in CHCl₃); TLC: R_f: 0.44 (60:40 Et₂O:hexanes with 1% v/v AcOH); ¹H-NMR (400 MHz, CDCl₃) δ 9.51 (s, 1H), 5.19 (dd, *J* = 10.1, 3.4 Hz, 2H), 5.17 (m, 1H), 5.13 (dd, *J* = 3.4, 1.8 Hz, 1H), 5.01 (t, *J* = 9.9 Hz, 1H), 4.81 (d, *J* = 1.7 Hz, 1H), 4.04 (m, 1H), 3.92 (dq, *J* = 9.9, 6.2 Hz, 1H), 2.61 (dd, *J* = 14.7, 6.0 Hz, 1H), 2.59 (m, 2H), 2.44 (dd, *J* = 15.6, 5.1 Hz, 1H), 2.11 (s, 3H), 2.00 (s, 3H), 1.94 (s, 3H), 1.66 – 1.45 (m, 4H), 1.22 (t, *J* = 10.3 Hz, 20H), 1.16 (d, *J* = 6.3 Hz, 3H), 0.84 (q, *J* = 6.2 Hz, 6H); ¹³C-NMR (101 MHz, CDCl₃) δ 175.7, 170.5, 170.2, 170.0, 96.1, 74.9, 71.1, 70.7, 70.3, 69.2, 66.7, 40.0, 38.7, 33.8, 33.1, 31.8, 31.7, 30.3, 29.5, 29.3, 29.1, 29.1, 25.1, 24.8, 22.6, 20.9, 20.7, 20.7, 17.2, 14.0.

(S)-3-(((S)-[3-2,3,4-Tri-O-acetyl- α -L-rhamnopyranosyl]decanoyl)oxy)decanoic acid (10c)

9b (230 mg, 0.319 mmol) was dissolved in 25 mL dry THF at room temperature in a flame-dried 100 mL round bottom flask, and a catalytic amount of 10 wt% Pd/C was added to the solution. The reaction mixture was placed in H₂ gas (1 atm) at room temperature, and the reaction was stirred for 2.5 h. The reaction mixture was diluted in CH₂Cl₂, filtered through Celite and concentrated. Purification was achieved via flash chromatography (1:1 Et₂O:hexanes with 1% v/v AcOH). Product **10c** was obtained as a clear oil; yield 38%; [α]D = -27.9° (c = 1.0 in CHCl₃); TLC: R_f: 0.22 (60:40 Et₂O:hexanes with 1% v/v AcOH); ¹H-NMR (400 MHz, CDCl₃) δ 9.45 (s, 1H), 5.28 (m, 1H), 5.16 (dd, *J* = 9.3, 3.2 Hz, 1H), 5.12 (m, 1H), 5.01 (t, *J* = 9.8 Hz, 1H), 4.86 (d, *J* = 1.3 Hz, 1H), 4.03 (dt, *J* = 8.2, 6.1 Hz, 1H), 3.89 (dq, *J* = 9.8, 6.3 Hz, 1H), 2.54 (m, 2H), 2.48 (dd, *J* = 16.0, 8.2 Hz, 1H), 2.41 (dd, *J* = 16.0, 4.3 Hz, 1H), 2.09 (s, 3H), 2.00 (s, 3H), 1.94 (s, 3H), 1.64 – 1.46 (m, 5H), 1.32 – 1.18 (m, 20H), 1.16 (d, *J* = 6.3 Hz, 3H), 0.83 (ddd, *J* = 11.5, 5.8, 3.1 Hz, 6H); ¹³C-NMR (101 MHz, CDCl₃) δ 173.5, 170.9, 170.8, 170.5, 170.0, 98.3, 70.9, 70.8, 70.1, 69.7, 66.6, 39.4, 39.1, 35.0, 34.1, 31.7, 31.7, 30.2, 29.4, 29.2, 29.1, 29.0, 25.1, 25.0, 22.6, 22.5, 20.9, 20.8, 20.7, 17.2, 14.0.

(R)-3-(((S)-[3-2,3,4-tri-O-acetyl- α -L-rhamnopyranosyl]decanoyl)oxy)decanoic acid (10d)

9b (230 mg, 0.319 mmol) was dissolved in 25 mL dry THF at room temperature in a flame-dried 100 mL round bottom flask, and a catalytic amount of 10 wt% Pd/C was added to the solution. The reaction mixture was placed in H₂ gas (1 atm) at room temperature, and the reaction was stirred for 2.5 h. The reaction mixture was diluted in CH₂Cl₂, filtered through Celite and concentrated. Purification was achieved via flash chromatography (1:1 Et₂O: hexanes with 1% v/v AcOH). Product **10d** was obtained as a colorless oil; yield 39%; [α]D = -31.8° (c = 1.0 in CHCl₃); TLC: R_f: 0.27 (60:40 Et₂O:hexanes with 1% v/v AcOH); ¹H-NMR (400 MHz, CDCl₃) δ 9.72 (s, 1H), 5.21 (dd, *J* = 10.1, 3.4 Hz, 1H), 5.17 (m, 1H), 5.09 (dd, *J* = 3.4, 1.8 Hz, 1H), 5.01 (t, *J* = 9.9 Hz, 1H), 4.85 (d, *J* = 1.6 Hz, 1H), 3.99 (m, 1H), 3.90 (dq, *J* = 9.9, 6.2 Hz, 1H), 2.56 (m, 2H), 2.48 (dd, *J* = 15.5, 6.9 Hz, 1H), 2.42 (dd, *J* = 15.4, 5.4 Hz, 1H), 2.10 (s, 3H), 2.00 (s, 3H), 1.93 (s, 3H), 1.66 – 1.47 (m, 4H), 1.23 (d, *J* = 5.3 Hz, 20H), 1.16 (d, *J* = 6.3 Hz, 3H), 0.84 (td, *J* = 6.8, 3.6 Hz, 6H); ¹³C-NMR (101 MHz, CDCl₃) δ 175.3, 170.3, 170.3, 170.0, 170.0, 97.2, 76.1, 71.1, 70.8, 70.3, 69.1,

66.7, 39.3, 38.8, 34.8, 33.8, 31.7, 30.3, 29.4, 29.2, 29.1, 29.0, 25.1, 25.1, 22.6, 22.5, 20.9, 20.7, 20.7, 17.2, 14.0.

Final Products: Deacetylation of Glycosides 10a, 10b, 10c and 10d

(R)-3-(((R)-3-[α -L-rhamnopyranosyl]decanoyl)oxy)decanoic acid (1)

10a (51 mg, 0.081 mmol) was dissolved in 5 mL dry CH₃OH at room temperature in a flame-dried 25 mL round bottom flask, and NaOCH₃ was added until the reaction mixture attained a pH 9-10. The reaction mixture was allowed to stir for 3 h at room temperature. The reaction was quenched with Dowex H⁺ resin, the resin was filtered, and the solution was concentrated. The residue was redissolved in CH₂Cl₂, the solid Na⁺ salts were filtered, and the solution was concentrated. No further purification was required. Product **1** was obtained as a colorless oil; yield 98%; [α]_D = -61.0° (c = 1.0 in CHCl₃); TLC: R_f: 0.45 (10% CH₃OH-CH₂Cl₂ with 1% v/v AcOH); ¹H-NMR (400 MHz, CDCl₃/CD₃OD) δ 5.47 (td, *J* = 11.9, 6.4 Hz, 1H), 4.92 (s, 1H), 4.33 – 4.24 (m, 1H), 3.87 (s, 1H), 3.83 – 3.73 (m, 2H), 3.46 – 3.37 (m, 2H), 2.68 – 2.52 (m, 4H), 1.75 – 1.58 (m, 4H), 1.39 (d, *J* = 5.8 Hz, 23H), 0.99 (t, *J* = 6.2 Hz, 6H); ¹³C-NMR (101 MHz, CDCl₃) δ 174.6, 171.4, 96.5, 72.4, 71.9, 71.4, 70.8, 70.2, 68.0, 39.8, 39.4, 33.8, 31.7, 31.2, 29.1, 28.8, 28.7, 28.6, 24.6, 24.0, 22.0, 16.5, 13.2; ESI-MS (neg.): *m/z*: 1007.0 (55, [2M - H]⁻), 503.3 (99, [M - H]⁻).

(S)-3-(((R)-3-[α -L-rhamnopyranosyl]decanoyl)oxy)decanoic acid (2)

10b (38 mg, 0.060 mmol) was dissolved in 5 mL dry CH₃OH at room temperature in a flame-dried 25 mL round bottom flask, and NaOCH₃ was added until the reaction mixture attained a pH 9-10. The reaction mixture was allowed to stir for 3 h at room temperature. The reaction was quenched with Dowex H⁺ resin, the resin was filtered, and the solution was concentrated. The residue was redissolved in CH₂Cl₂, the solid Na⁺ salts were filtered, and the solution was concentrated. No further purification was required. Product **2** was obtained as a colorless oil; yield 97%; [α]_D = -50.1° (c = 1.0 in CHCl₃); TLC: R_f: 0.43 (10% CH₃OH-CH₂Cl₂ with 1% v/v AcOH); ¹H-NMR (400 MHz, CDCl₃/CD₃OD) δ 5.50 (dt, *J* = 12.3, 6.0 Hz, 1H), 5.08 (d, *J* = 1.5 Hz, 1H), 4.29 – 4.21 (m, 1H), 4.07 (dd, *J* = 3.3, 1.6 Hz, 1H), 3.99 – 3.91 (m, 2H), 3.64 – 3.57 (m, 2H), 2.87 – 2.71 (m, 4H), 1.93 – 1.79 (m, 4H), 1.54 (d, *J* = 6.2 Hz, 23H), 1.14 (dd, *J* = 7.0, 5.5 Hz, 6H); ¹³C-NMR

(101 MHz, CDCl₃/CD₃OD) δ 174.9, 170.8, 98.8, 74.0, 72.3, 71.9, 70.7, 70.6, 68.3, 40.1, 40.0, 33.7, 32.9, 31.3, 29.1, 28.9, 28.8, 28.7, 24.8, 24.7, 22.1, 16.7, 13.4; ESI-MS (neg.): m/z : 1007.0 (55, [2M- H]⁻), 503.3 (99, [M - H]⁻).

(S)-3-(((S)-3-[α -L-rhamnopyranosyl]decanoyl)oxy)decanoic acid (3)

10c (77 mg, 0.122 mmol) was dissolved in 5 mL dry CH₃OH at room temperature in a flame-dried 25 mL round bottom flask, and NaOCH₃ was added until the reaction mixture attained a pH 9-10. The reaction was then allowed to stir for 3 h at room temperature. The reaction was quenched with Dowex H⁺ resin, the resin was filtered, and the solution was concentrated. The residue was redissolved in CH₂Cl₂, the solid Na⁺ salts were filtered, and the solution was concentrated. No further purification was required. Product **3** was obtained as a colorless oil; yield 98%; [α]_D = -5.19° (c = 1.0 in CHCl₃); TLC: R_f: 0.35 (10% CH₃OH-CH₂Cl₂ with 1% v/v AcOH); ¹H-NMR (400 MHz, CDCl₃/CD₃OD) δ 5.47 – 5.38 (m, 1H), 4.90 (d, J = 1.4 Hz, 1H), 4.21 – 4.13 (m, 1H), 3.88 (dd, J = 3.4, 1.7 Hz, 1H), 3.82 – 3.72 (m, 2H), 3.48 (t, J = 9.5 Hz, 1H), 2.61 (d, J = 6.2 Hz, 2H), 2.57 – 2.52 (m, 2H), 1.64 (ddd, J = 21.3, 16.5, 10.5 Hz, 4H), 1.35 (dd, J = 17.0, 5.0 Hz, 23H), 1.02 – 0.93 (m, 6H); ¹³C-NMR (101 MHz, CDCl₃/CD₃OD) δ 175.1, 171.6, 100.2, 75.5, 71.8, 71.7, 70.4, 70.3, 68.4, 40.1, 39.7, 34.9, 33.9, 31.3, 31.2, 29.6, 29.0, 28.9, 28.7, 28.6, 24.7, 22.1, 22.1, 16.6, 13.3; ESI-MS (neg.): m/z : 1007.0 (55, [2M- H]⁻), 503.3 (99, [M - H]⁻).

(R)-3-(((S)-3-[α -L-rhamnopyranosyl]decanoyl)oxy)decanoic acid (4)

10d (78 mg, 0.124 mmol) was dissolved in 5 mL dry CH₃OH at room temperature in a flame-dried 25 mL round bottom flask, and NaOCH₃ was added until the reaction mixture attained a pH 9-10. The reaction was then allowed to stir for 3 h at room temperature. The reaction was quenched with Dowex H⁺ resin, the resin was filtered, and the solution was concentrated. The residue was redissolved in CH₂Cl₂, the solid Na⁺ salts were filtered, and the solution was concentrated. No further purification was required. Product **4** was obtained as a colorless oil; yield 98%; [α]_D = -13.0° (c = 1.0 in CHCl₃); TLC: R_f: 0.33 (10% CH₃OH-CH₂Cl₂ with 1% v/v AcOH); ¹H-NMR (400 MHz, CDCl₃/CD₃OD) δ 5.51 – 5.41 (m, 1H), 4.98 (m, 1H), 4.23 – 4.11 (m, 1H), 4.02 – 3.96 (m, 1H), 3.84 (dd, J = 7.5, 5.8 Hz, 2H), 3.63 – 3.49 (m, 1H), 2.63 (ddd, J = 22.6, 16.9, 6.6

Hz, 4H), 1.92 – 1.65 (m, 4H), 1.46 (s, 23H), 1.12 – 1.00 (m, 6H); ^{13}C -NMR (101 MHz, $\text{CDCl}_3/\text{CD}_3\text{OD}$) δ 175.3, 170.9, 100.2, 75.7, 72.0, 72.0, 70.5, 70.5, 68.4, 40.0, 39.8, 34.9, 33.8, 31.4, 31.4, 29.2, 29.0, 28.8, 28.7, 24.8, 24.7, 22.2, 16.7, 13.4; ESI-MS (neg.): m/z : 1007.0 (55, $[2\text{M} - \text{H}]^-$), 503.3 (99, $[\text{M} - \text{H}]^-$).

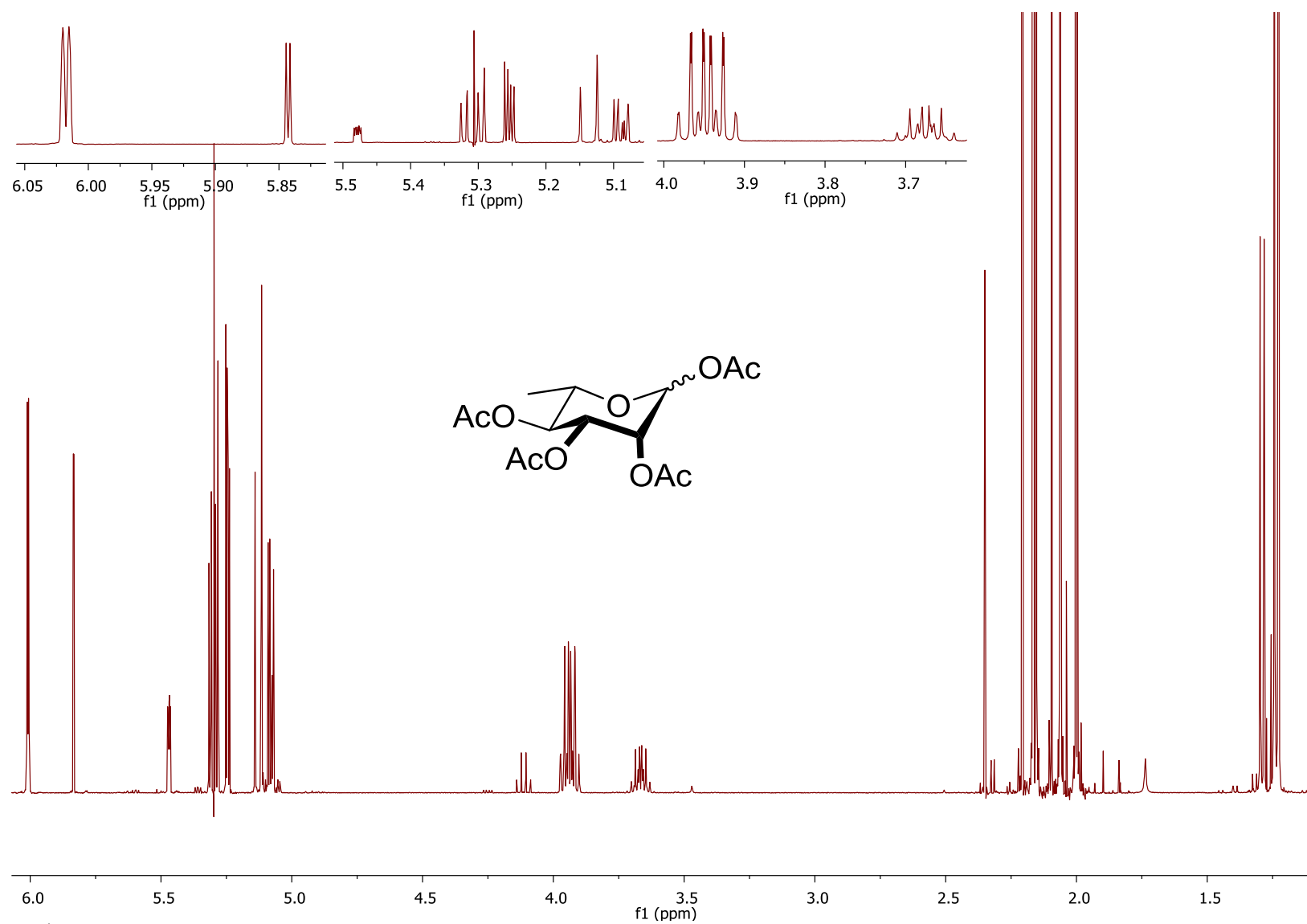


Figure S2. ^1H -NMR spectra of (α/β) -peracetylated rhamnose.

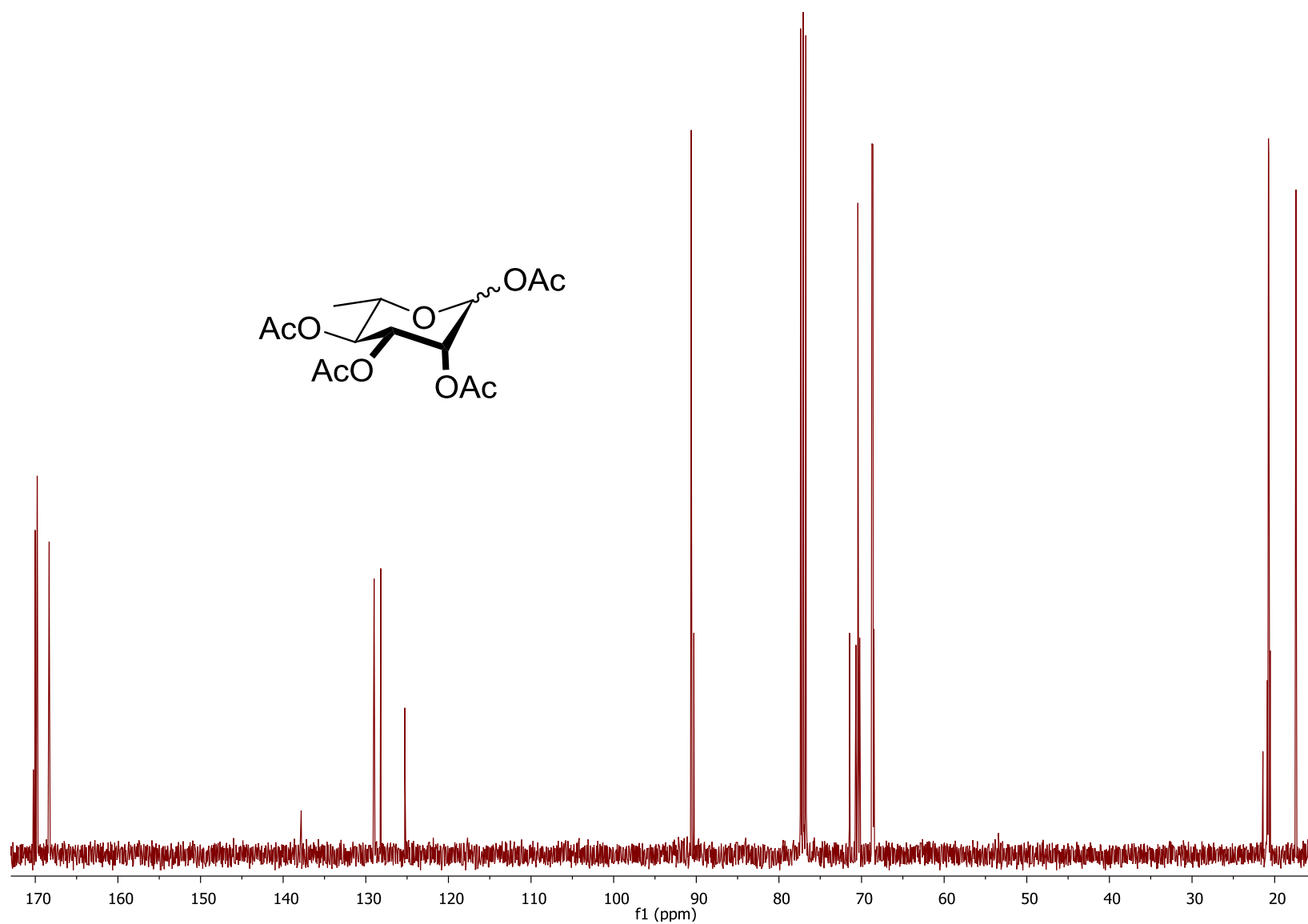


Figure S3. ^{13}C -NMR spectra of (α/β) -peracetylated rhamnose.

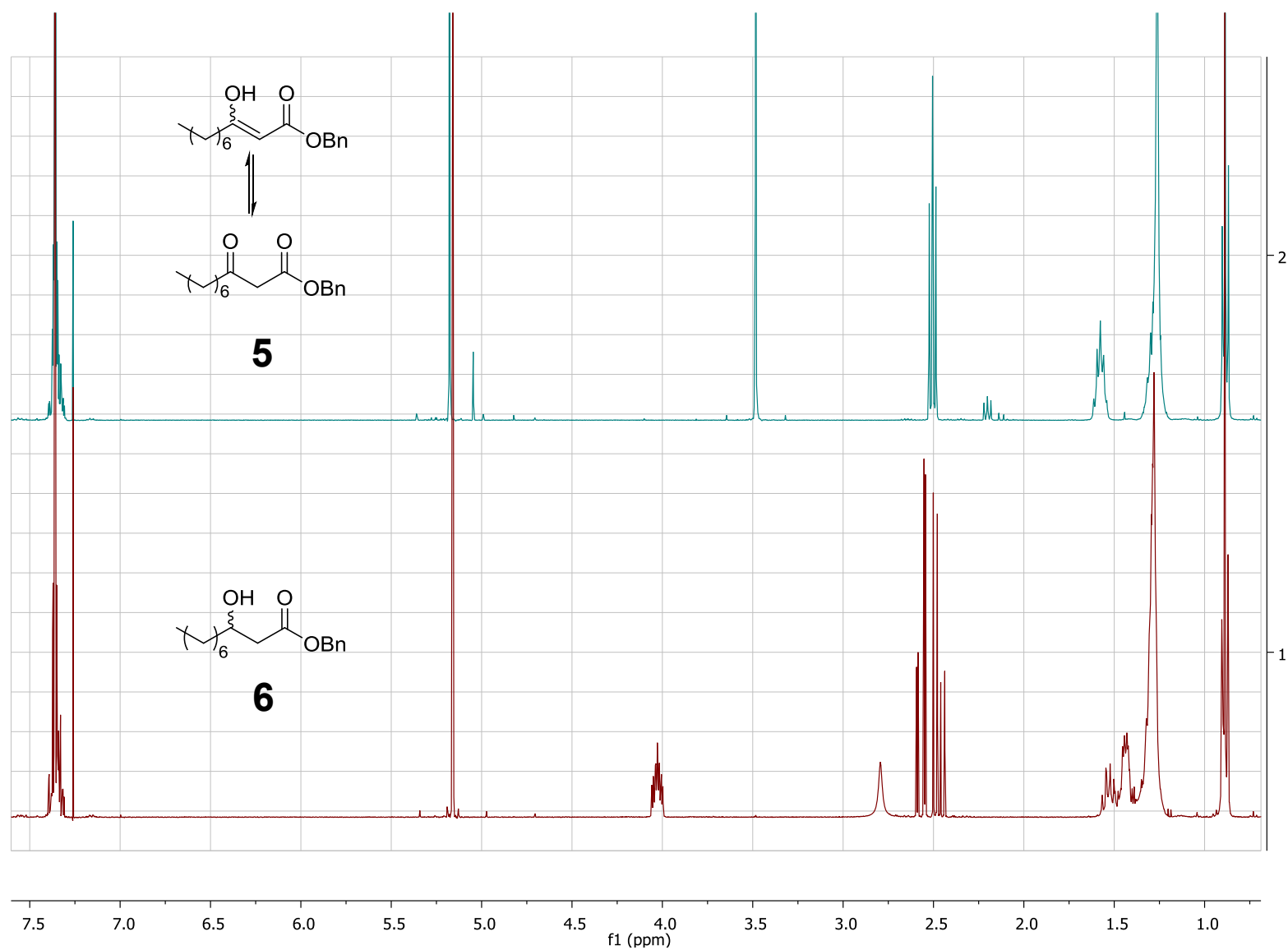


Figure S4. ¹H-NMR spectrum of benzyl 3-oxodecanoate ester (**5**) and benzyl 3-hydroxydecanoate ester (**6**).

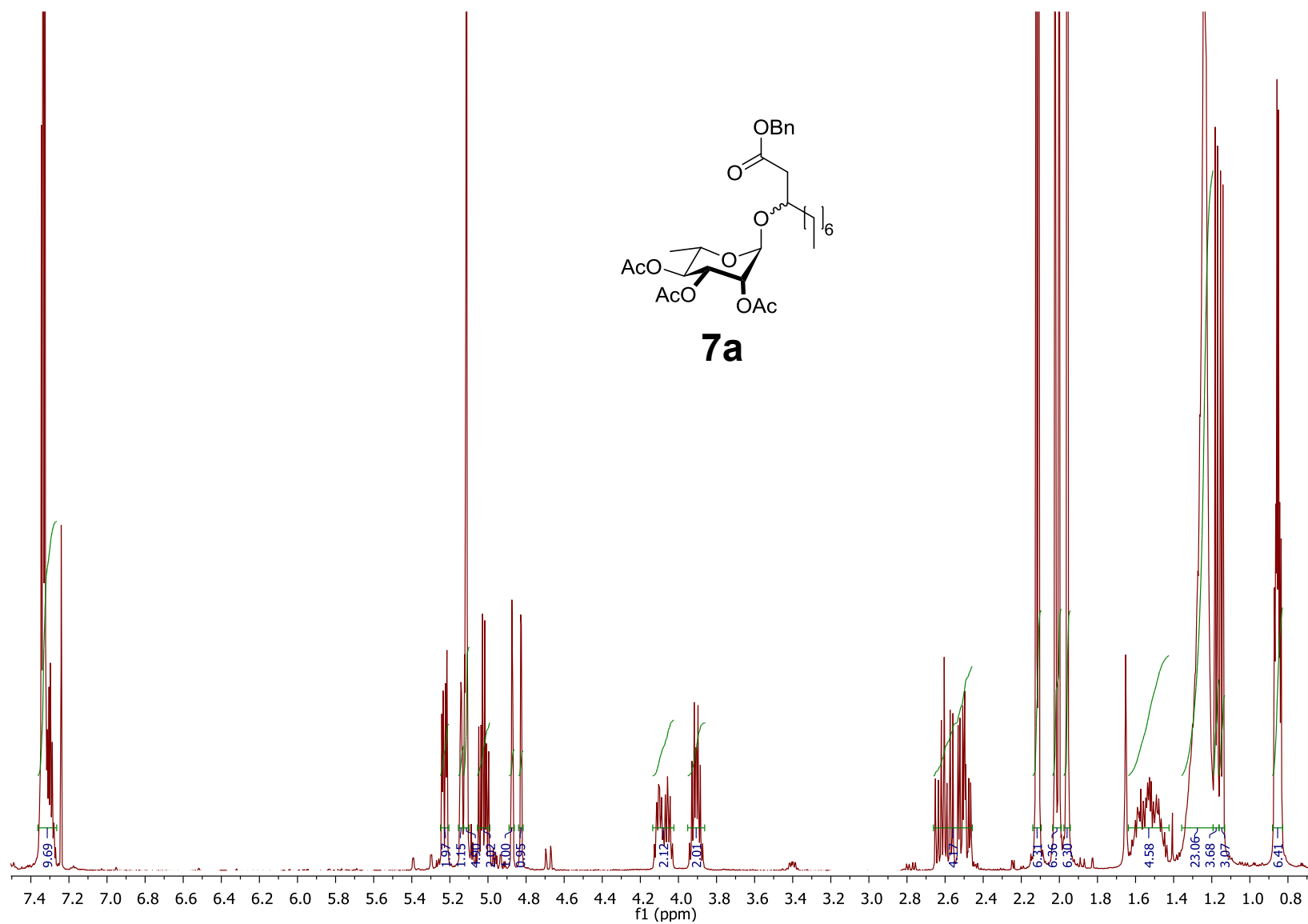


Figure S5. ^1H -NMR spectra of benzyl 3-O-(2,3,4-tri-O-acetyl- α -L-rhamnopyranosyl)decanoate (**7a**).

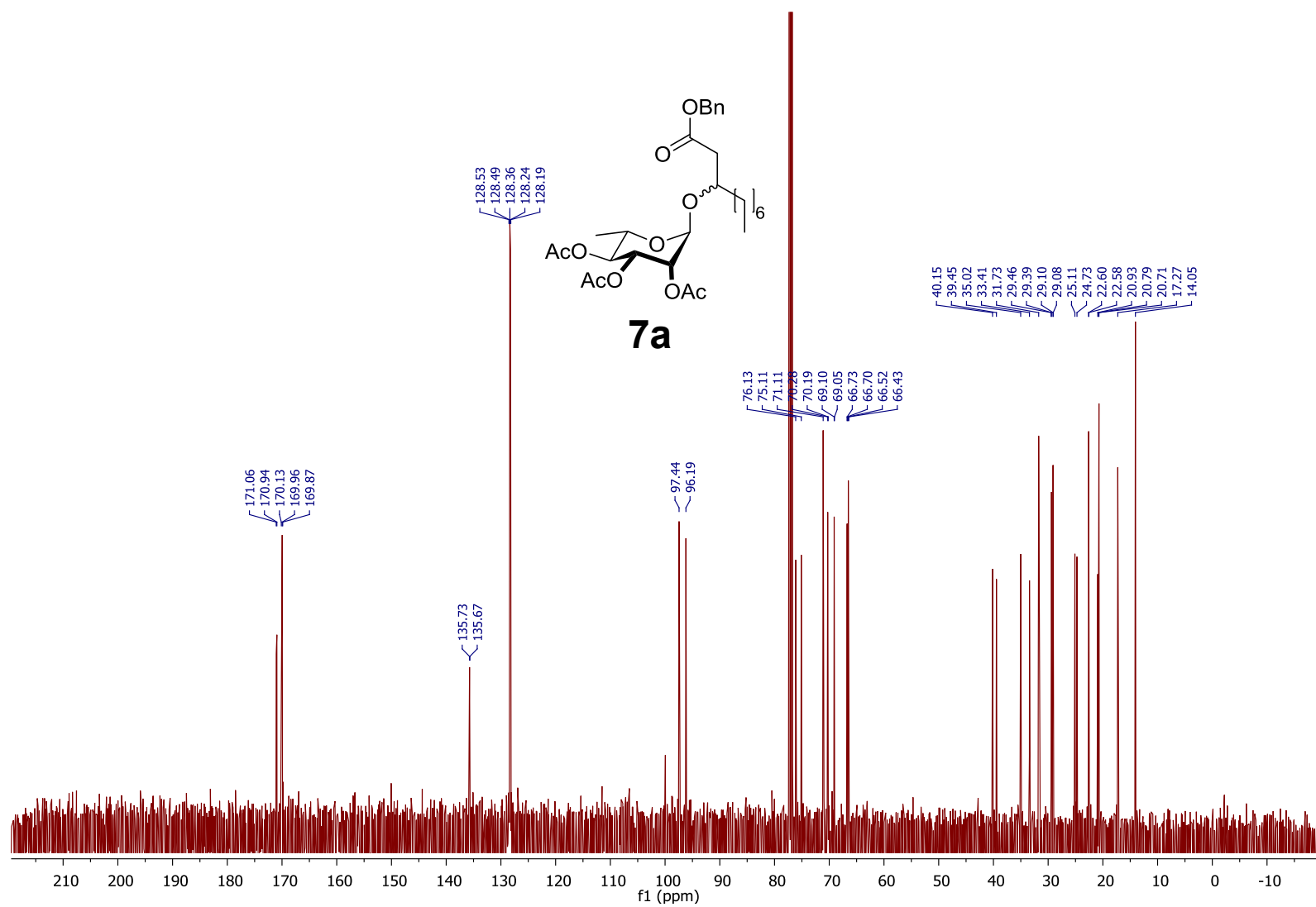


Figure S6. ^{13}C -NMR spectra of benzyl 3-O-(2,3,4-tri-O-acetyl- α -L-rhamnopyranosyl)decanoate (**7a**).

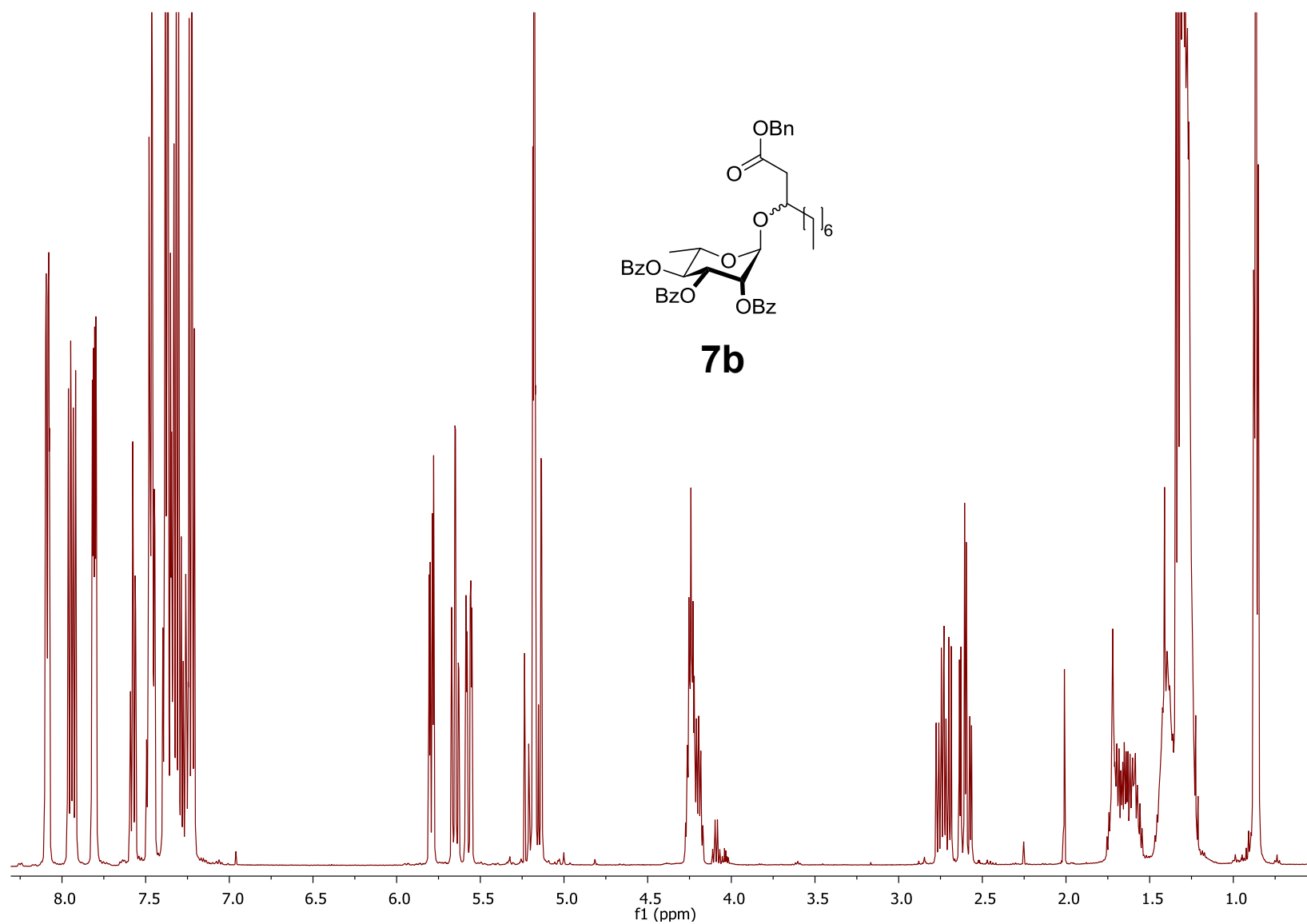


Figure S7 ^{13}C -NMR spectra of benzyl 3-O-(2,3,4-tri-O-benzoyl- α -L-rhamnopyranosyl)decanoate (**7b**).

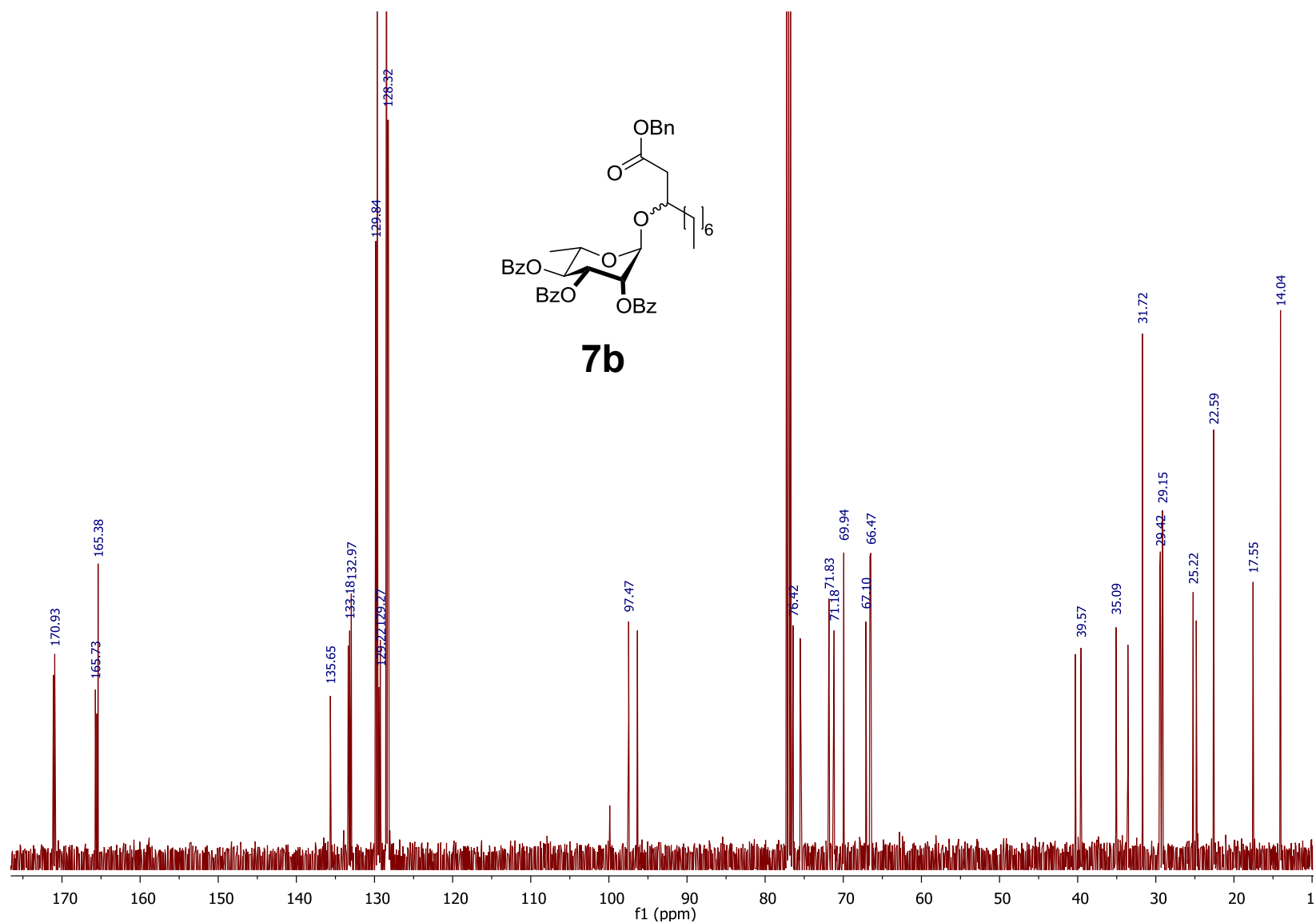
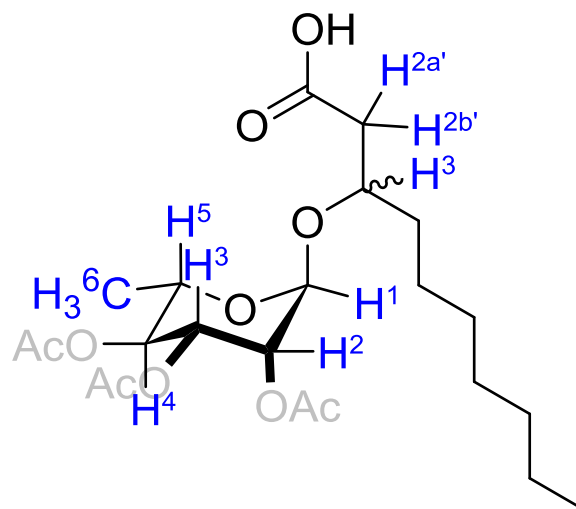


Figure S8 ¹³C-NMR spectra of benzyl 3-O-(2,3,4-tri-O-benzoyl-α-L-rhamnopyranosyl)decanoate (**7b**).

Table S1. ^1H -NMR and ^{13}C -NMR data for diastereomers **8a** and **8b**.

Compound	H-1	H-2	H-3	H-4	H-5	H-6	H-2a'	H-2b'	H-3'
^1H δ (ppm)	4.82 δ	5.14 δ	5.23 δ	5.02 δ	3.93 δ	1.15 δ	2.63 δ	2.52 δ	4.07 δ
8a	J (Hz)	$J_{1,2}$ 1.7	$J_{2,3}$ 3.4	$J_{3,4}$ 9.9	$J_{4,5}$ 9.9	$J_{5,6}$ 6.3	$J_{2a',3'}$ 7.6	$J_{2b',3'}$ 4.8	$J_{2a',3'}$ 7.5
		$J_{2,3}$ 3.4	$J_{3,4}$ 10.1	$J_{4,5}$ 9.9	$J_{5,6}$ 6.3		$J_{2a',2b'}$ 15.9	$J_{2a',2b'}$ 15.9	$J_{2b',3'}$ 5.9
^{13}C δ (ppm)	96.38 δ	70.30 δ	69.05 δ	71.10 δ	66.78 δ	17.15 δ	39.85 δ	39.85 δ	75.01 δ
^1H δ (ppm)	4.89 δ	5.12 δ	5.24 δ	5.03 δ	3.93 δ	1.18 δ	2.57 δ	2.49 δ	4.04 δ
8b	J (Hz)	$J_{1,2}$ 1.8	$J_{2,3}$ 3.5	$J_{3,4}$ 9.9	$J_{4,5}$ 9.8	$J_{5,6}$ 6.3	$J_{2a',3'}$ 7.5	$J_{2b',3'}$ 5.3	N/A
		$J_{2,3}$ 3.4	$J_{3,4}$ 10.1	$J_{4,5}$ 9.9	$J_{5,6}$ 6.3		$J_{2a',2b'}$ 15.8	$J_{2a',2b'}$ 15.8	$J_{2b',3'}$ 5.9
^{13}C δ (ppm)	97.50 δ	70.31 δ	69.07 δ	71.14 δ	66.79 δ	17.25 δ	39.31 δ	39.31 δ	76.20 δ



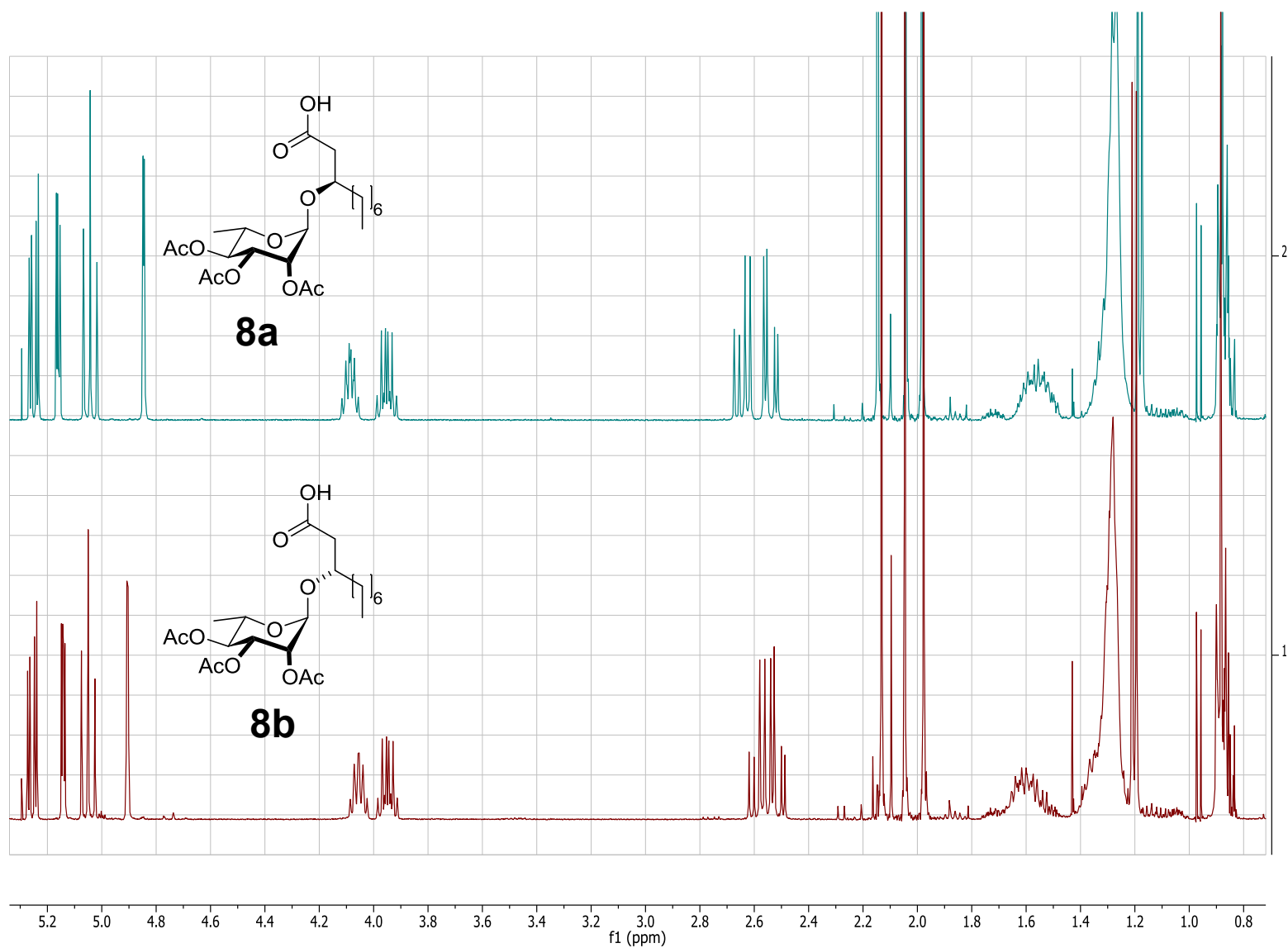


Figure S9. ¹H-NMR spectrum of (*R*)-3-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)decanoic acid (**8a**) and (*S*)-3-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)decanoic acid (**8b**).

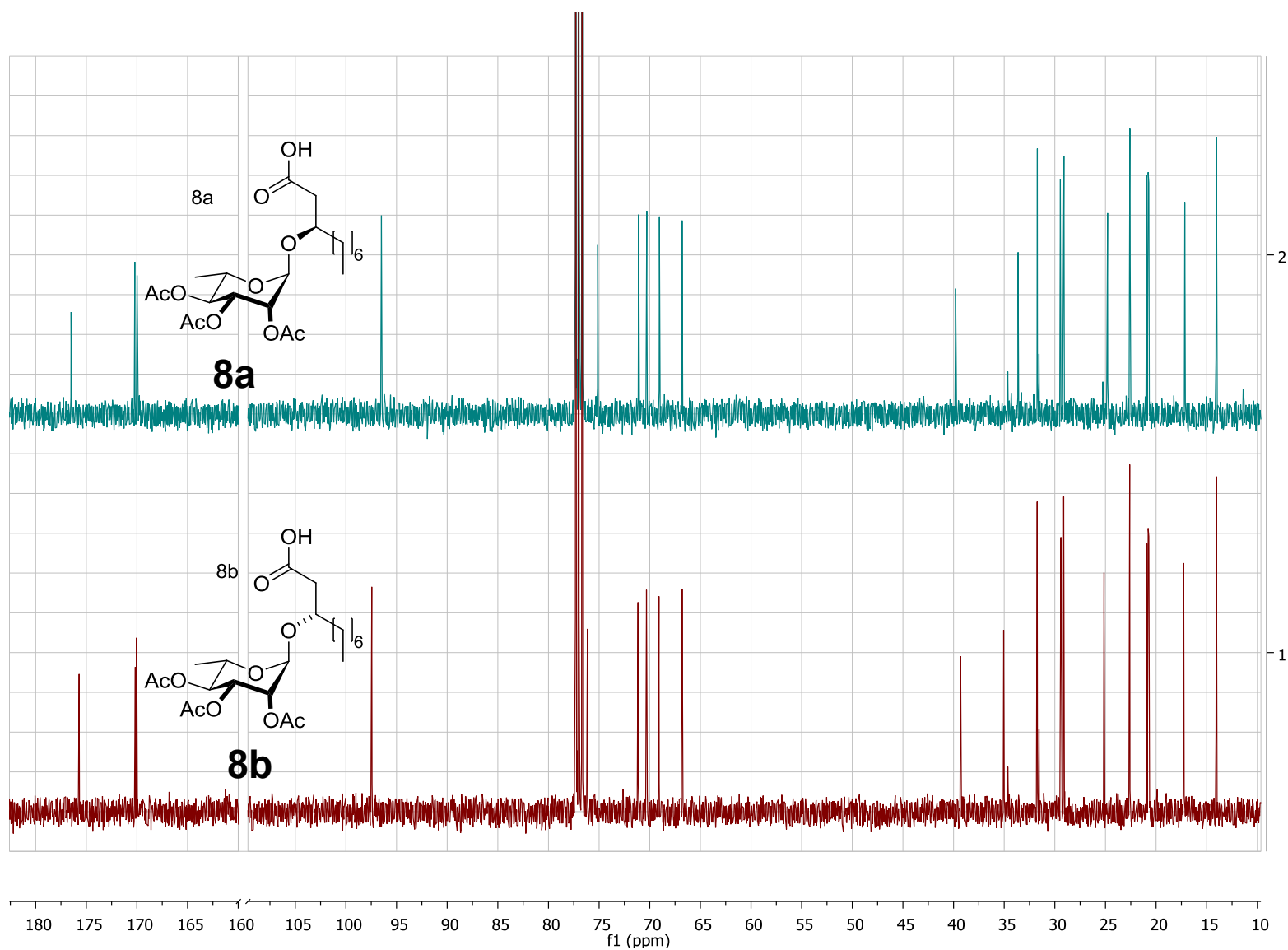


Figure S10. ^{13}C -NMR spectrum of (*R*)-3-O-(2,3,4-tri-*O*-acetyl- α -L-rhamnopyranosyl)decanoic acid (**8a**) and (*S*)-3-O-(2,3,4-tri-*O*-acetyl- α -L-rhamnopyranosyl)decanoic acid (**8b**).

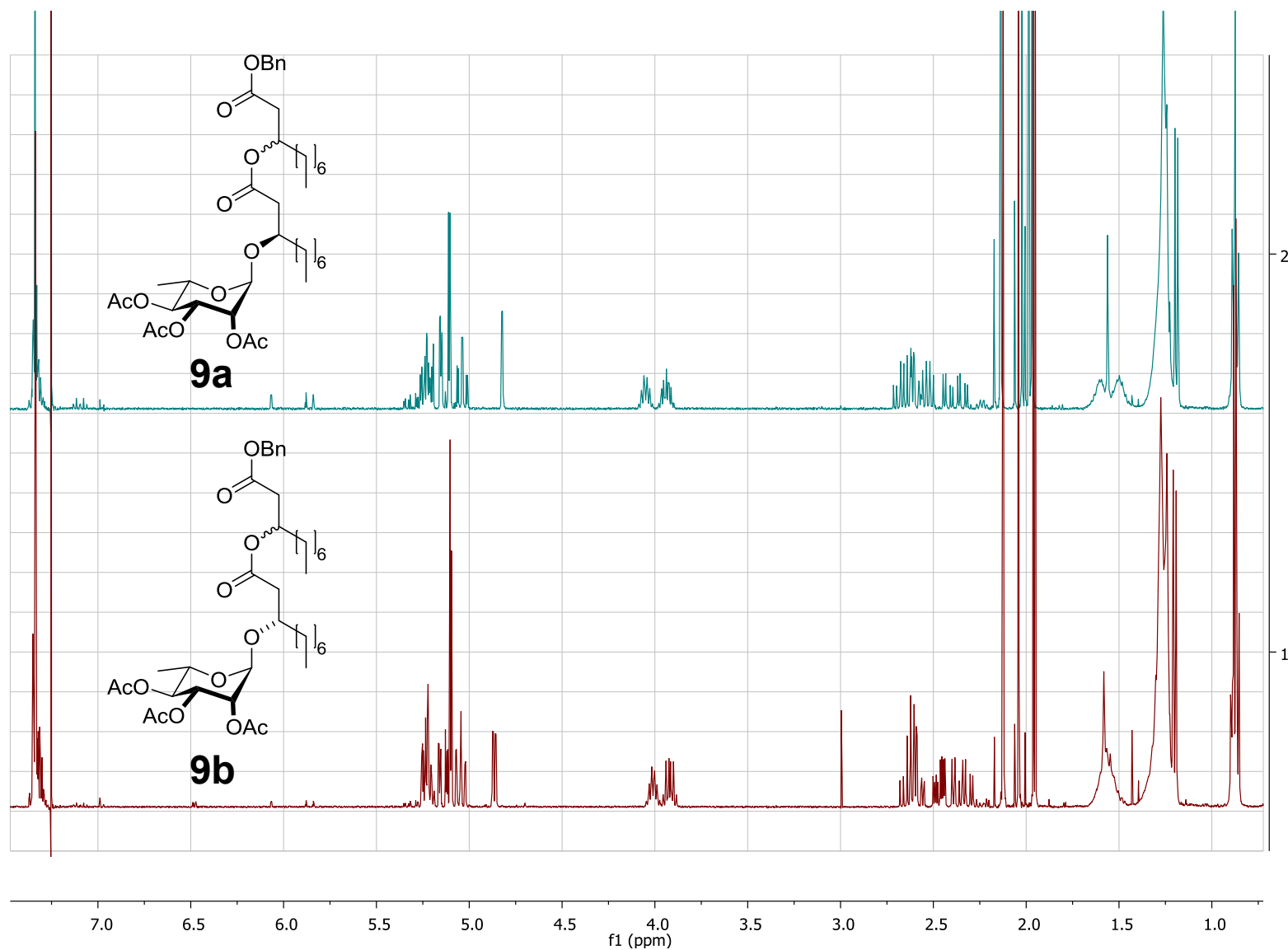


Figure S11. ^1H -NMR spectrum of benzyl (*R/S*)-3-*O*-[(*S*)-(3'-*O*-decyl)2,3,4-tri-*O*-acetyl- α -L-rhamnopyranosyl]decanoate (**9a**) and benzyl (*R/S*)-3-*O*-[(*S*)-(3'-*O*-decyl)2,3,4-tri-*O*-acetyl- α -L-rhamnopyranosyl]decanoate (**9b**).

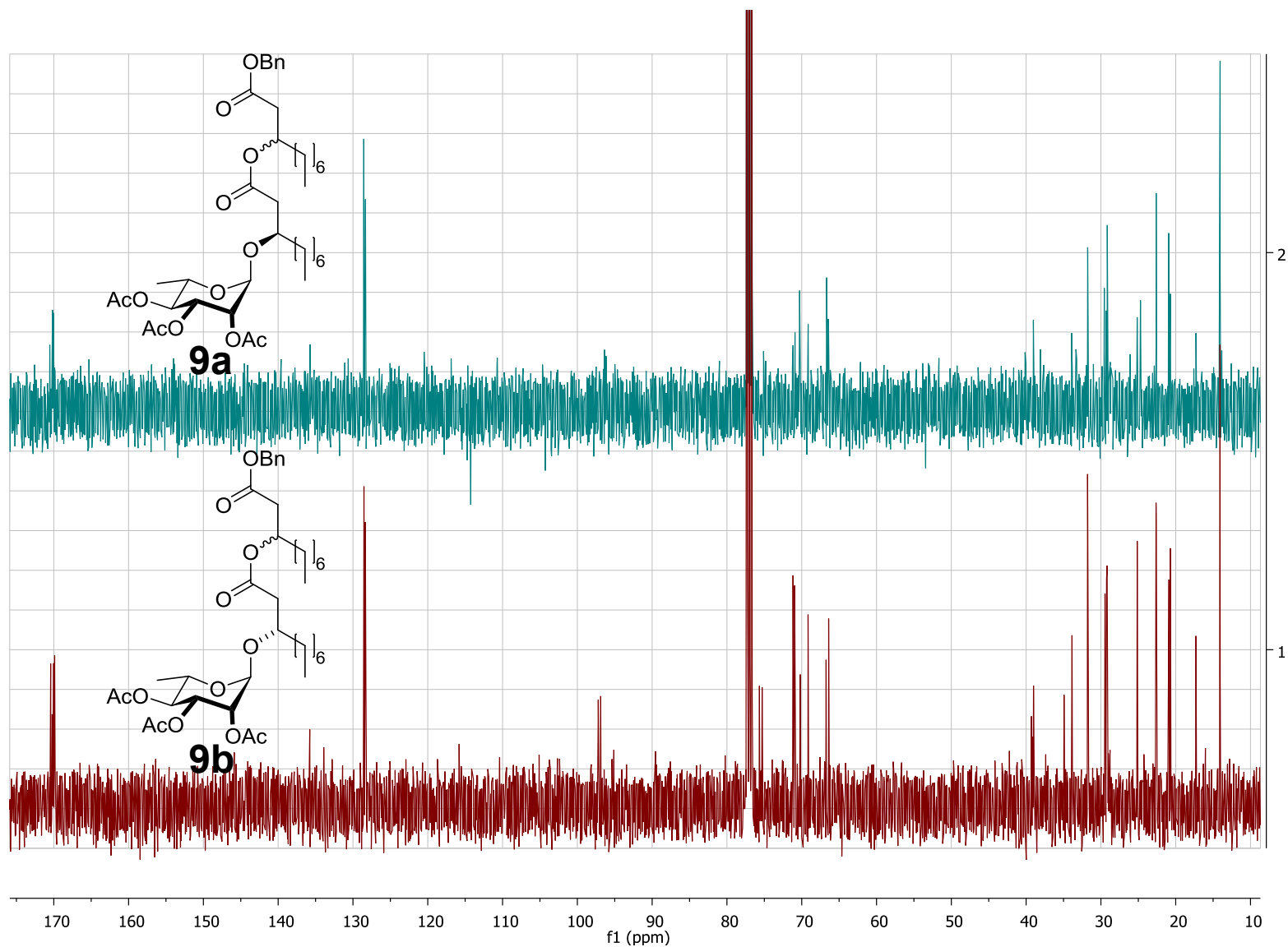
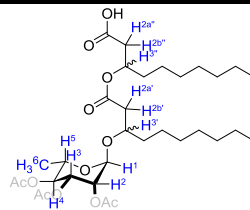


Figure S12. ¹³C-NMR spectrum of benzyl (*R/S*)-3-*O*-[(*S*)-(3'-*O*-decyl)2,3,4-tri-*O*-acetyl-α-*L*-rhamnopyranosyl]decanoate (**9a**) and benzyl (*R/S*)-3-*O*-[(*S*)-(3'-*O*-decyl)2,3,4-tri-*O*-acetyl-α-*L*-rhamnopyranosyl]decanoate (**9b**).

Table S2. ^1H -NMR and ^{13}C -NMR data for diastereomers **10a**, **10b**, **10c** and **10d**.

Compound	H-1	H-2	H-3	H-4	H-5	H-6	H-2a'	H-2b'	H-3'	H-2a''	H-2b''	H-3''	
10a	¹ H δ (ppm)	4.83 δ	5.10 δ	5.23 δ	5.00 δ	3.99 δ	1.18 δ	2.61 δ	2.44 δ	4.13 δ	2.54 δ	2.54 δ	5.30 δ
	J (Hz)	J _{1,2} 1.6	J _{1,2} 1.7	J _{2,3} 3.5	J _{3,4} 10.0	J _{4,5} 9.9	J _{5,6} 6.3	J _{2a',3'} 8.4	J _{2b',3'} 4.1	J _{2a',3'} 8.7	N/A	N/A	N/A
		J _{2,3} 3.4	J _{3,4} 10.1	J _{4,5} 10.0	J _{5,6} 6.2	J _{2a',2b'} 15.6	J _{2a',2b'} 15.7	J _{2b',3'} 4.5	N/A	N/A	N/A		
	¹³ C δ (ppm)	94.74 δ	70.34 δ	69.60 δ	71.17 δ	66.59 δ	17.22 δ	39.52 δ	39.52 δ	72.65 δ	39.08 δ	39.08 δ	70.79 δ
10b	¹ H δ (ppm)	4.81 δ	5.13 δ	5.19 δ	5.01 δ	3.92 δ	1.16 δ	2.61 δ	2.44 δ	4.04 δ	2.59 δ	2.59 δ	5.15 δ
	J (Hz)	J _{1,2} 1.7	J _{1,2} 1.8	J _{2,3} 3.4	J _{3,4} 9.9	J _{4,5} 9.9	J _{5,6} 6.3	J _{2a',3'} 6.0	J _{2b',3'} 5.1	N/A	N/A	N/A	N/A
		J _{2,3} 3.4	J _{3,4} 10.1	J _{4,5} 9.9	J _{5,6} 6.2	J _{2a',2b'} 14.7	J _{2a',2b'} 15.6	N/A	N/A	N/A	N/A		
	¹³ C δ (ppm)	96.11 δ	70.26 δ	69.18 δ	71.08 δ	66.65 δ	17.19 δ	39.97 δ	39.97 δ	74.89 δ	38.73 δ	38.73 δ	70.68 δ
10c	¹ H δ (ppm)	4.86 δ	5.12 δ	5.16 δ	5.01 δ	3.89 δ	1.16 δ	2.48 δ	2.41 δ	4.03 δ	2.54 δ	2.54 δ	5.28 δ
	J (Hz)	J _{1,2} 1.3	N/A	J _{2,3} 3.2	J _{3,4} 9.8	J _{4,5} 9.8	J _{5,6} 6.3	J _{2a',3'} 8.2	J _{2b',3'} 4.3	J _{2a',3'} 8.2	N/A	N/A	N/A
			N/A	J _{3,4} 9.3	J _{4,5} 9.8	J _{5,6} 6.3	J _{2a',2b'} 16.0	J _{2a',2b'} 16.0	J _{2b',3'} 6.1	N/A	N/A	N/A	
	¹³ C δ (ppm)	96.34 δ	70.14 δ	69.72 δ	70.93 δ	66.57 δ	17.18 δ	39.39 δ	39.39 δ	75.03 δ	39.09 δ	39.09 δ	70.79 δ
10d	¹ H δ (ppm)	4.85 δ	5.09 δ	5.21 δ	5.01 δ	3.90 δ	1.16 δ	2.48 δ	2.42 δ	3.99 δ	2.56 δ	2.56 δ	5.17 δ
	J (Hz)	J _{1,2} 1.6	J _{1,2} 1.8	J _{2,3} 3.4	J _{3,4} 9.9	J _{4,5} 9.9	J _{5,6} 6.3	J _{2a',3'} 6.9	J _{2b',3'} 5.4	N/A	N/A	N/A	N/A
		J _{2,3} 3.4	J _{3,4} 10.1	J _{4,5} 9.9	J _{5,6} 6.2	J _{2a',2b'} 15.5	J _{2a',2b'} 15.4	N/A	N/A	N/A	N/A		
	¹³ C δ (ppm)	97.50 δ	70.31 δ	69.07 δ	71.14 δ	66.79 δ	17.25 δ	39.31 δ	39.31 δ	76.20 δ	39.31 δ	39.31 δ	76.20 δ



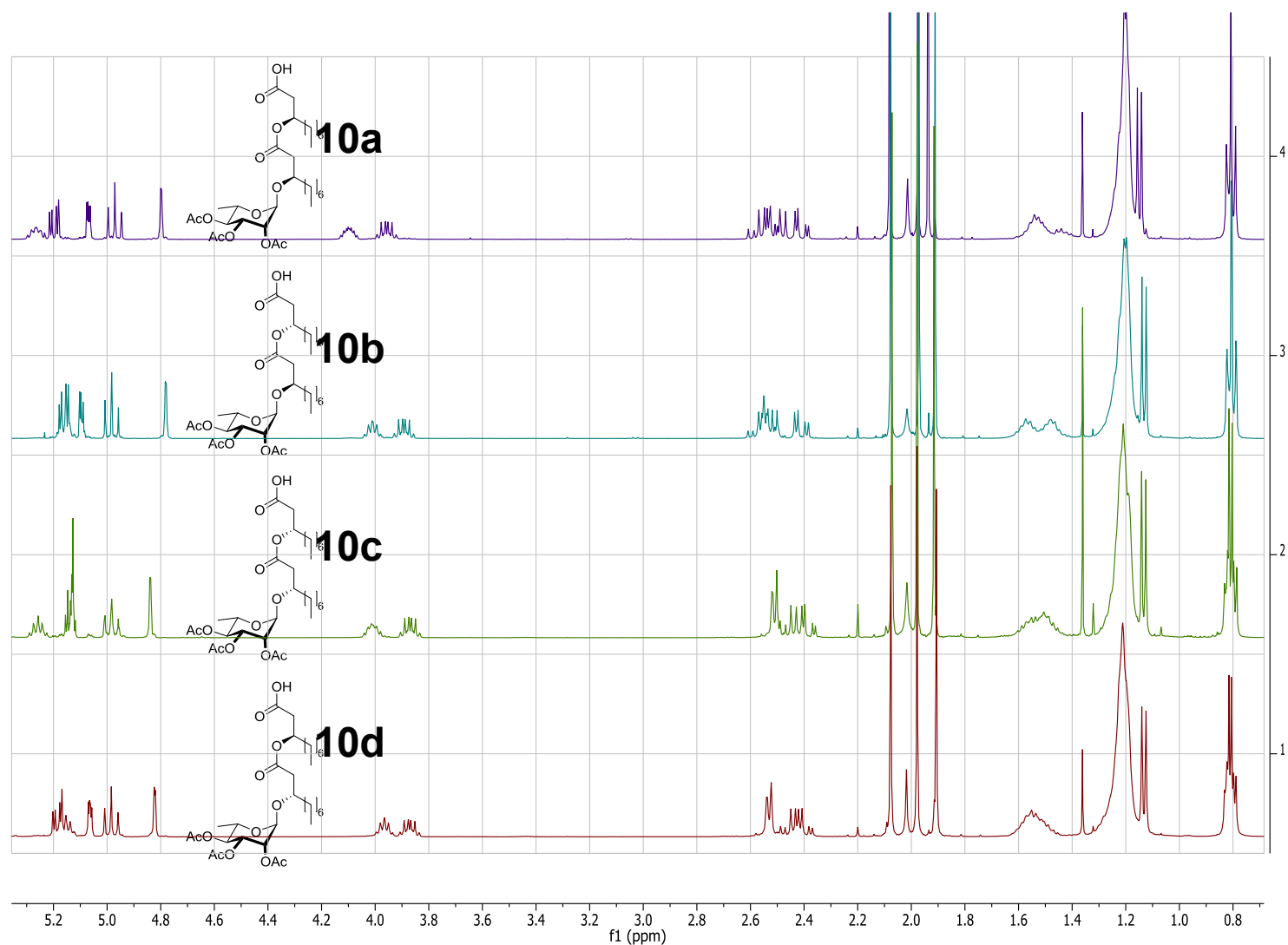


Figure S13. ^1H -NMR spectrum for (*R*)-3-(((*R*)-3-[2,3,4-tri-*O*-acetyl- α -L-rhamnopyranosyl]decanoyl)oxy)decanoic acid (**10a**); (*S*)-3-(((*R*)-[3-2,3,4-tri-*O*-acetyl- α -L-rhamnopyranosyl]decanoyl)oxy)decanoic acid (**10b**); (*S*)-3-(((*S*)-[3-2,3,4-tri-*O*-acetyl- α -L-rhamnopyranosyl]decanoyl)oxy)decanoic acid (**10c**); and (*R*)-3-(((*S*)-[3-2,3,4-Tri-*O*-acetyl- α -L-rhamnopyranosyl]decanoyl)oxy)decanoic acid (**10d**).

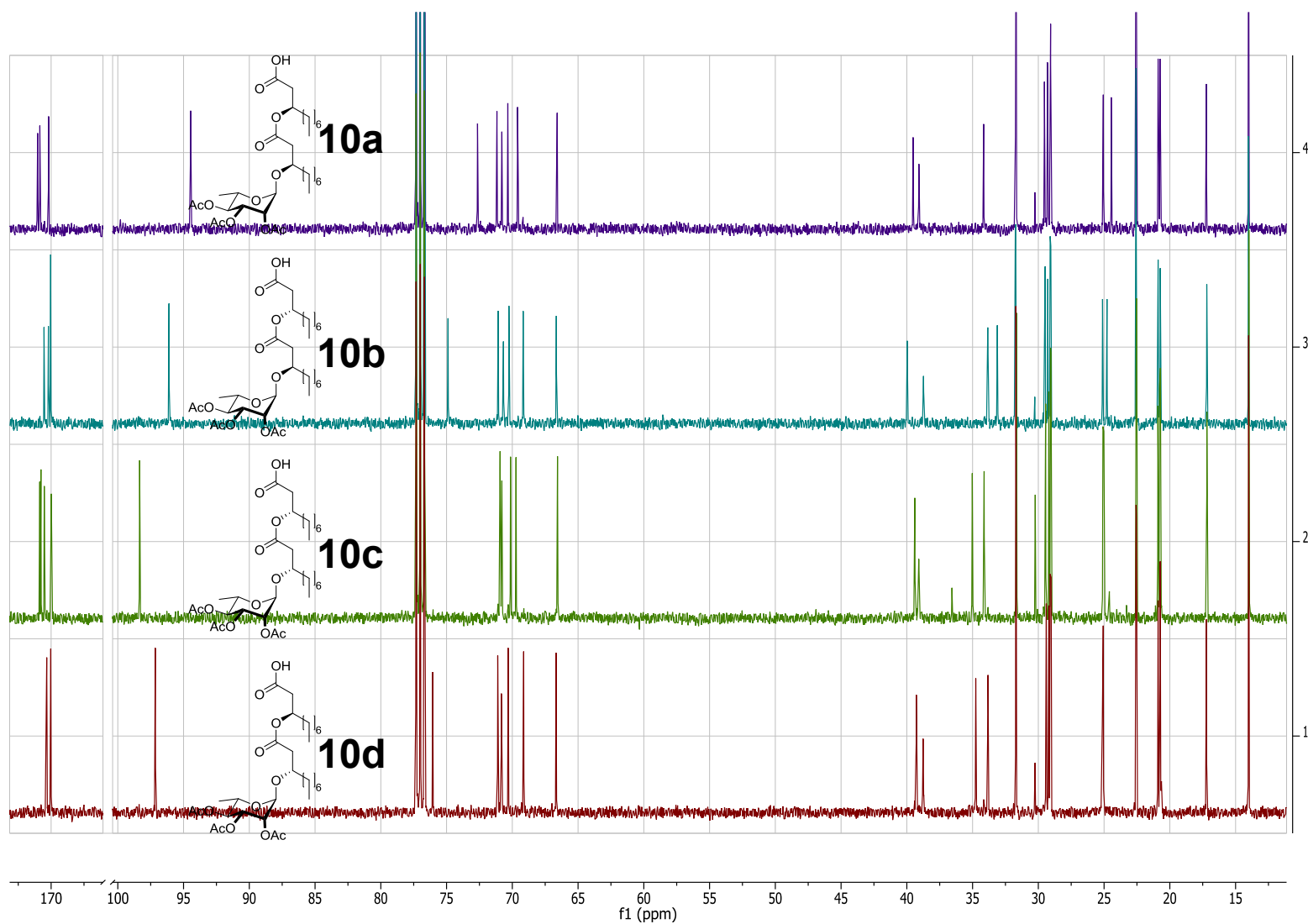


Figure S14. ^{13}C -NMR spectrum for (*R*)-3-(((*R*)-3-[2,3,4-tri-*O*-acetyl- α -L-rhamnopyranosyl]decanoyl)oxy)decanoic acid (**10a**); (*S*)-3-(((*R*)-[3-2,3,4-tri-*O*-acetyl- α -L-rhamnopyranosyl]decanoyl)oxy)decanoic acid (**10b**); (*S*)-3-(((*S*)-[3-2,3,4-tri-*O*-acetyl- α -L-rhamnopyranosyl]decanoyl)oxy)decanoic acid (**10c**); and (*R*)-3-(((*S*)-[3-2,3,4-tri-*O*-acetyl- α -L-rhamnopyranosyl]decanoyl)oxy)decanoic acid (**10d**).

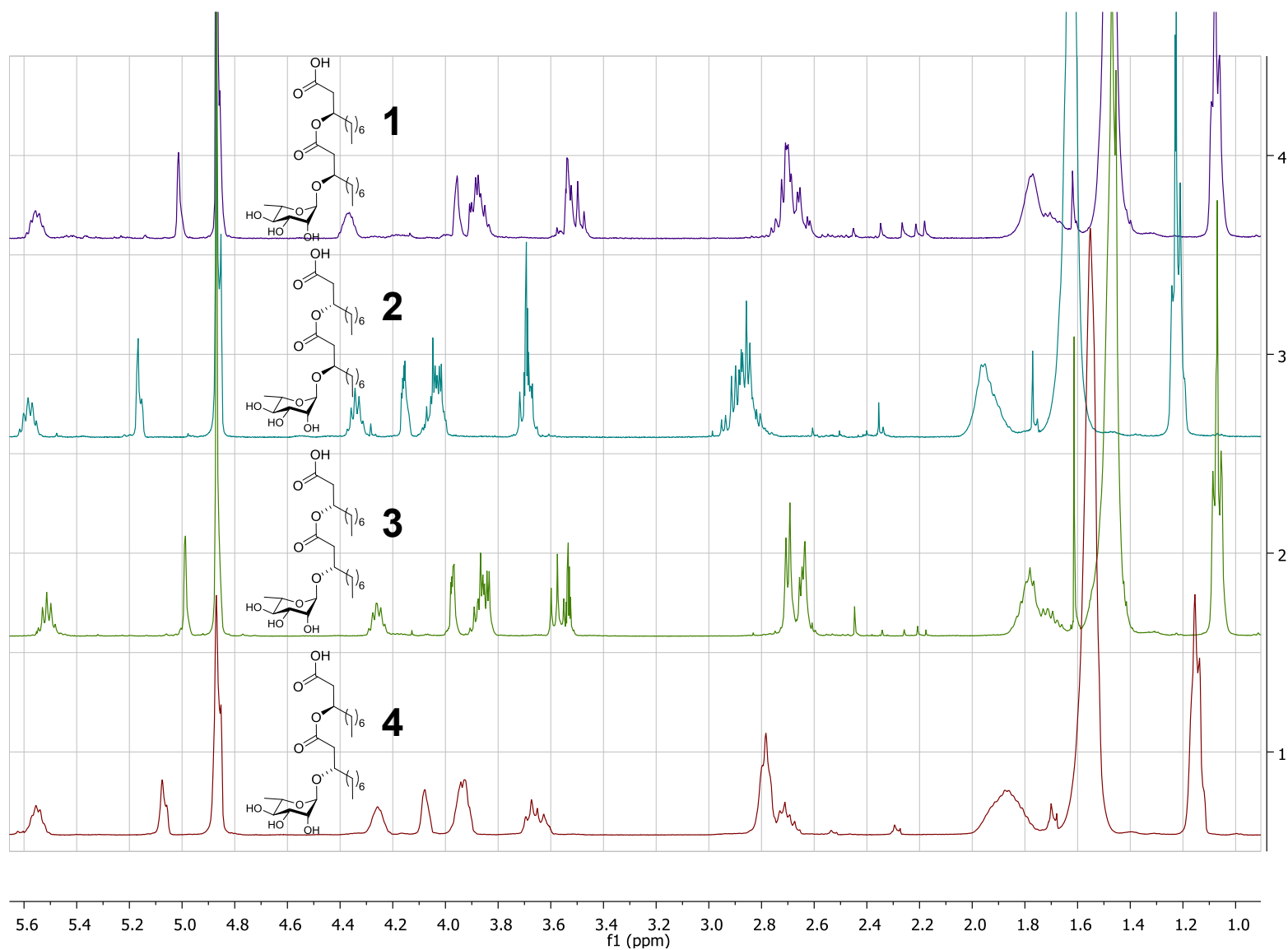


Figure S15. ^1H -NMR spectrum of (*R*)-3-(((*R*)-3-[α -L-rhamnopyranosyl]decanoyl)oxy)decanoic acid (**1**); (*S*)-3-(((*R*)-3-[α -L-rhamnopyranosyl]decanoyl)oxy)decanoic acid (**2**); (*S*)-3-(((*S*)-3-[α -L-rhamnopyranosyl]decanoyl)oxy)decanoic acid (**3**); and (*R*)-3-(((*S*)-3-[α -L-rhamnopyranosyl]decanoyl)oxy)decanoic acid (**4**).

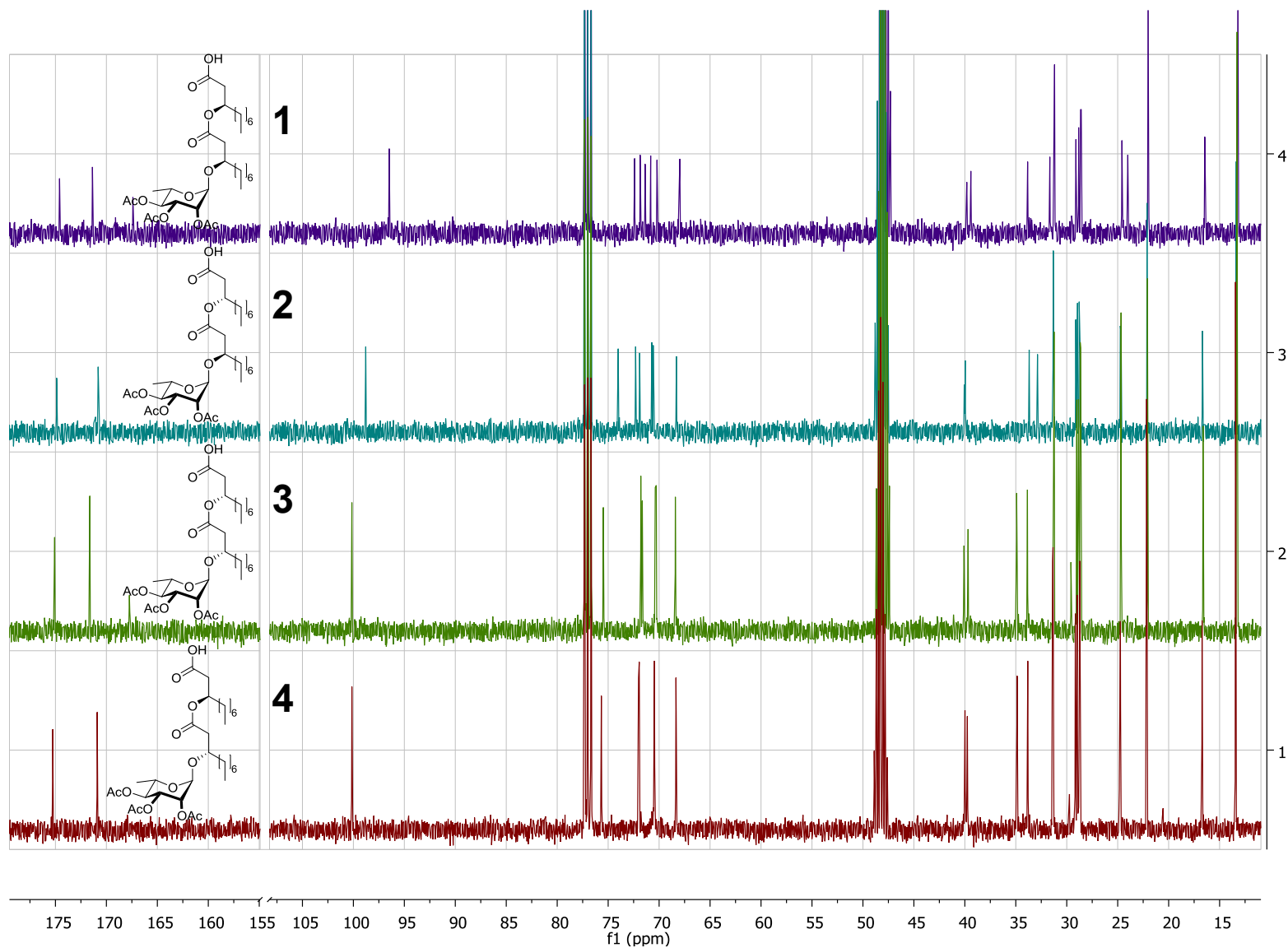


Figure S16. ^1H -NMR spectrum of (*R*)-3-(((*R*)-3-[α -L-rhamnopyranosyl]decanoyl)oxy)decanoic acid (**1**); (*S*)-3-(((*R*)-3-[α -L-rhamnopyranosyl]decanoyl)oxy)decanoic acid (**2**); (*S*)-3-(((*S*)-3-[α -L-rhamnopyranosyl]decanoyl)oxy)decanoic acid (**3**); and (*R*)-3-(((*S*)-3-[α -L-rhamnopyranosyl]decanoyl)oxy)decanoic acid (**4**).

Absolute Configuration Determination of the Carbinol Group of the Inner Lipid Tail by Mosher Ester Analysis

Methanolisys of Deacetylated **8a** and **8b** and Biosynthesized Rhamnolipids

Methyl 3-hydroxydecanoate (8a'**, **8b'** and **11**)**

In three different 25 mL round bottom flasks equipped with stir bars and condensers, the deacetylated diastereomers **8a** (49 mg, 0.146 mmol; R_f 0.38, 1:1 Et₂O:hexanes with 1% v/v AcOH) and **8b** (129.5 mg, 387 μ mol; R_f = 0.26, 1:1 Et₂O:hexanes with 1% v/v AcOH) and naturally produced rhamnolipids (154 mg, 0.305 mmol) were dissolved respectively in 10 mL of a 0.625 M methanolic solution of HCl_(aq) and stirred for 12 h at 70 °C. The reaction mixtures were allowed to cool, 10 mL of aqueous sat. NaHCO₃ were added, and the mixtures were extracted 5x with 10 mL of ethyl acetate. The organic layers were collected, dried with MgSO₄, filtered, and the solvent was removed at 35 °C by rotoevaporation at reduced pressure. The methyl esters were isolated from their respective pale yellowish oils by flash column chromatography in 25 g of silica columns equilibrated with hexanes. The mixtures were dissolved in 2 mL of a 1:1 hexanes/DCM mixture, loaded onto the column and eluted with 25 mL/min of 10% ethyl acetate/hexanes while monitoring absorbance at 200 nm to yield **8a'**, **8b'** and **11** as clear oils. Typical yields 88-90%. TLC R_f 0.38, 20% EtAcO/Hex.

8a': ¹H-NMR (400 MHz, Chloroform-d) δ 4.00 (ddq, J = 12.0, 7.8, 3.7 Hz, 1H), 3.71 (s, 3H), 2.85 (d, J = 4.0 Hz, 1H), 2.52 (dd, J = 16.4, 3.2 Hz, 1H), 2.41 (dd, J = 16.4, 9.0 Hz, 1H), 1.43 (dtd, J = 7.9, 4.3, 1.9 Hz, 2H), 1.37 – 1.26 (m, 10H), 0.92 – 0.84 (m, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 173.51, 68.03, 51.72, 41.09, 36.53, 31.79, 29.47, 29.22, 25.48, 22.64, 14.08.

8b': ¹H-NMR (400 MHz, Chloroform-d) δ 4.00 (ddt, J = 12.0, 7.4, 3.9 Hz, 1H), 3.71 (s, 3H), 2.89 (d, J = 3.9 Hz, 1H), 2.51 (dd, J = 16.3, 3.2 Hz, 1H), 2.41 (dd, J = 16.4, 9.0 Hz, 1H), 1.48 – 1.40 (m, 2H), 1.37 – 1.20 (m, 10H), 0.94 – 0.82 (m, 3H). ¹³C-NMR (101 MHz, CDCl₃) δ 173.48, 68.02, 51.70, 41.12, 36.53, 31.78, 29.46, 29.21, 25.47, 22.63, 14.07.

11: ^1H -NMR (400 MHz, Chloroform- d) δ 4.04 – 3.95 (m, 1H), 3.71 (s, 3H), 2.87 (d, J = 4.0 Hz, 1H), 2.51 (dd, J = 16.4, 3.2 Hz, 1H), 2.41 (dd, J = 16.4, 9.0 Hz, 1H), 1.48 – 1.40 (m, 2H), 1.39 – 1.21 (m, 10H), 0.91 – 0.84 (m, 3H); ^{13}C -NMR (101 MHz, CDCl_3) δ 173.50, 68.03, 51.72, 41.11, 36.53, 31.78, 29.47, 29.21, 25.47, 22.63, 14.07.

Mosher Ester Synthesis

Methyl (R)-3-(((R)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)oxy)decanoate (8a'-(R))

S-(–)-MTPA-Cl (23.18 mg, 92 μmol) was added to a room temperature stirred solution of the methyl 3-hydroxydecanoate ester **8a'** (10 mg, 46.2 μmol) and dry pyridine (11.31 mg, 0.143 mmol) in 1 mL dry CDCl_3 . The reaction progress was monitored by TLC on silica gel (20% ethyl acetate/hexanes). The reaction was stopped after 3 h, and the unreacted acid chloride was quenched with water and Et_2O . The aqueous layer was extracted with two additional portions of ether, and the combined organic layers were dried with MgSO_4 , filtered, and concentrated at 30 $^\circ\text{C}$ by rotoevaporation at reduced pressure. The Mosher's ester was isolated from the pale yellowish oil by flash column chromatography in a 25 g of silica equilibrated with hexanes. The mixture was dissolved in 1 mL of a 1:1 hexanes/DCM mixture, loaded onto a silica column equilibrated with 165 mL 5% EtOAc/Hex, and eluted at 25 mL/min with 33 mL 5% EtOAc/Hex with a gradient from 5%-40% over 330 mL, and 66 mL 40% EtOAc/Hex while monitoring absorbance at 200 nm to yield **8a'-(R)** as a clear oil: 64%, TLC R_f 0.67, 20% EtAcO/Hex. ^1H -NMR (400 MHz, Chloroform- d) δ 7.56 – 7.50 (m, 2H), 7.39 (dd, J = 5.1, 2.2 Hz, 3H), 5.48 (ddt, J = 7.8, 6.4, 5.2 Hz, 1H), 3.66 (s, 3H), 3.55 (s, 3H), 2.70 (dd, J = 15.9, 8.2 Hz, 1H), 2.61 (dd, J = 15.9, 4.7 Hz, 1H), 1.71 – 1.58 (m, 2H), 1.37 – 1.11 (m, 10H), 0.87 (t, J = 7.0 Hz, 3H); ^{13}C -NMR (101 MHz, CDCl_3) δ 170.51, 165.92, 132.31, 129.53, 128.30, 127.31, 121.84, 73.31, 55.45, 51.88, 38.60, 33.61, 31.63, 29.13, 29.01, 24.60, 22.59, 14.05.

Methyl (R)-3-(((S)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)oxy)decanoate (8a'-(S))

R-(–)-MTPA-Cl (23.18 mg, 92 μmol) was added to a room temperature stirred solution of the methyl 3-hydroxydecanoate ester **8a'** (10 mg, 46.2 μmol) and dry pyridine (11.31 mg, 0.143 mmol) in 1 mL dry CDCl_3 . The reaction progress was monitored by TLC on silica gel (20% ethyl

acetate/hexanes). The reaction was stopped after 3 h, and the unreacted acid chloride was quenched with water and Et₂O. The aqueous layer was extracted with two additional portions of ether, and the combined organic layers were dried with MgSO₄, filtered, and concentrated at 30 °C by rotoevaporation at reduced pressure. The Mosher's ester was isolated from the pale yellowish oil by flash column chromatography in a 25 g of silica equilibrated with hexanes. The mixture was dissolved in 1 mL of a 1:1 hexanes/DCM mixture, loaded onto a silica column equilibrated with 165 mL 5% EtOAc/Hex and eluted at 25 mL/min with 33 mL 5% EtOAc/Hex with a gradient from 5%-40% over 330 mL, and 66 mL 40% EtOAc/Hex while monitoring absorbance at 200 nm to yield **8a'-(S)** as a clear oil: 50%, TLC R_f 0.67, 20% EtAcO/Hex. ¹H-NMR (400 MHz, Chloroform-d) δ 7.54 – 7.50 (m, 2H), 7.43 – 7.36 (m, 3H), 5.48 (tt, *J* = 6.8, 5.3 Hz, 1H), 3.59 (s, 3H), 3.53 (q, *J* = 1.2 Hz, 3H), 2.65 (dd, *J* = 15.9, 8.0 Hz, 1H), 2.57 (dd, *J* = 15.9, 5.0 Hz, 1H), 1.67 (dddd, *J* = 22.3, 16.2, 11.3, 5.0 Hz, 2H), 1.38 – 1.25 (m, 10H), 0.88 (t, *J* = 6.7 Hz, 3H); ¹³C-NMR (101 MHz, CDCl₃) δ 170.32, 165.85, 132.18, 129.53, 128.33, 127.43, 121.85, 73.50, 55.36, 51.80, 38.44, 33.76, 31.67, 29.20, 29.06, 24.99, 22.60, 14.06.

Methyl (S)-3-(((R)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)oxy)decanoate (8b'-(R))

S-(–)-MTPA-Cl (22.3 mg, 88.7 μmol) was added to a room temperature stirred solution of the methyl 3-hydroxydecanoate ester **8b'** (9.6 mg, 44.3 μmol) and dry pyridine (10.8 mg, 137.6 μmol) in 1 mL dry CDCl₃. The reaction progress was monitored by TLC on silica gel (20% ethyl acetate/hexanes). The reaction was stopped after 3 h, and the unreacted acid chloride was quenched with water and Et₂O. The aqueous layer was extracted with two additional portions of ether, and the combined organic layers were dried with MgSO₄, filtered, and concentrated at 30 °C by rotoevaporation at reduced pressure. The Mosher's ester was isolated from the pale yellowish oil by flash column chromatography in a 25 g of silica equilibrated with hexanes. The mixture was dissolved in 1 mL of a 1:1 hexanes/DCM mixture, loaded onto a silica column equilibrated with 165 mL 5% EtOAc/Hex and eluted at 25 mL/min with 33 mL 5% EtOAc/Hex with a gradient from 5%-40% over 330 mL, and 66 mL 40% EtOAc/Hex while monitoring absorbance at 200 nm to yield **8b'-(R)** as a clear oil: 64%, TLC R_f 0.64, 20% EtAcO/Hex. ¹H-NMR (400 MHz, Chloroform-d) δ 7.53 (dd, *J* = 6.5, 2.9 Hz, 2H), 7.39 (dd, *J* = 5.2, 1.8 Hz, 3H), 5.53 –

5.42 (m, 1H), 3.59 (s, 3H), 3.53 (d, $J = 1.3$ Hz, 3H), 2.65 (dd, $J = 15.9, 7.9$ Hz, 1H), 2.57 (dd, $J = 15.9, 5.0$ Hz, 1H), 1.80 – 1.57 (m, 2H), 1.40 – 1.14 (m, 10H), 0.88 (t, $J = 6.8$ Hz, 3H); ^{13}C -NMR (101 MHz, CDCl_3) δ 170.31, 165.85, 132.18, 129.53, 128.33, 127.45, 121.85, 73.49, 55.38, 51.79, 38.44, 33.76, 31.67, 29.20, 29.06, 24.99, 22.60, 14.06.

Methyl (S)-3-(((S)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)oxy)decanoate (8b'-(S))

R-(–)-MTPA-Cl (26.1 mg, 103.5 μmol) was added to a room temperature stirred solution of the methyl 3-hydroxydecanoate ester **8b'** (11.2 mg, 51.7 μmol) and dry pyridine (12.7 mg, 160.5 μmol) in 1 mL dry CDCl_3 . The reaction progress was monitored by TLC on silica gel (20% ethyl acetate/hexanes). The reaction was stopped after 3 h, and the unreacted acid chloride was quenched with water and Et_2O . The aqueous layer was extracted with two additional portions of ether, and the combined organic layers were dried with MgSO_4 , filtered, and concentrated at 30 °C by rotoevaporation at reduced pressure. The Mosher's ester was isolated from the pale yellowish oil by flash column chromatography in a 25 g of silica equilibrated with hexanes. The mixture was dissolved in 1 mL of a 1:1 hexanes/DCM mixture, loaded onto a silica column equilibrated with 165 mL 5% EtOAc/Hex and eluted at 25 mL/min with 33 mL 5% EtOAc/Hex with a gradient from 5%-40% over 330 mL, and 66 mL 40% EtOAc/Hex while monitoring absorbance at 200 nm to yield **8b'-(S)** as a clear oil: 64%, TLC R_f 0.64, 20% EtAcO/Hex. ^1H -NMR (400 MHz, Chloroform- d) δ 7.53 (dd, $J = 6.9, 2.9$ Hz, 2H), 7.39 (dd, $J = 5.1, 2.0$ Hz, 3H), 5.52 – 5.42 (m, 1H), 3.66 (s, 3H), 3.54 (d, $J = 1.4$ Hz, 3H), 2.70 (dd, $J = 16.0, 8.2$ Hz, 1H), 2.60 (dd, $J = 15.9, 4.6$ Hz, 1H), 1.73 – 1.50 (m, 2H), 1.29 – 1.23 (m, 10H), 0.87 (t, $J = 7.0$ Hz, 3H).

Methyl (R)-3-(((S)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)oxy)decanoate (11-(S))

R-(–)-MTPA-Cl (27.0 mg, 107.2 μmol) was added to a room temperature stirred solution of the methyl 3-hydroxydecanoate ester **11** (11.6 mg, 53.6 μmol) and dry pyridine (13.1 mg, 166.2 μmol) in 1 mL dry CDCl_3 . The reaction progress was monitored by TLC on silica gel (20% ethyl acetate/hexanes). The reaction was stopped after 3 h, and the unreacted acid chloride was quenched with water and Et_2O . The aqueous layer was extracted with two additional portions of ether, and the combined organic layers were dried with MgSO_4 , filtered, and concentrated at

30 °C by rotoevaporation at reduced pressure. The Mosher's ester was isolated from the pale yellowish oil by flash column chromatography in a 25 g of silica equilibrated with hexanes. The mixture was dissolved in 1 mL of a 1:1 hexanes/DCM mixture, loaded onto a silica column equilibrated with 165 mL 5% EtOAc/hexanes and eluted at 25 mL/min with 33 mL 5% EtOAc/Hex with a gradient from 5%-40% over 330 mL, and 66 mL 40% EtOAc/Hex while monitoring absorbance at 200 nm to yield **11-(S)** as a clear oil: 64%, TLC R_f 0.67, 20% EtAcO/Hex. $^1\text{H-NMR}$ (400 MHz, Chloroform- d) δ 7.53 (dd, J = 6.8, 2.9 Hz, 2H), 7.42 – 7.38 (m, 3H), 5.48 (tt, J = 6.7, 5.4 Hz, 1H), 3.59 (s, 3H), 3.53 (s, 2H), 2.65 (dd, J = 15.9, 7.9 Hz, 1H), 2.57 (dd, J = 15.9, 5.0 Hz, 1H), 1.80 – 1.61 (m, 2H), 1.38 – 1.16 (m, 10H), 0.88 (t, J = 6.8 Hz, 3H); $^{13}\text{C-NMR}$ (101 MHz, CDCl_3) δ 170.31, 165.85, 132.17, 129.53, 128.33, 127.44, 121.84, 73.49, 55.36, 51.79, 38.44, 33.76, 31.66, 29.19, 29.05, 24.98, 22.59, 14.06.

Methyl (R)-3-(((R)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)oxy)decanoate (11-(R))

S-(–)-MTPA-Cl (34.2 mg, 135.9 μmol) was added to a room temperature stirred solution of the methyl 3-hydroxydecanoate ester **11** (14.7 mg, 67.9 μmol) and dry pyridine (16.6 mg, 210.6 μmol) in 1 mL dry CDCl_3 . The reaction progress was monitored by TLC on silica gel (20% ethyl acetate/hexanes). The reaction was stopped after 3 h, and the unreacted acid chloride was quenched with water and Et_2O . The aqueous layer was extracted with two additional portions of ether, and the combined organic layers were dried with MgSO_4 , filtered, and concentrated at 30 °C by rotoevaporation at reduced pressure. The Mosher's ester was isolated from the pale yellowish oil by flash column chromatography in a 25 g of silica equilibrated with hexanes. The mixture was dissolved in 1 mL of a 1:1 hexanes/DCM mixture, loaded onto a silica column equilibrated with 165 mL 5% EtOAc/Hexanes and eluted at 25 mL/min with 33 mL 5% EtOAc/Hex with a gradient from 5%-40% over 330 mL, and 66 mL 40% EtOAc/Hex while monitoring absorbance at 200 nm to yield **11-(R)** as a clear oil: 64%, TLC R_f 0.67, 20% EtAcO/Hex. $^1\text{H-NMR}$ (400 MHz, Chloroform- d) δ 7.53 (dd, J = 6.8, 2.9 Hz, 2H), 7.39 (dd, J = 5.1, 2.0 Hz, 3H), 5.47 (tt, J = 6.5, 5.0 Hz, 1H), 3.66 (s, 3H), 3.55 (s, 3H), 2.70 (dd, J = 15.9, 8.1 Hz, 1H), 2.61 (dd, J = 15.9, 4.7 Hz, 1H), 1.68 – 1.58 (m, 2H), 1.31 – 1.23 (m, 10H), 0.90 – 0.84 (m, 3H); $^{13}\text{C-NMR}$

NMR (101 MHz, CDCl₃) δ 170.52, 165.92, 132.31, 129.53, 128.30, 127.30, 121.84, 73.32, 55.45, 51.88, 38.60, 33.61, 31.63, 29.13, 29.01, 24.60, 22.59, 14.05.

Mosher Ester Analysis

Table S3. ¹H-NMR spectral data for the **S**- and **R**-MTPA esters pairs: **8a'-(S)** and **8a'-(R)**; **8b'-(S)** and **8b'-(R)**; and **11-(S)** and **11-(R)** which are derivatives of the product of the methanolysis of the acetylated single chain rhamnolipids **8a** (TLC R_f = 0.38) and **8b** (TLC R_f = 0.26), and natural rhamnolipids respectively. The $\Delta\delta^{SR} = (\delta_S - \delta_R)$ data are presented in ppm and Hertz. See text for the interpretation of its meaning for each pair.

	δ S-ester	δ R-ester	$\Delta\delta^{SR} = (\delta_S - \delta_R)$		δ S-ester	δ R-ester	$\Delta\delta^{SR} = (\delta_S - \delta_R)$		δ S-ester	δ R-ester	$\Delta\delta^{SR} = (\delta_S - \delta_R)$	
	(8a'-(S))	(8a'-(R))	ppm	Hz (400 Mhz)	(8b'-(S))	(8b'-(R))	ppm	Hz (400 Mhz)	(11-(S))	(11-(R))	ppm	Hz (400 Mhz)
	(ppm)	(ppm)			(ppm)	(ppm)			(ppm)	(ppm)		
Me-	0.88	0.87	+0.01	+4	0.87	0.88	-0.01	-4	0.88	0.86	+0.02	+8
-(CH₂)₅-	1.28	1.22	+0.06	+24	1.26	1.27	-0.01	-4	1.28	1.23	+0.05	+20
γ-H₂	1.67	1.63	+0.04	+16	1.61	1.69	-0.08	-32	1.70	1.63	+0.07	+28
β-H	5.48	5.48	0.00	0.00	5.47	5.47	0.00	0.00	5.48	5.47	0.01	+4
α-H'	2.57	2.61	-0.04	-16	2.60	2.57	+0.03	+12	2.57	2.61	-0.04	-16
α-H'	2.65	2.70	-0.05	-20	2.70	2.65	+0.05	+20	2.65	2.70	-0.05	-20
MeOCO-	3.59	3.66	-0.07	-28	3.66	3.59	+0.07	+28	3.59	3.66	-0.07	-28

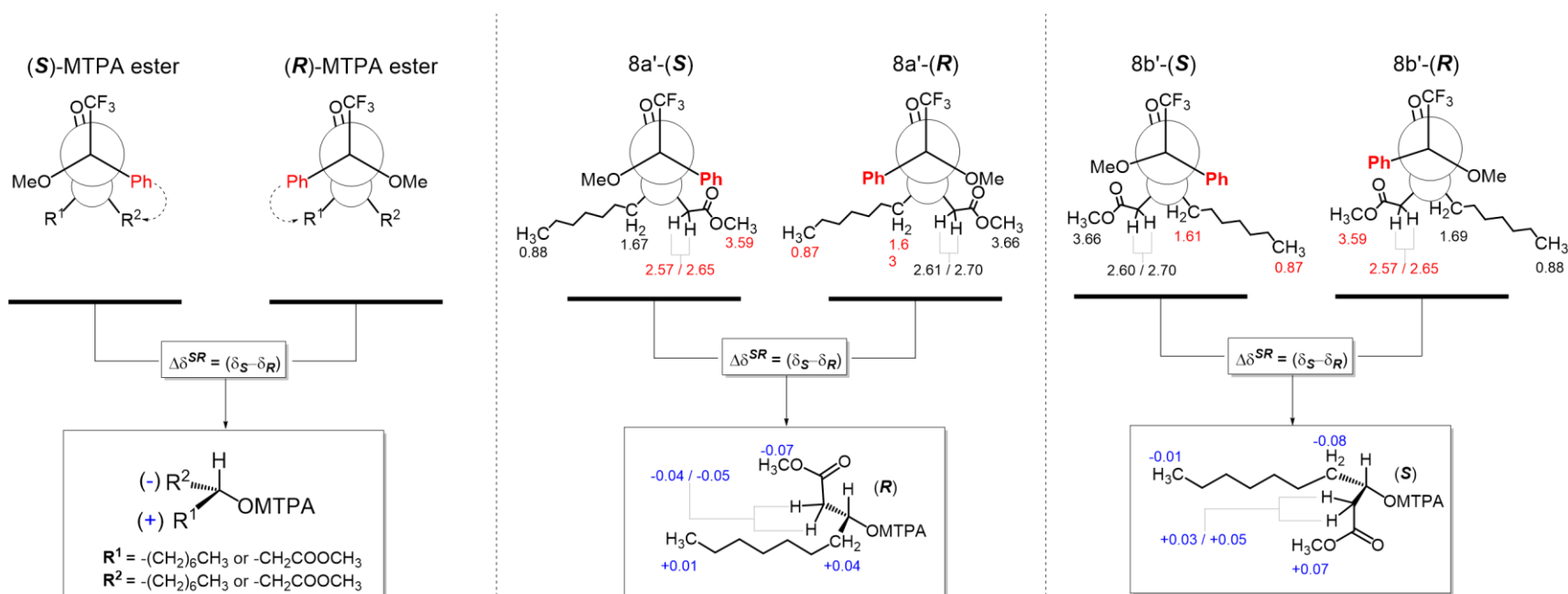


Figure S17. Absolute configuration assignments by Mosher ester analysis of the carbinols of the lipid esters product of the methanolysis of **8a** and **8b**. The Newman's projections of the empirically based conformational picture are shown in which the ester adopts the usual s-trans arrangement about the O-CO bond, and both the trifluoromethyl substituent, the methine proton of the carbinol are syn-coplanar with the carbonyl group. The anisotropic shielding effect of the aryl group of the MTPA moiety results in an upfield chemical shift for the affected protons in the NMR spectrum. ¹H-NMR spectral data were taken from table S1 and shown in ppm next to the relevant protons. The signs of $\Delta\delta^{SR}$ values for protons residing in R¹ will be positive and those on R² will be negative as depicted in the left panel. At the bottom of each panel, the absolute configuration of the carbinol in the lipid tail is presented based on these data, which for **8a** is **R** and for **8b** is **S**. Natural rhamnolipid was used as control, since it has been reported elsewhere (Nakagawa and Matsuyama, *FEMS Microbiol. Lett.*, **1993**, *108*, 99-102) that the absolute configuration of the carbinols of its lipid tails are **R**, which is congruent with our studies and the biochemistry of the microorganism.

Absolute Configuration Determination of the Outer Lipid Tail by Optical Rotation

Cleavage of Outer Lipid Tails of Deacetylated Rha-C10-C10 Diastereomers

Methyl 3-hydroxydecanoate (1a, 2a, 3a, 4a)

To four 25 mL round bottom flasks distinctively labeled and equipped with stir bars, the diastereomers **1** (247.6 mg, 490.9 μmol , $R_f = 0.36$, 1:1 Et₂O: hexanes with 1% v/v AcOH), **2** (200 mg, 396.5 μmol , $R_f = 0.44$, 1:1 Et₂O: hexanes with 1% v/v AcOH), **3** (227.2 mg, 450.5 μmol , $R_f = 0.22$, 1:1 Et₂O:hexanes with 1% v/v AcOH), and **4** (220.9 mg, 438.0 μmol , $R_f = 0.27$, 1:1 Et₂O: hexanes with 1% v/v AcOH) were dissolved in 3.0 mL of a 3.0 M aqueous solution of NaOH and stirred at room temperature overnight. The solutions were neutralized with drop-wise additions of 1.0 M HCl while monitoring the pH with pH-paper. The neutral solutions were extracted with 5x 10 mL of ethyl acetate. The organic layers were dried with MgSO₄, filtered and concentrated at 35 °C by rotoevaporation at reduced pressure. The white solids obtained were transferred to four flame-dried 25 mL round bottom flasks distinctively labeled and equipped with stir bars. The hydrolysates were dissolved in 16 mL of a 15% methanolic solution of BF₃ and 2,2-dimethoxypropane (1.1 equivalents). The reaction mixture was allowed to stir for 20 min at 50 °C, cooled down to room temperature, quenched with 8 mL of water, and extracted 5x with 10 mL of hexanes. The organic layers were collected, dried with MgSO₄, filtered and concentrated at 35 °C by rotoevaporation at reduced pressure. The methyl ester is isolated from the pale yellowish oil by flash column chromatography in a 25 g of silica equilibrated with hexanes. The mixture was dissolved in 2 mL of a 1:1 hexanes/DCM mixture, loaded onto the column, and eluted with 25 mL/min of 10% ethyl acetate/hexanes while monitoring absorbance at 200 nm to yield a clear oil, typical yield 40%. %, TLC: $R_f = 0.39$ (20% ethyl acetate/hexanes).

1a (R): $[\alpha]_D = -8.58 \pm 0.16 \text{ deg cm}^2 \text{ g}^{-1}$ ($c = 0.875$ in CHCl₃); ¹H-NMR (400 MHz, Chloroform-d) δ 4.03 (dddd, $J = 12.1, 8.8, 5.9, 3.5 \text{ Hz}$, 1H), 3.74 (s, 3H), 2.89 (d, $J = 4.0 \text{ Hz}$, 1H), 2.54 (dd, $J = 16.4, 3.2 \text{ Hz}$, 1H), 2.44 (dd, $J = 16.4, 9.0 \text{ Hz}$, 1H), 1.50 – 1.43 (m, 2H), 1.39 – 1.23 (m, 10H), 1.00 – 0.82

(m, 3H); ^{13}C -NMR (101 MHz, CDCl_3) δ 173.50, 68.03, 51.72, 41.10, 36.53, 31.79, 29.47, 29.22, 25.48, 22.64, 14.08.

2a (S): $[\alpha]_{\text{D}} = +9.24 \pm 0.08 \text{ deg cm}^2 \text{ g}^{-1}$ ($c = 0.8967$ in CHCl_3); ^1H -NMR (400 MHz, Chloroform- d) δ 4.04 – 3.96 (m, 1H), 3.71 (s, 3H), 2.90 (d, $J = 3.9$ Hz, 1H), 2.51 (dd, $J = 16.3, 3.2$ Hz, 1H), 2.41 (dd, $J = 16.4, 9.0$ Hz, 1H), 1.43 (ddq, $J = 7.7, 4.7, 1.6$ Hz, 2H), 1.34 – 1.24 (m, 10H), 0.94 – 0.83 (m, 3H); ^{13}C -NMR (101 MHz, CDCl_3) δ 173.48, 68.02, 51.70, 41.12, 36.54, 31.78, 29.46, 29.21, 25.47, 22.63, 14.06.

3a (S): $[\alpha]_{\text{D}} = +10.29 \pm 0.24 \text{ deg cm}^2 \text{ g}^{-1}$ ($c = 0.9433$ in CHCl_3); ^1H -NMR (400 MHz, Chloroform- d) δ 4.00 (ddt, $J = 12.0, 7.4, 3.9$ Hz, 1H), 3.71 (s, 3H), 2.89 (d, $J = 3.9$ Hz, 1H), 2.51 (dd, $J = 16.3, 3.2$ Hz, 1H), 2.41 (dd, $J = 16.4, 9.0$ Hz, 1H), 1.48 – 1.40 (m, 2H), 1.37 – 1.20 (m, 10H), 0.94 – 0.82 (m, 3H); ^{13}C -NMR (101 MHz, CDCl_3) δ 173.48, 68.02, 51.70, 41.12, 36.53, 31.78, 29.46, 29.21, 25.47, 22.63, 14.07.

4a (R): $[\alpha]_{\text{D}} = -6.70 \pm 0.62 \text{ deg cm}^2 \text{ g}^{-1}$ ($c = 0.525$ in CHCl_3); ^1H -NMR (400 MHz, Chloroform- d) δ 4.00 (ddq, $J = 12.0, 7.8, 3.7$ Hz, 1H), 3.71 (s, 3H), 2.85 (d, $J = 4.0$ Hz, 1H), 2.52 (dd, $J = 16.4, 3.2$ Hz, 1H), 2.41 (dd, $J = 16.4, 9.0$ Hz, 1H), 1.43 (dtd, $J = 7.9, 4.3, 1.9$ Hz, 2H), 1.37 – 1.26 (m, 10H), 0.92 – 0.84 (m, 3H); ^{13}C -NMR (101 MHz, CDCl_3) δ 173.51, 68.03, 51.72, 41.09, 36.53, 31.79, 29.47, 29.22, 25.48, 22.64, 14.08.

Synthesis of Methyl (R)-3-hydroxydecanoate by Noyori's Enantioselective Hydrogenation as Control for Optical Rotation

Methyl (R)-3-hydroxydecanoate (12)

Under a controlled atmosphere saturated with argon inside a glovebox, 15 mL of 0.24 M degassed ethanolic solution of **5**, and 40 mg of Noyori's type catalyst (CAS# 199684-47-4, purchased from Strem Chemicals) was added to an oven dried 500 mL pyrex bottle suitable for a 3911 Parr[®] shaker hydrogenation reactor. The ethanol was freshly bi-distilled over CaH_2 and filtrated before used. Solution degassing was accomplished by three freeze-pump-thaw cycles.

The bottle with the reaction mixture saturated with argon was capped and secured on the Parr Shaker. The bottle was subjected to three H₂ saturation-evacuation cycles before increasing the pressure to 45 psi in H₂, setting the temperature to 115 °C and shaking for 72 h. The temperature was controlled using a J-type thermocouple connected to a 4833 Parr[®] temperature controller and a glass fabric heating mantle attached around the 500 mL glass bottle. The bottle was evacuated at low pressure while cooling down, celite and methylene chloride were added, and the mixture was filtered and concentrated at 40 °C by rotoevaporation at reduced pressure. The crude dark reddish oil obtained was purified by flash column chromatography in 100 g of silica equilibrated with 5% ethyl acetate/hexanes. The mixture was dissolved in 3 mL of a 1:1 hexanes/DCM mixture, loaded onto the column, and eluted with 50 mL/min of 132 mL of 5% ethyl acetate/hexanes and a gradient of 5% to 40% over a volume of 1320 mL to yield a yellowish oil composed of an inseparable mixture of 70% benzyl and 30% ethyl 3-hydroxydecanoate esters. TLC: R_f 0.67 (30% ethyl acetate/hexanes); ¹H-NMR (400 MHz, Chloroform-d) δ 7.40 – 7.29 (m, 5H), 5.15 (s, 2H), 4.17 (q, *J* = 7.1 Hz, 0.8H), 4.01 (dt, *J* = 8.3, 4.9 Hz, 1H), 2.93 (s, 0.4H), 2.84 (s, 1H), 2.56 (dd, *J* = 16.4, 3.2 Hz, 1H), 2.46 (dd, *J* = 16.4, 8.9 Hz, 1H), 2.50 (dd, *J* = 16.4, 3.1 Hz, 0.4H), 2.39 (dd, *J* = 16.4, 9.0 Hz, 0.4H), 1.47 – 1.21 (m, 13.2H), 0.90 – 0.84 (m, 4.2H). ¹³C-NMR (101 MHz, CDCl₃) δ 173.13, 172.87, 135.60, 128.62, 128.38, 128.26, 68.05, 66.48, 60.66, 41.36, 41.30, 36.53, 31.79, 29.49, 29.47, 29.23, 29.21, 25.48, 25.46, 22.64, 14.19, 14.09.

An amount of 52 mg of this mixture of esters was added to a 25 mL round bottom flask containing 10 mL of a 0.625 M methanolic solution of HCl_(aq) and stirred for 12 h at 70 °C. The reaction mixture was allowed to cool, and 10 mL of aqueous saturated NaHCO₃ were added. The mixture was extracted 5x with 10 mL of ethyl acetate, the organic layers were collected, dried with MgSO₄ and filtered. The solvent was removed at 35 °C by rotoevaporation at reduced pressure. The methyl ester was isolated from the crude pale yellowish oil by flash column chromatography in a 25 g of silica equilibrated with hexanes. The mixture was dissolved in 2 mL of a 1:1 hexanes/DCM mixture, loaded onto the column and eluted with 25 mL/min of 10% ethyl acetate/hexanes while monitoring absorbance at 200 nm to yield a clear oil: 47% TLC:

R_f 0.39 (20% ethyl acetate/hexanes); [α]_D = -11.51 \pm 0.54 deg cm² g⁻¹ (c = 0.82 in CHCl₃); ¹H-NMR (400 MHz, Chloroform-d) δ 4.04 – 3.93 (m, 1H), 3.71 (s, 3H), 2.89 (d, *J* = 2.5 Hz, 1H), 2.51 (dd, *J* = 16.4, 3.2 Hz, 1H), 2.41 (dd, *J* = 16.4, 9.0 Hz, 1H), 1.43 (dtdd, *J* = 5.7, 4.7, 3.8, 2.1 Hz, 2H), 1.37 – 1.23 (m, 10H), 0.93 – 0.73 (m, 3H); ¹³C-NMR (101 MHz, CDCl₃) δ 173.49, 68.02, 51.71, 41.11, 36.53, 31.78, 29.46, 29.21, 25.47, 22.63, 14.07.

Optical Rotation

Table S4. Specific optical rotation measurements in dry chloroform of methyl 3-hydroxydecanoate esters obtained from saponification of the outer lipid tails of **1**, **2**, **3** and **4**.

	Sample	%w/v (g/100mL)	[α] _D ^T (deg cm ² g ⁻¹) ^a	T (°C)	Absolute Configuration
	1a	0.87	-8.58 \pm 0.16	24.1	(<i>R</i>)
	2a	0.89	+9.24 \pm 0.08	25.1	(<i>S</i>)
	3a	0.94	+10.29 \pm 0.24	24.6	(<i>S</i>)
	4a	0.52	-6.70 \pm 0.62	25.0	(<i>R</i>)
Contr ols	12	0.82	-11.51 \pm 0.54	25.2	(<i>R</i>)
	8b'	1.77	+11.58 \pm 0.19	25.2	(<i>S</i>)
Literature values	Ref 1 ^d	2.50	-21 ^o _c	RT (?)	(<i>R</i>)
	Ref 2	0.75	-12.9 ^b	20	(<i>R</i>)
	Ref 3	1.17	-15.5 ^b	30	(<i>R</i>)
	Ref 4	1.14	-19.6 ^b	23	(<i>R</i>)
	Ref 5	1.00	-20 ^o _c	20	(<i>R</i>)
	Ref 6	Neat	-4.5 ^o _c	20	(<i>R</i>)

^a Results are average of 100 readings; errors are standard deviations.

Absolute configurations assigned by comparing their values to optical rotation values of methyl 3-(*R*)-hydroxydecanoate ester obtained by Noyori's asymmetric hydrogenation (**12**), methyl 3-(*S*)-hydroxydecanoate ester (**8b'**) with confirmed absolute configuration by Mosher's esters analysis, and the specific^b and observed^c rotation values reported in literature. ^d Ref 1: JACS, **1949**, 71, 4124-6; Ref 2: J. Org. Chem. **2015**, 80 (9), 4419–4429; Ref 3: Biotechnol. Bioeng. **2002**, 77 (6), 717–722; Ref 4: J. Org. Chem. **1990**, 55 (12), 3917–3921; Ref 5: Carbohydr. Res. **1988**, 180 (2), 195–205; Ref 6: Bull. Chem. Soc. Jpn. **2002**, 75 (2), 355–363.

Absolute Configuration Determination Summary

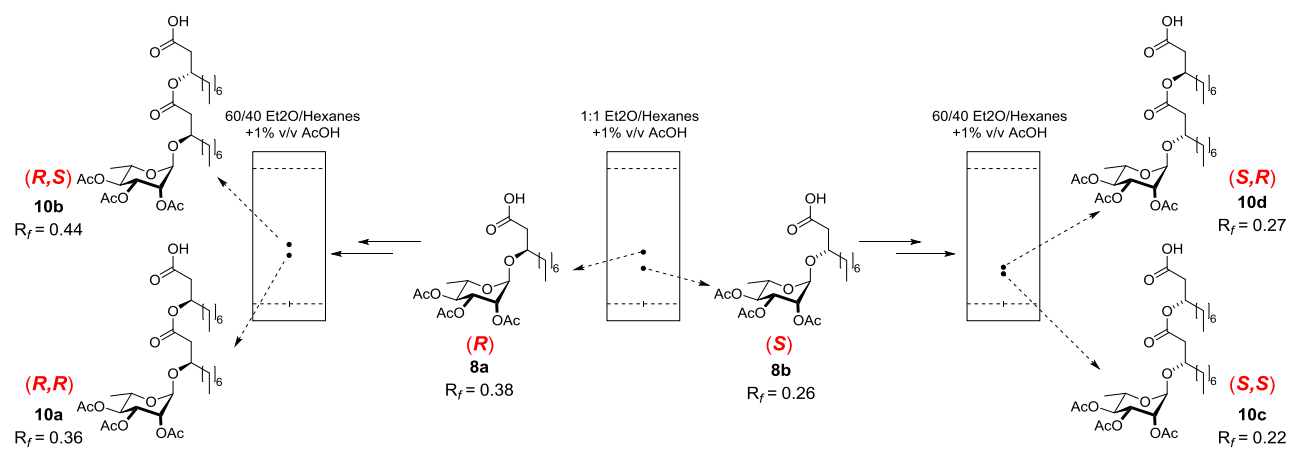


Figure S18. Absolute configuration assignments of the free acid glycolipids **8a** and **8b**; and **10a**, **10b**, **10c** and **10d** in terms of their TLC R_f 's. The stereochemistry is listed from right to left, starting with the carbinol closer to the rhamnopyranosyl ring (*Inner lipid tail, outer lipid tail*).

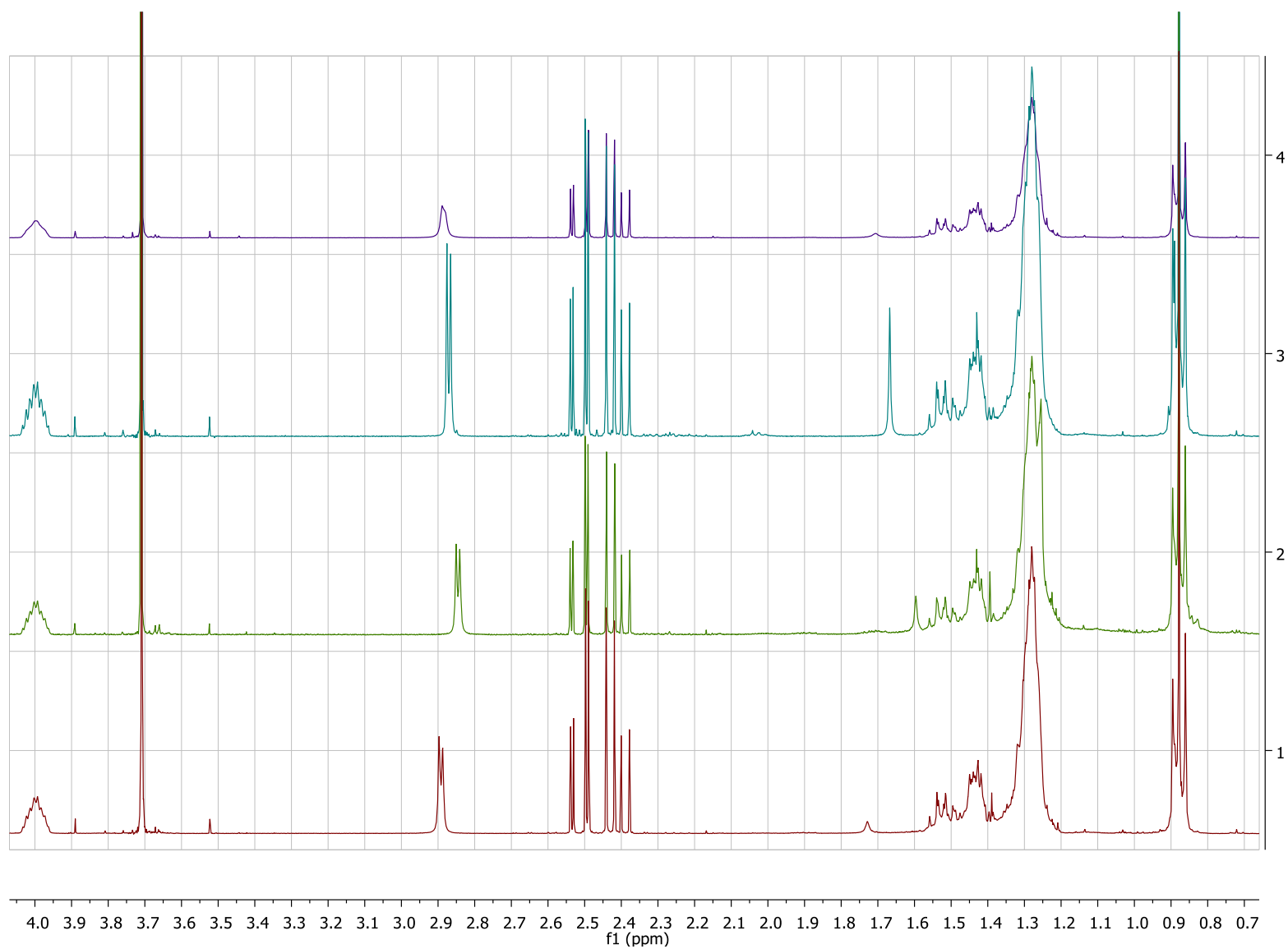


Figure S19. ^1H -NMR spectral data for methyl 3-hydroxydecanoate esters enantiomers.

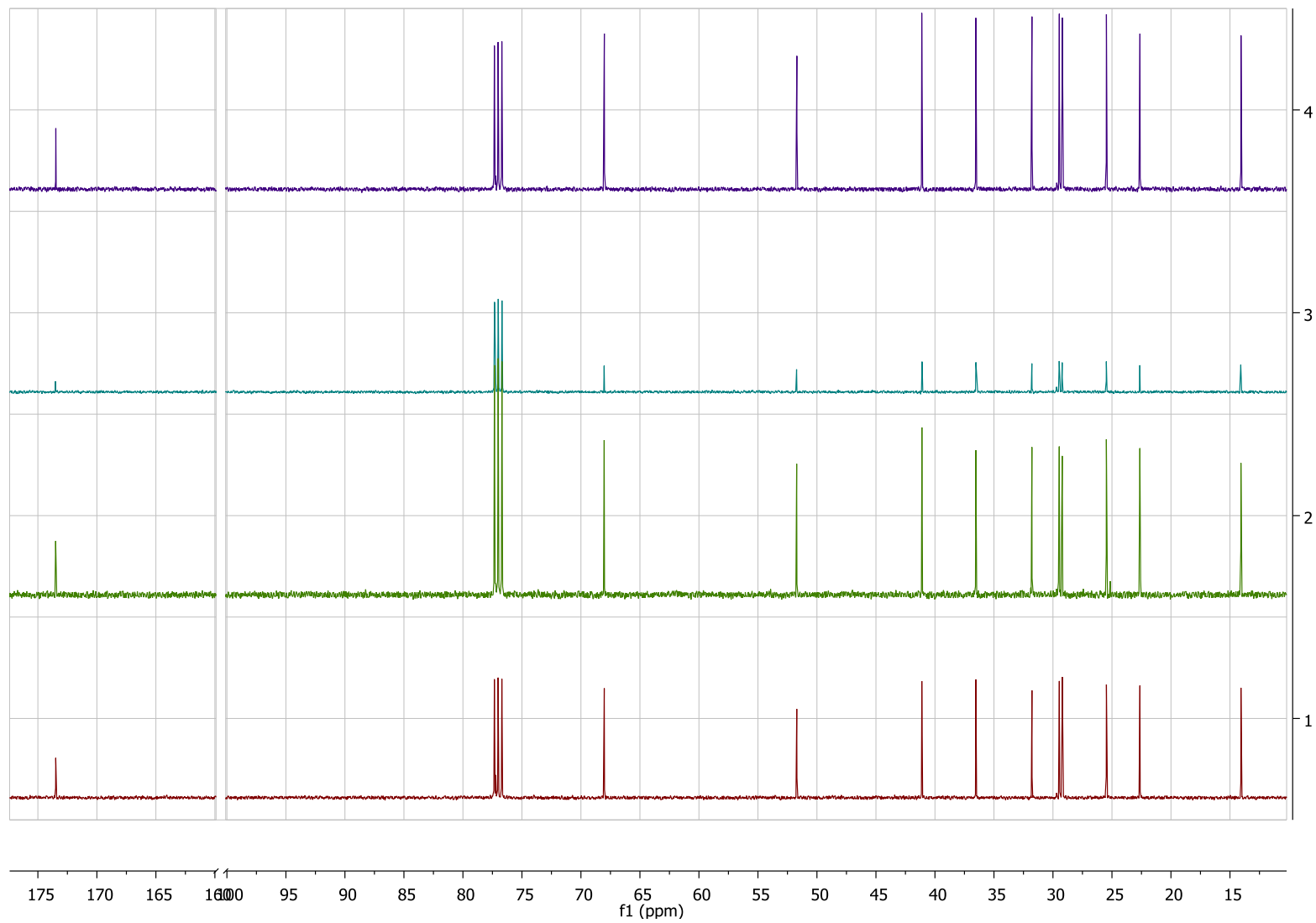


Figure S20. ^{13}C -NMR spectral data for methyl 3-hydroxydecanoate esters enantiomers.

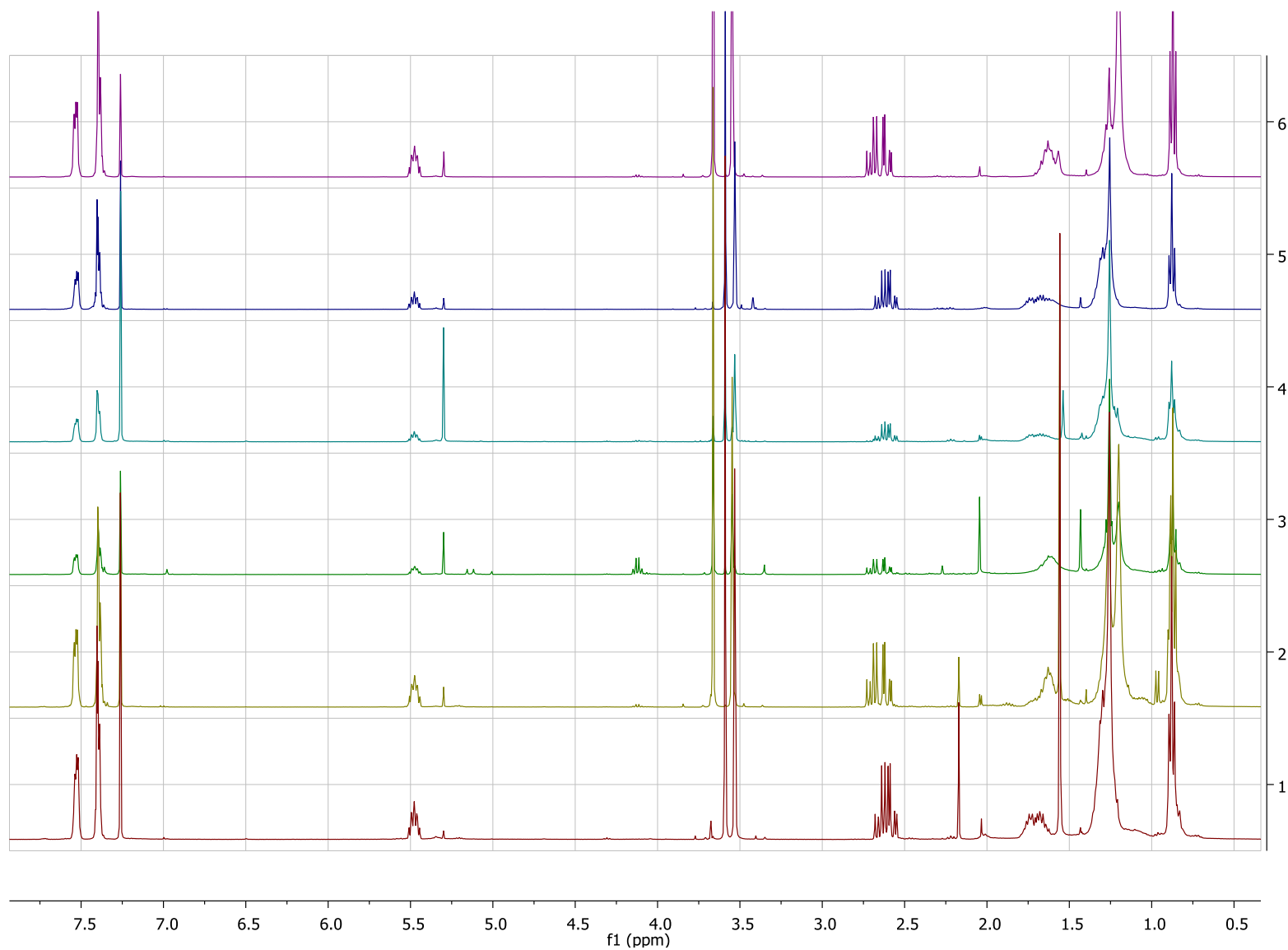


Figure S21. ^1H -NMR spectral data for Mosher esters of methyl 3-hydroxydecanoate esters diastereomers.

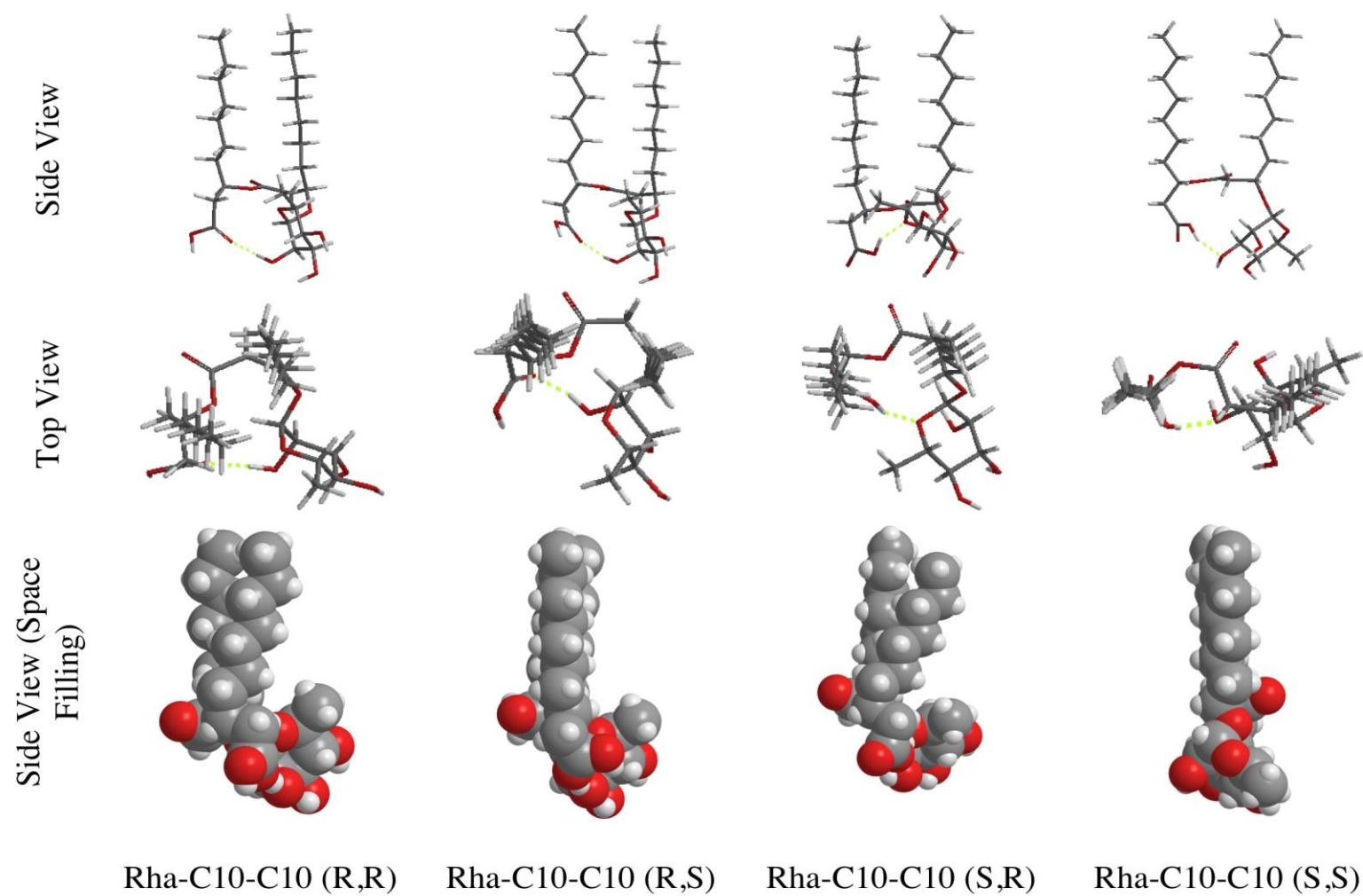


Figure S22. Energy minimized ChemDraw® 3D structures of the four Rha-C10-C10 diastereomers.