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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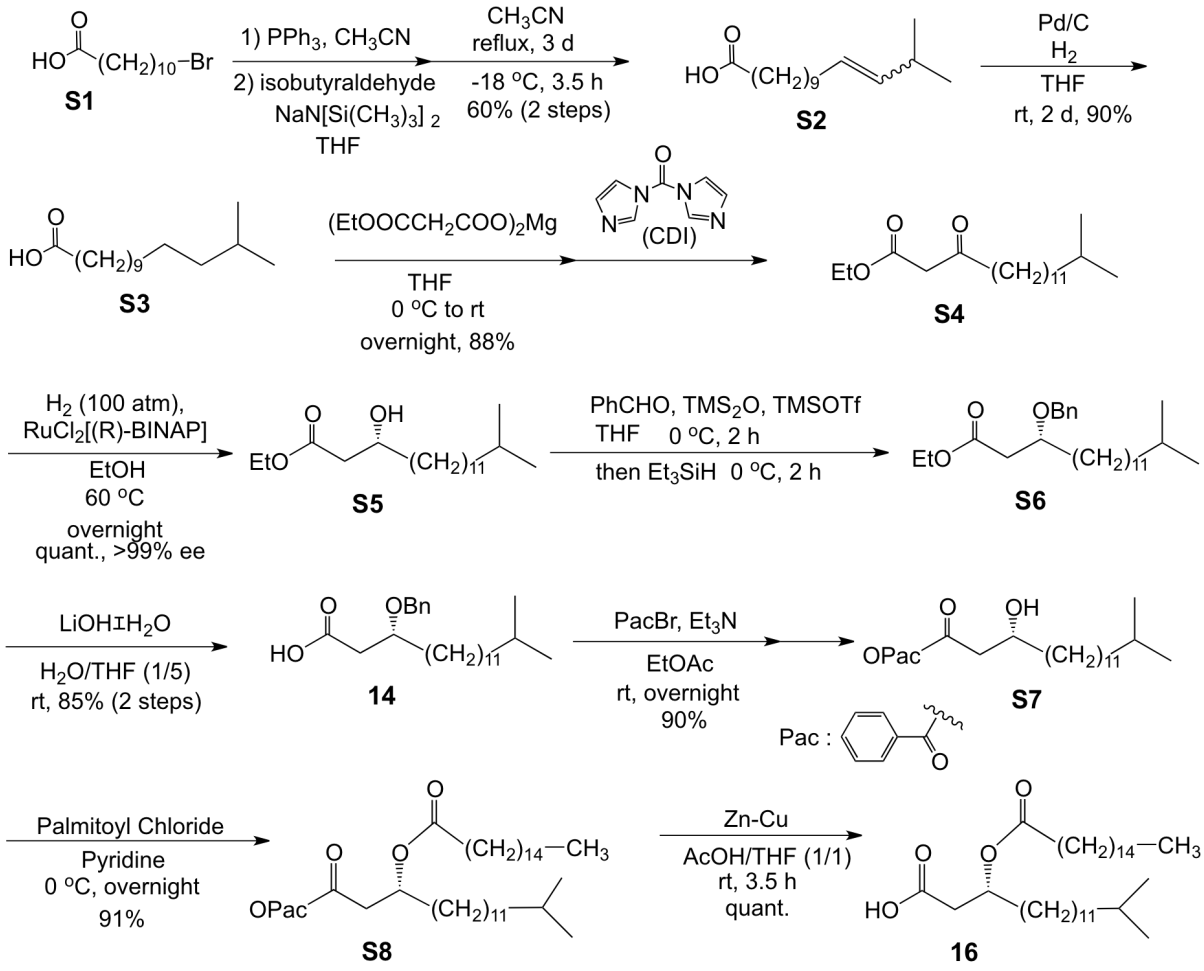
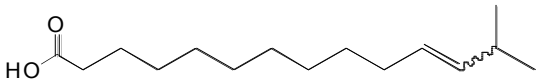
Materials and methods

Chemical synthesis of fatty acids



Scheme 1. Synthesis of β-hydroxy carboxylic acids

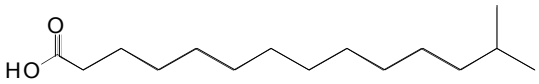


13-methltetradecan-11-enoic acid (S2)

1-bromoundecanoic acid **S1** (20 g, 75.4 mmol) and triphenylphosphine (29.6 g, 113 mmol) were dissolved in dry CH_3CN (220 mL) under Ar. The mixture was heated at 100 ºC under reflux for 3 d, and then the solution was concentrated in vacuo. The residue was dissolved in CH_3CN, and the solution was extracted with *n*-Hexane to remove triphenylphosphine. The solution of CH_3CN 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 ºC under Ar. The color of the reaction mixture solution turned orange red. After the mixture was stirred for 1 h, isobutyraldehyde (1.65 mL, 18.2 mmol) was added dropwise at -20 ºC and the mixture was discolored. After being stirred for 3.5 h at -20 ºC, 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_2SO_4 and concentrated in vacuo. The residue was purified by silica-gel column chromatography ($CHCl_3$ / Acetone = 40/1) to give **S2** (2.5 g, 60% for 2 steps) as a yellow oil.

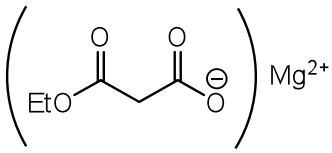
ESI-MS (negative) m/z 239.19 $[M-H]^-$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ = 5.26-5.16 (m, 2H, -C<u>H</u>=C<u>H</u>-CH-$(CH_3)_2$), 2.63-2.55 (m, 1H, -CH=CH-C<u>H</u>-$(CH_3)_2$), 2.35 (t, 2H, *J* = 7.48Hz, HOOC-C<u>H_2</u>-), 2.03 (q, 2H, *J* = 6.87Hz, -C<u>H_2</u>-CH=CH-), 1.67-1.61 (m, 2H, HOOC-CH_2-C<u>H_2</u>-), 1.32-1.20 (m, 12H, HOOC-CH_2-CH_2-(C<u>H_2</u>)$_6$-), 0.95 (d, 6H, *J* = 6.71Hz, -CH=CH-CH-(C<u>H_3</u>)$_2$).



13-methyltetradecanoic acid (S3)

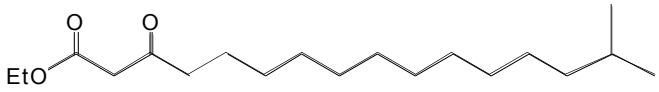
To a solution of **S2** (2.7 g, 11.2 mmol) in THF (10 mL) was added Pd/C (10% Pd) (2.80 g) at room temperature, and stirred under 0.1 MPa of H_2 for 2 d. The mixture was filtered through membrance-filter and the filtrate was concentrated in vacuo to give **S3** (2.45 g, 90%) as a white solid.

ESI-MS (negative) m/z 241.22 $[M-H]^-$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ = 2.39 (t, 2H, *J* = 7.48Hz, HOOC-C<u>H_2</u>-), 1.70-1.60 (m, 2H, HOOC-CH_2-C<u>H_2</u>-), 1.59-1.53 (m, 1H, -C<u>H</u>-$(CH_3)_2$), 1.52-1.20 (m, 18H, HOOC-CH_2-CH_2-(C<u>H_2</u>)$_9$-), 0.91 (d, 6H, *J* = 6.71Hz, -CH-(C<u>H_3</u>)$_2$).



Diethyl magnesium malonate

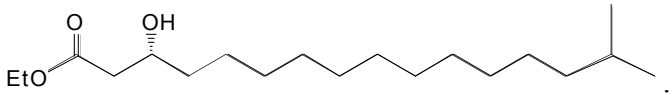
Magnesium ethoxide (1.50 g, 13.1 mmol) was added to a solution of monoethyl malonate (3.09 mL, 26.2 mmol) in anhydrous THF (31.5 mL) under Ar. The resulting mixture was stirred at room temperature for 2 h. The solvent was removed in vacuo and the gummy residue was triturated with ether to give **Diethyl 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 $NaHCO_3$ and brine, dried over Na_2SO_4, 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 $[M+H]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ = 4.19 (q, 2H, *J* = 7.17Hz, $CH_3C\underline{H}_2OOC$-CH_2-), 3.42 (s, 2H, EtOOC-C$\underline{H}_2$-), 2.52 (t, 2H, *J* = 7.32Hz, EtOOC-CH_2-OC-C$\underline{H}_2$), 1.59-1.49 (m, 3H, -C$\underline{H}_2$-$(CH_2)_8$-C$\underline{H}$-), 1.29-1.25 (m, 19H, C$\underline{H}_3CH_2OOC$-, -$(C\underline{H}_2)_8$- CH-$(CH_3)_2$), 0.86 (d, 6H, *J* = 6.56Hz, -CH-$(C\underline{H}_3)_2$).



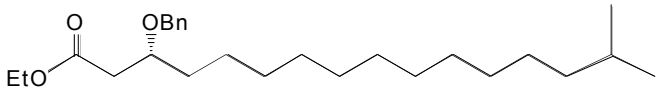
Ethyl (*R*)-3-hydroxy-15-methylhexadecanoate (S5)

A dry 20-mL Schlenk tube containing a Teflon-coated stirring bar was charged with $[RuCl_2PhH]_2$(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 ºC 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

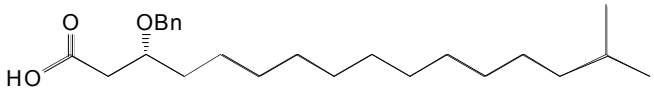
added above freshly prepared $RuCl_2$[(*R*)-BINAP] quickly. The bottle was capped above immediately, and connected to a hydrogen cylinder. Hydrogen was pressurized to 100 atm. The solution was stirred at 60 ºC overnight. The mixture was concentrated in vacuo. The residue was purified by silica-gel column chromatography (toluene / EtOAc = 8/1) to give **S5** (20.1 g, quant.) as a white crystal.

ESI-MS (positive) m/z 315.18 $[M+H]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ = 4.17 (q, 2H, *J* = 7.17Hz, $CH_3C\underline{H}_2OOC$-CH_2-), 4.00 (brs, 1H, CH_3CH_2OOC-CH_2-$C\underline{H}$(OH)-), 2.88 (brs, 1H, O<u>H</u>), 2.55 (dd, 1H, *J* =16.30Hz, 3.20Hz, $C\underline{H}_2$-CH(OH)-), 2.39 (dd, 1H, *J* =16.34Hz, 9.16Hz, $C\underline{H}_2$-CH(OH)-), 1.54-1.48 (m, 2H, -CH_2-CH(OH)-$C\underline{H}_2$), 1.44-1.39 (m, 2H, -CH_2-CH(OH)-CH_2-$C\underline{H}_2$), 1.34-1.26 (m, 20H, $C\underline{H}_3CH_2OOC$-, -$(C\underline{H}_2)_8$-CH_2- $C\underline{H}$-$(CH_3)_2$), 1.16-1.13 (m, 2H, -$C\underline{H}_2$-CH-$(CH_3)_2$), 0.87(d, 6H, *J* = 6.56Hz, -CH-$(C\underline{H}_3)_2$).



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 TMS_2O (8.73 mL, 85.9 mmol) and TMSOTf (10.4 mL, 57.2 mmol) at 0 ºC 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 Et_3SiH (13.7 mL, 85.9 mmol).The resulting mixture was stirred at 0 ºC for 2 h. The mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with $CHCl_3$. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, 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.

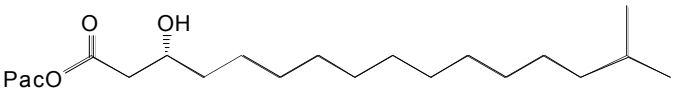


(*R*)-3-benzyloxy-15-methylhexadecanoic acid (16)

To a solution of **S6** (13.1 g, 32.4 mmol) in THF/H_2O (100 mL/20 mL) was added LiOH·H_2O (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 $CHCl_3$. The combine organic layer was washed with 1 M HCl, dried over Na_2SO_4, concentrated in vacuo. The residue was purified by silica-gel column chromatography ($CHCl_3$ / MeOH = 50/1) to give **14** (7.0 g, 60%) as yellow oil.

ESI-MS (positive) m/z 315.18 $[M+H]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ = 7.17-7.26 (m, 5H, $C_6\underline{H}_5CH_2$-), 4.51 (d, 1H, J_{gem} = 11.5Hz, PhC<u>H</u>$_2$OCH-), 4.48 (d, 1H, J_{gem} = 11.50Hz, PhC<u>H</u>$_2$OCH-), 3.81 (m, 1H, *J* = 6.10Hz, PhC<u>H</u>$_2$OC<u>H</u>-), 2.57 (dd, 1H, *J* = 15.42Hz, 7.20Hz, CH_3CH_2OOC-$C\underline{H}_2$-CH(OBn)-), 2.47 (dd, 1H, *J* = 15.38Hz, 5.20Hz, CH_3CH_2OOC-$C\underline{H}_2$-CH(OBn)-), 1.61-1.55 (m, 1H, -$C\underline{H}$-$(CH_3)_2$), 1.52-1.09 (m, 22H,

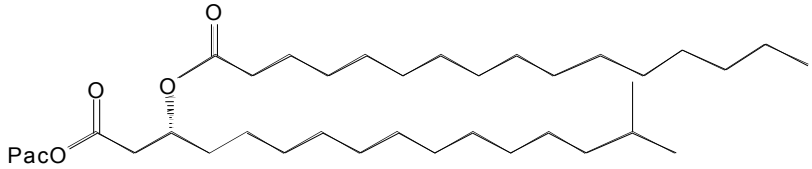
$(CH_3)_2$-CH- $(C\underline{H}_2)_{11}$-), 0.80 (d, 6H, J = 6.6Hz, -CH-$(C\underline{H}_3)_2$).



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 insoluble materials were filtered off and the filtrate was concentrated in vacuo. The residue was extracted with $CHCl_3$. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, 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.

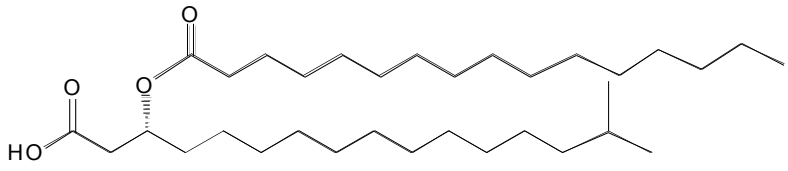
^{1}H-NMR (500 MHz, $CDCl_3$) δ = 7.90-7.47 (m, 5H, $\underline{Ph}COCH_2$-), 5.48 (d, 1H, J_{gem}= 16.50Hz, $PhCOC\underline{H}_2$-), 5.37 (d, 1H, J_{gem}= 16.40Hz, $PhCOC\underline{H}_2$-), 4.13 (brs, 1H, $PacOOCCH_2C\underline{H}(OH)$-), 2.69 (dd, 1H, J = 15.10Hz, 3.00Hz, $PacOOCC\underline{H}_2CH(OH)$-), 2.57 (dd, 1H, J = 15.10Hz, 9.30Hz, $PacOOCC\underline{H}_2CH(OH)$-), 1.54-1.49 (m, 22H, $(CH_3)_2$-$C_{11}\underline{H}_{22}$-), 0.86 (d, 6H, J =6.60Hz, $(C\underline{H}_3)_2$-$C_{11}H_{22}$-)



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 ºC, 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_2SO_4, 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, $\underline{Ph}COCH_2$-), 5.33 (s, 2H, $PhCOC\underline{H}_2$-), 2.77 (dd, 1H, J_{gem} = 15.40Hz, 7.50Hz, $PacOOCC\underline{H}_2$), 2.72 (dd, 1H, J_{gem} = 15.40Hz, 5.50Hz, $PacOOCC\underline{H}_2$), 1.59-1.16 (m, 48H, $CH_3C_{13}\underline{H}_{26}CH_2COO)CHC_{11}\underline{H}_{22}$-), 0.89-0.86 (m, 9H, J = 6.50Hz, $(C\underline{H}_3)_2C_{11}H_{22}(C\underline{H}_3C_{13}H_{26}CH_2COO)CH$-).



(*R*)-3-Hexadecanoyloxy-15-methylhexadecanoic acid (14)

After being exposed to ultrasonic wave for 1 h, Zn (6.0 g) in water was added few drops of aqueous $CuSO_4$ 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 ($CHCl_3$ only) to give **14** (970 mg, quant.) as non-color oil.

ESI-MS (positive) m/z 525.50 $[M+H]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$) δ = 5.21 (m, 1H, *J* = 6.30Hz, $C_{15}H_{30}COOC\underline{H}$-), 2.63 (dd, 1H, *J* = 15.80Hz, 7.30Hz, CH_3CH_2OOC-$C\underline{H}_2$-$CH(OOCC_{15}H_{30})$-), 2.47 (dd, 1H, *J* = 16.00Hz, 5.50Hz, $CH_3CH_2OOCC\underline{H}_2$-), 2.28 (t, 2H, *J* = 7.50Hz, $C_{14}H_{29}C\underline{H}_2COO$-), 1.54-1.13 (m, 48H, -$(CH_3C_{13}\underline{H}_{26}CH_2COO)CH$-$C_{11}\underline{H}_{22}$-), 1.51 (m, 1H, *J* = 6.50Hz, -CH_2-$C\underline{H}(OOCC_{15}H_{30})$-), 0.89-0.86 (m, 9H, $(C\underline{H}_3)_2$-$C_{11}H_{22}$-$(C\underline{H}_3C_{13}H_{26}CH_2COO)$-). Found: C, 75.28; H, 12.27% Calc. for $C_{33}H_{64}O_4$: C, 75.52; H, 12.29%.