Electronic Supplementary Information for:

**Innate immunomodulation by lipophilic termini of lipopolysaccharide; synthesis of lipid A from *Porphyromonas gingivalis* and other bacteria and their immunomodulative response**

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**Materials and methods**

**Chemical synthesis of fatty acids**

\[ S1 \rightarrow S2 \rightarrow S3 \rightarrow S4 \rightarrow S5 \rightarrow S6 \rightarrow S7 \rightarrow S8 \rightarrow 16 \]

Scheme 1. Synthesis of β-hydroxy carboxylic acids
**13-methyltetradecan-11-enoic acid (S2)**

1-bromoundecanoic acid S1 (20 g, 75.4 mmol) and triphenylphosphine (29.6 g, 113 mmol) were dissolved in dry CH<sub>3</sub>CN (220 mL) under Ar. The mixture was heated at 100 °C under reflux for 3 d, and then the solution was concentrated in vacuo. The residue was dissolved in CH<sub>3</sub>CN, and the solution was extracted with n-Hexane to remove triphenylphosphine. The solution of CH<sub>3</sub>CN was concentrated in vacuo to give crude 10-carboxydecyltriphenylphosphoniumbromide (40.2g). The compound was used without further purification.

Crude compound 10-carboxydecyltriphenylphosphoniumbromide was dried under reduced pressure, and isobutyraldehyde was dried by MS4A before the reaction. To a solution of 10-carboxydecyltriphenylphosphoniumbromide (9.6 g, ca. 18.2 mmol) in dry THF (200 mL) was added dropwise NaHMDS 1 M THF solution (18.5 mL, 18.5 mmol) at -20 °C under Ar. The color of the reaction mixture solution turned orange red. After the mixture was stirred for 3.5 h at -20 °C, the reaction was quenched with 1 M HCl, and the mixture was extracted with diethylether. The organic layer was washed with 1 M HCl, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. The residue was purified by silica-gel column chromatography (CHCl<sub>3</sub>/ Acetone = 40/1) to give S2 (2.5 g, 60% for 2 steps) as a yellow oil.

ESI-MS (negative) m/z 239.19 [M-H]; <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ = 5.26-5.16 (m, 2H, -CH=CH-CH-(CH<sub>3</sub>)<sub>2</sub>), 2.63-2.55 (m, 1H, -CH=CH-CH-(CH<sub>3</sub>)<sub>2</sub>), 2.35 (t, 2H, J = 7.48Hz, HOOC-CH<sub>2</sub>-), 2.03 (q, 2H, J = 6.87Hz, -CH<sub>2</sub>-CH=CH-), 1.67-1.61 (m, 2H, HOOC-CH<sub>2</sub>-CH<sub>2</sub>-), 1.32-1.20 (m, 12H, HOOC-CH<sub>2</sub>-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>6</sub>-), 0.95 (d, 6H, J = 6.71Hz, -CH=CH-CH-(CH<sub>3</sub>)<sub>2</sub>).

**13-methyltetradecanoic acid (S3)**

To a solution of S2 (2.7 g, 11.2 mmol) in THF (10 mL) was added Pd/C (10% Pd) (2.80 g) at room temperature, and stirred under 0.1 MPa of H<sub>2</sub> for 2 d. The mixture was filtered through membrane-filter and the filtrate was concentrated in vacuo to give S3 (2.45 g, 90%) as a white solid.

ESI-MS (negative) m/z 241.22 [M-H]; <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ = 2.39 (t, 2H, J = 7.48Hz, HOOC-CH<sub>2</sub>-), 1.70-1.60 (m, 2H, HOOC-CH<sub>2</sub>-CH<sub>2</sub>-), 1.59-1.53 (m, 1H, -CH-(CH<sub>3</sub>)<sub>2</sub>), 1.52-1.20 (m, 18H, HOOC-CH<sub>2</sub>-CH<sub>2</sub>-(CH<sub>2</sub>)<sub>6</sub>-), 0.91 (d, 6H, J = 6.71Hz, -CH-(CH<sub>3</sub>)<sub>2</sub>).
Diethyl magnesium malonate
Magnesium ethoxide (1.50 g, 13.1 mmol) was added to a solution of monoethyl malonate (3.09 mL, 26.2 mmol) in anhydrous THF (31.5 mL) under Ar. The resulting mixture was stirred at room temperature for 2 h. The solvent was removed in vacuo and the gummy residue was triturated with ether to give Diethyl magnesium malonate (3.68 g, 12.8 mmol) as a white solid.

Ethyl 3-oxo-15-methylhexadecanoate (S4)
To a solution of S3 (1.83 g, 7.55 mmol) in dry THF (36 mL) was added CDI (1.29 g, 7.93 mmol) and the mixture was stirred for 3.5 h. To the solution was added diethyl magnesium malonate (2.60 g, 9.06 mmol) and the resulting mixture was stirred overnight at room temperature. The reaction mixture was quenched with 1 M HCl and extracted with EtOAc. The combined organic layer was washed with saturated aqueous NaHCO₃ and brine, dried over Na₂SO₄, filtered and evaporated in vacuo. The residue was purified by silica-gel column chromatography (Hexane / EtOAc = 10/1) to give S4 (2.11 g, 88%) as a white crystal.

ESI-MS (positive) m/z 313.27 [M+H]⁺; ¹H-NMR (500 MHz, CDCl₃) δ = 4.19 (q, 2H, J = 7.17Hz, CH₃CH₂OOC-CH₂-), 3.42 (s, 2H, EtOOC-CH₂-), 2.52 (t, 2H, J = 7.32Hz, EtOOC-CH₂-OC-CH₂-), 1.59-1.49 (m, 3H, -CH₂-(CH₂)₈-CH-), 1.29-1.25 (m, 19H, CH₃CH₂OOC-, -(CH₂)₉-CH-(CH₃)₂), 0.86 (d, 6H, J = 6.56Hz, -CH-(CH₃)₂).

Ethyl (R)-3-hydroxy-15-methylhexadecanoate (S5)
A dry 20-mL Schlenk tube containing a Teflon-coated stirring bar was charged with [RuCl₂PhH]₂(41.0 mg, 82.3 mmol), (R)-BINAP (100 mg, 161mmol), and DMF (3 mL, deaerated before use), and the bottle was capped securely. The resulting reddish brown solution was heated at 100 °C for 20 min to give a clear reddish brown solution. The reaction mixture was cooled to room temperature, and then concentrated at 60 °C under high vacuum with vigorous stirring. The resulting reddish orange solid was used for asymmetric hydrogenation without purification.
A autoclave charged with ethanol (20 mL) and S4 (20.0 g, 64.0mmol). To the solution was
added above freshly prepared RuCl₂[(R)-BINAP] quickly. The bottle was capped above immediately, and connected to a hydrogen cylinder. Hydrogen was pressurized to 100 atm.

The solution was stirred at 60 °C overnight. The mixture was concentrated in vacuo. The residue was purified by silica-gel column chromatography (toluene / EtOAc = 8/1) to give S5 (20.1 g, quant.) as a white crystal.

ESI-MS (positive) m/z 315.18 [M+H]^+; ¹H-NMR (500 MHz, CDCl₃) δ = 4.17 (q, 2H, J = 7.17Hz, CH₃CH₂OOC-CH₂-), 4.00 (brs, 1H, CH₃CH₂OOC-CH₂-CH(OH)-), 2.88 (brs, 1H, OH), 2.55 (dd, 1H, J₁=16.30Hz, 3.20Hz, CH₃-CH(OH)-), 2.39 (dd, 1H, J₂=16.34Hz, 9.16Hz, CH₂-CH(OH)-), 1.54-1.48 (m, 2H, -CH₂-CH(OH)-CH₂-), 1.44-1.39 (m, 2H, -CH₂-CH(OH)-CH₂-CH₂-), 1.34-1.26 (m, 20H, CH₃CH₂OOC-), -(CH₃)₈CH₂- CH-(CH₃)₂), 1.16-1.13 (m, 2H, -CH₂-CH-(CH₃)₂), 0.87(d, 6H, J = 6.56Hz, -CH-(CH₃)₂).

Ethyl (R)-3-benzyloxy-15-methylhexadecanoate (S6)

To a solution of S5 (9.00 g, 28.6 mmol) in dry THF (10 mL) was added TMS₂O (8.73 mL, 85.9 mmol) and TMSOTf (10.4 mL, 57.2 mmol) at 0 °C under Ar. After stirring for 15 min, the mixture was added PhCHO (8.73 g, 85.9 mmol). After stirring for 2 h, the mixture was added Et₃SiH (13.7 mL, 85.9 mmol). The resulting mixture was stirred at 0 °C for 2 h. The mixture was quenched with saturated aqueous NaHCO₃ and extracted with CHCl₃. The organic layer was washed with saturated aqueous NaHCO₃ and brine, dried over Na₂SO₄, concentrated in vacuo. The residue was purified by silica-gel column chromatography (Hexane / EtOAc = 20/1) to give S6 (17.3 g) as yellow oil.

(R)-3-benzyloxy-15-methylhexadecanoic acid (16)

To a solution of S6 (13.1 g, 32.4 mmol) in THF/H₂O (100 mL/20 mL) was added LiOH·H₂O (3.88 mL, 162.1 mmol) at room temperature, and stirred under Ar for 2 d. The mixture was quenched with 1 M HCl and extracted with CHCl₃. The combine organic layer was washed with 1 M HCl, dried over Na₂SO₄, concentrated in vacuo. The residue was purified by silica-gel column chromatography (CHCl₃ / MeOH = 50/1) to give 14 (7.0 g, 60%) as yellow oil.

ESI-MS (positive) m/z 315.18 [M+H]^+; ¹H-NMR (500 MHz, CDCl₃) δ = 7.17-7.26 (m, 5H, C₆H₅CH₂-), 4.51 (d, 1H, J₁ = 11.5Hz, PhCH₆OCH-), 4.48 (d, 1H, J = 11.50Hz, PhCH₂OCH-), 3.81 (m, 1H, J = 6.10Hz, PhCH₂OCH-), 2.57 (dd, 1H, J₁ = 15.42Hz, 7.20Hz, CH₃CH₂OOC-CH₂-CH(OBn)-), 2.47 (dd, 1H, J = 15.38Hz, 5.20Hz, CH₃CH₂OOC-CH₂-CH(OBn)-), 1.61-1.55 (m, 1H, -CH-(CH₃)₂), 1.52-1.09 (m, 22H,
(CH$_3$)$_2$-CH- (CH$_2$)$_{11}$-, 0.80 (d, 6H, $J$ = 6.6Hz, -CH-(CH$_3$)$_2$).

**Phenacyl (R)-3-hydroxy-15-methylhexadecanoate (S7)**

To a solution of 14 (613.7 mg, 2.14 mmol) in dry EtOAc (20 mL) was added 2-bromoacetphenone (296.1 mL, 2.35 mmol) at room temperature, and stirred under Ar overnight. The mixture was quenched with 1 M HCl and extracted with CHCl$_3$. The organic layer was washed with saturated aqueous NaHCO$_3$ and brine, dried over Na$_2$SO$_4$, and concentrated in vacuo. The residue was purified by silica-gel column chromatography (CHCl$_3$ only) to give S7 (789.2 mg, 90%) as white solid.

$^1$H-NMR (500 MHz, CDCl$_3$) δ = 7.90-7.47 (m, 5H, PhCOCH$_2$-), 5.48 (d, 1H, $J_{gem}$ = 16.50Hz, PhCOCH$_2$-), 5.37 (d, 1H, $J_{gem}$ = 16.40Hz, PhCOCH$_2$-), 4.13 (brs, 1H, PacOOCC$_2$HCH(OH)-), 2.69 (dd, 1H, $J$ = 15.10Hz, 3.00Hz, PacOOCC$_2$HCH(OH))-), 2.57 (dd, 1H, $J$ = 15.10Hz, 9.30Hz, PacOOCC$_2$HCH(OH)-), 1.54-1.49 (m, 22H, (CH$_3$)$_2$-C$_{11}$H$_{22}$-), 0.86 (d, 6H, $J$ = 6.60Hz, (CH$_3$)$_2$-C$_{11}$H$_{22}$-).

**Phenacyl (R)-3-hexadecanoyloxy-15-methylhexadecanoate (S8)**

To a solution of S7 (611.1 mg, 1.51 mmol) in pyridine (10 mL) was added hexadecanoyl chloride (687.4 mL, 2.27 mmol) at 0 °C, and stirred under Ar overnight. The mixture was quenched with 1 M HCl and extracted with CHCl$_3$. The combine organic layer was washed with 1 M HCl and brine, dried over Na$_2$SO$_4$, and concentrated in vacuo. The residue was purified by silica-gel column chromatography (CHCl$_3$ only) to give S8 (885.4 mg, 91%) as non-color solid.

$^1$H-NMR (500 MHz, CDCl$_3$) δ = 7.90-7.47 (m, 5H, PhCOCH$_2$-), 5.33 (s, 2H, PhCOCH$_2$-), 2.77 (dd, 1H, $J_{gem}$ = 15.40Hz, 7.50Hz, PacOOCC$_2$H$_2$), 2.72 (dd, 1H, $J_{gem}$ = 15.40Hz, 5.50Hz, PacOOCC$_2$H$_2$), 1.59-1.16 (m, 48H, CH$_3$C$_{13}$H$_{26}$CH$_2$COO)CHC$_{11}$H$_{22}$-), 0.89-0.86 (m, 9H, $J$ = 6.50Hz, (CH$_3$)$_2$C$_{11}$H$_{22}$((CH$_3$)$_2$C$_{13}$H$_{26}$CH$_2$COO)CH-).
After being exposed to ultrasonic wave for 1 h, Zn (6.0 g) in water was added few drops of aqueous CuSO₄ to make Zn-Cu. The mixture was filtered and the residue was added to the solution of S₈ (1.19 g, 1.85 mmol) in AcOH/THF (7 mL / 7 mL). After being stirred at room temperature for 3 h, the insoluble materials were filtered off and the filtrate was concentrated in vacuo. The residue was purified by silica-gel column chromatography (CHCl₃ only) to give 14 (970 mg, quant.) as non-color oil.

ESI-MS (positive) m/z 525.50 [M+H]+; ¹H-NMR (500 MHz, CDCl₃) δ = 5.21 (m, 1H, J = 6.30Hz, C₁₅H₃₀COOCH⁻), 2.63 (dd, 1H, J = 15.80Hz, 7.30Hz, CH₃CH₂OOC-CH₂-CH(OOCC₁₅H₃₀⁻), 2.47 (dd, 1H, J = 16.00Hz, 5.50Hz, CH₃CH₂OOCCH₂⁻), 2.28 (t, 2H, J = 7.50Hz, C₁₄H₂₉CH₂COO⁻), 1.54-1.13 (m, 48H, -(CH₃)₁₃H₂₆CH₂COO)-(CH-C₁₁H₂₂⁻), 1.51 (m, 1H, J = 6.50Hz, -CH₂CH(OOCC₁₅H₃₀⁻), 0.89-0.86 (m, 9H, (CH₃)₂-C₁₁H₂₂-(CH₂C₁₃H₂₆CH₂COO⁻). Found: C, 75.28; H, 12.27% Calc. for C₃₃H₆₄O₄: C, 75.52; H, 12.29%.