Supporting Information

A cardiolipin from *Muribaculum intestinale* induces antigen-specific cytokine responses

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Experimental Methods

General Experimental Procedures. High resolution electrospray ionization mass spectrometry (HRESIMS) was carried out using an Agilent 6530 LC-q-TOF mass spectrometer (Agilent Technologies, Santa Clara, CA) equipped with an Agilent 1290 UHPLC system. GCMS spectra were obtained using an Agilent system. NMR spectra were obtained using Bruker NEO NMR system (^{1}H: 600 MHz, ^{13}C: 150 MHz) (Bruker, Billerica, MA) with CD_3OD or $CDCl_3$ (Cambridge Isotope Laboratories, Inc., Tewksbury, MA). HPLC for purification were carried out on an Agilent 1200 system equipped with an ELSD using a Phenomenex Luna C_8 (2) column (5 μm, 250 × 10 mm). All chemicals were HPLC grade.

Bacterial Cultivation and Extraction. *Muribaculum intestinale* strain S24-7 (DSM 28989) was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). *M. intestinal* S24-7 was inoculated in yeast casitone fatty acids with carbohydrates (YCFAC) agar. After incubation for 3 days under anaerobic conditions at 37 ℃, the single colony was transferred into 5 mL of YCFAC broth and incubated in the same condition for 5 days. Then, 5 mL of seed culture was inoculated into 500 ml of YCFAC broth and incubated in the same condition for 7 days. Bacterial cultures were centrifuged at 8000 rpm for 30 min for the partition into cell pellets and supernatants. Harvested cell pellets were extracted with $CHCl_3$/MeOH (1:1) and stirred for 24 hr at room temperature. The extract was filtered by Whatman Grade 3 filter paper and evaporated under reduced pressure at room temperature to yield a crude extract (Figure S1).

Bioassay-guided Isolation of MiCL-1. The curde extract (50 mg) was fractionated by Sephadex LH-20 column eluted with $CHCl_3$/MeOH (1:1) to obtain six fractions (C1-C6). Fraction C2 highly detected the pro-inflammatory cytokine TNF-α production in mouse bone marrow dendritic cells (mBMDCs) and was narrowed down for further purification. Fraction C2 (23 mg) was subjected to Sephadex LH-20 column eluted with MeOH 100%, followed by purification using reversed-phase HPLC (Phenomenex, Luna C8 (2), 5 μm, 250 × 10 mm, flow rate 2.0 mL/min) with a gradient of H_2O-MeOH: 0–10 min, 10–50% B; 10–35 min, 50% B; 35–45 min, 50–90% B; 45–65 min, 90–100% B; 65–90 min, 100% B (Figure S1). MiCL-1 (**1**) was eluted at 85 min (6.3 mg).

MiCL-1 (**1**): amorphous white solid; ^{1}H NMR (600 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) see table S1; APCI-HRMS *m/z*: $[M-H]^-$ calcd. for $C_{77}H_{145}O_{17}P_2$, 1403.9962; found, 1403.9913.

Fatty Acid Analysis. 0.1 mg of MiCL-1 was prepared in a 4 mL vial and dried under vacuum overnight. Then, 100 μL of toluene was added into the prepared vial and stirred at room temperature. After 10 min, 200 μL of 0.5 M sodium methoxide solution was added. The reaction mixture was stirred at 50 ℃ for 10 mim and quenched by the addition of glacial acetic acid. The fatty acid methyl ester (FAME) products were extracted with hexane and water. The organic layer was injected into a gas chromatography combined with HP-5ms Ultra Inert column (0.25 mm x 30 m) and anlyzed with a gradient of temperature: 150 °C for 3 min, 150–250 °C at 6 °C/min, and 250 °C for 3 min. All FAME derivatives of MiCL-1 were identified as palmitic acid (16:0) and oleic acid (18:1) by comparing with those of standards.[1-2]

mBMDC Cytokine Assay. These assays were done as described previously.[3] Bone marrow-derived DCs (BMDCs) were collected from the femurs and tibias of C75BL/6 mice wild-type mice, TLR2$^{-/-}$, or TLR4$^{-/-}$ mice. The cells were incubated in RPMI 1640 (Gibco) supplemented with 10% heat-inactivated FBS (Invitrogen), 1% Penicillin-Streptomycin (Gibco), and mGM-CSF (PeproTech). The suspended cells were plated into petri dishes and incubated in 5% CO_2 and 37℃. On day 5, floating and loosely attached cells were collected, representing the BMDCs. Cells were seeded into 96 well plates with ~ 100,000 cells/well and then stimulated by adding with fractions or single molecule at a final concentration of 50 μg mL^{-1}. A final concentration of LPS (InvivoGen tlrl-b5lps) of 3-625 ng mL^{-1} and Pam3CSK4 (InvivoGen tlrl-pms) of 0.250-1.562 μg mL^{-1} were used as controls. The plates were incubated in 5% CO_2 and 37℃ for 16 h and then centrifuged. The supernatant was analyzed following mouse TNF-α uncoated ELISA kit's instructions (Invitrogen by ThermoFisher). SoftMax Pro v.6.2.1 (SpectraMax, Molecular Devices) was used as reader control and data analysis of ELISA plate.

Human MDDCs Cytokine Assay. Human cells were collected from healty donors at the Research Blood Components LLC. The Massachusetts Brigham Institutional Review Board approved these experiments in compliance with all relevant ethical regulations and followed protocol 2018P001504. Peripheral blood mononuclear cells (PBMCs) were enriched by incubation with the RosetteSep™ Human Monocyte Enrichment Cocktail (STEMCELL Technologies) for 20 min at room temperature with shaking. Human monocytes were isolated by density gradient separation using Ficoll-Paque PLUS density gradient media (GE Healthcare) and SepMate tubes (STEMCELL Technologies) according to manufacturer's instructions. Human monocyte-derived dendritic cells (hMDDCs) were differentiated by 20 ng mL^{-1} human GM-CSF (PeproTech Inc) from PBMCs. On day 5, human MDDCs were collected and stimulated with purified compounds at 50 μg mL^{-1} in RPMI-1640 media (Gibco) containing 10% FBS (Invitrogen) and 1% penicillin- streptomycin (Gibco). LPS at a final concentration of 3-625 ng mL^{-1} and Pam3CSK4 at a final concentration of 0.250-1.562 μg mL^{-1} were used as controls. After overnight incubation at 5% CO_2 and 37℃, the supernatant was analyzed following Human cytometric bead array kit's instructions (BioLegend). Flow cytometer (Agilent) was used as reader, and data were collected by NovoExpress 1.4.1 and analyzed by FlowJo v10.7.

CRISPR targeting. PBMCs were isolated from buffy coats using Sepmate tubes (STEMCELL Technologies) and ammonium–chloride–potassium lysis buffer following the manufacturer's instruction. Human MDDCs were harvested from PBMCs by negative selection using RosetteSep human Monocyte Enriched Cocktail (STEMCELL Technologies) according to the manufacturer's instructions. Alt-R sgRNAs were reconstituted to 100 μmol L^{-1} with Nuclease-Free Duplex Buffer (IDT). In a sterile polymerase chain reaction strip, the sgRNAs were mixed with Cas9 (IDT) at a molar ratio of 2:1 for each reaction and incubated at room temperature for over 20 min. MDDCs were washed twice with 5 mL of PBS and counted. Then 2×10^6 cells per reaction were resuspended in 16 μL of P3 primary nucleofection solution (Lonza). The cells in P3 buffer was added to each Cas9–ribonucleoprotein complex. The cell–ribonucleoprotein mix was then immediately loaded into the supplied nucleofector cassette strip (Lonza) and nucleofected using 4D-Nucleofector with CM-137 program. Then 180 μL of prewarmed medium was immediately added into each cassette well. A volume of 1×10^5 cells was seeded into a 96-well plate with RPMI-1640 media supplemented with GlutaMAX Supplement, beta-mercaptoethanol, 10% heat-inactivated FBS, and hGM-CSF. After

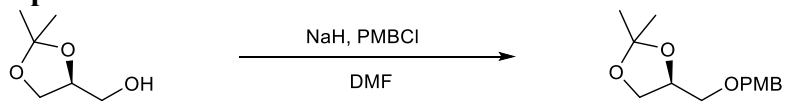
incubation for 5 day, MDDCs were stimulated with single molecule at a final concentration of 50 μg mL^{-1} as well as LPS at a final concentration of 3-625 ng mL^{-1} and Pam3CSK4 at a final concentration of 0.250-1.562 μg mL^{-1} as the controls for 18 h. The supernatant was analyzed following human TNF-α uncoated ELISA kit's instructions (Invitrogen by ThermoFisher). SoftMax Pro v.6.2.1 (SpectraMax, Molecular Devices) was used as reader control and data analysis of ELISA plate. The sgRNA sequences used were as follows:

Non targeting control (CGTTAATCGCGTATAATACG)
Human TLR1 (GGTCTTAGGAGAGACTTATG)
Human TLR2 (GACCGCAATGGTATCTGCAA)
Human TLR6 (ATTCATTTCCGTCGGAGAAC)

Experimental Procedures for Synthesis

Reagents, Instruments, and Techniques. High resolution electrospray ionization mass spectrometry (HRESIMS) was carried out using an Agilent 6530 LC-q-TOF mass spectrometer (Agilent Technologies, Santa Clara, CA) equipped with an Agilent 1290 UHPLC system. NMR spectra were obtained using Bruker Advance (^{1}H: 400 MHz, ^{13}C: 100 MHz) or Bruker NEO NMR system (^{1}H: 600 MHz, ^{13}C: 150 MHz) (Bruker, Billerica, MA) with CD_3OD or $CDCl_3$ (Cambridge Isotope Laboratories, Inc., Tewksbury, MA). HPLC for purification were carried out on an Agilent 1200 system equipped with an ELSD using a Phenomenex Luna C_8 (2) column (5 μm, 250 × 10 mm). Thin layer chromatography (TLC) was performed using silica gel 60 F_{254} aluminum, together with the detection of ultraviolet and permanganate staining. All chemicals were HPLC grade.

Synthesis of Compound 4.

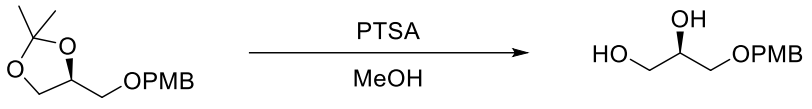


3 **4**

(+)-1,2-*O*-isopropylidene-*sn*-glycerol (**3**, 1.0 g, 7.6 mmol) was prepared in oven-dried round bottom flask and dissolved in dry DMF (25 mL) under argon. The solution was cooled down at 0 °C and then slowly added with NaH (394 mg, 9.8 mmol, 1.3 equiv). The mixture was stirred at room temperature for 30 min. *p*-Methoxybenzyl chloride (1.4 g, 8.3 mmol, 1.1 equiv) in dry DMF (5 mL) was added subsequently. The mixture was stirred at room temperature overnight, quenched by the addition of H_2O, and diluted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified using silica gel flash chromatographty (*n*-hexane/EtOAc = 12:1) to give PMB ether (**4**).[4]

^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.25 (2H, dd, J = 8.8, 3.0 Hz), 6.87 (2H, dd, J = 8.8, 3.0 Hz), 4.52 (1H, d, J = 17.4 Hz), 4.47 (1H, d, J = 17.4 Hz), 4.27 (1H, m), 4.04 (1H, dd, J = 12.0, 9.6Hz), 3.80 (3H, s), 3.72 (2H, dd, J = 12.6, 9.6 Hz), 3.53 (2H, dd, J = 15.0, 8.4 Hz), 3.43 (2H, dd, J = 15.0, 8.4 Hz), 1.41 (3H, s), 1.35 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 159.4, 130.1, 129.9, 114.0, 113.9, 109.5, 71.6, 70.9, 67.0, 64.2, 55.4, 26.9, 25.5; ESI-MS *m*/*z*: $[M + Na]^+$ found 275.1.

Synthesis of Compound 5.

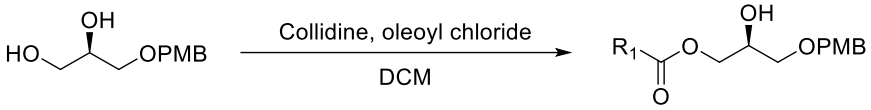


4 5

PMB protected ether **4** (1.2 g, 4.8 mmol) in MeOH (50 mL) was added with *p*-TsOH (48 mg, 0.24 mmol, 0.05 equiv). The mixture was stirred at room temperature overnight and evaporated. Then, the mixture was redissolved in EtOAc and successively washed with 1.5% K_2CO_3 and brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated. The residue was purified using silica gel flash chromatography (*n*-hexane/EtOAc = 9:1) to give 3-(4-methoxybenzyl)-*sn*-glycerol (**5**).[5]

^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.26 (2H, dd, J = 8.8, 2.4 Hz), 6.89 (2H, dd, J = 8.8, 2.4 Hz), 4.49 (2H, s), 3.88 (1H, m), 3.81 (3H, s), 3.69 (1H, dd, J = 11.4, 3.6 Hz), 3.62 (1H, dd, J = 11.4, 5.4 Hz), 3.55 (1H, dd, J = 10.2, 1.8 Hz), 3.51 (1H, dd, J = 9.6, 6.6 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 159.5, 129.9, 129.7, 129.6, 114.0, 73.4, 71.6, 70.7, 64.2, 55.4; ESI-MS *m*/*z*: $[M + Na]^+$ found 235.1.

Synthesis of Compound 6.

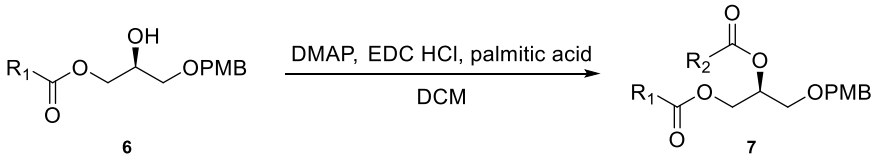


5 6

3-(4-Methoxybenzyl)-*sn*-glycerol (**5**, 500 mg, 2.4 mmol) was dissolved in dry CH_2Cl_2 (25 mL) at room temperature under argon and cooled to -78 °C. 2,4,6-Trimethylpryidine (858 mg, 7.1 mmol, 3.0 equiv) was added under argon and the mixture was stirred for 10 min. Oleoyl chloride (746.3 mg, 2.5 mmol, 1.5 equiv) in dry CH_2Cl_2 (5 mL) was added dropwise and stirred for 2 hr. The reaction was quenched by the addition of MeOH and washed with 0.5 M HCl, 1.5% K_2CO_3, and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified using silica gel flash chromatography (*n*-hexane/EtOAc = 4:1) to give 1-*O*-oleoyl-3-(4-methoxybenzyl)-*sn*-glycerol (**6**).[6]

^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.25 (2H, d, J = 8.4 Hz), 6.88 (2H, d, J = 8.1 Hz), 5.34 (2H, m), 4.49 (2H, s), 4.17 (1H, dd, J = 11.4, 4.2 Hz), 4.12 (1H, dd, J = 11.4, 6.0 Hz), 4.02 (1H, m), 3.81 (3H, s), 3.52 (1H, dd, J = 9.6, 4.2 Hz), 3.46 (1H, dd, J = 9.6, 6.0 Hz), 2.32 (2H, m), 2.01 (2H, m), 1.61 (2H, m), 1.28 (22H, m), 0.88 (3H, t, J = 7.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 174.1, 159.5, 130.2, 129.9, 114.0, 73.3, 70.7, 69.1, 65.5, 55.4, 34.3, 32.1, 29.9-29.2, 27.4, 27.3, 25.0, 22.8, 14.3; APCI-HRMS *m*/*z*: $[M + Na]^+$ calcd for $C_{29}H_{48}O_5Na$ 499.3396; found, 499.3394.

Synthesis of Compound 7.



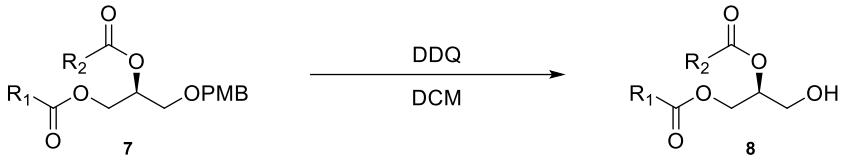
6 7

To a solution of 1-*O*-oleoyl-3-(4-methoxybenzyl)-*sn*-glycerol (**6**, 390 mg, 0.82 mmol) in CH_2Cl_2 (25 mL) was added DMAP (30.5 mg, 0.5 mmol, 0.3 equiv), EDC HCl (423.6 mg, 2.2

mmol, 2.7 equiv), and palmitic acid (484.6 mg, 1.9 mmol, 2.3 equiv) in CH_2Cl_2 (5 mL). After reaction for 48 hr at room temperature, the mixture was diluted with CH_2Cl_2 and washed with 0.5 M HCl, 1.5% K_2CO_3, and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified using silica gel flash chromatography (*n*-hexane/EtOAc = 15:1) to obtain 1-*O*-oleoyl-2-*O*-plamtitoyl-3-(4-methoxybenzyl)-*sn*-glycerol (**7**).[6]

^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.23 (2H, dd, J = 9.0, 2.4 Hz), 6.87 (2H, d, J = 8.4, 1.8 Hz), 5.34 (2H, m), 5.22 (1H, m), 4.49 (1H, d, J = 12.0 Hz), 4.44 (1H, d, J = 12.0 Hz), 4.32 (1H, dd, J = 12.0, 3.6 Hz), 4.17 (1H, dd, J = 12.0, 6.6 Hz), 3.80 (3H, s), 3.55 (2H, m), 2.29 (4H, m), 2.01 (4H, m), 1.60 (4H, m), 1.27 (42H, m), 0.88 (3H, t, J = 7.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.6, 173.3, 159.5, 130.2, 129.9, 129.8, 114.0, 73.1, 70.2, 70.1, 68.0, 62.9, 55.4, 34.5, 34.3, 32.1, 32.0, 29.9-29.2, 27.4, 27.3, 25.1, 25.0, 22.8, 14.3; APCI-HRMS *m*/*z*: [M + Na]$^+$ calcd for $C_{45}H_{78}O_6Na$ 737.5688; found,737.5691.

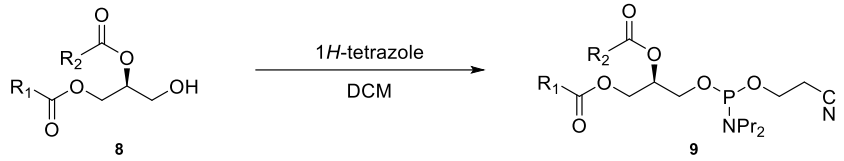
Synthesis of Compound 8.



To a solution of the PMB-protected diacylglycerol (**7**, 500 mg, 0.7 mmol) in CH_2Cl_2 (50 mL) was added DDQ (397.3 mg, 1.75 mmol, 2.5 equiv). After reaction for 48 hr at room temperature, the mixture was additionally diluted with CH_2Cl_2, washed with 1.5% K_2CO_3, and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified using silica gel flash chromatography (*n*-hexane/EtOAc = 7:1) to give 1-*O*-oleoyl-2-*O*-plamtitoyl-*sn*-glycerol (**8**).[7]

^{1}H NMR (600 MHz, $CDCl_3$) δ: 5.34 (2H, m), 5.08 (1H, m), 4.32 (1H, dd, J = 12.0, 4.8 Hz), 4.24 (1H, dd, J = 12.0, 5.4 Hz), 3.73 (2H, m), 2.33 (4H, m), 2.01 (4H, m), 1.62 (4H, m), 1.28 (42H, m), 0.88 (6H, t, J = 7.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 174.1, 173.9, 130.2, 129.8, 72.2, 68.5, 65.2, 62.1, 61.7, 34.4, 34.2, 32.1, 32.0, 29.9-29.2, 27.4, 27.3, 25.1, 25.0, 22.8, 14.3; APCI-HRMS *m*/*z*: [M + Na]$^+$ calcd for $C_{37}H_{70}O_5Na$ 617.5112; found 617.5115.

Synthesis of Compound 9.

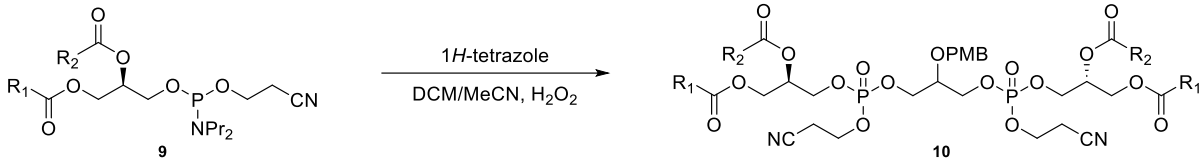


A mixture of diacylglycerol **8** (300 mg, 0.5 mmol), 0.45 M 1*H*-tetrazole in dry MeCN (3.3 mL, 1.5 mmol, 3.0 equiv), 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphophordiamidite (452.1 mg, 1.5 mmol, 3.0 equiv) and dry CH_2Cl_2 was stirred at room temperature for 2 h. The reaction was quenched with 1.5% K_2CO_3 and dried over Na_2SO_4, filtered, and concentrated. The residue was purified using silica gel column chromatography (*n*-hexane/EtOAc = 9:1, 3% Et_3N) to give diacylglycerol-phophoramidite (**9**).[8]

^{1}H NMR (600 MHz, $CDCl_3$) δ: 5.34 (2H, m), 5.19 (1H, m), 4.34 (1H, m), 4.16 (1H, m), 3.85 (1H, m), 3.78 (2H, m), 3.69 (1H, m), 3.59 (2H, m), 2.63 (2H, m), 2.31 (4H, m), 2.00 (4H, m), 1.61 (4H, m), 1.28 (42H, m), 1.17 (12H, m), 0.88 (6H, t, J = 7.2 Hz); ^{13}C NMR (125 MHz,

$CDCl_3$) δ: 173.5, 173.2, 130.1, 129.9, 117.7, 70.8, 62.5, 61.9, 61.8, 61.7, 61.6, 58.7, 58.6, 58.5, 58.4, 43.3, 43.2, 34.5, 34.2, 32.1, 32.0, 29.9-29.3, 27.4, 27.3, 25.1, 25.0, 24.8, 24.7, 22.8, 20.5, 20.4, 14.3; APCI-HRMS *m/z*: $[M + Na]^+$ calcd for $C_{46}H_{87}N_2O_6PNa$ 817.6220; found 817.6194.

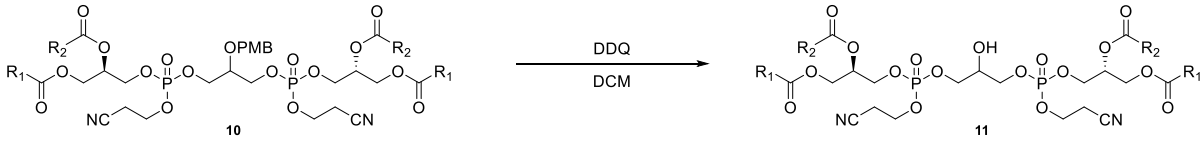
Synthesis of Compound 10.



A mixture of diacylglycerol-phophoramidite **9** (200 mg, 0.25 mmol, 2.2 equiv), PMB-protected glycerol (24.0 mg, 0.11 mmol), 0.45 M 1*H*-tetrazole in MeCN (0.75 mL, 0.33 mmol, 3.0 equiv), and dry CH_2Cl_2/MeCN (v/v = 2:1, 0.16 mL) was stirred under argon at room temperature. 1.0 equiv of 1*H*-tetrazole solution was added every hour during the reaction of 5 hr. H_2O_2 aq. (30 wt %, 80 μL) was then added to the mixture, and the reaction mixture was stirred for 30 min. The reaction was quenched with saturated $Na_2S_2O_3$ solution, and the whole was extracted with CH_2Cl_2. The organic extract was washed with saturated NH_4Cl solution, 1.5% K_2CO_3, and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified using silica gel flsh chromatography (*n*-hexane/EtOAc = 3:1) to give PMB/cyanoethyl-protected cardiolipin (**10**).[8]

^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.29 (2H, m), 6.88 (2H, d, J = 8.4 Hz), 5.34 (4H, m), 5.24 (2H, m), 4.59 (2H, m), 4.30 (4H, m), 4.18 (12H, m), 3.83 (1H, m), 3.80 (3H, s), 2.70 (4H, m), 2.32 (8H, m), 2.01 (8H, m), 1.60 (8H, m), 1.26 (84H, m), 0.88 (12H, t, J = 7.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.4, 173.0, 159.7, 130.2, 129.9, 114.1, 75.1, 72.2, 69.4, 69.3, 66.1, 66.0, 62.4, 62.3, 61.7, 55.4, 34.3, 34.1, 32.1, 32.0, 29.9-29.3, 27.4, 27.3, 25.0, 24.9, 22.8, 19.7, 14.3; APCI-HRMS *m/z*: $[M + Na]^+$ calcd For $C_{91}H_{160}N_2O_{18}P_2Na$ 1654.1022; found 1654.1034.

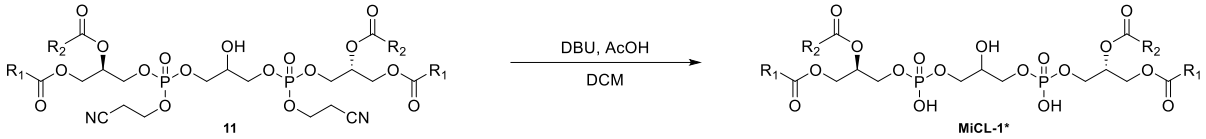
Synthesis of Compound 11.



To a solution of PMB/cyanoethyl-protected cardiolipin **10** (70 mg, 0.04 mmol) in CH_2Cl_2 (15 mL) was added DDQ (31.8 mg, 0.14 mmol, 3.5 equiv), the reaction mixture was stirred at room temperature for 20 hr. The mixture was additionally diluted with CH_2Cl_2, washed with 1.5% K_2CO_3, and brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified using silica gel flash chromatography ($CHCl_3$/MeOH = 20:1) to give cyanoethyl-protected cardiolipin (**11**).[8]

^{1}H NMR (600 MHz, $CDCl_3$) δ: 5.34 (4H, m), 5.26 (2H, m), 4.33 (2H, dd, J = 12.0, 4.2 Hz), 4.22 (14H, m), 4.10 (1H, m), 2.79 (4H, m), 2.33 (8H, m), 2.00 (8H, m), 1.61 (8H, m), 1.27 (84H, m), 0.87 (12H, t, J = 7.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 173.5, 173.1, 130.2, 129.8, 116.6, 69.4, 69.1, 68.6, 68.5, 66.3, 66.2, 62.6, 62.5, 61.7, 34.3, 34.1, 32.1, 32.0, 29.9-29.2, 27.4, 27.3, 25.0, 24.9, 22.8, 19.8, 19.79, 14.3; APCI-HRMS *m/z*: $[M + Na]^+$ calcd for $C_{83}H_{152}N_2O_{17}P_2Na$ 1534.0440; found,1534.0458.

Synthesis of MiCL-1*.



To a solution of cyanoethyl phosphate **11** (30 mg, 0.02 mmol) in dry CH_2Cl_2 (0.3 mL) was added DBU (18.3 µL, 0.12 mmol, 6.0 equiv). The reaction was stirred at room temperature for 5 min after which it was acidified with glacial acetic acid (24 µL, 0.4 mmol, 20.0 equiv). The volatile was evaporated in vacuo and the crude was purified using reversed-phase HPLC (Phenomenex, Luna C8 (2), 5 µm, 250 × 10 mm, flow rate 2.0 mL/min) with a gradient of H_2O-MeOH: 0–10 min, 10–50% B; 10–35 min, 50% B; 35–45 min, 50–90% B; 45–65 min, 90–100% B; 65–90 min, 100% B to give pure MiCL-1*.[9]

^{1}H NMR (600 MHz, $CDCl_3$) δ: 5.33 (4H, m), 5.22 (1H, m), 4.39 (2H, br d, J = 10.2 Hz), 4.17 (2H, br s), 3.91 (10H, m), 2.91 (8H, m), 2.00 (8H, m), 1.57 (8H, m), 1.33-1.25 (92H, m), 0.88 (12H, t, J = 7.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 174.0, 173.7, 130.2, 129.9, 72.3, 68.6, 65.2, 62.2, 61.8, 34.5, 34.3, 32.1-29.3, 27.4, 27.3, 25.2, 25.1, 22.9, 14.3; APCI-HRMS *m*/*z*: $[M + H]^+$ calcd. for $C_{77}H_{146}O_{17}P_2$, 1403.9913; found, 1403.9962.

Supplementary Figures

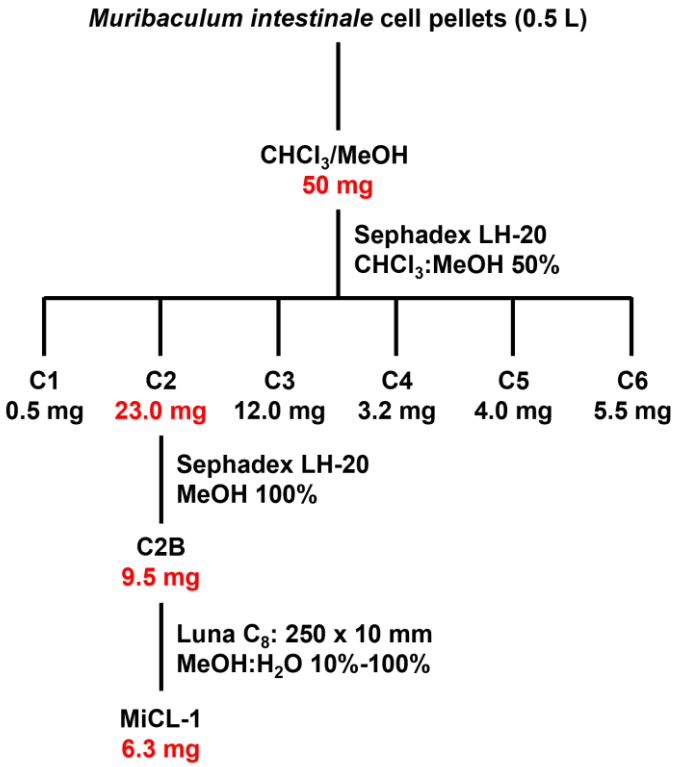


Figure S1. Isolation scheme for MiCL-1 (**1**)

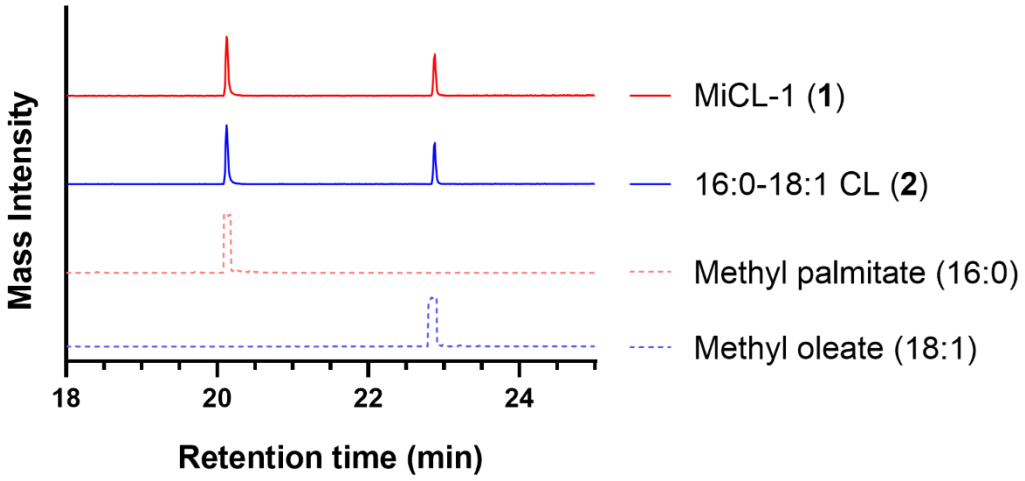


Figure S2. GC/MS chromatograms of FAMEs for MiCL-1 (**1**)

Spectral Data

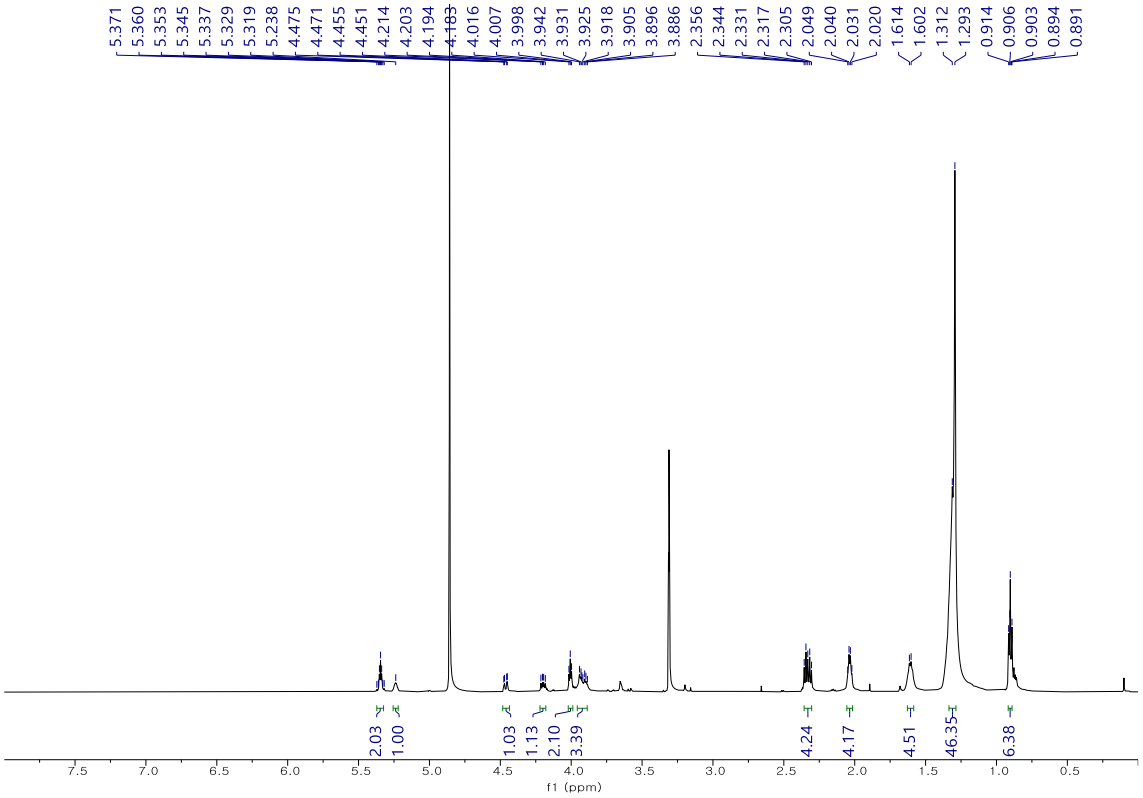


Figure S3. ^{1}H NMR spectrum for MiCL-1 (**1**) (600 MHz, CD_3OD)

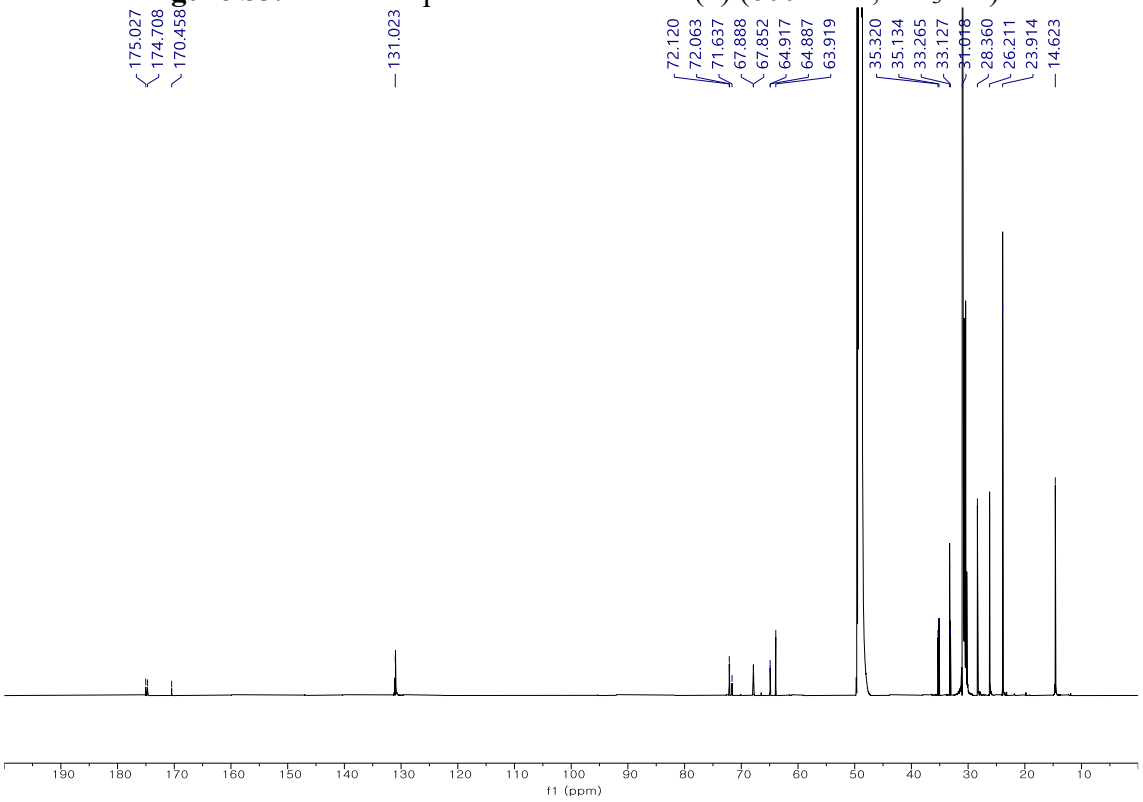


Figure S4. ^{13}C NMR spectrum for MiCL-1 (**1**) (150 MHz, CD_3OD)

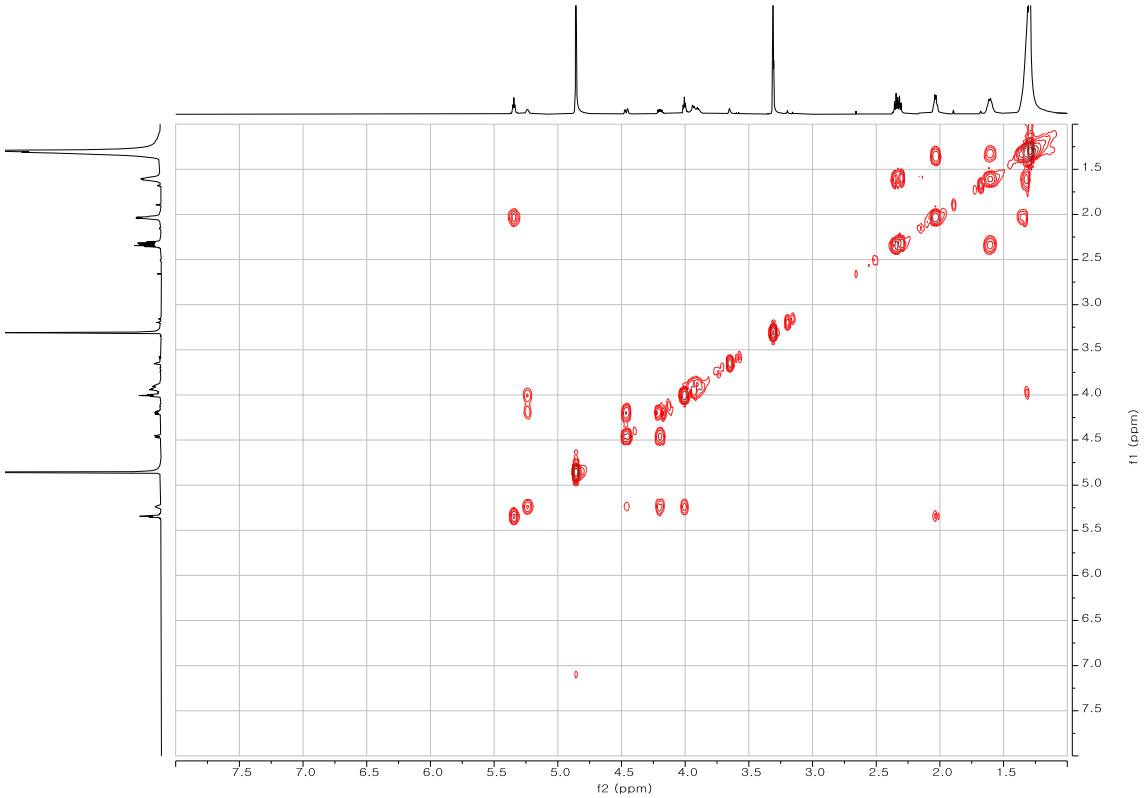


Figure S5. COSY NMR spectrum for MiCL-1 (**1**) (600 MHz, CD_3OD)

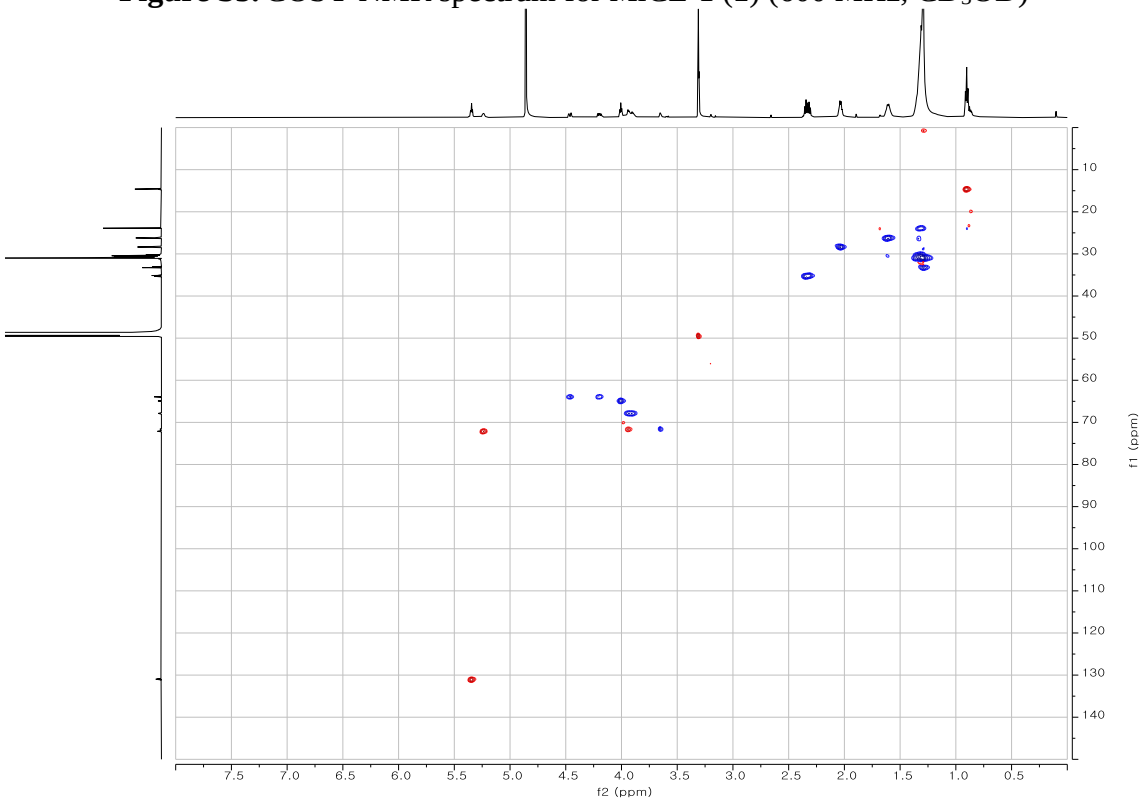


Figure S6. HSQC NMR spectrum for MiCL-1 (**1**) (600 MHz, CD_3OD)

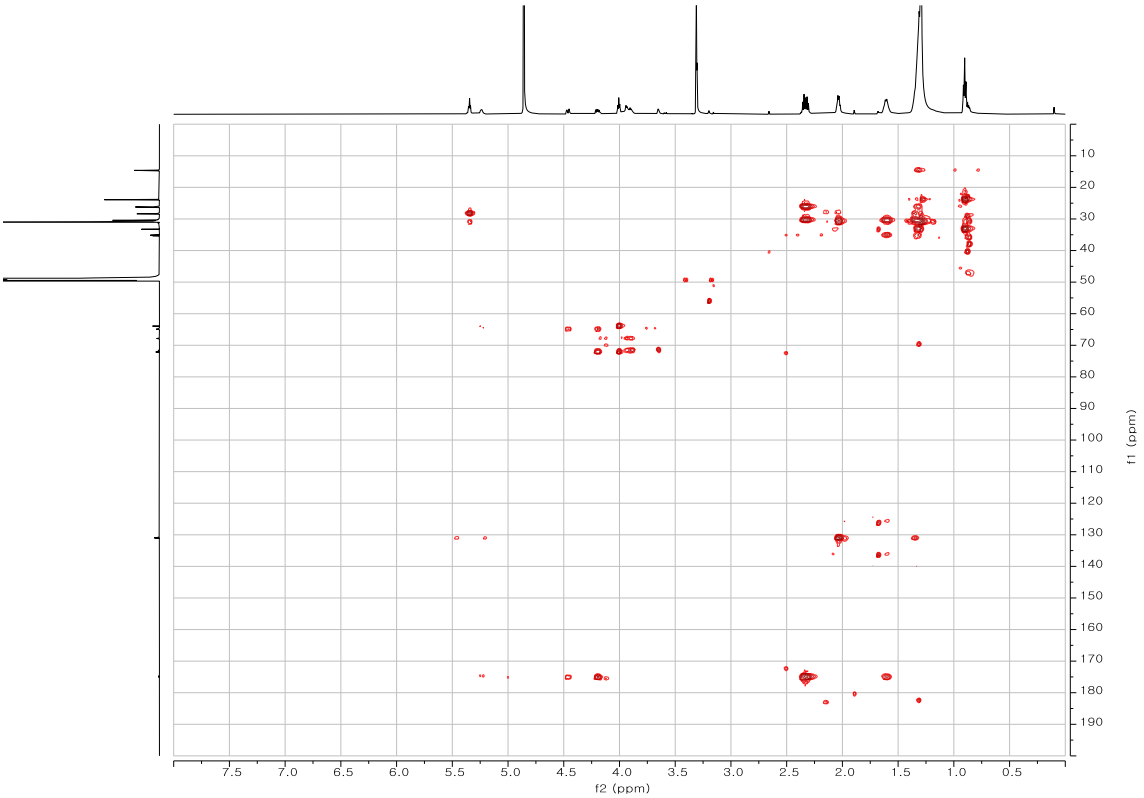


Figure S7. HMBC NMR spectrum for MiCL-1 (**1**) (600 MHz, CD_3OD)

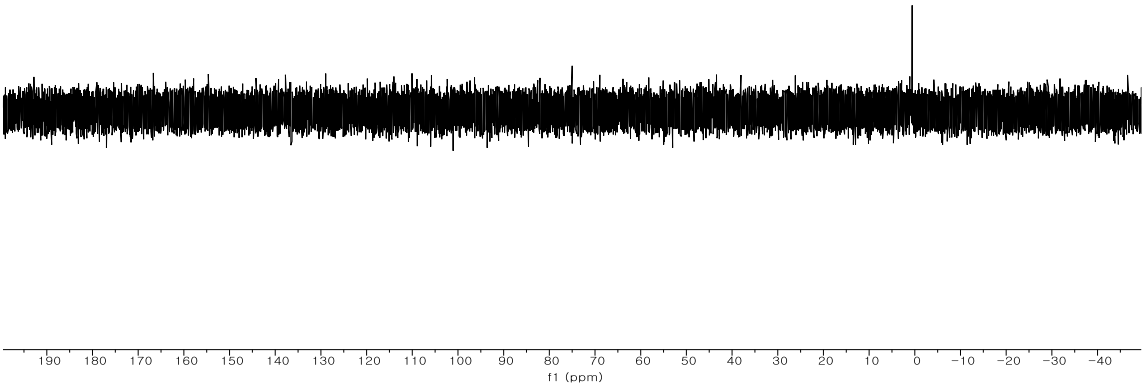
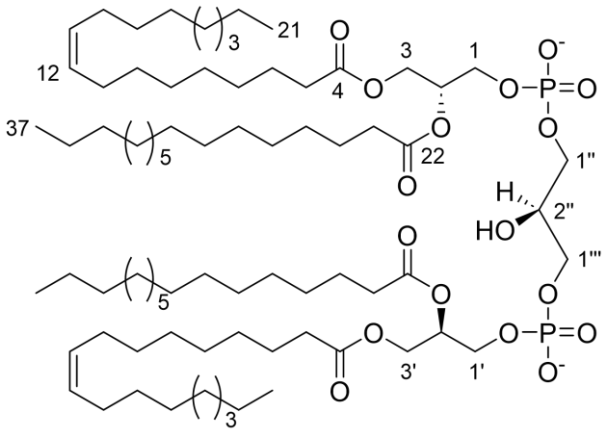


Figure S8. ^{31}P NMR spectrum for MiCL-1 (**1**) (400 MHz, CD_3OD)

Table S1. ^{1}H and ^{13}C NMR data of MiCL-1 (**1**) in CD_3OD[a]

Position	δ_C	δ_H (multi, *J* in Hz)
1/1'	64.9/64.8	4.01 (m)
2/2'	72.1/72.0	5.24 (m)
3/3'	63.9	4.46 (dd, 12.0, 2.4),4.20 (dd, 12.0, 6.6)
4/4'	174.7	
5/5'	35.1	2.32 (t, 7.2)
6/6'	26.2	1.61 (m)
7-10/7'-10'	33.3-23.9	1.34-1.28 (m)
11/11'	28.4	2.04 (m)
12/12'	131.0/130.9	5.35 (m)
13/13'	131.0/130.9	5.35 (m)
14/14'	28.4	2.04 (m)
15-20/15'-20'	33.3-23.9	1.34-1.28 (m)
21/21'	14.6	0.90 (t, 7.2)
22/22'	175.0	
23/23'	35.3	2.34 (t, 7.2)
24/24'	26.2	1.61 (m)
25-36/25'-36'	33.3-23.9	1.34-1.28 (m)
37/37'	14.6	0.90 (t, 7.2)
1"/1"'	67.9/67.8	3.92 (m)
2"	71.6	3.92 (m)

[a] ^{1}H and ^{13}C NMR were measured at 600 and 150 MHz, respectively.



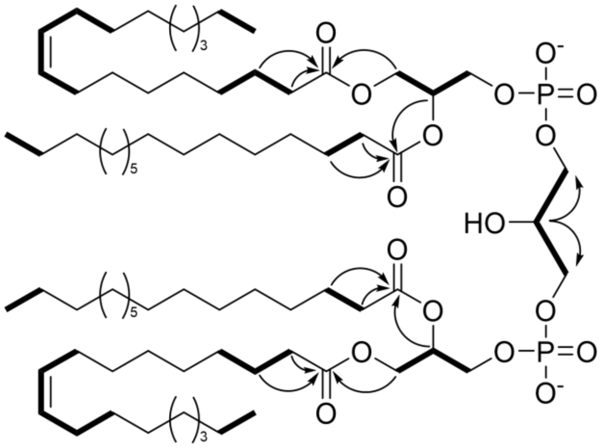


Figure S9. Key COSY and HMBC correlations of MiCL-1 (**1**)

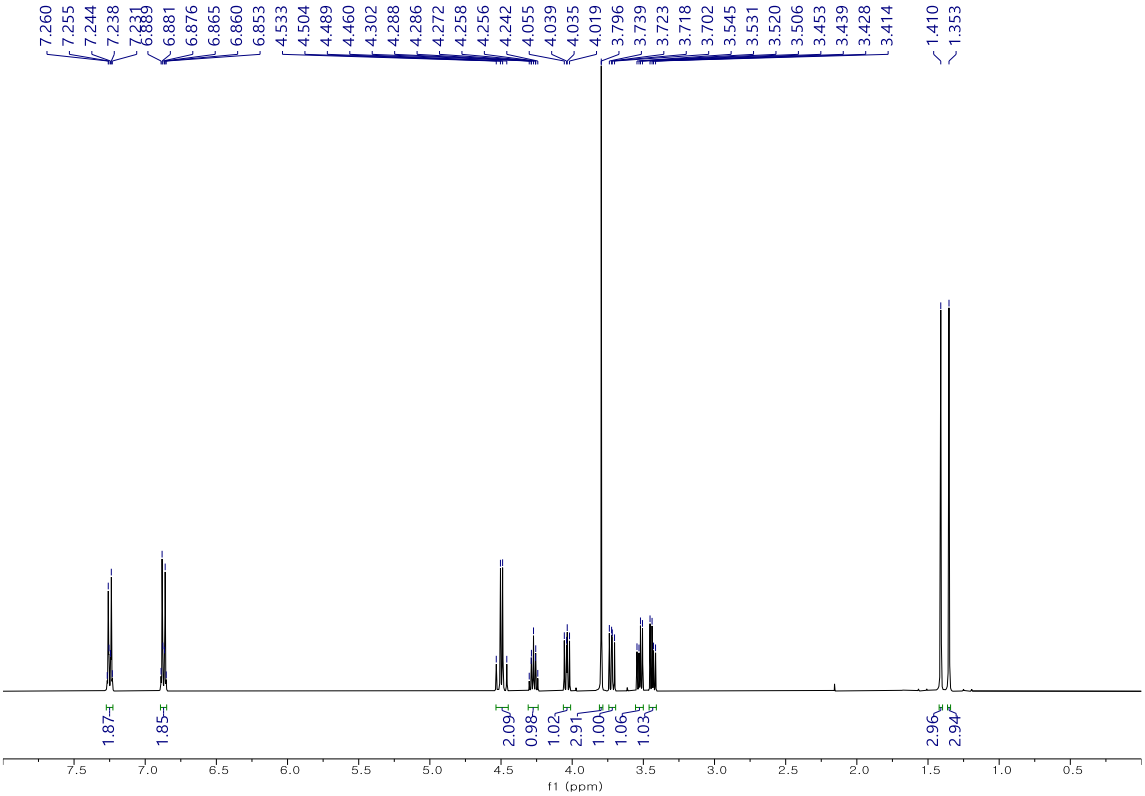


Figure S10. ^{1}H NMR spectrum for compound **4** (600 MHz, $CDCl_3$)

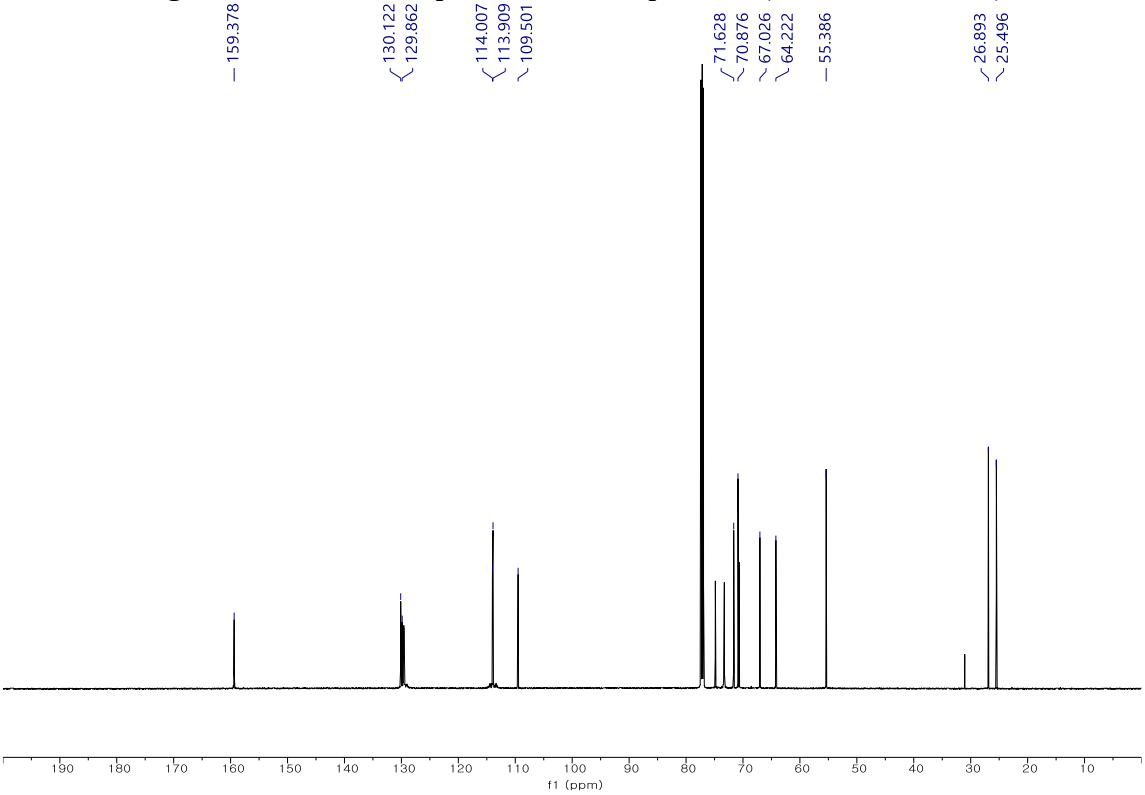


Figure S11. ^{13}C NMR spectrum for compound **4** (150 MHz, $CDCl_3$)

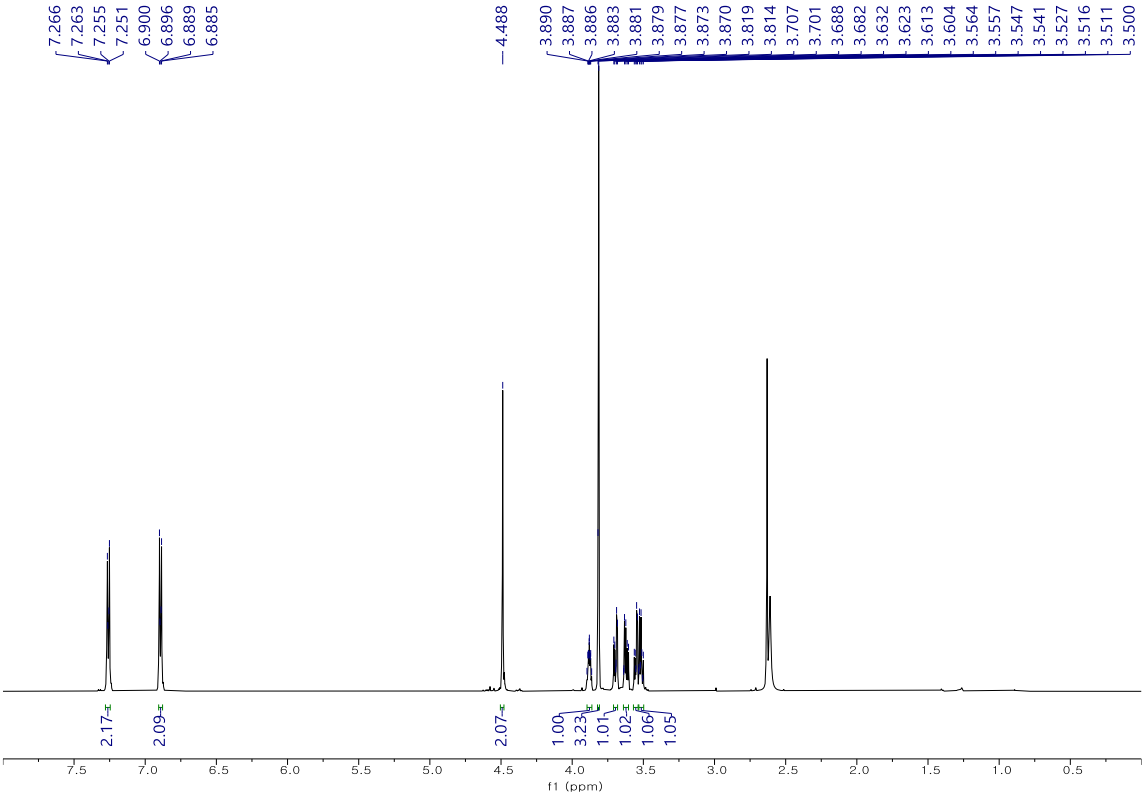


Figure S12. ^{1}H NMR spectrum for compound **5** (600 MHz, $CDCl_3$)

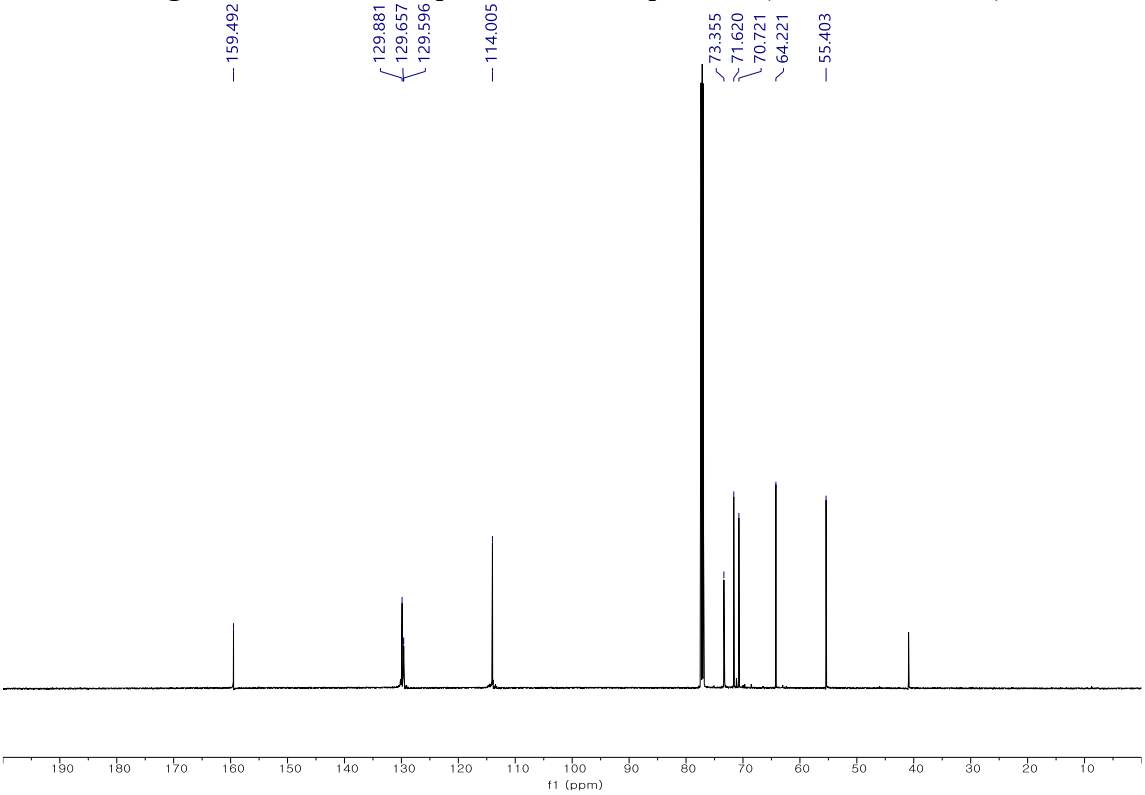


Figure S13. ^{13}C NMR spectrum for compound **5** (150 MHz, $CDCl_3$)

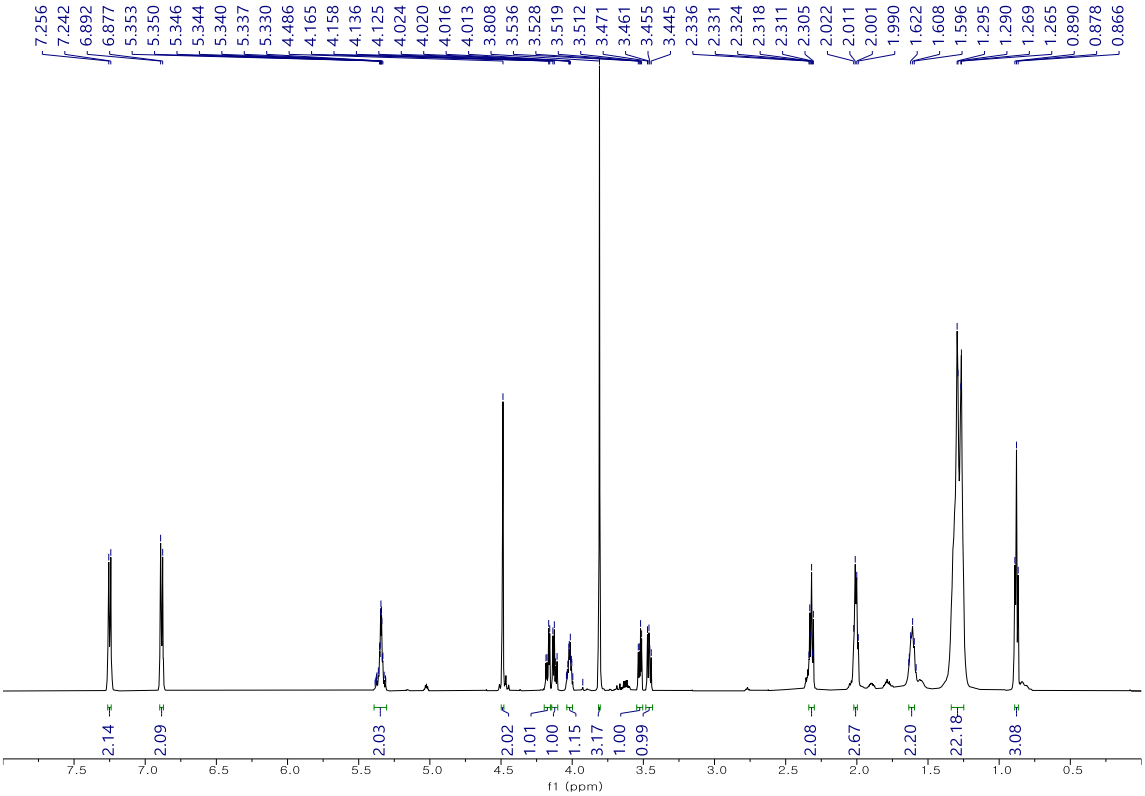


Figure S14. ^{1}H NMR spectrum for compound **6** (600 MHz, $CDCl_3$)

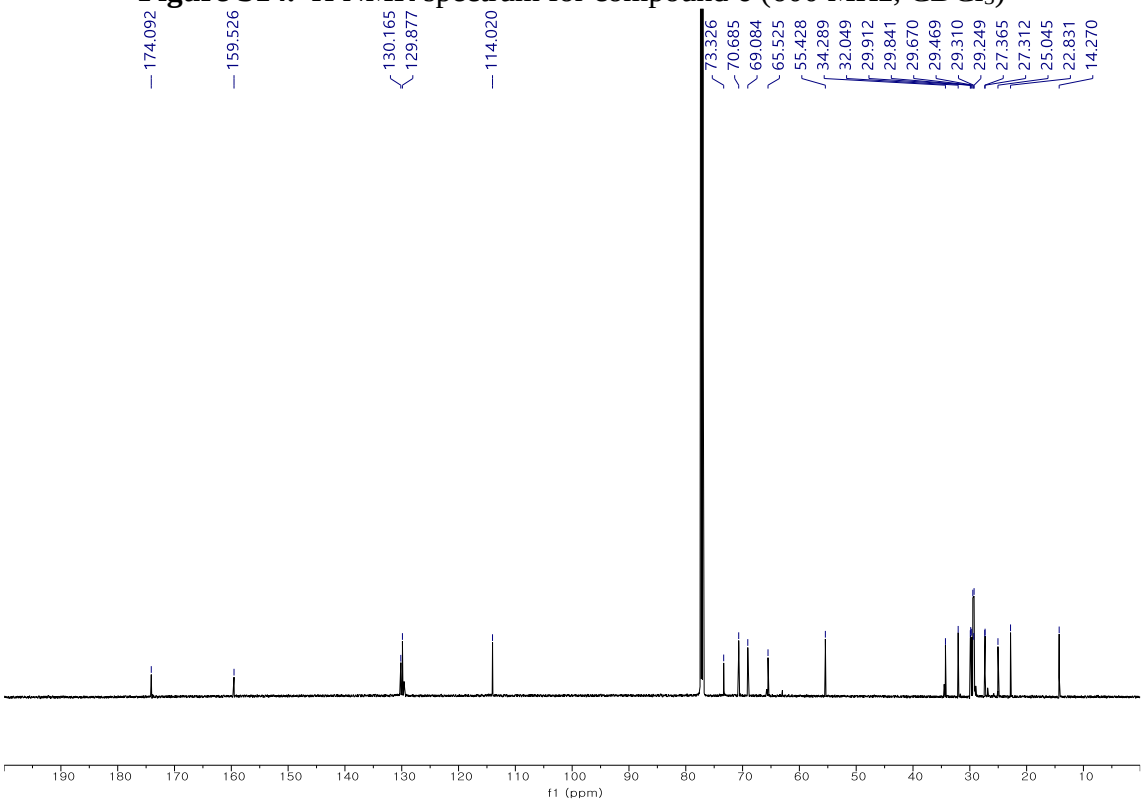


Figure S15. ^{13}C NMR spectrum for compound **6** (150 MHz, $CDCl_3$)

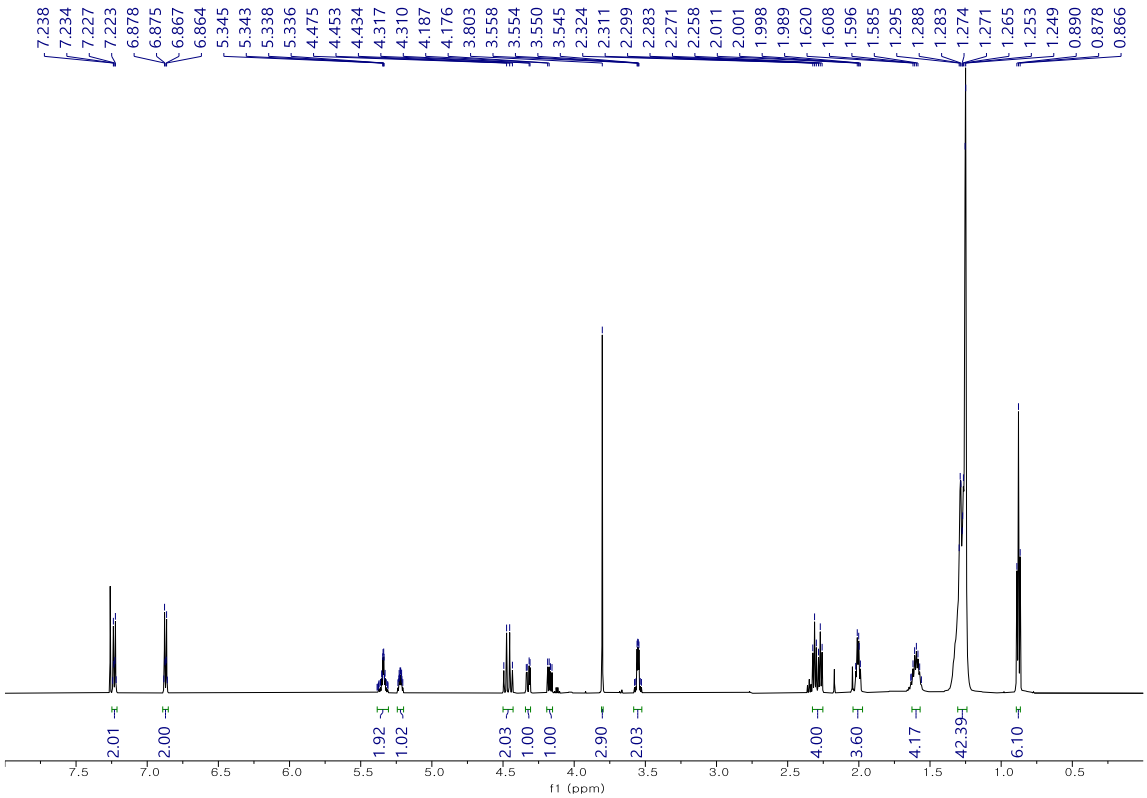


Figure S16. ^{1}H NMR spectrum for compound **7** (600 MHz, $CDCl_3$)

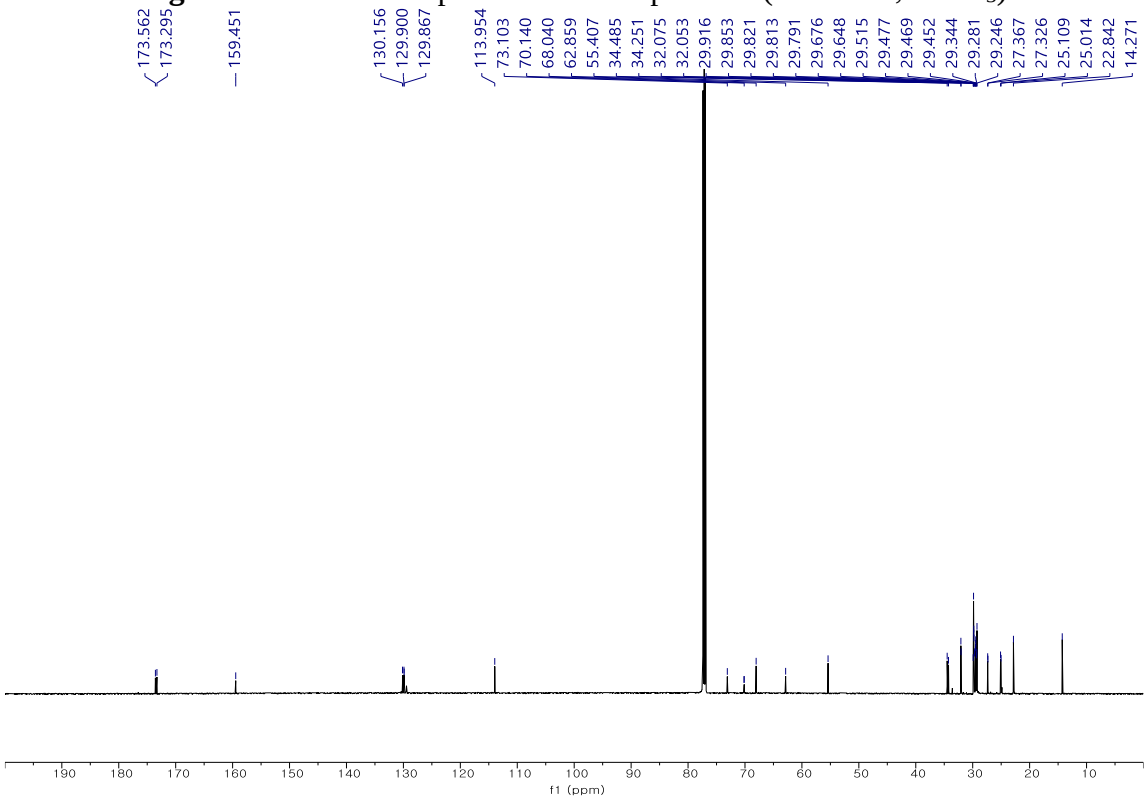


Figure S17. ^{13}C NMR spectrum for compound **7** (150 MHz, $CDCl_3$)

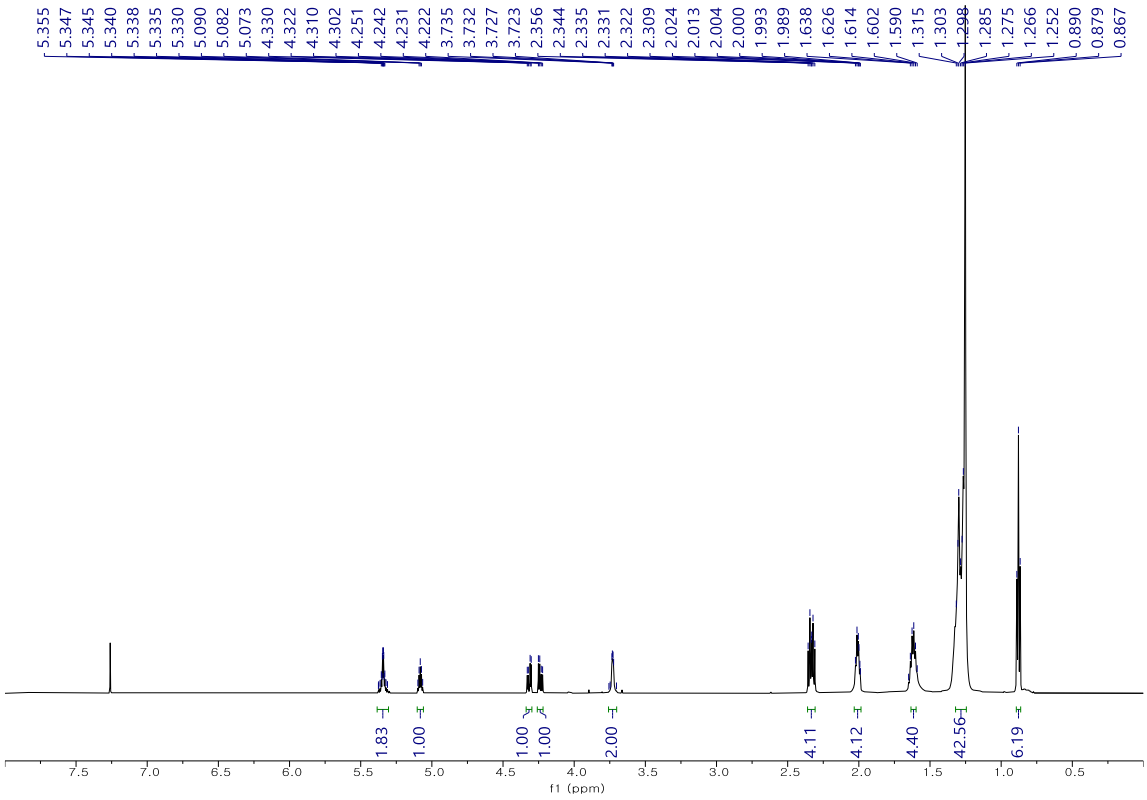


Figure S18. ^{1}H NMR spectrum for compound **8** (600 MHz, $CDCl_3$)

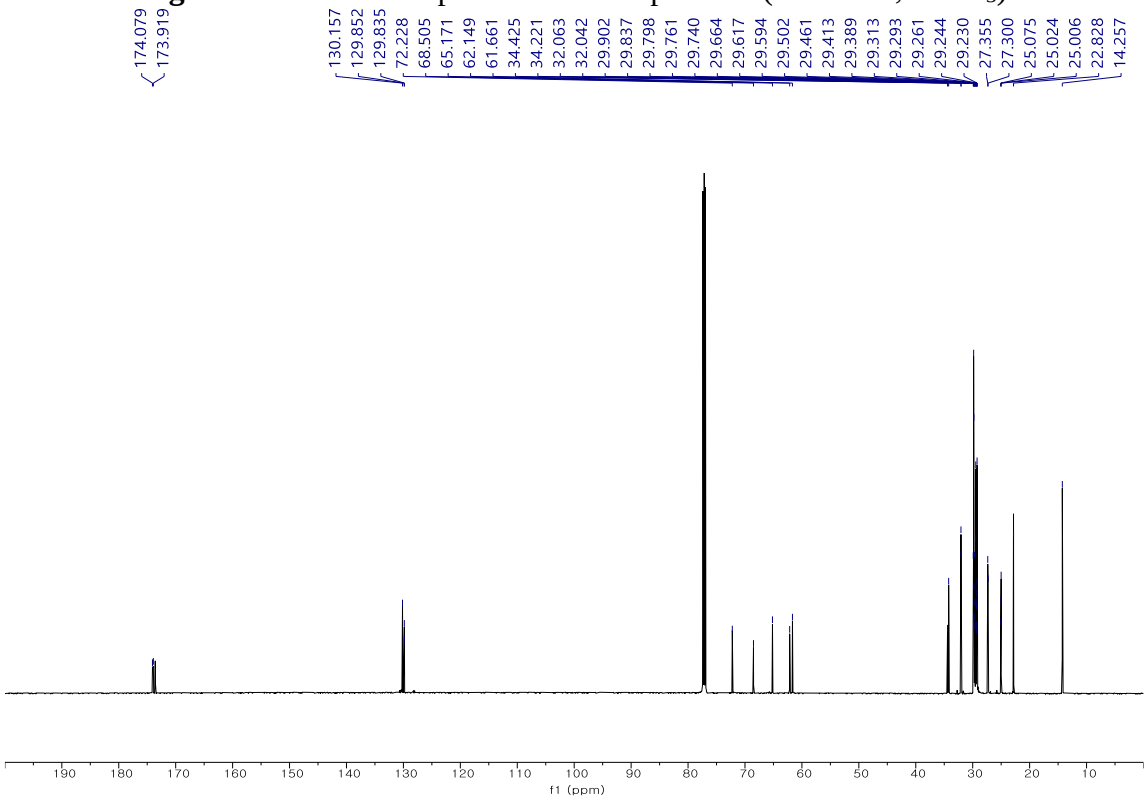


Figure S19. ^{13}C NMR spectrum for compound **8** (150 MHz, $CDCl_3$)

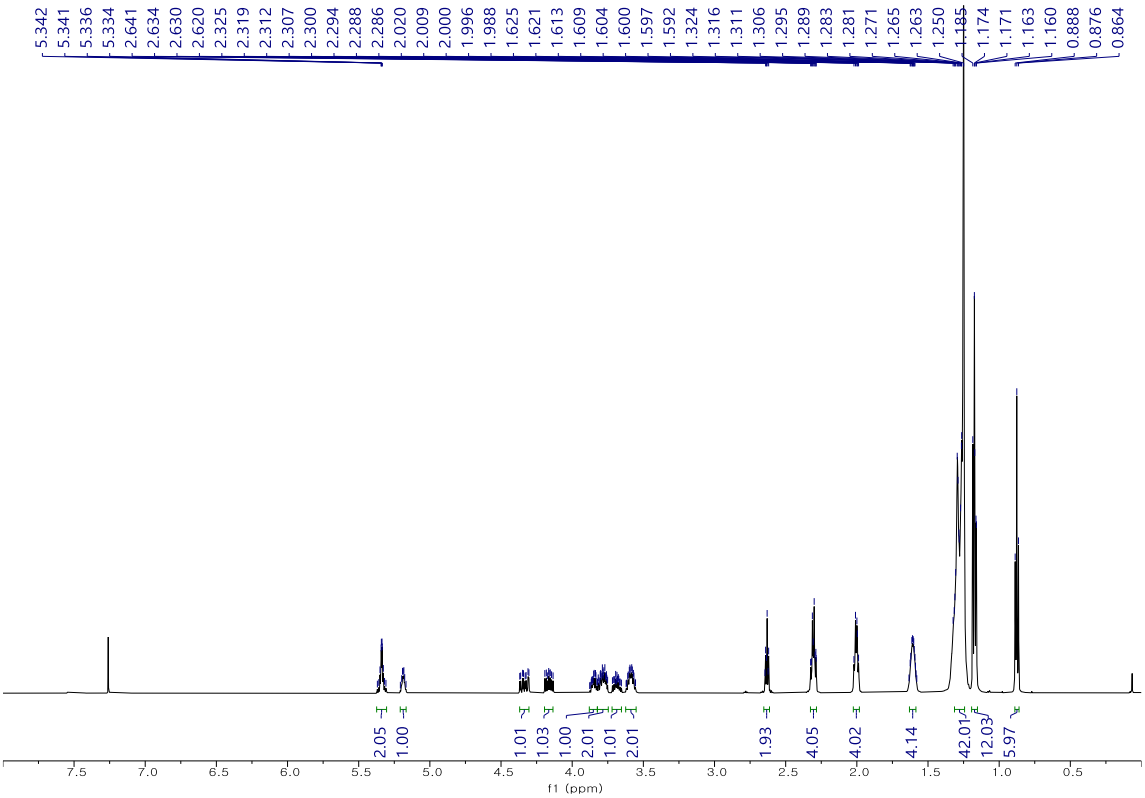


Figure S20. ^{1}H NMR spectrum for compound **9** (600 MHz, $CDCl_3$)

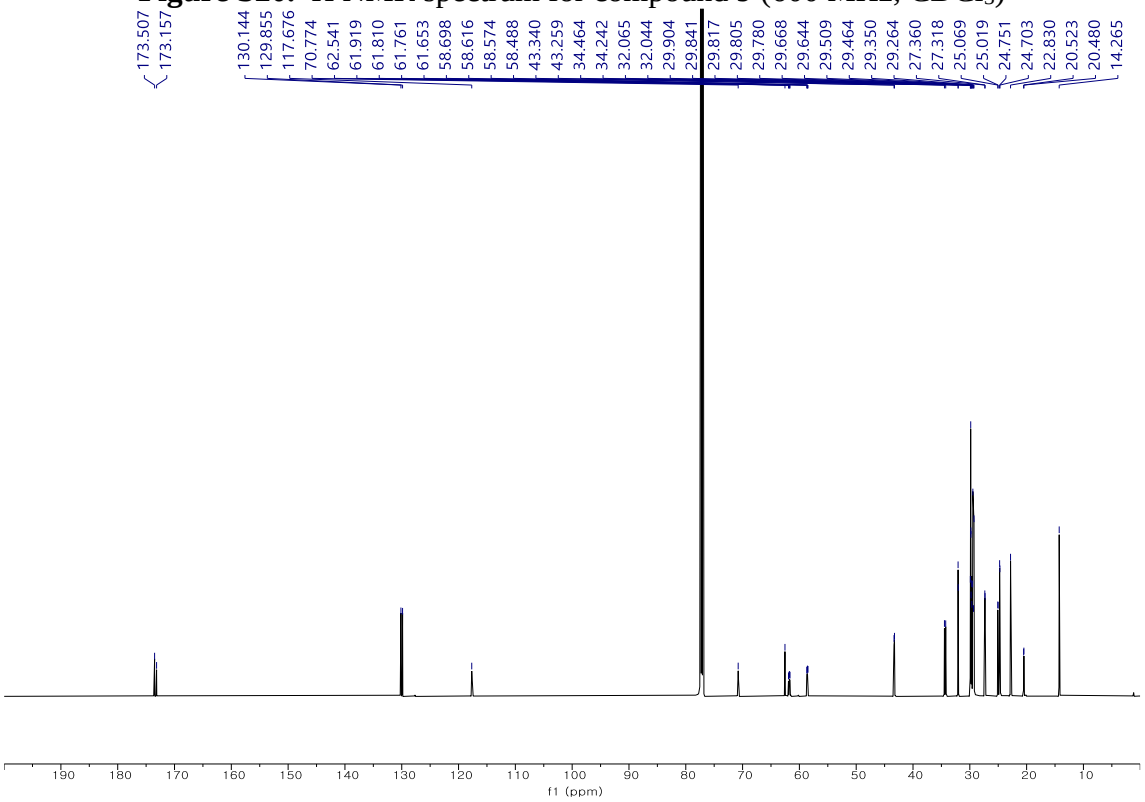


Figure S21. ^{13}C NMR spectrum for compound **9** (150 MHz, $CDCl_3$)

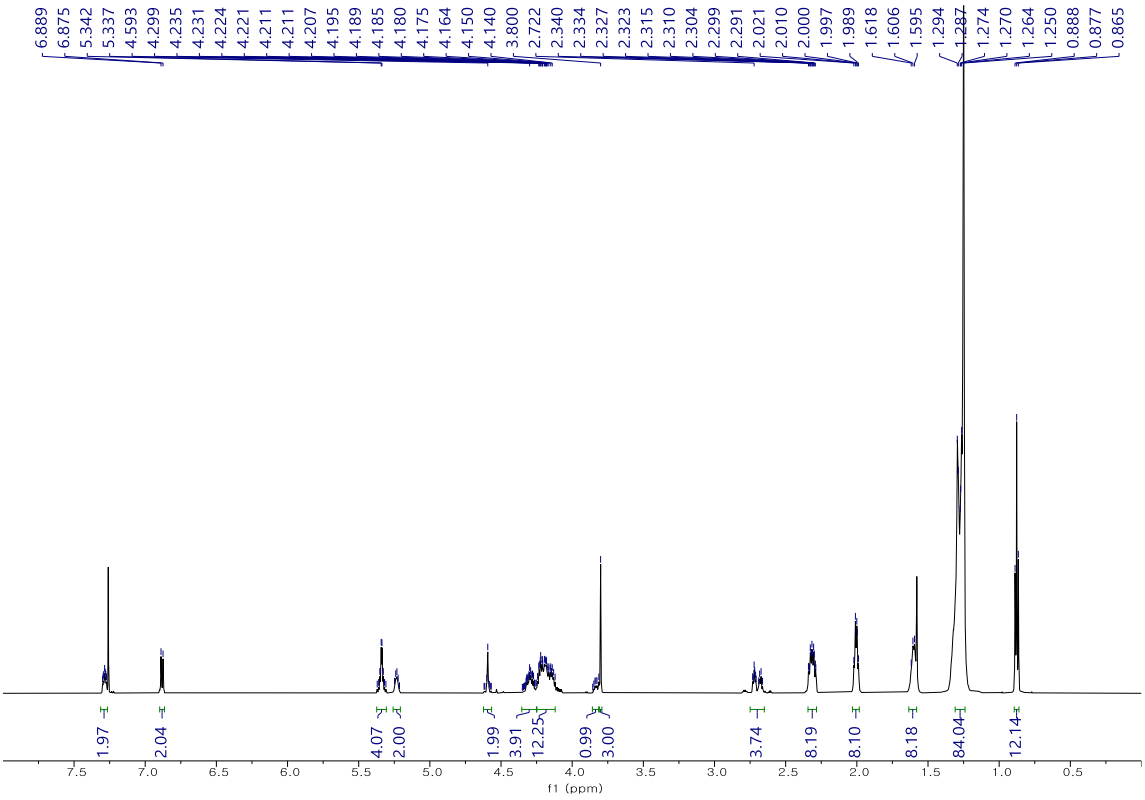


Figure S22. ^{1}H NMR spectrum for compound **10** (600 MHz, $CDCl_3$)

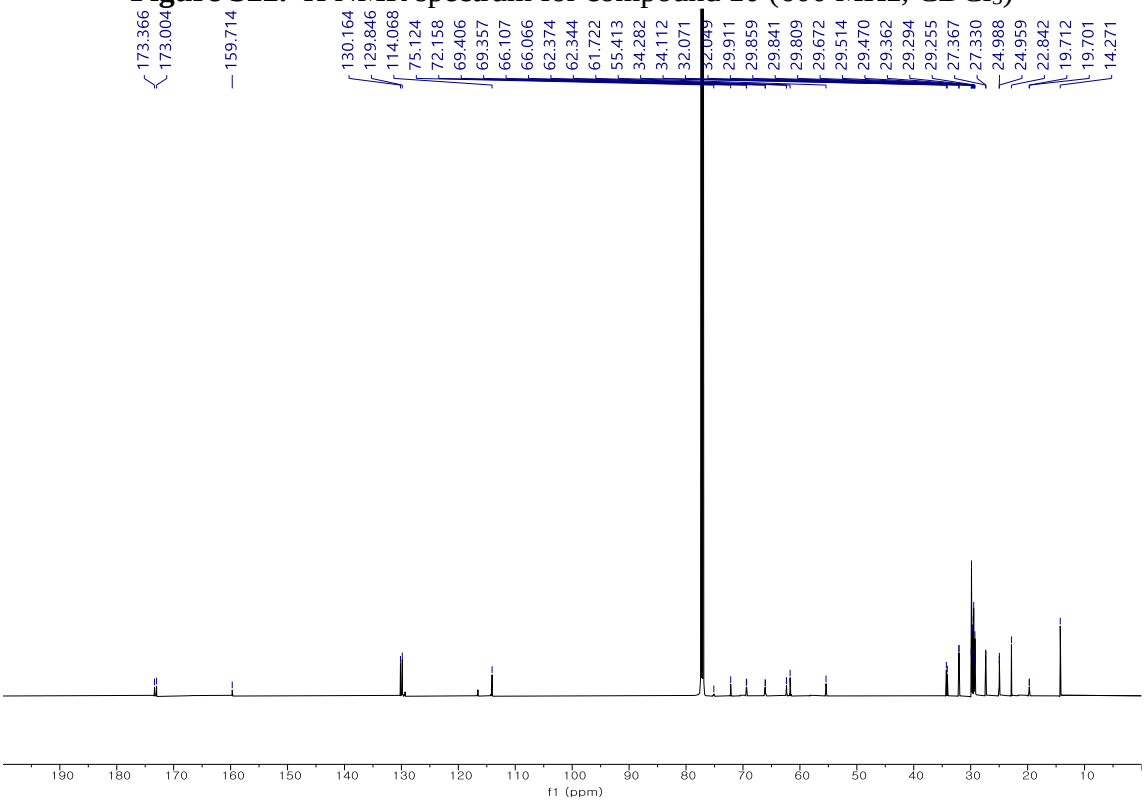


Figure S23. ^{13}C NMR spectrum for compound **10** (150 MHz, $CDCl_3$)

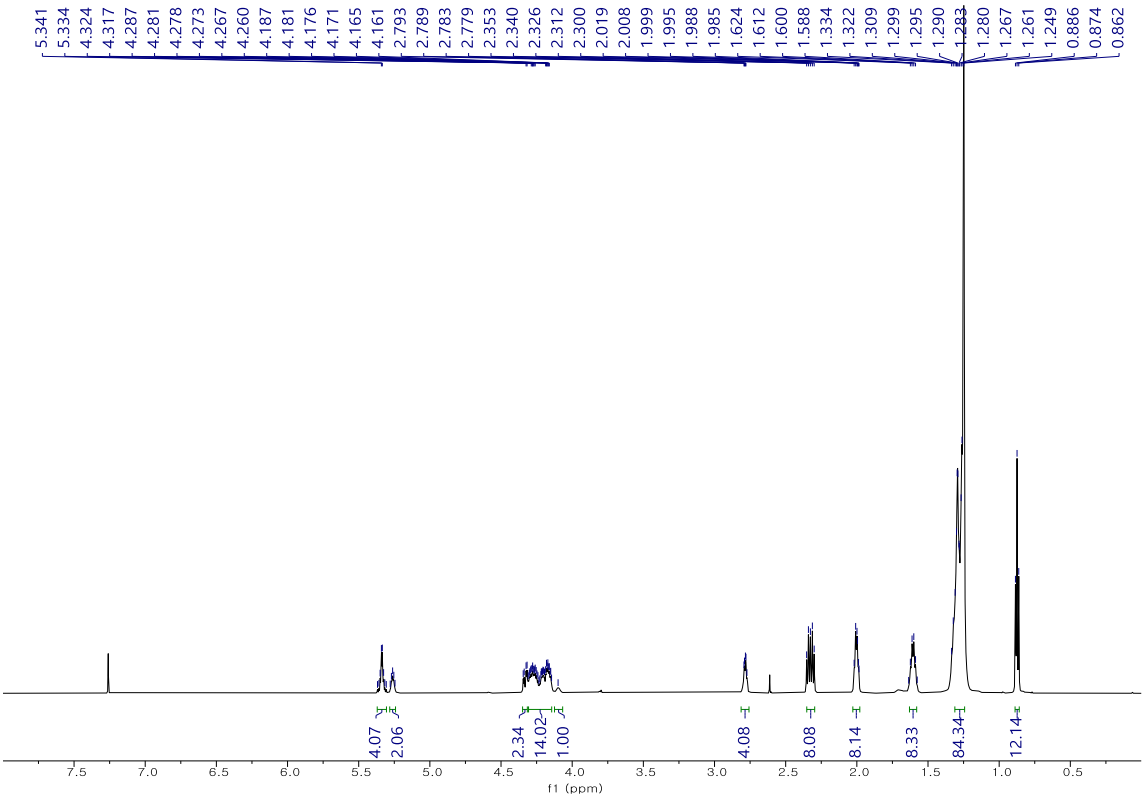


Figure S24. ^{1}H NMR spectrum for compound **11** (600 MHz, $CDCl_3$)

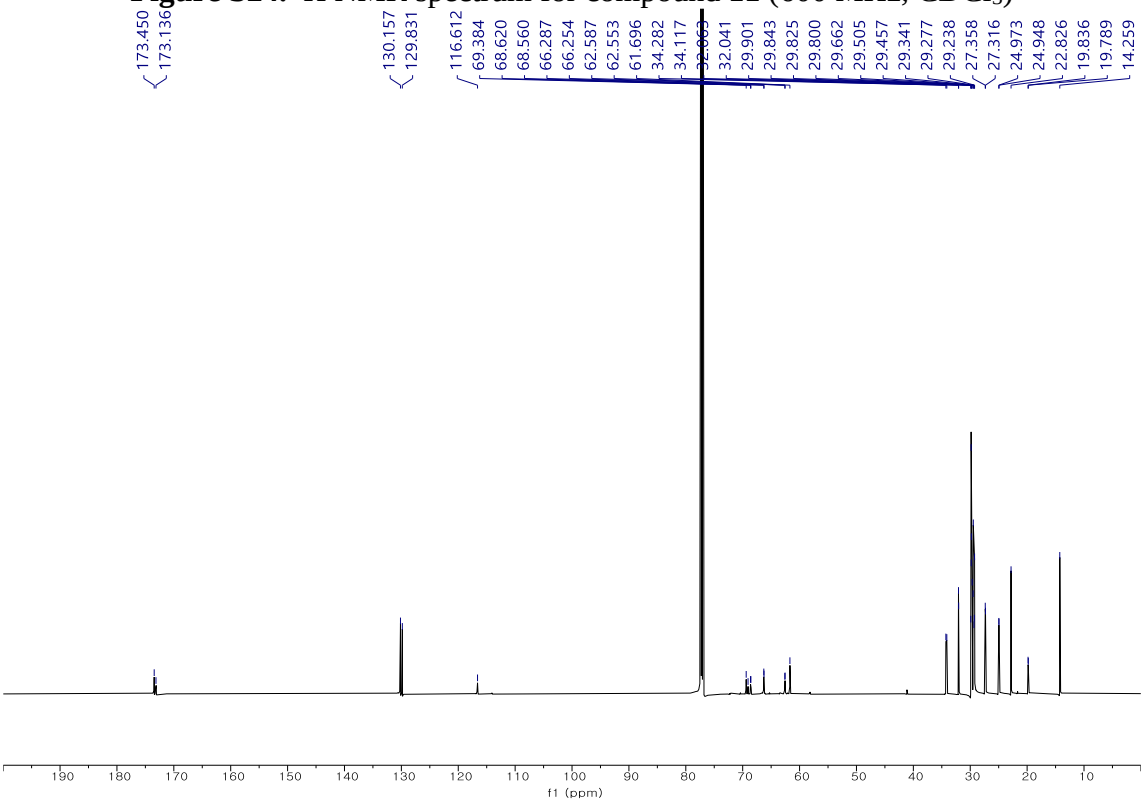


Figure S25. ^{13}C NMR spectrum for compound **11** (150 MHz, $CDCl_3$)

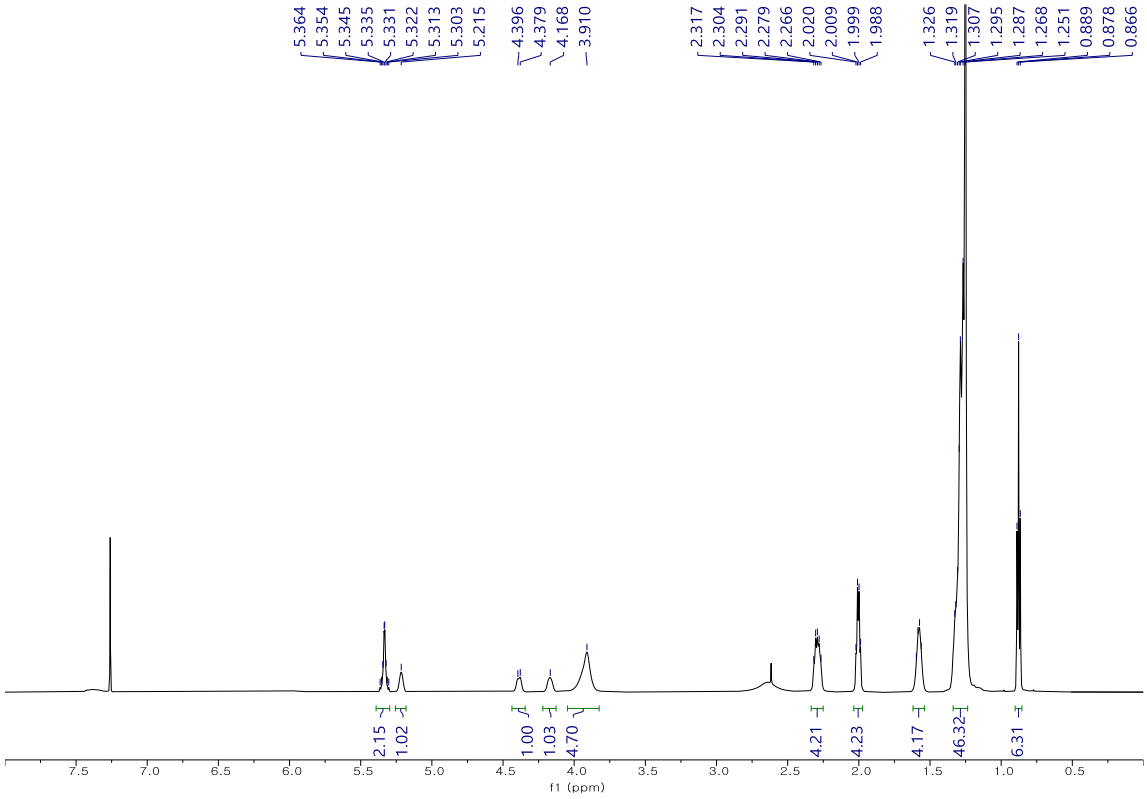


Figure S26. ^{1}H NMR spectrum for MiCL-1* (600 MHz, $CDCl_3$)

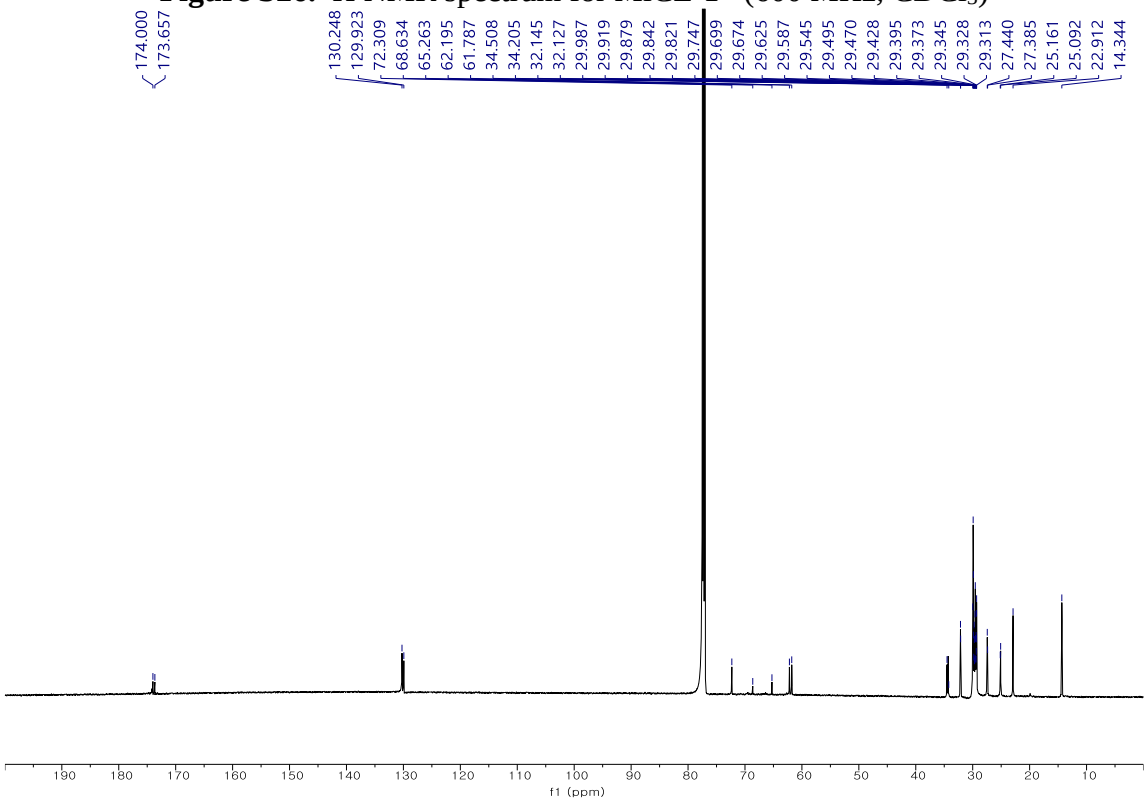


Figure S27. ^{13}C NMR spectrum for MiCL-1* (150 MHz, $CDCl_3$)

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