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**SUPPLEMENTARY CONTENT**

# Synthesis of glycerolipids containing simple linear acyl chains or aromatic rings and evaluation of their Mincle signaling activity

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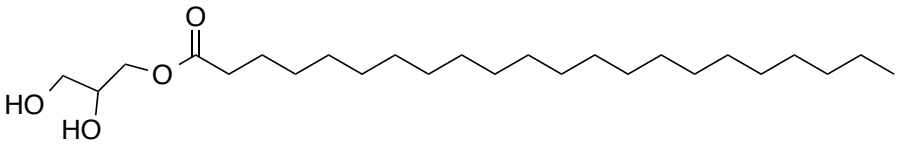
## GENERAL METHODS

$^1$H NMR spectra were recorded at 400 MHz or 500 MHz and $^{13}$C NMR spectra were recorded at 100 MHz or 125 MHz on JEOL ECS400, ECX400, and ECA500 instruments. The chemical shifts are expressed in ppm downfield from the internal solvent peaks CDCl3 (7.26 ppm, $^1$H NMR), CD3OD (3.31 ppm, $^1$H NMR), CDCl3 (77.0 ppm $^{13}$C NMR), and CD3OD (49.0 ppm $^{13}$C NMR), and J values are expressed in hertz. The coupling patterns are expressed as s (singlet), d (doublet), t (triplet), m (multiplet), br (broad) or brs (broad singlet). ESI mass spectra were recorded on a Waters ACQUITY UPLC/Xevo G2-S QTof or electron spray ionization quadrupole time of flight (ESI-QTOF) mass spectrometer (micrOTOF-QII-HC; BRUKER). Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60F254 plates. Flash column chromatography was performed on Fji Sylysia silica gel PSQ 60B.

### 2,3-Dihydroxypropyl behenate (2a)



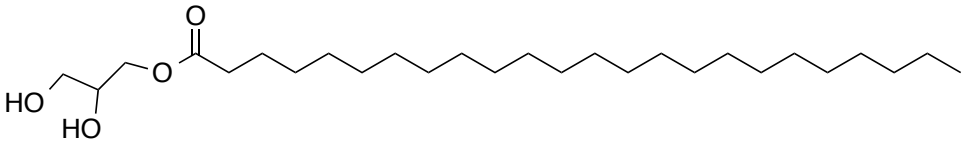

To a stirred solution of behenic acid (500 mg, 1.47 mmol) in toluene (1.5 mL) was added $SOCl_2$ (19.9 μL, 2.20 μmol) and 1 drop of DMF. After stirring for 2 h at 40 ˚C, the mixture was evaporated, and toluene was added and evaporated again three times. The residue was concentrated under reduced pressure to give the desired acid chloride, which was used for the next reaction without further purification.

To a stirred solution of (2,2-dimethyl-1,3-dioxolan-4-yl) methanol (182 μL, 1.47 mmol) in DCM (5 mL) was added pyridine (237 μL, 2.94 mmol) and the afforded acid chloride at 0 ˚C. After stirring overnight at the same temperature, the mixture was supplemented with 1 M HCl and extracted with $CHCl_3$. The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to give a crude product, which was purified by short silica gel column chromatography (n-hexane/EtOAc = 8:1) to yield an isopropylidene glycerolipid.

To a stirred solution of isopropylidene glycerolipid in DCM (1.0 mL) and MeOH (1.0 mL) was added amberlyst (100 mg). After stirring for 12 h at room temperature, the mixture was filtered and washed with MeOH. The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to give a crude product, which was purified

by silica gel column chromatography (n-hexane/EtOAc = 6:1) to yield a mixture of mono acyl glycerolipids (35.6 mg, 29% over 2 steps). The major compound is a title compound: $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm); 4.23-4.13 (ddd, $J$ = 3.6, 9.2, 15.2 Hz, 2H), 3.96-3.91 (m, 1H), 3.72-3.68 (m, 1H), 3.62-3.57 (m, 1H), 2.35 (t, $J$ = 7.3 Hz, 2H), 1.63 (m, 2H), 1.30-1.25 (complex m, 36 H), 0.88 (t, $J$ = 6.8 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ (ppm); 174.4, 70.2, 65.2, 63.3, 34.1, 31.9, 29.7-29.1 (many unresolved C's), 24.9, 22.7, 14.1; HRMS-ESI (*m/z*): $[M+Na]^+$ calcd $C_{31}H_{62}O_4Na$, 437.3601 found, 437.3609.

**2,3-Dihydroxypropyl tetracosanoate (2b)**

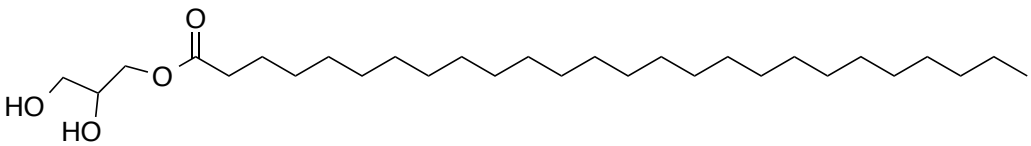



To a stirred solution of tetracosanoic acid (100 mg, 271 μmol) in toluene (271 μL) was added $SOCl_2$ (43.0 μL, 596.7 μmol) and 1 drop of DMF. After stirring for 2.5 h at 40 ˚C, the mixture was evaporated, and toluene was added and evaporated again. The residue was concentrated under reduced pressure to give the desired acid chloride, which was used for the next reaction without further purification.

To a stirred solution of (2,2-dimethyl-1,3-dioxolan-4-yl) methanol (30.5 μL, 247 μmol) in DCM (4.9 mL) was added pyridine (21.9 μL, 271 μmol) and the afforded acid chloride at 0 ˚C. After stirring for 14.5 h at room temperature, the mixture was supplemented with 1 M HCl and extracted with $CHCl_3$.The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to give a crude product, which was purified by short silica gel column chromatography (*n*-hexane/EtOAc = 6:1) to yield an isopropylidene glycerolipid.

To a stirred solution of isopropylidene glycerolipid in DCM (440 μL) and MeOH (440 μL) was added amberlyst (86 mg). After stirring for 1 h at 60 ˚C, the reaction was cooled to room temperature. The mixture was filtered and washed with MeOH. The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (*n*-hexane/EtOAc = 6:1) to yield a mixture of mono acyl glycerolipids (41.5 mg, 38% over 2 steps). Major compound is a title compound:$^1$H NMR (500 MHz, $CDCl_3$) δ (ppm); 4.23-4.13 (m, 2H), 3.93 (m, 1H), 3.69 (m, 1H), 3.61 (m, 1H), 2.35 (t, $J$ = 7.5 Hz, 2H), 1.63 (m, 2H), 1.29-1.22 (complex m, 40 H), 0.88 (t, $J$ = 7.5 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ (ppm); 174.4, 70.2, 65.2, 63.3, 34.1, 31.9, 29.7-29.1 (many unresolved C's), 24.9, 22.7, 14.1; HRMS-ESI (*m/z*): $[M+Na]^+$ calcd $C_{27}H_{54}O_4Na$, 465.3914 found, 465.3927.

**2,3-Dihydroxypropyl hexacosanoate (2c)**

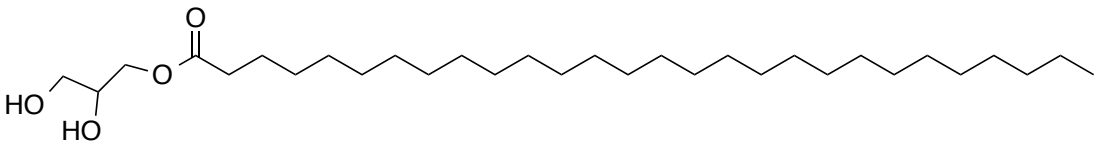



To a stirred solution of tetracosanoic acid (100 mg, 252 μmol) in toluene (252 μL) was added $SOCl_2$ (40.0 μL, 555 μmol) and 1drop of DMF. After stirring for 1.5 h at 40 ˚C, the mixture was evaporated, and toluene was added and evaporated again. The residue was concentrated under reduced pressure to give the desired acid chloride, which was used for the next reaction without further purification.

To a stirred solution of (2,2-dimethyl-1,3-dioxolan-4-yl) methanol (28.3 μL, 229 μmol) in DCM (4.6 mL) was added pyridine (18.5 μL, 229 μmol) and the afforded acid chloride at 0 ˚C. After stirring for 15.5 h at room temperature, the mixture was supplemented with 1 M HCl and extracted with $CHCl_3$. The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to yield a crude product, which was purified by short silica gel column chromatography (*n*-hexane/EtOAc = 6:1) to give an isopropylidene glycerolipid.

To a stirred solution of isopropylidene glycerolipid in DCM (200 μL) and MeOH (200 μL) was added amberlyst (38 mg). After stirring for 3 h at 60˚C, the reaction was cooled to room temperature. The mixture was filtered and washed by MeOH. The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (*n*-hexane/EtOAc = 3:2) to yield a mixture of mono acyl glycerolipids (22.4 mg, 21% over 2 steps). The major compound is a title compound: $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm); 4.23-4.13 (m, 2H), 3.93 (m, 1H), 3.70 (m, 1H), 3.60 (m, 1H), 2.35 (t, *J* = 7.3 Hz, 2H), 1.63 (m, 2H), 1.30-1.25 (complex m, 44 H), 0.88 (t, *J* = 7.3 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ (ppm); 174.5, 70.4, 65.3, 63.5, 34.3, 32.1, 29.9-29.3 (many unresolved C's), 25.1, 22.9, 14.3; HRMS-ESI (*m/z*): $[M+Na]^+$ calcd $C_{29}H_{58}O_4Na$, 493.4227 found, 493.4240.

**2,3-Dihydroxypropyl octacosanoate (2d)**

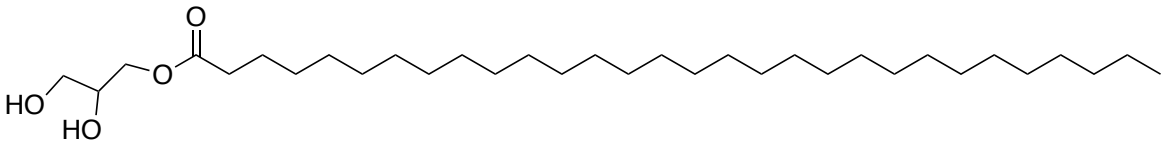



To a stirred solution of octaconsanoic acid (92.5 mg, 218 μmol) in toluene (500 μL) was added $SOCl_2$ (34.6 μL, 518 μmol) and 1drop of DMF. After stirring for 1.5 h at 40 ˚C, the mixture was evaporated, and toluene was added and evaporated again. The residue was concentrated under reduced pressure to yield the desired acid chloride, which was used for the next reaction without further purification.

To a stirred solution of (2,2-dimethyl-1,3-dioxolan-4-yl) methanol (24.5 μL, 198 μmol) in DCM (4 mL) was added pyridine (16.0 μL, 198 μmol) and the afforded acid chloride at 0 ˚C. After stirring for 15.5 h at room temperature, the mixture was supplemented with 1 M HCl and extracted with $CHCl_3$. The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to give a crude product, which was purified by short silica gel column chromatography (*n*-hexane/EtOAc = 8:1) to yield a mixture of isopropylidene glycerolipid.

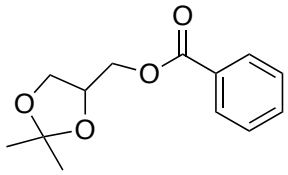
To a stirred solution of isopropylidene glycerolipid in DCM (300 μL) and MeOH (300μL) was added amberlyst (37 mg). After stirring for 3 h at 60 ˚C, the reaction was cooled to room temperature. The mixture was filtered and washed with MeOH. The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (*n*-hexane/EtOAc = 4:1) to yield a mixture of mono acyl glycerolipids (9.8 mg, 10% over 2 steps). The major compound is a title compound; $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm); 4.23-4.13 (m, 2H), 3.93 (m, 1H), 3.68 (m, 1H), 3.61 (m, 1H), 2.35 (t, *J* = 7.3 Hz, 2H), 1.63 (m, 2H), 1.30-1.25 (complex m, 48 H), 0.88 (t, *J* = 6.8 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ (ppm); 174.4, 70.2, 65.2, 63.3, 34.1, 31.9, 29.7-29.1 (many unresolved C's), 24.9, 22.7, 14.1; HRMS-ESI (*m/z*): $[M+Na]^+$ calcd $C_{31}H_{62}O_4Na$, 521.4540 found, 521.4545.

**2,3-Dihydroxypropyl triacontanoate (2e)**



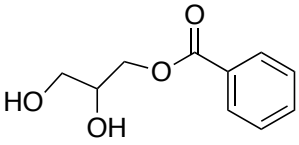
To a stirred solution of mellisic acid (21.3 mg, 47.0 μmol) in DCM (500 μL) was added (2,2-dimethyl-1,3-dioxolan-4-yl) methanol (5.8 μL, 47.0 μmol), DMAP (0.3 mg, 0.24 μmol) and WSCI (10.0 mg, 52.3 μmol). The reaction mixture was stirred overnight at room temperature. The reaction mixture was purified by short column chromatography ($CHCl_3$) to afford an acylated compound (9.4 mg, 35%). To a stirred solution of acylated compound (7.9 mg, 13.9 μmol) in a mixture of AcOH (0.75 mL) and $H_2O$ (0.25 mL) was heated to 65 ˚C and stirred at overnight. After cooling to room temperature, the solvent was removed in vacuo. The residue was purified by flash column chromatography (Hex/EtOAc 1/1) to yield a mixture of mono acyl glycerolipid (4.0 mg, 55%). The major compound is a title compound: $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm); 4.24-4.13 (m, 2H), 3.96-3.91 (m, 1H), 3.70 (m, 1H), 3.72-3.68 (dd, $J$ = 4.0, 11.6 Hz, 1H), 3.62-3.58 (dd, $J$ = 6.0, 11.6 Hz, 1H), 2.35 (t, $J$ = 7.6 Hz, 2H), 1.63 (m, 2H), 1.25-1.30 (complex m, 42H), 0.88 (t, $J$ = 6.8 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ (ppm); 174.9, 70.4, 65.7, 63.4, 34.1, 31.9, 29.9-29.3 (many unresolved C's), 24.9, 22.7, 14.1; HRMS-ESI (*m/z*): $[M+Na]^+$ calcd $C_{33}H_{66}O_4Na$, 549.4853 found, 549.4860.

**(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl benzoate (4a)**



To a stirred solution of (2,2-dimethyl-1,3-dioxolan-4-yl) methanol (500 mg, 3.78 mmol) in DCM (75.6 mL) was added pyridine (335 μL, 4.16 mmol) and benzoyl chloride (479 μL, 4.16 mmol) at 0 ˚C. After stirring for 26 h at room temperature, the mixture was supplemented with 1 M HCl and extracted with $CHCl_3$, and the obtained organic layer was washed by $H_2O$. The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to give a crude product, which was purified by short silica gel column chromatography (*n*-hexane/EtOAc = 2:1) to yield a title compound (206.8 mg, 23% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm); 8.05 (m, 2H), 7.57 (m, 1H), 7.45 (t, 2H), 4.40 (complex m, 3H), 4.16 (m, 1H), 3.89 (m, 1H), 1.46 (s, 3H), 1.40 (s, 3H), HRMS-ESI (*m/z*): $[M+Na]^+$ calcd $C_{10}H_{12}O_4Na$, 219.0628 found, 219.0832.

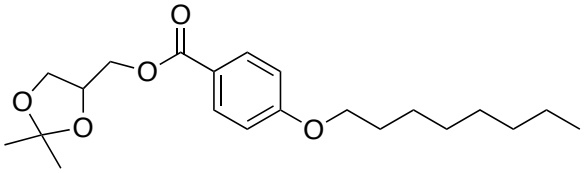
**2,3-Dihydroxypropyl benzoate (5a)**



To a stirred solution of (2,2-dimethyl-1,3-dioxolan-4-yl)methyl benzoate in DCM (936 μL) and MeOH (936 μL) was added amberlyst (185 mg). After stirring for 1 h at 60˚C, the reaction was cooled to room temperature. The mixture was filtered and washed with MeOH. The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (*n*-hexane/EtOAc = 1:1) to yield a mixture of mono acyl glycerolipids (80.4 mg, 85% yield). The major compound is a title compound: $^1$H NMR(400

MHz, $CD_3OD$) δ (ppm); 8.07 (m, 2H) , 7.61 (m, 1H), 7.48 (m, 2H), 4.41 (m, 2H), 4.31 (m, 1H), 3.97(m, 1H), 3.65 (m, 2H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ (ppm); 167.0, 133.3, 129.7 (2C), 129.5, 128.4 (2C), 70.3, 65.7, 63.4; HRMS-ESI (*m/z*): $[M+Na]^+$ calcd $C_{10}H_{12}O_4Na$, 219.0628 found, 219.0632.

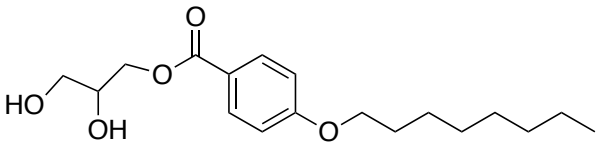
**(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 4-(octyloxy)benzoate (4b)**



To a stirred solution of 4-(octyloxy) benzoic acid (214.0 mg, 809.5 μmol) in Toluene (809 μL) was added $SOCl_2$ (128.4 μL, 1.78 mmol) and 1 drop of DMF. After stirring for 2.5 h at 40 ˚C, the mixture was evaporated, and added Toluene and evaporated again. Then the residue was concentrated under reduced pressure to give a desired acid chloride, which was used to the next reaction without further purification.

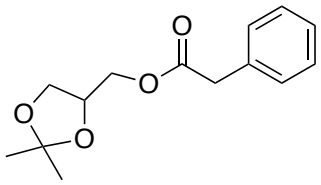
To a stirred solution of (2,2-dimethyl-1,3-dioxolan-4-yl) methanol (90.9 μL, 735.9 μmol) in DCM (7.4 mL) was added pyridine (89.5 μmol) and 4-(octyloxy)benzoyl chloride at 0 ˚C. After stirring for 19 h at room temperature, the mixture was supplemented with 1 M HCl and extracted with $CHCl_3$, and the obtained organic layer was washed by $H_2O$. The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to give a crude product, which was purified by short silica gel column chromatography (*n*-hexane/EtOAc = 2:1) to yield a title compound (73.4 mg, 63%). $^1H$ NMR (500 MHz, $CDCl_3$) δ (ppm); 7.99 (d, *J* = 9.2 Hz, 2H), 6.91 (d, *J* = 8.6 Hz, 2H), 4.38 (complex m, 3H), 4.14 (dd, *J* = 6.3, 1.7 Hz, 1H,), 4.00 (m, 2H), 3.87 (dd, *J* = 5.7, 2.3 Hz, 1H), 1.80 (m, 2H), 1.50-1.22 (complex m, 16H), 0.89 (t, *J* = 6.9 Hz, 3H) HRMS-ESI (*m/z*): $[M+Na]^+$ calcd $C_{21}H_{32}O_5Na$, 387.2142 found, 387.2147.

**2,3-Dihydroxypropyl 4-(octyloxy)benzoate (5b)**



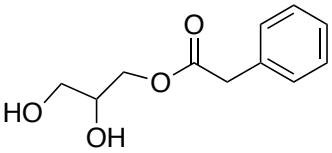
To a stirred solution of isopropylidene glycerolipid (31.2 mg, 85.6 μmol) in DCM (251 μL) and MeOH (251 μL) was added amberlyst (50 mg). After stirring for 2 h at 70˚C, the reaction was cooled to room temperature. The mixture was filtered and washed with MeOH. The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (*n*-hexane/EtOAc = 1:1) to yield a mixture of mono acyl glycerolipids (13.1 mg, 47% yield). The major compound is a title compound; $^1H$ NMR (500 MHz, $CDCl_3$) δ (ppm);7.99 (d, *J* = 8.6 Hz, 2H), 6.91 (d, *J* = 8.6 Hz, 2H), 4.41 (m, 2H), 4.02 (complex m, 3H), 3.75 (m, 1H), 3.68 (m, 1H), 1.80 (m. 2H), 1.46 (m, 2H), 1.4-1.23 (complex m, 10H), 0.89 (t, *J* = 6.9 Hz, 3H); $^{13}C$ NMR (100 MHz, $CDCl_3$) δ (ppm)166.8, 163.6, 131.9 (2C), 121.4, 114.2 (2C), 70.2, 68.3, 65.5, 63.4, 31.8, 29.3, 29.2, 29.1, 26.0, 22.6, 13.9; HRMS-ESI (*m/z*): $[M+Na]^+$ calcd $C_{18}H_{28}O_5Na$, 347.1829 found, 347.1838.

**(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 2-phenylacetate (7a)**



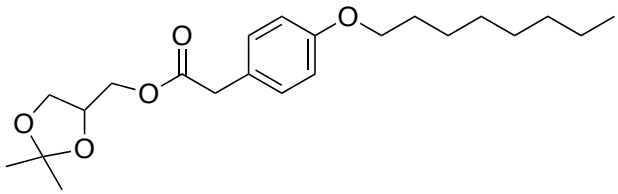
To a stirred solution of (2,2-dimethyl-1,3-dioxolan-4-yl) methanol (500 mg, 3.78 mmol) in DCM (75.6 mL) was added pyridine (335 μL, 4.16 mmol) and obtained phenylacetyl chloride (549 μL, 4.16 mmol) at 0 ˚C. After stirring for 16 h at room temperature, the mixture was supplemented with 1 M HCl and extracted with $CHCl_3$, and the obtained organic layer was washed by $H_2O$. The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to give a crude product, which was purified by short silica gel column chromatography (*n*-hexane/EtOAc = 3:1) to yield a title compound (768.0 mg, 81% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ7.23 (complex m, 5H), 4.31 (m, 1H), 4.16 (m, 2H), 4.03 (m, 1H), 3.69 (complex m, 3H), 1.41 (s, 3H), 1.36 (s, 3H), HRMS-ESI (*m/z*): $[M+Na]^+$ calcd $C_{14}H_{18}O_4Na$, 273.1097 found, 273.1103.

**2,3-Dihydroxypropyl 2-phenylacetate (8a)**



To a stirred solution of (2,2-dimethyl-1,3-dioxolan-4-yl)methyl 2-phenylacetate (328 mg, 1.31 mmol) in DCM (3.5 mL) and MeOH (3.5 mL) was added amberlyst (689 mg). After stirring for 1 h at 60˚C, the reaction was cooled to room temperature. The mixture was filtered and washed by MeOH. The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (*n*-hexane/EtOAc = 1:1) to yield a mixture of mono acyl glycerolipids (145.1 mg, 53% yield). The major compound is a title compound: $^1$H NMR (500 MHz, $CD_3OD$) δ (ppm); 7.26 (complex m, 5H), 4.17 (m, 1H), 4.09 (m, 1H), 3.82 (m, 1H), 3.67 (m, 2H), 3.52 (m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ (ppm); 172.1, 133.6, 129.3 (2C), 128.6 (2C), 127.2, 69.9, 65.4, 63.2, 41.0.

**(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 2-(4-(octyloxy)phenyl)acetate (7b)**

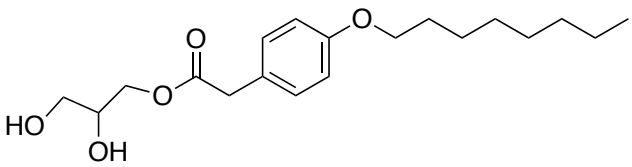


To a stirred solution of 2-(4-(octyloxy)phenyl)acetic acid (214.0 mg, 809.5 μmol) in Toluene (809 μL) was added $SOCl_2$ (128.4 μL, 1.78 mmol) and 1drop of DMF. After stirring for 2.5 h at 40 ˚C, the mixture was evaporated, and added Toluene and evaporated again. Then the residue was concentrated under reduced pressure to give a desired acid chloride, which was used to the next reaction without further purification.

To a stirred solution of (2,2-dimethyl-1,3-dioxolan-4-yl) methanol (90.9 μL, 735.9 μmol) in DCM (7.4 mL) was added pyridine (89.5 μmol) and obtained 4-(octyloxy)benzoyl chloride at room temperature. After stirring for 19 h at the same temperature, the mixture was supplemented with 1 M HCl and extracted with $CHCl_3$. The obtained organic layer was washed with $H_2O$. The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to give

a crude product, which was purified by short silica gel column chromatography (*n*-hexane/EtOAc = 2:1) to yield a title compound (165.1 mg, 59% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.18 (d, 2H, *J* = 8.6 Hz), 6.84 (d, *J* = 8.6 Hz, 2H), 4.30 (m, 1H),4.14 (m, 2H), 4.04 (dd, *J* = 6.9, 1.7 Hz, 1H), 3.93 (dd, *J* = 6.3 Hz, 2H), 3.70 (dd, *J* = 6.3, 2.3 Hz, 1H), 3.59 (s, 2H), 1.76 (m, 2H), 1.46-1.22 (complex m, 16H) 0.88 (t, *J* = 7.2 Hz, 3H), HRMS-ESI (*m/z*): $[M+Na]^+$ calcd $C_{22}H_{34}O_5Na$, 401.2298 found, 401.2304.

**2,3-Dihydroxypropyl 2-(4-(octyloxy)phenyl)acetate (8b)**




To a stirred solution of isopropylidene glycerolipid (82.5 mg, 218.1 μmol) in DCM (651 μL) and MeOH (651 μL) was added amberlyst (128 mg). After stirring for 2 h at 70˚C, the reaction was cooled to room temperature. The mixture was filtered and washed by MeOH. The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure to give a crude product, which was purified by silica gel column chromatography (*n*-hexane/EtOAc = 1:2) to yield a mixture of mono acyl glycerolipids (61.1 mg, 83% yield). The major compound is a title compound: $^1$H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.17 (d, *J* = 9.0 Hz , 2H), 6.85 (d, *J* = 8.5 Hz, 2H), 4.19 (m, 2H), 3.91 (complex m, 3H), 3.59 (complex m, 4H), 1.77 (m, 2H), 1.44 (m, 2H), 1.22-1.39 (complex m, 8H), 0.88 (t, *J* = 6.7 Hz, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.4, 158.3, 130.2 (2C), 125.3, 114.6 (2C), 70.1, 68.0, 65.5, 63.2, 40.2, 31.8, 29.3, 29.2 (2C), 26.0, 22.6, 14.1.; HRMS-ESI (*m/z*): $[M+Na]^+$ calcd $C_{19}H_{30}O_5Na$, 361.1985 found, 361.1992.

**The calculation of the rate of 2-acyl compound in each mono acyl glycerol (2a-2e) described in Fig. 2A**

We calculated the rate of the 2-acyl compounds in each inseparable mixture with the integrational ratio of the peak at 3.84 ppm. The peak is ascribed to the methylene (4H) next to the hydroxyl group of 2-acyl glycerol. In the case of the glycerolipid (C22) described in Fig. 2A, the integrational ratio of the peak at 3.84 ppm indicated 0.17 compared to the integrational ratio of hydrogen from the 1-acyl compound in $^1$H NMR (Fig. S1); the existing rate of the 2-acyl compound was calculated as 4.1% in the mixture. With this method, we calculated the percentage of the 2-acyl compounds in each C22-C30 mono acyl glycerol (**2a-2e**) shown in **Table S1**.

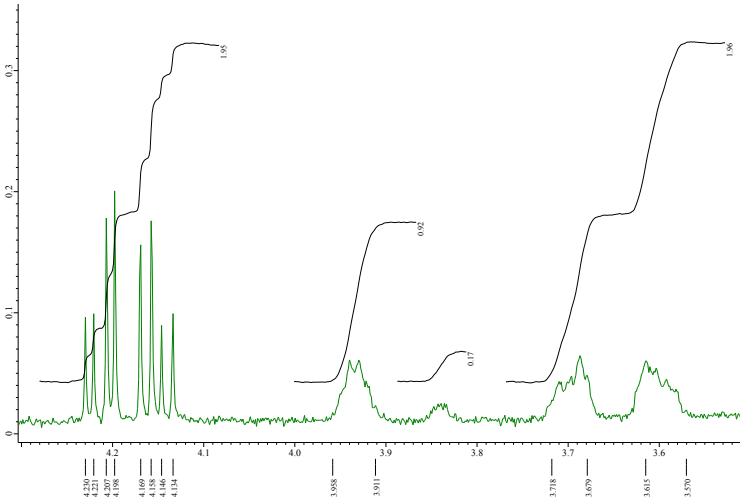



**Fig. S1** The enlarged view of 1H-NMR spectra of the mixture of dihydroxypropyl behenate (C22).

**Table S1**

| The lengths of acyl chain | The integrational ratio of the peak at 3.84 ppm | The percentage of the 2-acyl compounds |
|---|---|---|
| C22 | 0.17 | 4.1% |
| C24 | 0.31 | 7.2% |
| C26 | 0.40 | 9.1% |
| C28 | 0.42 | 9.5% |
| C30 | 0.64 | 13.8% |

**Complex model**

The structural model of Mincle in **Fig. 4** was prepared by homology modeling. The high-resolution crystal structures of bovine Mincle in complex with trehalose (PDB code: 4KZV), which coordinates the loop of N170-D177 to open the hydrophobic groove, was used as the starting model. The homology modeling was performed by referring to the amino acid sequence of human Mincle by prime (Schrödinger, LLC). The sequence of C78-E208 in bovine Mincle was used to the homology modeling. The amino acid sequence numbers in the main text and Fig. 4 are based on the corresponding amino acid residues from bovine Mincle. After the ligand was set up on the homology model as mentioned in the text, the ligand performed the minimization by prime (Schrödinger, LLC). The protein surfaces are colored as shown below; dark green: hydrophobic residues (ALA, CYS, ILE, LEU, MET, PHE, TRP, TYR, VAL, PRO), cyan: polar uncharged (SER, THR, HIS, GLN, ASN), blue: positively charged (LYS, ARG), and red: negatively charged (ASP, GLU).

**Reporter cell assay**

Reporter cell assay was performed as previous reported.[1] NFAT-GFP reporter cells expressing human or murine Mincle with FcRγ were prepared. Each glycolipid dissolved in chloroform:methanol (2:1) at 1 mg/ml was diluted in isopropanol, added to 96-well plates at 20 μl per well, followed by the evaporation of the solvent. After drying out, each reporter cell was added to 96-well plates in 200 μl per well (ca. 60000 cells per well). Reporter cells were stimulated for 18 h. Reporter activity was measured by flow cytometry. Error bar indicates ± standard deviation of three independent experiments.

**Reference**

(1) Yamasaki, S.; Ishikawa, E.; Sakuma, M.; Hara, H.; Ogata, K.; Saito, T. Mincle Is an ITAM-Coupled Activating Receptor That Senses Damaged Cells. *Nat. Immunol.* **2008**, *9* (10), 1179–1188.