

Supplementary Information for Publication

9.8 MAG. A new host lipid for *in meso* (lipid cubic phase) crystallization of integral membrane proteins

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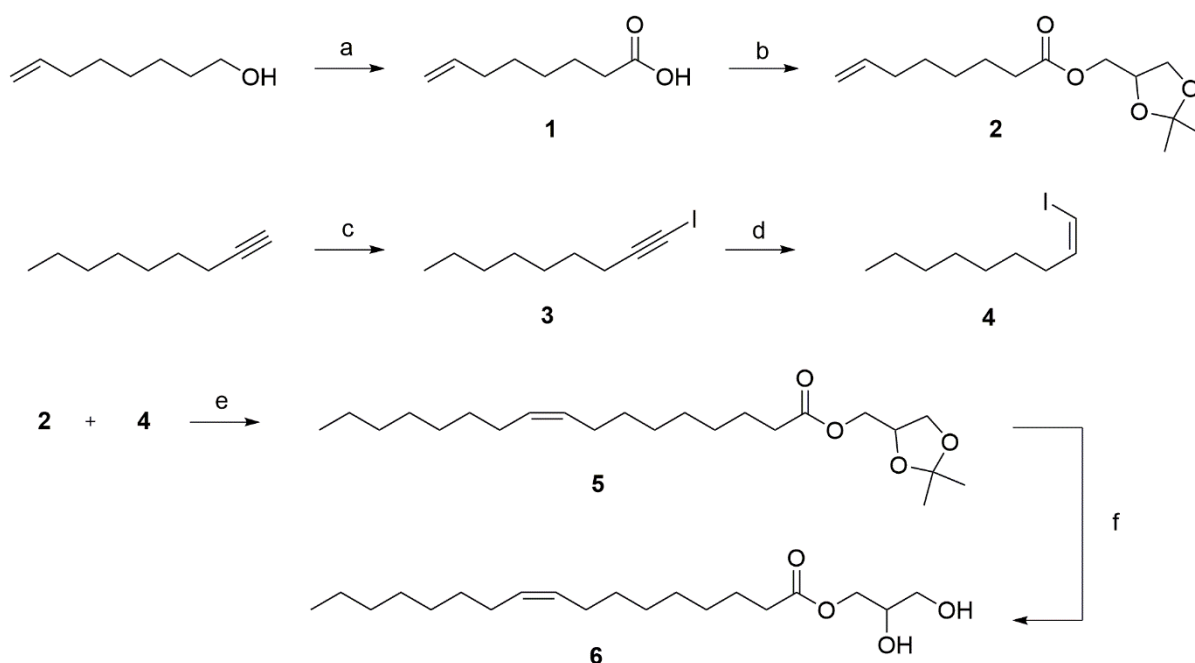
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9.8 MAG Synthesis and Purification

General procedure. The procedure followed in the current study is a slight modification of method reported in reference.⁴¹ Reagents were purchased from commercial sources and used without further purification. Thin layer chromatography analysis was performed using Merck F₂₅₄ silica gel 60 plates (175 – 225 μm thickness). Nuclear magnetic resonance (NMR) spectra were obtained using a Bruker Avance 400 spectrometer. Unless otherwise noted, ^1H NMR (400.13 MHz) spectra were recorded in CDCl_3 relative to residual solvent protons ($\delta_{\text{H}} = 7.26$ ppm); coupling constants were reported in Hz. Unless otherwise noted, ^{13}C NMR (100.6 MHz) measurements were made in CDCl_3 relative to residual solvent carbon ($\delta_{\text{C}} = 77.2$ ppm). Chemical shifts are reported in parts per million (ppm, δ) downfield from tetramethylsilane. Mass spectra were recorded using an electrospray ionisation (ESI) ion-trap. Unless otherwise indicated, procedures were carried out at room temperature which varied from 19.5 to 20.5 $^{\circ}\text{C}$.

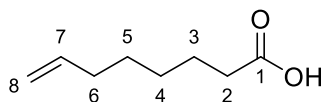
Synthesis of 9.8 MAG

Scheme 1. Synthesis pathway



Reaction conditions: (a) H_5IO_6 , PCC, ACN; (b) DMAP, DCC, solketal, CH_2Cl_2 ; (c) I_2 , TBAB, CuI, TEA, H_2O ; (d) BH_3 .DMS, cyclohexene, Et_2O , AcOH; (e) **1** 9-BBN, THF; **2** AsPh_3 , Cs_2CO_3 , Pd(dppf)Cl_2 . CH_2Cl_2 , DMF, H_2O ; (f) AcOH, H_2O .

Synthesis of oct-7-enoic acid



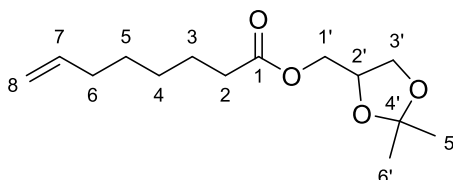
Periodic acid (37.78 g, 165.74 mmol, 2.5 eq.) was dissolved in acetonitrile (150 mL) and stirred vigorously for 15 min. The solution was brought to 0 $^{\circ}\text{C}$ and oct-7-enol (8.50 g, 66.30 mmol, 1.0 eq.) added dropwise via syringe. A solution of pyridinium chlorochromate (0.72 g, 3.32 mmol, 0.05 eq.) in acetonitrile (50 mL) was added dropwise via syringe over 10 min. The resulting solution was stirred at 20 $^{\circ}\text{C}$ for 24 h. The mixture was added to deionised water (300 mL) and the product extracted with

ethyl acetate (2 x 200 mL). The combined organic layers were washed with deionised water (200 mL) and sat. aq. sodium thiosulfate (200 mL), dried over magnesium sulfate and concentrated under vacuum to yield a colourless oil (9.22 g, 98%).

¹H-NMR (CDCl₃, 400 MHz, ppm): δ = 1.30-1.45 (m, 4H, H₄, H₅), 1.60-1.68 (m, 2H, H₃), 2.01-2.08 (m, 2H, H₆), 2.35 (t, J = 7.52 Hz, 2H, H₂), 4.90-5.03 (m, 2H, H₈), 5.73-5.85 (m, 1H, H₇), 11.11 (br s, 1H, OH).

¹³C-NMR (CDCl₃, 100 MHz, ppm): δ = 24.50, 28.49, 33.54, 34.06, 114.48, 138.75, 180.46.

Synthesis of (2,2-dimethyl-1,3-dioxolan-4-yl)methyl oct-7-enoate

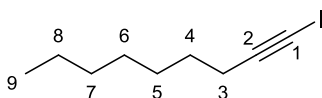


In an oven dried 500 mL round bottom flask, 4-dimethylaminopyridine (7.06 g, 57.83 mmol, 1.0 eq.) was dissolved in anhydrous dichloromethane (100 mL). Oct-7-enoic acid (8.22 g, 57.83 mmol, 1.0 eq.) and solketal (8.41 g, 63.62 mmol, 1.1 eq.) were added sequentially, via syringe, under a positive pressure of argon. The solution was brought to 0 °C and a solution of N,N'-dicyclohexylcarbodiimide (13.13 g, 63.62 mmol, 1.1 eq.) in anhydrous dichloromethane (50 mL) was added dropwise over 10 min. The reaction was allowed to heat to 20 °C and stirred for 16 h. The resulting mixture was cooled to -20 °C and the precipitate filtered by vacuum filtration. The filtrate was concentrated under vacuum and purified by column chromatography over silica gel, eluting with hexanes:ethyl acetate (85:15 by vol.) to yield a colourless oil (10.54 g, 71%).

¹H-NMR (CDCl₃, 400 MHz, ppm): δ = 1.31-1.45 (m, 4H, H₄, H₅), 1.36 (s, 3H, H_{6'}), 1.42 (s, 3H, H_{5'}), 1.58-1.67 (m, 2H, H₃), 2.00-2.07 (m, 2H, H₆), 2.34 (t, J = 7.55 Hz, 2H, H₂), 3.70-3.76 (dd, J_1 = 8.46 Hz, J_2 = 6.16 Hz, 1H, H_{3'}), 4.04-4.19 (m, 3H, H_{1'}, H_{3'}), 4.27-4.34 (m, 1H, H_{2'}), 4.90-5.02 (m, 2H, H₈), 5.73-5.84 (m, 1H, H₇).

¹³C-NMR (CDCl₃, 100 MHz, ppm): δ = 24.73, 25.40, 26.69, 28.48, 28.55, 33.53, 34.04, 64.55, 66.35, 73.66, 109.82, 114.44, 138.76, 173.53.

Synthesis of 1-iodonon-1-yne

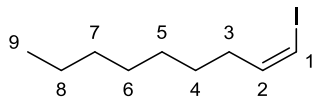


To a 500 mL round bottom flask charged with deionised water (200 mL) was added tetra-n-butylammonium bromide (21.85 g, 67.80 mmol, 1.0 eq.) and copper iodide (0.13 g, 0.67 mmol, 0.01 eq.) and the mixture stirred vigorously for 10 min. Iodine (34.40 g, 135.60 mmol, 2.0 eq.) was added, followed by triethylamine (28.3 mL, 203.40 mmol, 3.0 eq.) added via syringe and the mixture stirred vigorously for 10 min. Non-1-yne (11.13 mL, 67.80 mmol, 1.0 eq.) was added via syringe and the mixture stirred vigorously for 24 h. The product was extracted with ethyl acetate (3 x 250 mL), the combined organic layers washed with sat. aq. sodium thiosulfate (3 x 200 mL) and sat. aq. sodium chloride (200 mL), dried over magnesium sulfate and concentrated under vacuum. The crude was purified by column chromatography over silica gel, eluting with 100% hexanes to yield a pale-yellow oil (13.72 g, 81%).

¹H-NMR (CDCl₃, 400 MHz, ppm): δ = 0.88 (m 3H, H₉), 1.22-1.41 (m, 8H, H₅, H₆, H₇, H₈), 1.46-1.55 (m, 2H, H₄), 2.35 (t, J = 7.10 Hz, 2H, H₃).

¹³C-NMR (CDCl₃, 100 MHz, ppm): δ = -7.67, 14.10, 20.83, 22.63, 28.50, 28.76, 31.71, 94.89.

Synthesis of (Z) 1-iodonon-1-ene

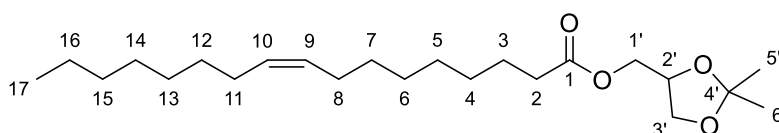


To an oven dried 250 mL 3 neck round bottom flask charged with anhydrous diethyl ether (50 mL), was added borane dimethyl sulphide complex (2 M in tetrahydrofuran, 30.18 mL, 60.36 mmol, 1.1 eq.) via syringe under argon. The solution was cooled to 0 °C and anhydrous cyclohexene (12.24 mL, 120.72 mmol, 2.2 eq.) was added dropwise via syringe over 10 min. The mixture was stirred at 0 °C for 15 min, heated to 20 °C and stirred for 1 h. The mixture was cooled to 0 °C and 1-iodonon-1-yne (13.72 g, 54.87 mmol, 1.0 eq.) added dropwise via syringe, the mixture was stirred at 0 °C for 30 min, heated to 20 °C and stirred for 2h. The mixture was cooled to 0 °C and acetic acid (50 mL, 16 eq.) added via syringe over 15 min, the mixture heated to 20 °C and stirred for 2.5 h. The mixture was diluted with diethyl ether (50 mL) and deionised water (100 mL) and the organic layer washed with deionised water (2 x 50 mL) and sat. aq. sodium chloride (2 x 50 mL), dried over magnesium sulfate, swirled with ethanolamine (25 mL) and the resulting white precipitate filtered. The filtrate was concentrated under vacuum and purified by column chromatography over silica gel, eluting with 100% hexane to yield a pale-yellow oil (9.78 g, 65%).

¹H-NMR (CDCl₃, 400 MHz, ppm): δ = 0.89 (m 3H, H₉), 1.20-1.47 (m, 10H, H₄, H₅, H₆, H₇, H₈), 2.09-2.18 (m 2H, H₃), 6.13-6.20 (m, 2H, H₁, H₂).

¹³C-NMR (CDCl₃, 100 MHz, ppm): δ = 14.09, 22.63, 27.94, 29.05, 29.10, 31.76, 34.67, 82.10, 141.47.

Synthesis of (2,2-dimethyl-1,3-dioxolan-4-yl)methyl (Z) septadec-9-enoate



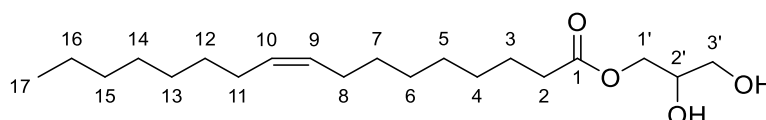
To an oven-dried 500 mL 3-neck round bottom flask charged with anhydrous tetrahydrofuran (75 mL) was added (2,2-dimethyl-1,3-dioxolan-4-yl)methyl oct-7-enoate (5.93 g, 23.14 mmol, 1.0 eq.). The solution was cooled to 0 °C and 9-borabicyclo[3.3.1]nonane (0.5 M in tetrahydrofuran, 50.9 mL, 25.45 mmol, 1.1 eq.) added dropwise via syringe. The reaction was allowed to heat to 20 °C and stirred for 16 h. The reaction vessel was then quenched with deionised water (75 mL) for 2 h. To a separate oven-dried round bottom flask charged with dimethylformamide (150 mL) was added triphenylarsine (1.42 g, 4.63 mmol, 0.2 eq.), caesium carbonate (11.31 g, 34.71 mmol, 1.5 eq.) and 1-iodonon-1-ene (7.00 g, 27.76 mmol, 1.2 eq.). Both vessels were degassed via the freeze-pump-thaw method and sparged with argon for 15 min. To the vessel containing dimethylformamide was added [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium.dichloromethane complex (0.95 g, 1.16 mmol, 0.05 eq.) and the solution sparged with argon for 15 min. The tetrahydrofuran mixture was transferred to the dimethylformamide vessel via canula over 15 min and the reaction stirred for 16 h. The mixture was added to sat. aq. sodium chloride (400 mL), the product extracted with diethyl ether (3 x 200 mL), the combined organic layers were washed with deionised water (200 mL) sat. aq. sodium chloride (200 mL), dried over magnesium sulfate and concentrated under vacuum. The crude product was purified

by column chromatography over silica gel, eluting with hexanes:ethyl acetate (95:5 by vol.) to yield a pale yellow oil (4.07 g, 46%).

¹H-NMR (CDCl₃, 400 MHz, ppm): δ = 0.87 (m, 3H, H17), 1.21-1.35 (m, 18H, H4, H5, H6, H7, H12, H13, H14, H15, H16), 1.36 (s, 3H, H6'), 1.43 (s, 3H, H5'), 1.57-1.66 (m, 2H, H3), 1.96-2.04 (m, 4H, H8, H11), 2.33 (m, 2H, H2), 3.70-3.75 (m, 1H, H3'), 4.04-4.18 (m, 3H, H1', H3'), 4.26-4.34 (m, 1H, H2'), 5.28-5.38 (m, 2H, H9, H10).

¹³C-NMR (CDCl₃, 100 MHz, ppm): δ = 14.10, 22.69, 24.88, 27.16, 27.22, 29.09, 29.16, 29.22, 29.30, 29.69, 29.77, 31.90, 34.13, 64.51, 66.34, 73.64, 109.80, 129.74, 130.02, 173.62.

Synthesis of 2,3-dihydroxypropyl (Z) septadec-9-enoate (9.8 MAG)



To a 250 mL round bottom flask charged with acetic acid:water (75 mL, 4:1 by vol.) was added (2,2-dimethyl-1,3-dioxolan-4-yl)methyl (Z) septadec-9-enoate (2.50 g, 6.55 mmol, 1.0 eq.). The mixture was heated to 50 °C and stirred for 2 h. The mixture was quenched with sat. aq. sodium bicarbonate to a pH of 7. The product was extracted with diethyl ether (3 x 200 mL), the combined organic layers washed with deionised water (200 mL) and sat. aq. sodium chloride (200 mL), dried over magnesium sulfate and concentrated under vacuum. The crude was purified by column chromatography over silica gel, eluting with gradient elution from hexanes:ethyl acetate (3:1 by vol.) to hexanes:ethyl acetate:acetone (2:1:1 by vol.) to yield a pale yellow waxy solid (1.23 g, 55 %).

¹H-NMR (CDCl₃, 400 MHz, ppm): δ = 0.87 (m, 3H, H17), 1.21-1.37 (m, 18H, H4, H5, H6, H7, H12, H13, H14, H15, H16), 1.59-1.67 (m, 2H, H3), 1.97-2.04 (m, 4H, H8, H11), 2.34 (m, 2H, H2), 3.57-3.72 (m, 2H, H3'), 3.90-3.96 (m, 1H, H2'), 4.12-4.23 (m, 2H, H1'), 5.29-5.38 (m, 2H, H9, H10).

¹³C-NMR (CDCl₃, 100 MHz, ppm): δ = 14.06, 22.63, 24.84, 27.11, 27.17, 29.04, 29.06, 29.11, 29.18, 29.23, 29.64, 29.71, 31.83, 34.10, 63.32, 65.06, 70.23, 129.66, 129.98, 174.33.

HRMS (m/z ES⁺): Found 343.2837 (M⁺ + H). C₂₀H₃₉O₄: Calculated 343.2843.

Quality characterization of synthetic 9.8 MAG

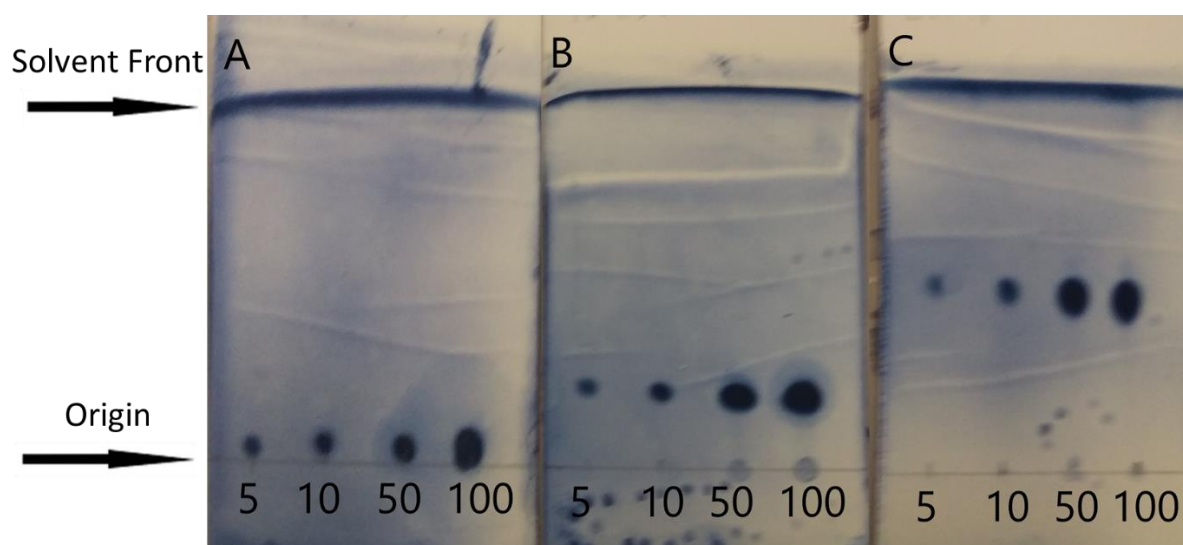


Figure S1. Thin layer chromatographic analysis of synthetic 9.8 MAG. Lipid loadings in micrograms are indicated at the bottom of each lane. Eluting solvent systems are as follows: (A) hexanes:ethyl acetate (75:25, by vol.); (B) hexanes:acetone:ethyl acetate (74:25:1 by vol.); (C) hexanes:acetone:ethyl acetate (50:25:25 by vol.). Lipid was placed on the plate as a solution in hexanes. Spots were visualized by staining with a 0.04 M ammonium molybdate solution followed by charring with a heat gun.

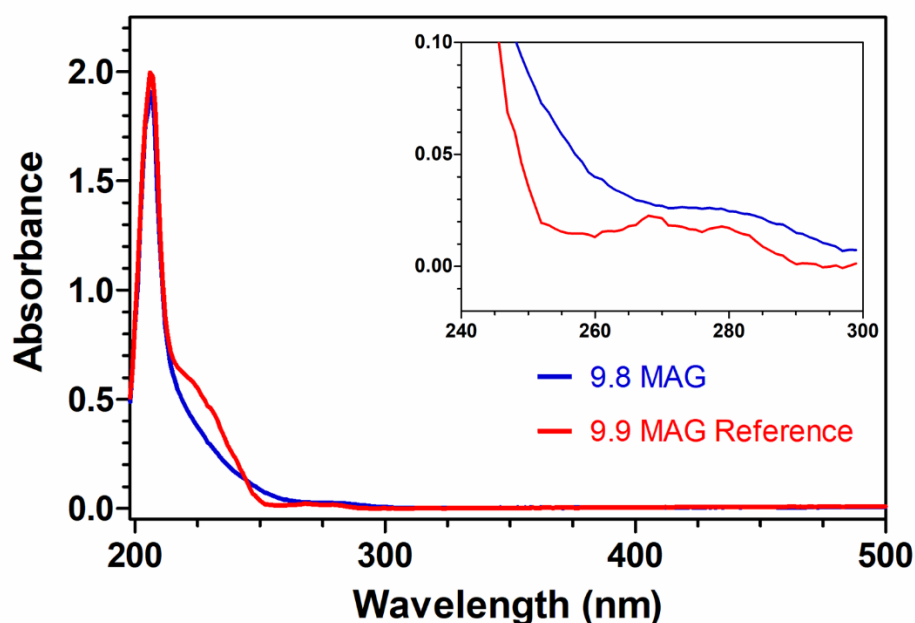


Figure S2. UV-visible absorption spectrum of synthetic 9.8 MAG and commercial 9.9 MAG.

Measurements were made in 1 cm quartz cuvettes at a lipid concentration of 14 mM in ethanol. The commercial lipid was obtained from Nu Chek (M239-025-C). Ultraviolet (UV) absorbance by the lipid can impact on its use in spectrophotometric measurements of reconstituted membrane proteins in the cubic phase, where the lipid is present at a concentration of ~2 M. These spectra were collected as part of a bigger quality control study. The reference spectrum for commercial 9.9 MAG shown here was included in.³⁷

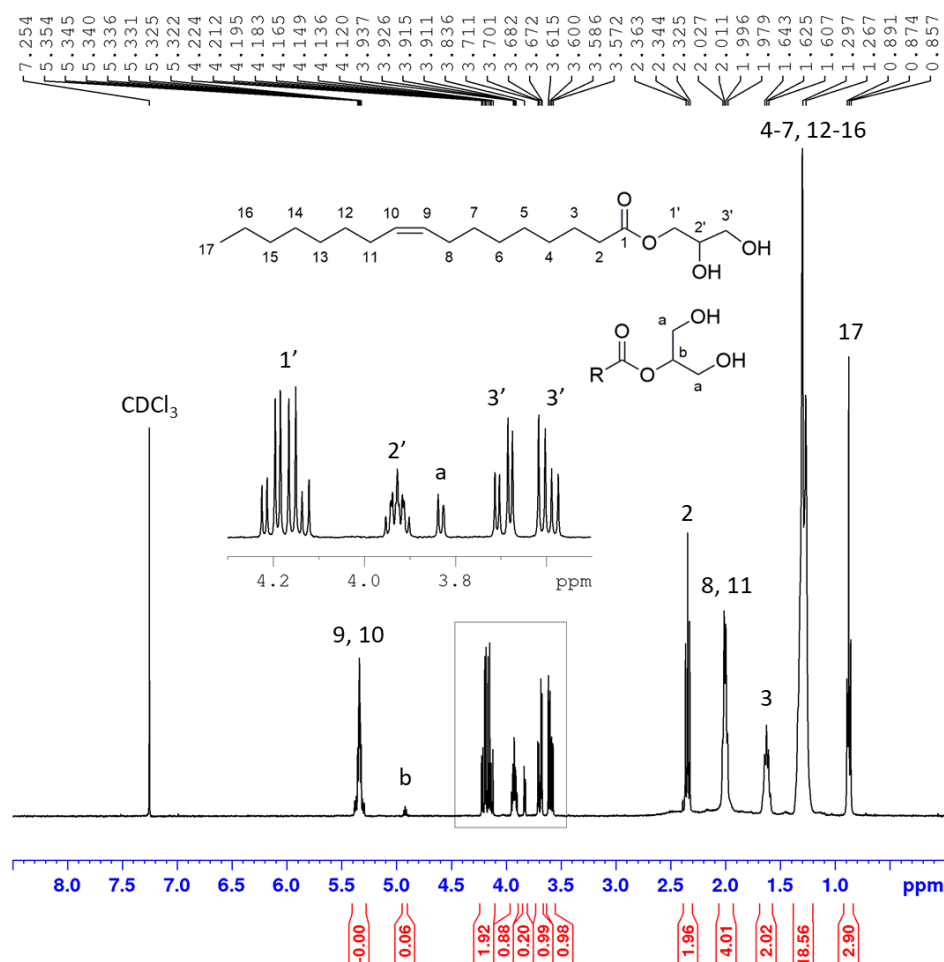


Figure S3. ^1H NMR of synthetic 9.8 MAG. Data was collected on a Bruker Avance 400 (400.13 MHz) with a sample of 5 mg synthetic 9.8 MAG in 600 μL of deuterated chloroform (CDCl_3). The sample contains both the 1-MAG and 2-MAG isomers, the structure or partial structure of which are included in the figure. The inset shows an expanded view of the glycerol region, which contains tell-tale resonances associated with the 2-MAG. Protons are labelled by numbers, with letters indicating the 2-MAG resonances. The spectrum has a signal-to-noise ratio of $>2,000$. This, coupled with the lack of unexplained resonances, indicates that the sample is of very high purity. The lipid had a pale yellow color that cannot be accounted for by additional resonances in the NMR spectrum. The mol% of 2-MAG in the mixture of isomers is estimated by NMR, as follows. The doublet of resonances at ~ 3.85 ppm arises from 4 methylene protons in the 2-MAG isomer (indicated by 'a' in the 2-MAG partial structure) which integrates to 0.2008 (red numbers on the x-axis at ~ 3.85 ppm). This value has been scaled relative to the integrated value for the doublet of doublet resonances centered at ~ 3.7 ppm arising from one of the diastereotopic protons at the *sn*-3 carbon of the 1-MAG glycerol unit (indicated by 3' in the 1-MAG structure), which has been normalized to an integrated value of 1.0 (0.99, red numbers at 3.7 ppm). This means that the signal at 3.85 ppm for the 2-MAG isomer representing 4 protons is 20.08% of the signal for the 1 proton in the 1-MAG isomer at 3.7 ppm. To calculate the 2-MAG concentration as a mol% of the 1- isomer content in the sample, its integrated value (20.08%) must be divided by 4. In this case, the 2-MAG content is $(20.08/4 =) 5.02$ mol%. Accordingly, the mol% of 2-MAG in the mixture of isomers is $(5.02 \text{ mol\% 2-MAG}) \times 100 / [105.02 \text{ mol\% of 1-MAG} + 2\text{-MAG}] =) 4.78 \text{ mol\% 2-MAG}$.

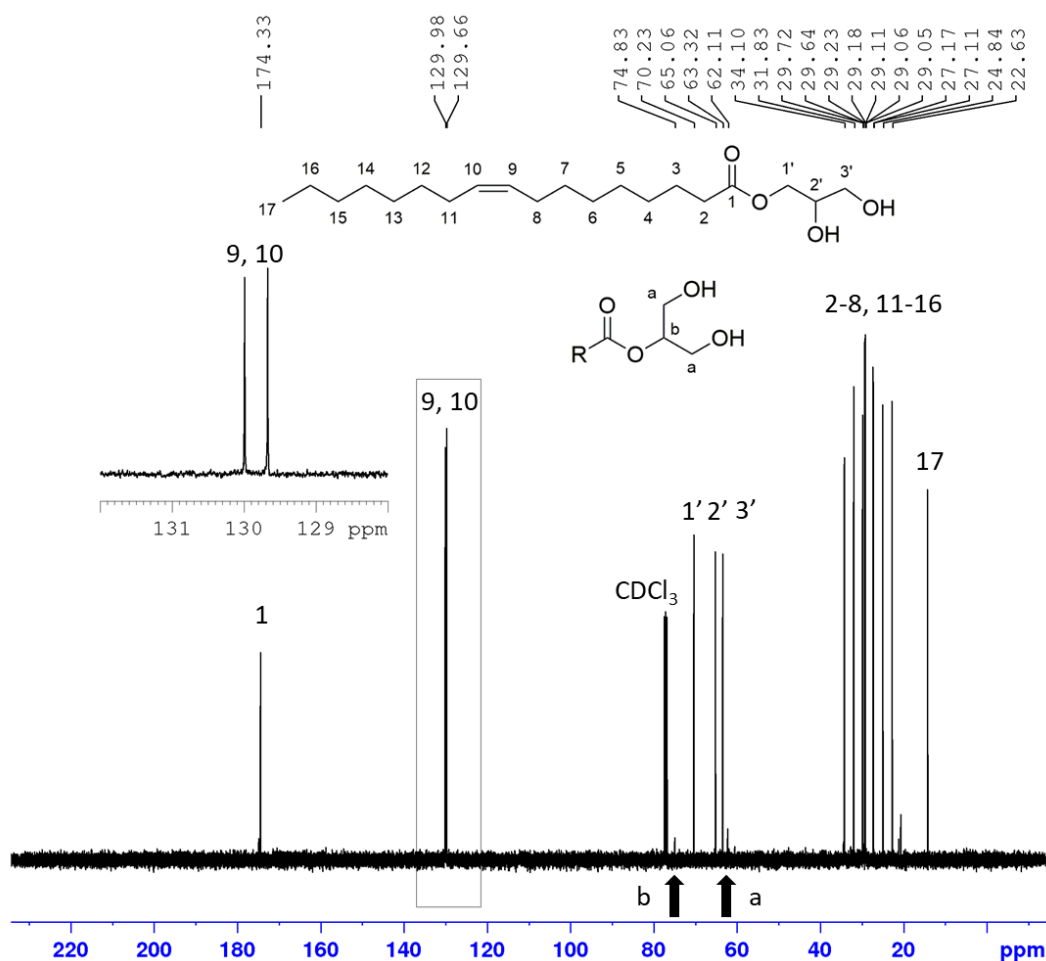


Figure S4. ^{13}C NMR spectrum of synthetic 9.8 MAG. Data was collected on a Bruker Avance 400 (400.13 MHz) with a sample of 5 mg synthetic 9.8 MAG in 600 μL deuterated chloroform (CDCl_3). The sample contains both the 1-MAG and 2-MAG isomers, the structure or partial structure of which are shown in the figure. Carbons are labelled by numbers, with letters indicating the 2-MAG resonances at 62.1 and 74.8 ppm. The lipid was synthesized *via* a Suzuki-Miyaura cross coupling, designed to give only the *cis*-alkene. The inset shows an expanded view of the alkene region. The *trans*-alkene (here not distinguishable from noise) would appear at approximately 130.6 and 130.9 ppm.⁴⁰ The *trans*-alkene content is estimated at ≤ 0.7 mol% based on a signal-to-noise ratio of 139.5 calculated using the Bruker Topspin processing software.

References:

40. Lyons, J. A.; Aragao, D.; Slattery, O.; Pisiakov, A. V.; Soulimane, T.; Caffrey, M. Structural Insights into Electron Transfer in Caa3-Type Cytochrome Oxidase. *Nature* 2012, 487, 514–518.
41. Coleman, B. E.; Cwynar, V.; Hart, D. J.; Havas, F.; Jakkam, M. M.; Patterson, S.; Ridenour, S.; Schmidt, M.; Smith, E.; Wells, A. J. Modular Approach to the Synthesis of Unsaturated 1-Monoacyl Glycerols. *Synlett* 2004, 8, 1339–1342.