

## Photoswitchable diacylglycerols enable optical control of protein kinase C

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DOI:

[10.1038/nchembio.2141](https://doi.org/10.1038/nchembio.2141)

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*Document Version*

Peer reviewed version

*Citation for published version (Harvard):*

Frank, J, Yushchenko, D, Hodson, D, Lipstein, N, Nagpal, J, Rutter, G, Rhee, J-S, Gottschalk, A, Brose, N, Schultz, C & Trauner, D 2016, 'Photoswitchable diacylglycerols enable optical control of protein kinase C', *Nature Chemical Biology*, vol. 12, pp. 755-762. <https://doi.org/10.1038/nchembio.2141>

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# SUPPLEMENTARY INFORMATION

## Photoswitchable diacylglycerols enable optical control of protein kinase C

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# SUPPLEMENTARY RESULTS

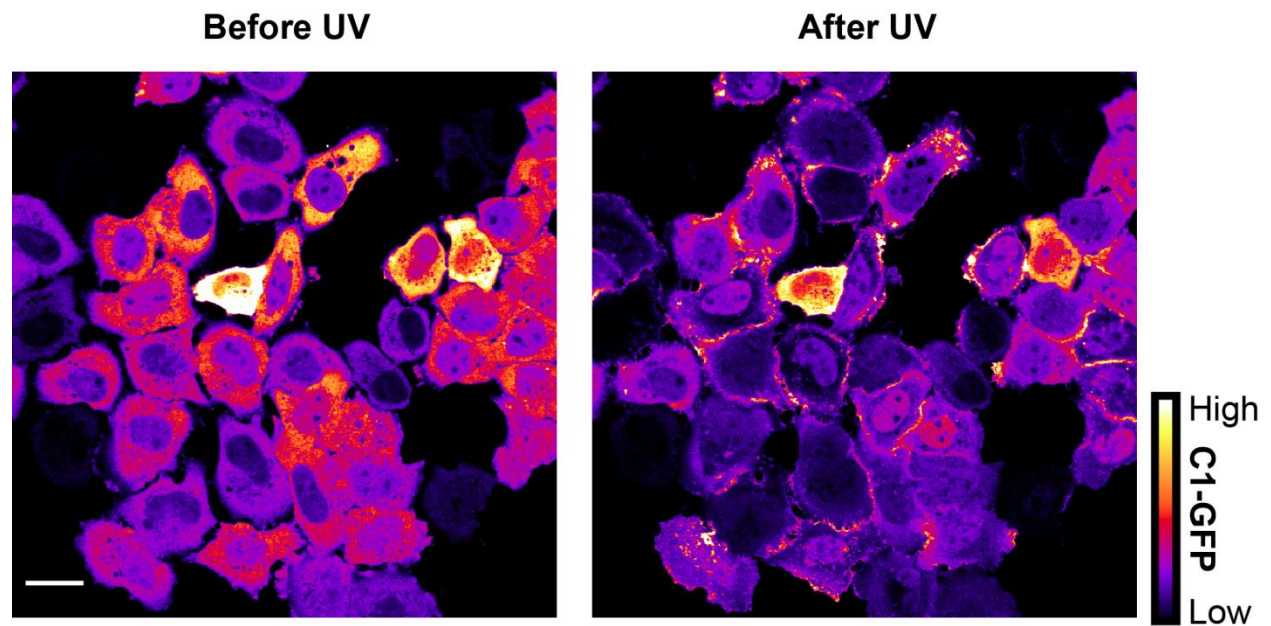
## SUPPLEMENTARY TABLES

Supplementary Table 1 | List of utilized cDNA constructs.

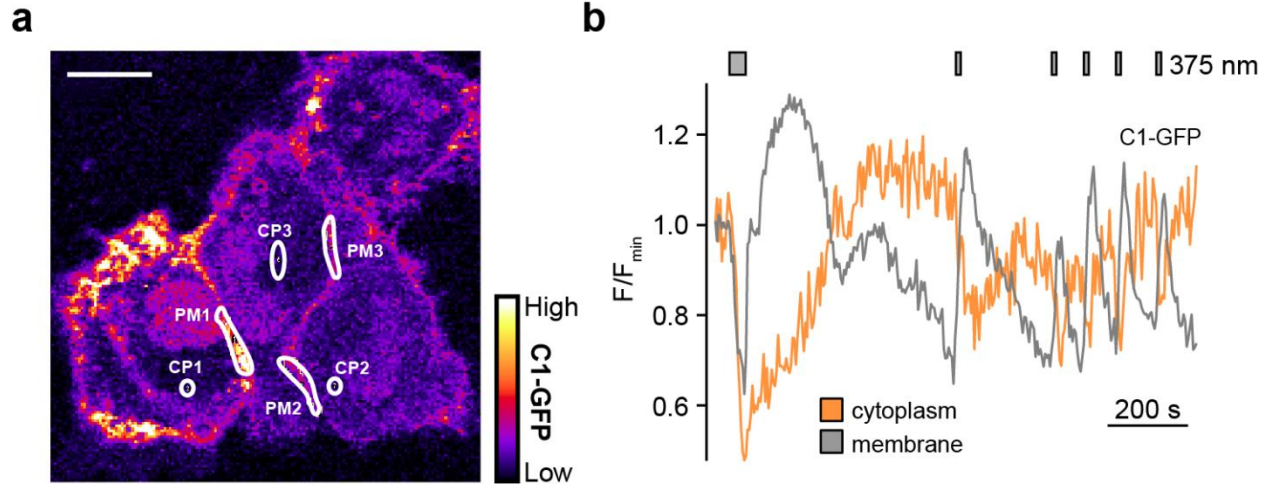
Name	Characterization
R-GECO <sup>24</sup>	Red intensimetric Ca <sup>2+</sup> sensor
C1-GFP <sup>21</sup>	Green DAG-sensing translocation probe
PKC $\delta$ -RFP*	Protein kinase C $\delta$ reporter
PKC $\alpha$ -GFP <sup>30</sup>	Protein kinase C $\alpha$ reporter
CKAR <sup>32</sup>	Cytosolic C kinase activity reporter
Munc13-1-GFP <sup>10</sup>	Munc13-1 translocation reporter
Munc13-1 <sup>H567K</sup> -GFP <sup>10</sup>	Munc13-1 <sup>H567K</sup> mutant translocation reporter

*\*The PKC $\delta$ -RFP construct was prepared by exchanging the eGFP from the original construct<sup>62</sup> with mRFP via the EcoRI and EcoRV sites in the MCS of pcDNA3 backbone.*

SUPPLEMENTARY FIGURES

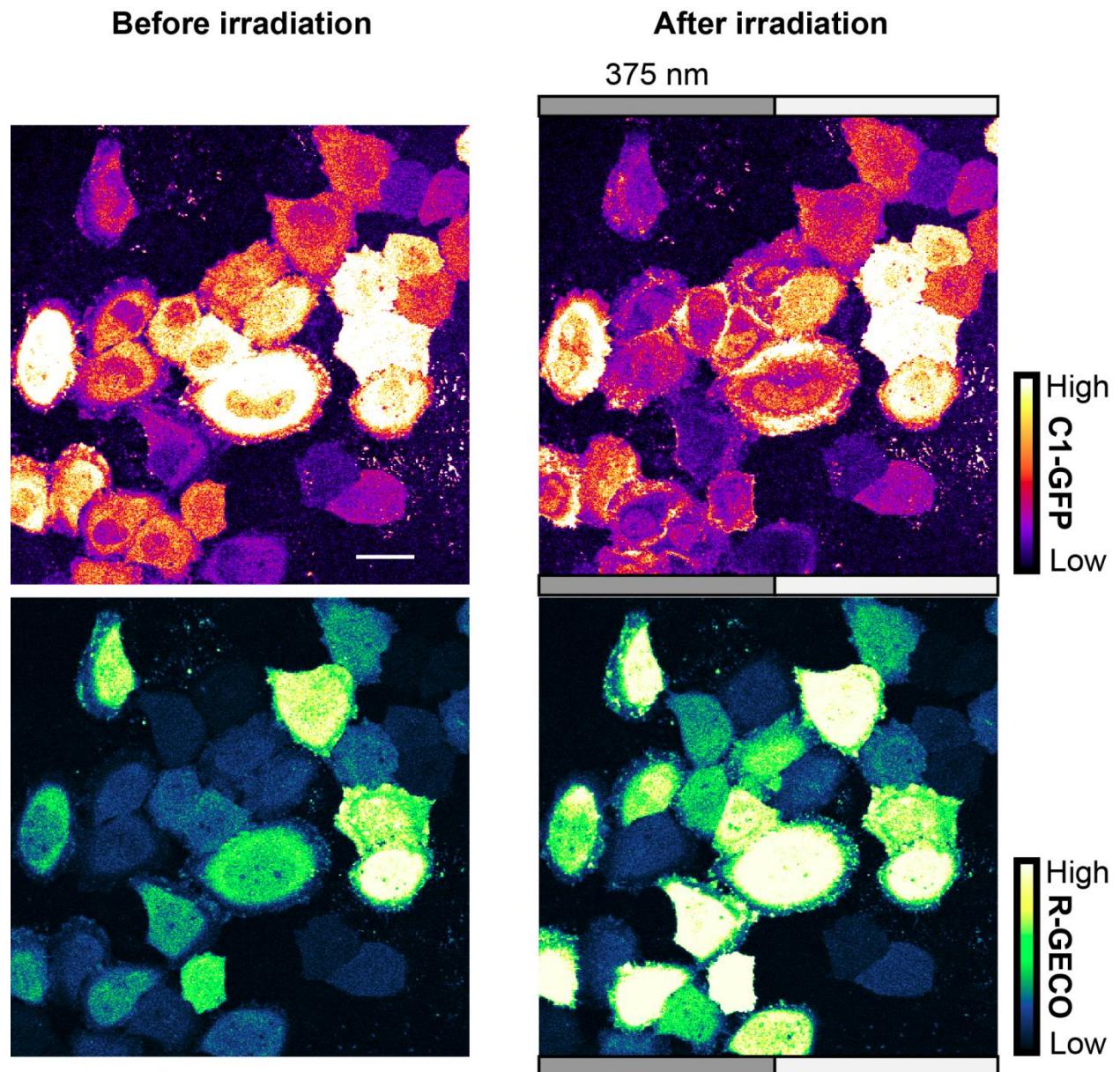


**Supplementary Figure 1 | Optical control of C1-GFP translocation.** In HeLa cells expressing C1-GFP, **PhoDAG-1** (150  $\mu$ M) triggered the translocation of C1-GFP towards the plasma membrane on irradiation with  $\lambda = 375$  nm laser light. Scale bar = 20  $\mu$ M.

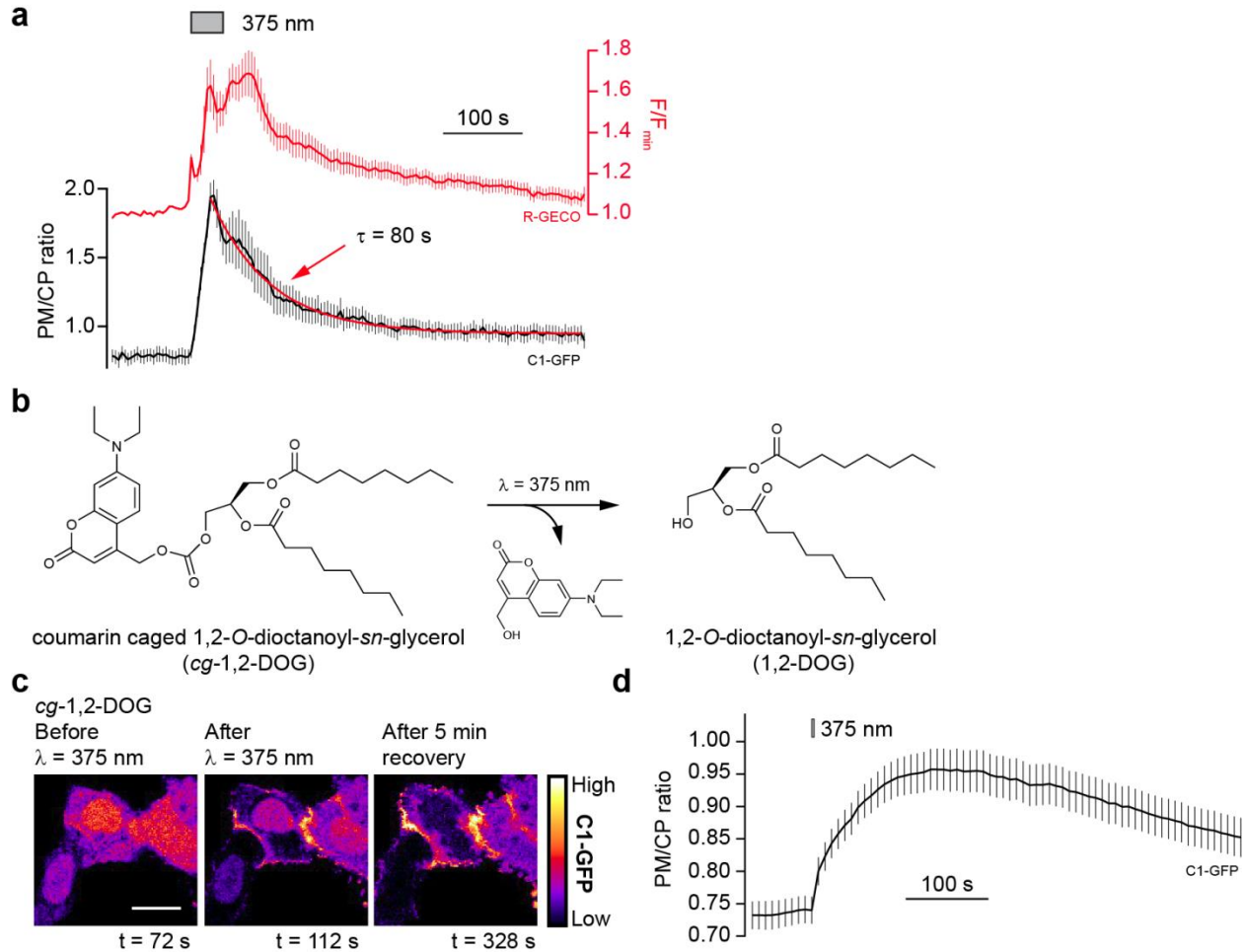


**Supplementary Figure 2 | Quantification of C1-GFP translocation.** (a) A representative fluorescence image detailing the cytoplasmic (CP) and plasma membrane (PM) sample regions used for translocation quantification. Displayed here is C1-GFP fluorescence after the application of **PhoDAG-1** (400  $\mu$ M) and  $\lambda = 375$  nm irradiation. Scale bar = 20  $\mu$ M. (b) The GFP fluorescence intensities at the plasma membrane and cytoplasm of a representative cell after the application of **PhoDAG-1** (200  $\mu$ M).

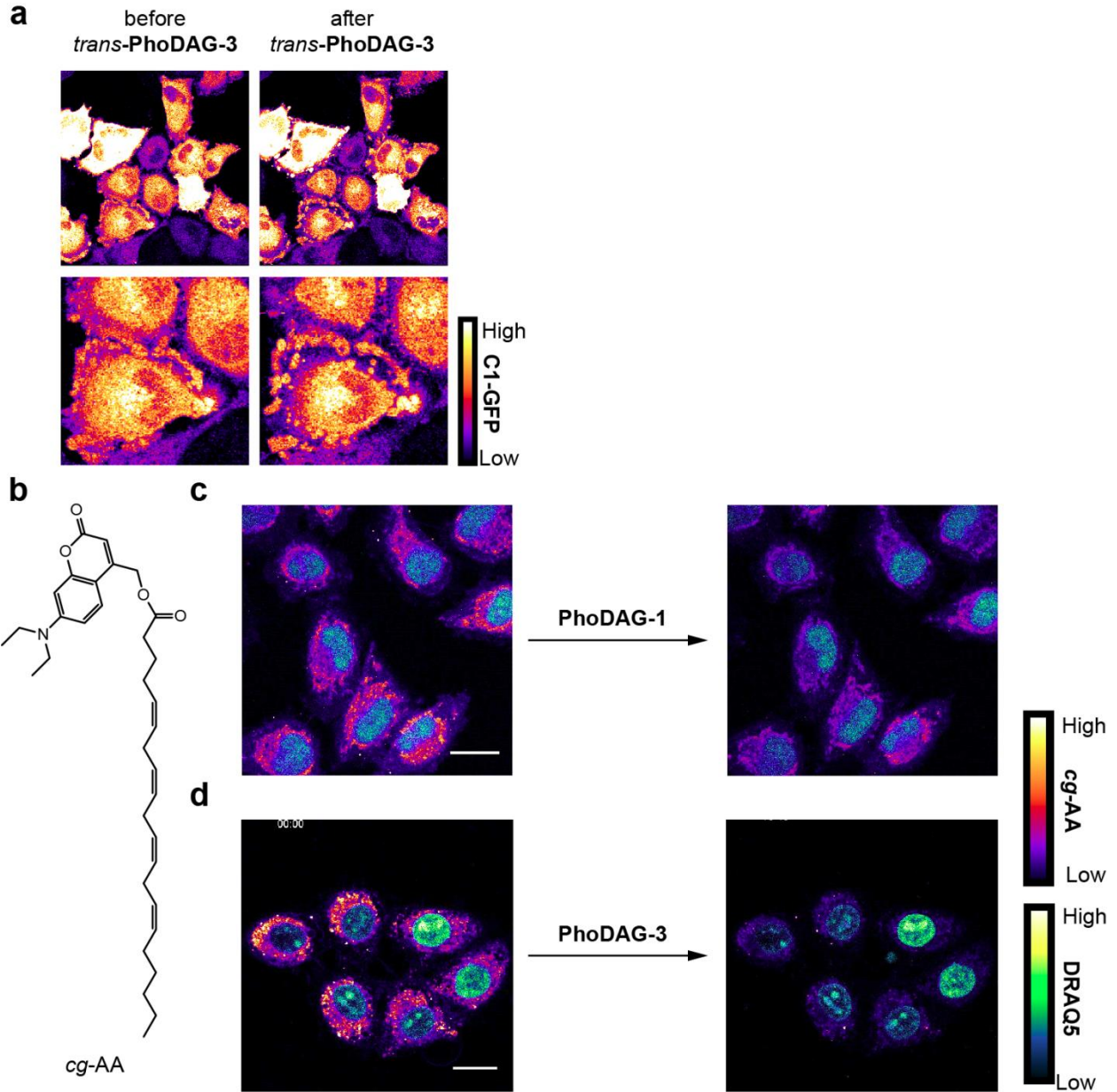




**Supplementary Figure 3 | PhoDAG-1 enables spatial control of DAG signaling.** In HeLa cells expressing C1-GFP and R-GECO, **PhoDAG-1** (150  $\mu\text{M}$ ) activation could be targeted to specific cells. In this case, only the left half of the field of view (gray bar) was photoactivated with  $\lambda = 375$  nm irradiation. In these cells, C1-GFP translocated towards the plasma membrane, alongside an increase in intracellular  $\text{Ca}^{2+}$  levels. The right half of the frame remained largely unaffected (white bar). Scale bar = 20  $\mu\text{M}$ .

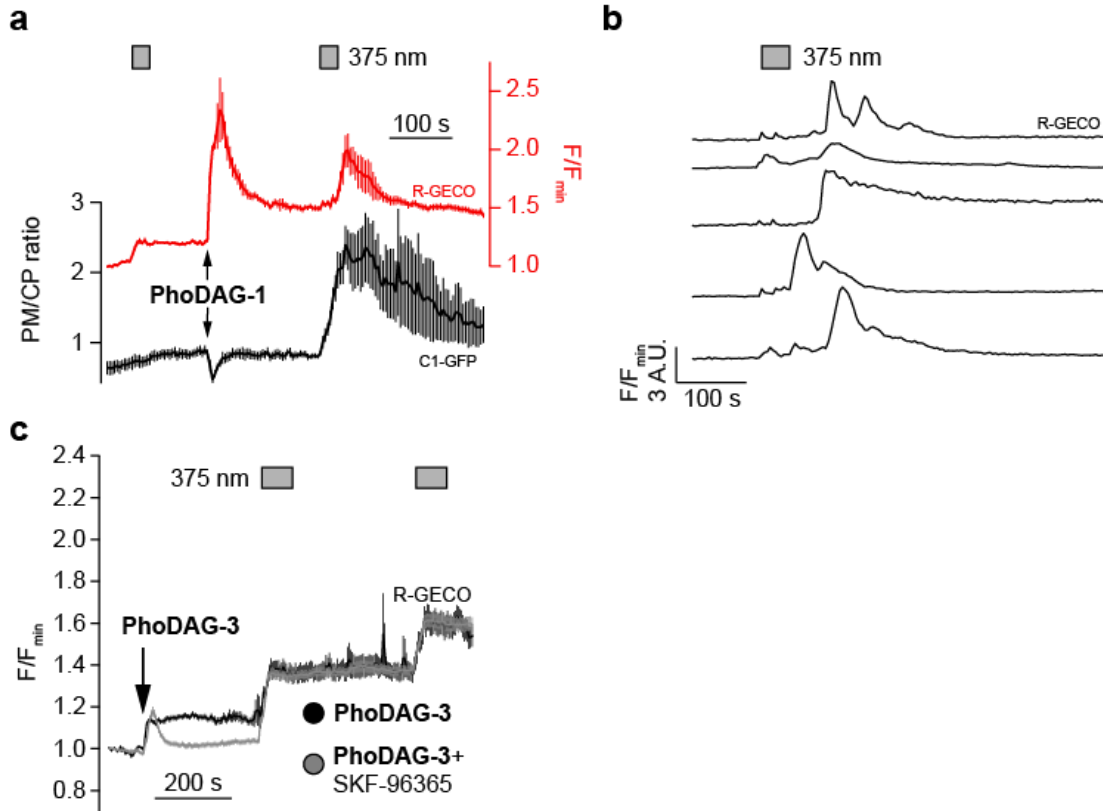


**Supplementary Figure 4 | Optical control of C1-GFP translocation in HeLa cells.** (a) HeLa cells expressing C1-GFP and R-GECO were incubated **PhoDAG-1** (150  $\mu\text{M}$ ) followed by washing with buffer to remove extracellular compound. Translocation of C1-GFP towards the plasma membrane was observed on irradiation with  $\lambda = 375$  nm light. An increase in intracellular  $\text{Ca}^{2+}$  was also observed in the same cells ( $n = 12$ ). (b) The chemical structure of 1,2-*O*-dioctanoyl-*sn*-glycerol (1,2-DOG) being liberated from caged 1,2-DOG (*cg*-1,2-DOG) on irradiation with  $\lambda = 375$  nm laser light. (c,d) The uncaging of *cg*-1,2-DOG (100  $\mu\text{M}$ ) led to C1-GFP translocation towards the plasma membrane in HeLa cells. After uncaging, the recovery was relatively slow. Shown are (c) fluorescence images of two representative cells, and (d) data averaged from multiple cells ( $n = 25$ ), displayed as the plasma membrane to cytoplasm (PM/CP) fluorescence ratio. Error bars were calculated as s.e.m. Scale bar = 20  $\mu\text{M}$ .

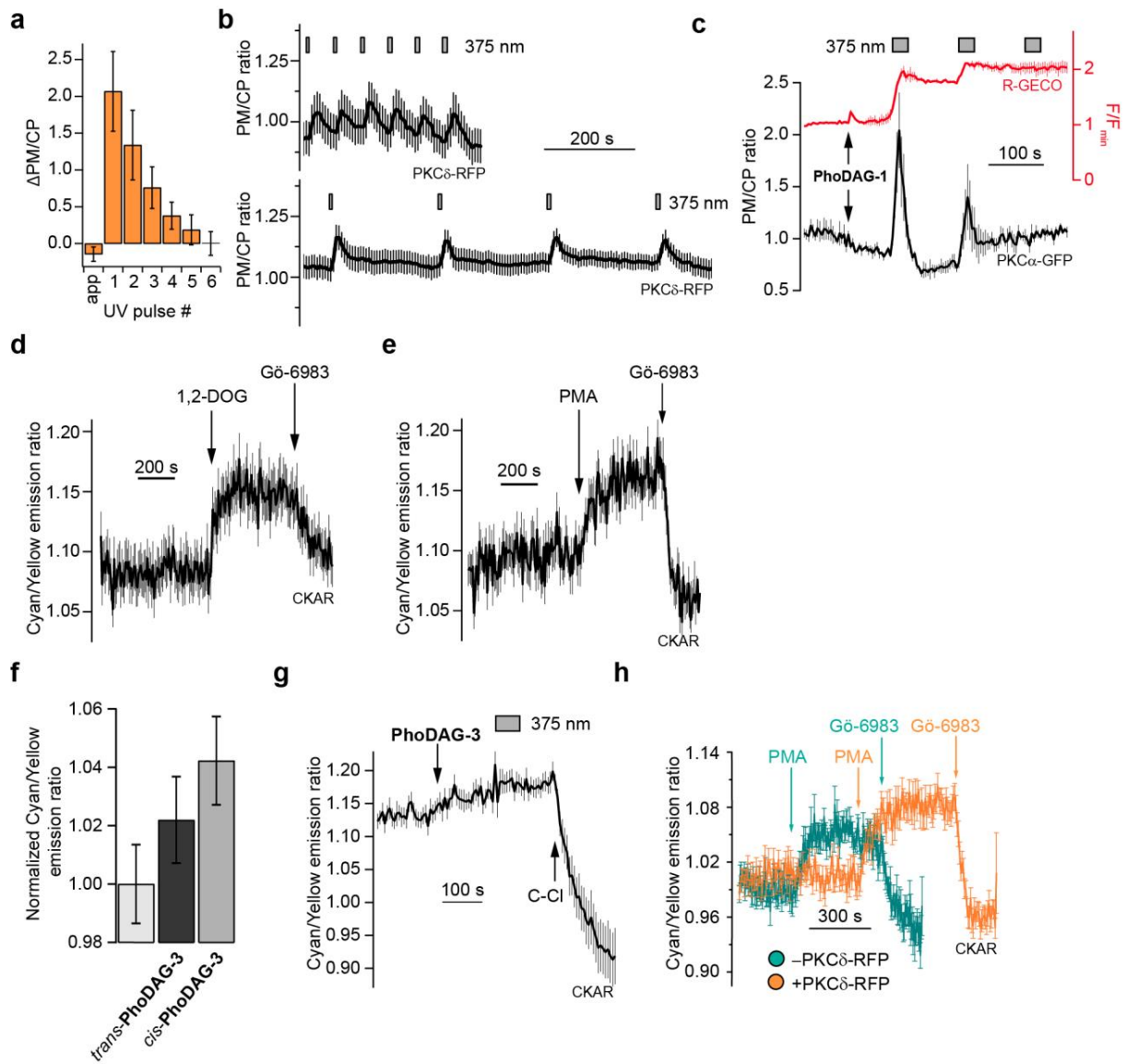


**Supplementary Figure 5 | Lipid chain length determines the localization and activity of the PhoDAGs.** (a) In HeLa cells, *trans*-PhoDAG-3 (150  $\mu$ M) caused translocation of C1-GFP towards internal membranes on application. (b) The chemical structure of coumarin-labeled arachidonic acid (cg-AA), which localizes in internal membranes. (c,d) Representative fluorescence images showing the localization of cg-AA (100  $\mu$ M, fire) before (left) and after (right) the addition of (c) **PhoDAG-1** (150  $\mu$ M) or (d) **PhoDAG-3** (150  $\mu$ M). **PhoDAG-3** quenched the coumarin fluorescence significantly more quickly than **PhoDAG-1**. Cell nuclei were stained with DRAQ-5 (green) as a reference. Scale bar = 20  $\mu$ M.

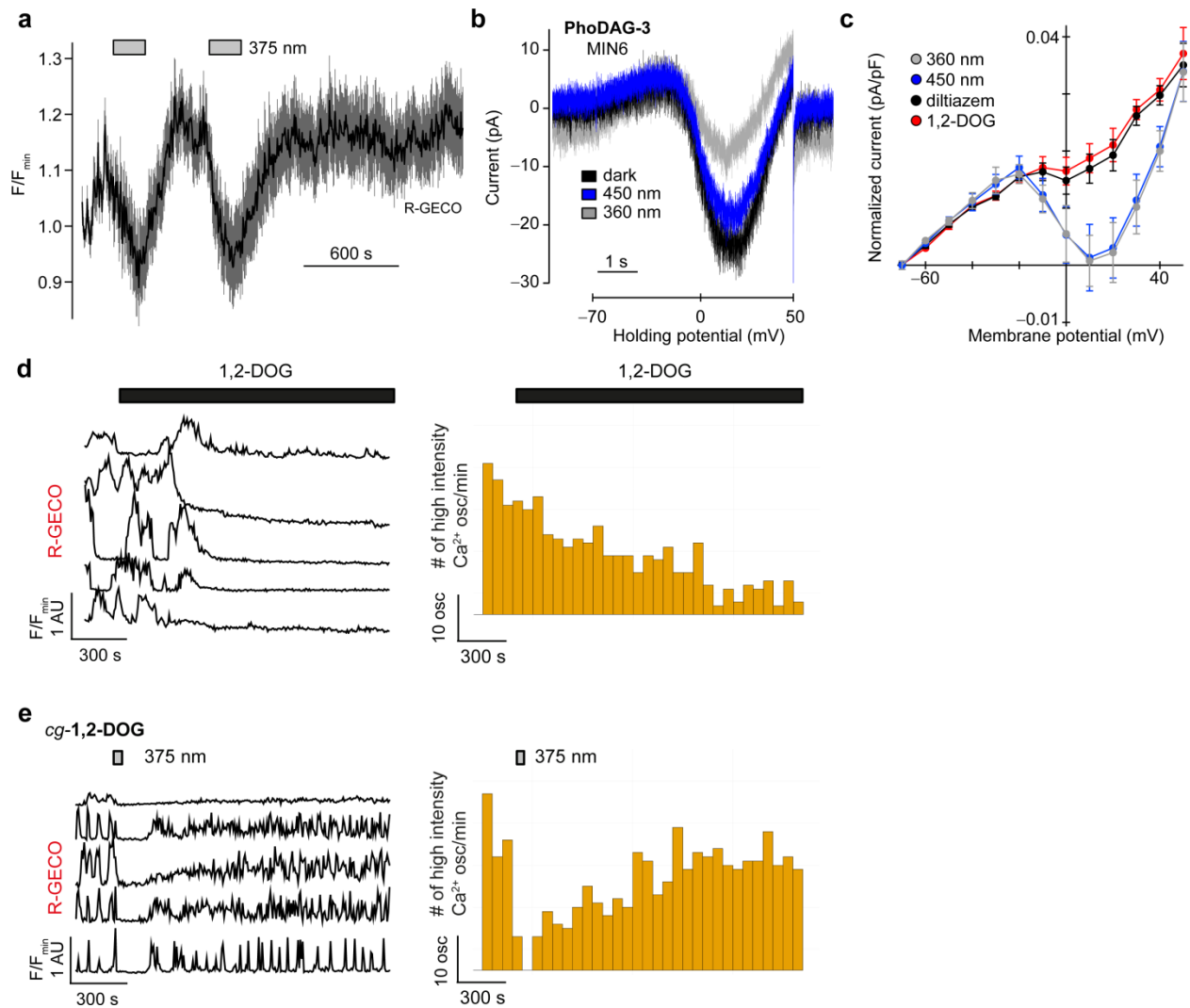




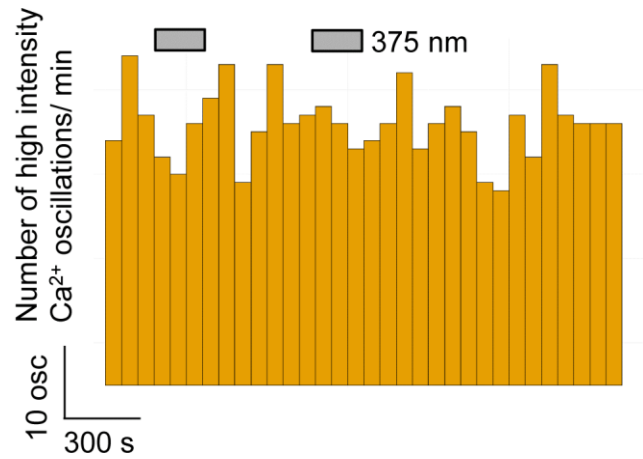
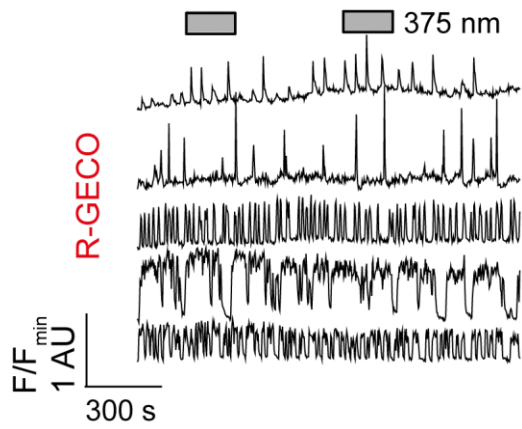
**Supplementary Figure 6 | PhoDAG-1 induces an increase in intracellular  $\text{Ca}^{2+}$  in HeLa cells.** HeLa cells were transfected with C1-GFP and R-GECO. (a) Before the application of **PhoDAG-1**, UV-A irradiation alone did not catalyze C1-GFP translocation. Application of *trans*-**PhoDAG-1** (300  $\mu\text{M}$ ) caused an increase in intracellular  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_i$ ) levels, however no translocation was observed until triggered by  $\lambda = 375$  nm irradiation ( $n = 6$ ). (b) On photoactivation, a small increase in  $[\text{Ca}^{2+}]_i$  was observed, followed by a delayed, larger  $\text{Ca}^{2+}$  increase, displayed as representative traces from individual cells. (c) The TRPC channel inhibitor SKF-96365 (50  $\mu\text{M}$ ) slightly decreased the  $\text{Ca}^{2+}$  influx after application of **PhoDAG-3** (150  $\mu\text{M}$ ), but not on photoactivation ( $n = 54$ , gray), when compared to **PhoDAG-3** alone ( $n = 16$ , black). Translocation is represented as the plasma membrane to cytoplasm (PM/CP) C1-GFP fluorescence ratio, while  $\text{Ca}^{2+}$  levels were plotted as the R-GECO fluorescence intensity normalized to the baseline fluorescence ( $F/F_{\text{min}}$ ). Error bars were calculated as s.e.m.



**Supplementary Figure 7 | Optical control of protein kinase C.** (a) In HeLa cells expressing PKC $\delta$ -RFP, translocation efficiency induced by **PhoDAG-1** (100  $\mu$ M) decayed over multiple cycles of irradiation of the same length (28 s pulses at  $\lambda = 375$  nm, n = 11). (b) Changing the time between UV-A pulses did not affect the magnitude of the translocation. Both the 60 s (n = 13) and 240 s (n = 15) intervals showed similar efficiencies. (c) PKC $\alpha$ -GFP translocated towards the plasma membrane on isomerization to *cis*-**PhoDAG-1** (150  $\mu$ M) (n = 3). Sequential photostimulations led to diminished efficiency, corresponding to a reduced Ca<sup>2+</sup> response. [Ca<sup>2+</sup>]<sub>i</sub> levels (R-GECO) were displayed as the RFP fluorescence intensity and normalized to the baseline fluorescence ( $F/F_{min}$ ). (d,e) As shown by the cytosolic C Kinase Activity Reporter (CKAR), the application of (d) 1,2-DOG (300  $\mu$ M, n = 32) and (e) phorbol 12-myristate 13-acetate (PMA, 5  $\mu$ M, n = 31) produced an increase in the CFP/YFP emission ratio, indicating kinase activation. In both cases, the FRET change was immediately reversed on addition of the broadband PKC inhibitor Gö-6983 (10  $\mu$ M). (f,g) **PhoDAG-3** (15  $\mu$ M) caused an increase in the CFP/YFP emission ratio on application, and a further increase on photoactivation. This effect was reversed by the application of chelerythrine chloride (C-Cl, 100  $\mu$ M) (n = 14). (h) The CKAR response in HeLa cells was compared between cells with (n = 37 orange) and without (n = 20, teal) the expression of exogenous PKC $\delta$ -RFP. Overexpression of PKC $\delta$ -RFP increased the CKAR response by 1.2 fold when compared to endogenous PKC expression only. Error bars were calculated as s.e.m.

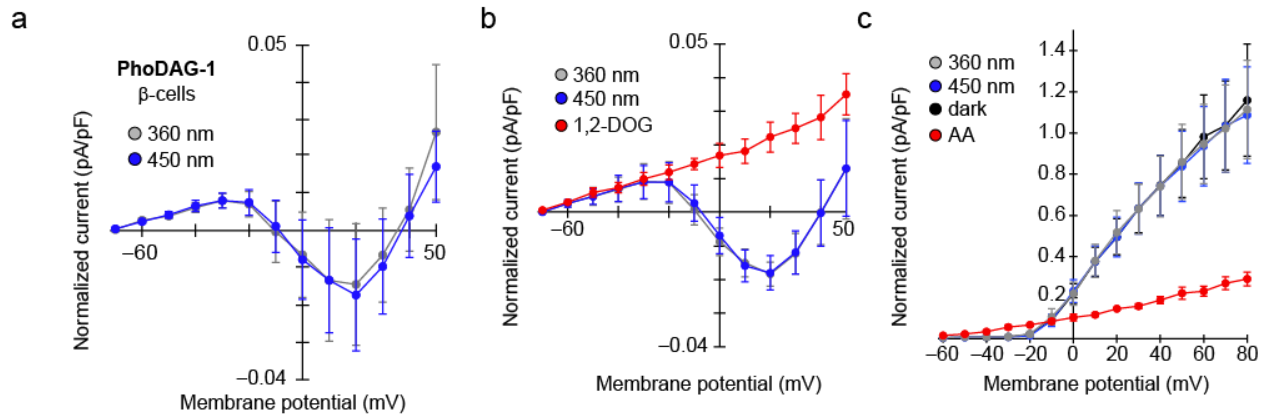


**Supplementary Figure 8 | Diacylglycerol generation decreases  $\text{Ca}^{2+}$  oscillations in MIN6 cells.**  $\text{Ca}^{2+}$  oscillations in glucose stimulated (20 mM) MIN6 cells were monitored using R-GECO (a) **PhoDAG-1** (300  $\mu\text{M}$ ) caused a decrease in the overall  $[\text{Ca}^{2+}]_i$  level on photoactivation. Data was averaged over multiple cells ( $n = 23$ ). (b) **PhoDAG-3** (35  $\mu\text{M}$ ) triggered a decrease in the whole-cell voltage activated L-type  $\text{Ca}^{2+}$  channel ( $\text{Ca}_v$ ) current, as shown by voltage ramps ( $-70$  to  $+50$  mV over 5 s) from a representative cell. (c) UV-A or blue irradiation alone did not affect the  $\text{Ca}_v$  current ( $n = 8$ ), while the application of 1,2-DOG (100  $\mu\text{M}$ ,  $n = 3$ ) and the L-type  $\text{Ca}^{2+}$  channel blocker diltiazem (500  $\mu\text{M}$ ,  $n = 3$ ) decreased the magnitude of the whole-cell  $\text{Ca}_v$  current. (d) The application of 1,2-*O*-dioctanoyl-*sn*-glycerol (1,2-DOG, 100  $\mu\text{M}$ ) led to a slow and permanent decrease in the frequency and intensity of  $\text{Ca}^{2+}$  oscillations ( $n = 25$ ). (e) After the uncaging of caged 1,2-DOG (*cg*-1,2-DOG, 100  $\mu\text{M}$ ), the frequency and intensity of the  $\text{Ca}^{2+}$  oscillations was immediately reduced, but recovered gradually over 20-30 min ( $n = 47$ ). Bar graphs represent the number of detected high intensity  $\text{Ca}^{2+}$  oscillations ( $>50\%$  of highest transient in each trace) within every 60 s interval. Error bars were calculated as s.e.m.

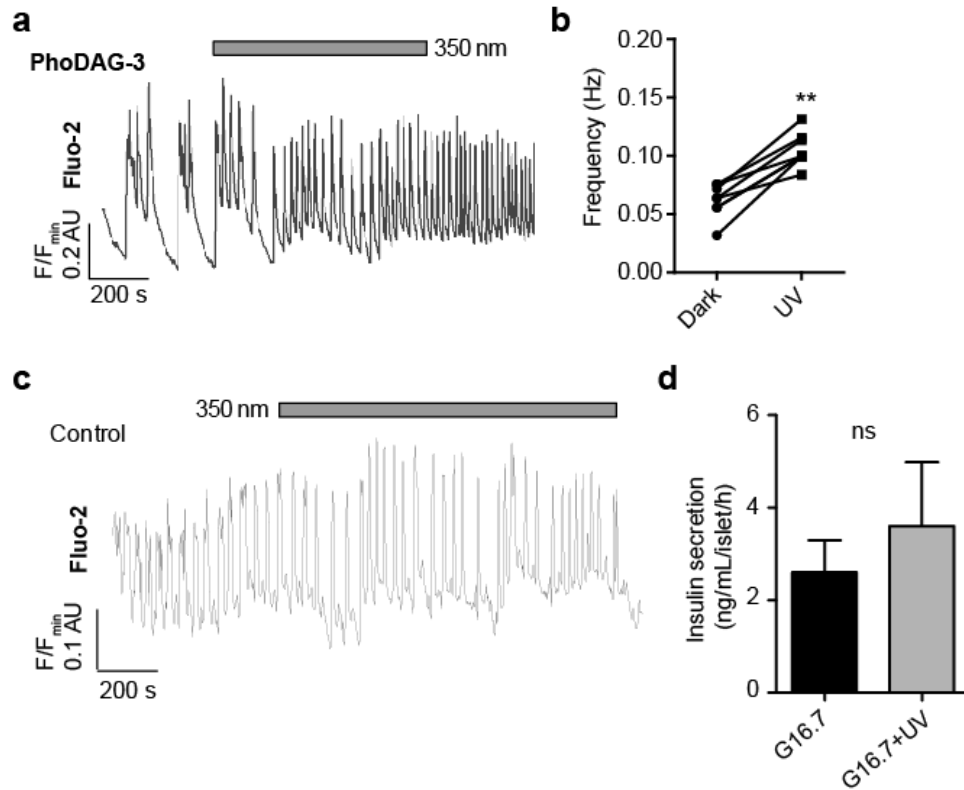


**Supplementary Figure 9 | UV-A illumination of MIN6 cells alone has no significant effect on Ca<sup>2+</sup> oscillations.** Ca<sup>2+</sup> oscillations in glucose-stimulated (20 mM) MIN6 cells were monitored using R-GECO. Shown are individual Ca<sup>2+</sup> traces from representative cells (left), and a statistical analysis of the oscillation frequency averaged over multiple cells (right). Bar graphs represent the number of detected high intensity Ca<sup>2+</sup> oscillations (>50% of highest transient in each trace) within every 60 s interval.

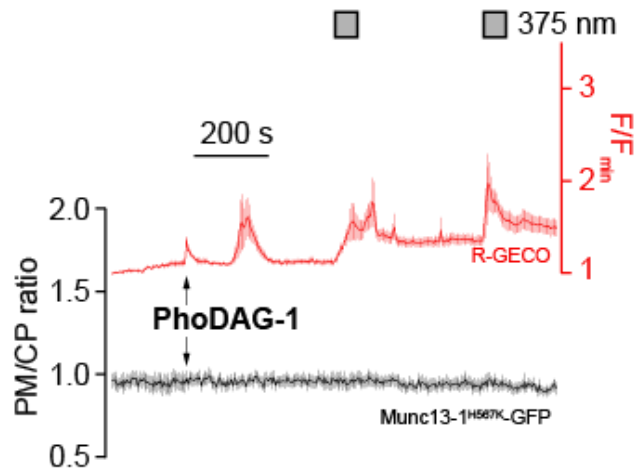




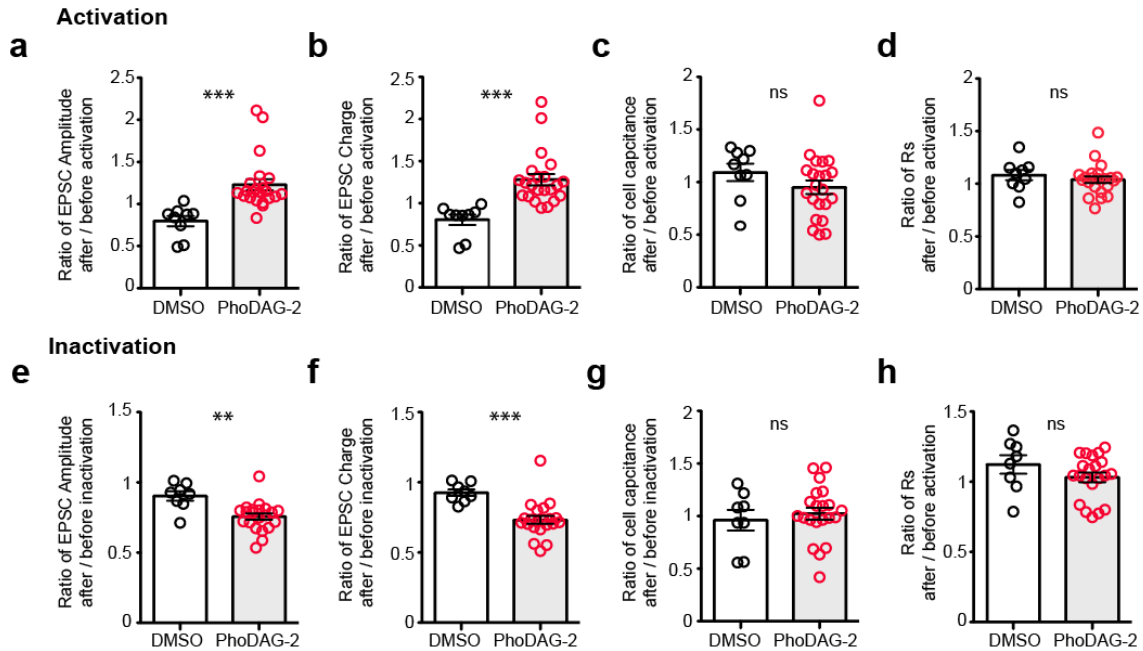
**Supplementary Figure 10 | Control experiments for electrophysiological studies in dissociated  $\beta$ -cells.** In primary mouse  $\beta$ -cells: **(a)** PhoDAG-1 (200  $\mu$ M) had no effect on the  $\text{Ca}_v$  current ( $n = 5$ ). **(b)** UV-A or blue-irradiation did not affect the  $\text{Ca}_v$  current ( $n = 8$ ), while the application of 1,2-DOG (15  $\mu$ M) decreased the magnitude of the  $\text{Ca}_v$  current ( $n = 3$ ). **(c)** UV-A or blue-irradiation also did not affect the delayed rectifier voltage-gated  $\text{K}^+$  channel ( $\text{K}_v$ ) current ( $n = 4$ ). Arachidonic acid (AA, 10  $\mu$ M) decreased the magnitude of the  $\text{K}_v$  current ( $n = 4$ ). Error bars were calculated as s.e.m.



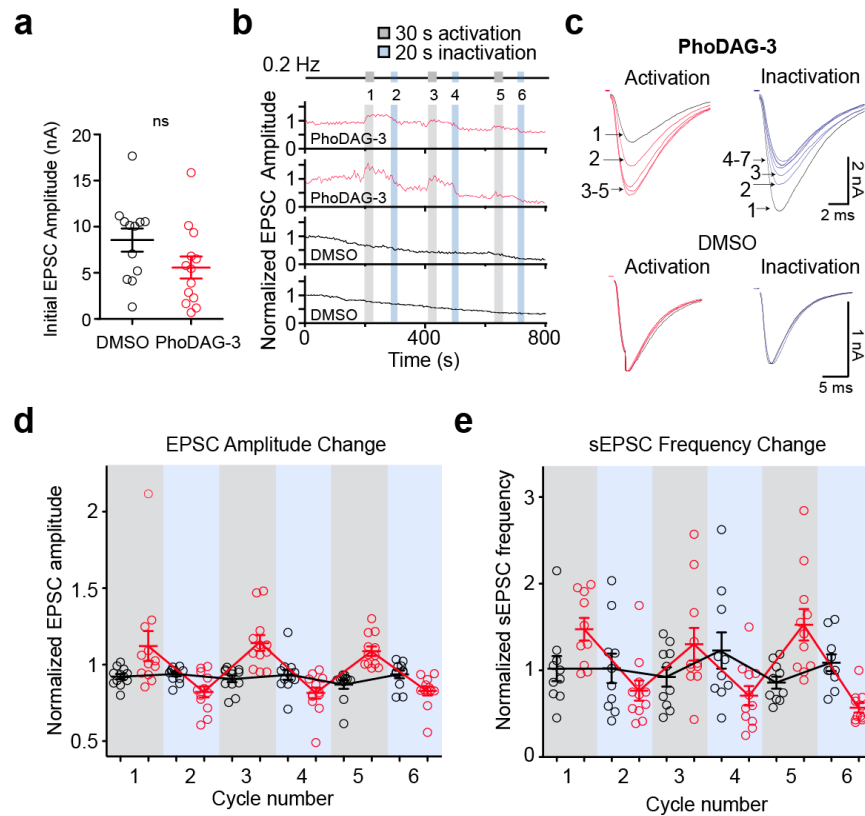
**Supplementary Figure 11 | UV-A irradiation alone does not alter glucose-stimulated pancreatic islet activity.** Ca<sup>2+</sup> oscillations in intact mouse pancreatic islets were monitored by Fluo-2. **(a,b)** Photoactivation of **PhoDAG-3** (100 μM) triggered an increase in the oscillation frequency, displayed as **(a)** a representative trace from a single islet and **(b)** the average oscillation frequencies for several islets before and after λ = 350 nm irradiation (n = 8). **(c,d)** UV-A irradiation (λ = 350 nm) alone did not affect **(c)** the speed of the Ca<sup>2+</sup> oscillations in islets stimulated by 11 mM glucose or **(d)** the insulin secretion stimulated by 16.7 mM glucose (G16.7) (n = 3 experiments from 3 animals). ns = not significant P>0.05, P>0.05. Error bars were calculated as s.e.m.



**Supplementary Figure 12 | A C1 domain point mutant abolishes the sensitivity of Munc13-1 to PhoDAG-1.** In HeLa cells, The DAG-insensitive Munc13-1<sup>H567K</sup>-GFP mutant translocation reporter did not translocate on the application of **PhoDAG-1** (150  $\mu$ M) or after photoactivation with  $\lambda = 375$  nm light, even though an increase in  $[Ca^{2+}]_i$  levels was observed (n = 13).



**Supplementary Figure 13 | PhoDAG-2 application does not affect the pipette series resistance in patch-clamp experiments.** PhoDAG-2 and PhoDAG-3 are lipid compounds, and therefore might affect the series resistance of the patch pipette, which could lead to artifacts in the EPSC amplitude. Moreover, the compound integrates in the cell membrane where it might lead to changes in the cell size or membrane conductance. To exclude these effects, we performed a control experiment with **PhoDAG-2** and monitored series resistance and membrane capacitance, before and after illumination (n represents the number of illumination cycles; DMSO, black, for activation n = 9, for inactivation n = 8; **PhoDAG-2**, red, for activation n = 22, for inactivation n = 21). (a) The ratio of the change in the EPSC amplitude was calculated by dividing the average amplitude after photoactivation, by that before photoactivation. (b) The ratio of the change in EPSC charge was calculated by dividing the average charge after activation by that before activation. (c) The ratio of the change in the cell capacitance, a measure of the cell body size and conductance, was calculated by dividing the average capacitance after activation by that before activation. (d) The ratio of the change in series resistance was calculated by dividing the average capacitance after activation by that before activation. (e) The ratio of the change in EPSC amplitude after inactivation. (f) The ratio of the change in EPSC charge after inactivation (g) The ratio of the change in cell capacitance after inactivation. (h) The ratio of the change in series resistance after inactivation. Error bars were calculated as s.e.m.; ns = not significant  $P > 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .



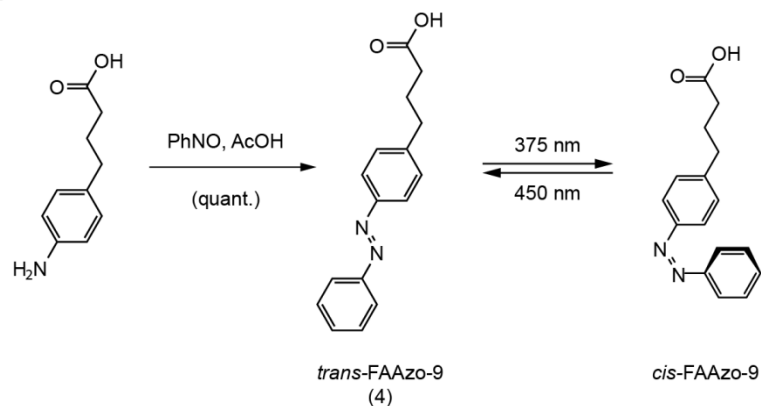
### Supplementary Figure 14 | PhoDAG-3 modulates synaptic transmission in hippocampal neurons.

WT mouse hippocampal neurons were pre-incubated at 37 °C in the extracellular recording solution containing 500  $\mu$ M **PhoDAG-3** or DMSO. The neurons were then whole-cell voltage clamped at room temperature and stimulated at 0.2 Hz. The evoked EPSCs were monitored. (a) The initial EPSC amplitudes measured in control neurons (black, n = 12) or in neurons where *trans*-**PhoDAG-3** was applied (red, n = 13) are not significantly different. (b) The normalized EPSC amplitude is plotted for two neurons preincubated with **PhoDAG-3** (red) and for two control neurons (black). (c) Example traces of EPSCs during activation and inactivation for **PhoDAG-3**. (d) Activation and inactivation by **PhoDAG-3** could be repeated over several cycles, corresponding to an increase and decrease in the EPSC amplitude, respectively (red = **PhoDAG-3**, n = 13; black = vehicle, n = 12). (e) The sEPSC frequency could also be increased by photoactivation with UV-A light, and decreased by inactivation with blue light (red = **PhoDAG-3**, n=11; black = vehicle, n=10). Data from individual neurons are presented by open circles, summaries are presented as mean  $\pm$  s.e.m. The Mann-Whitney test was used to determine statistical significance. ns = not significant P>0.05.

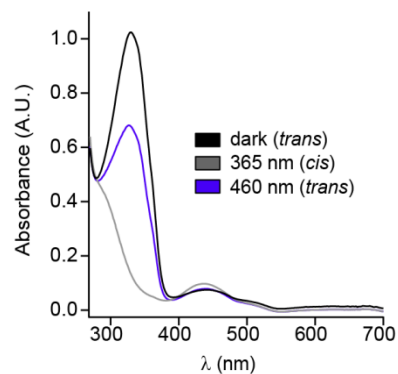


## SUPPLEMENTARY NOTES

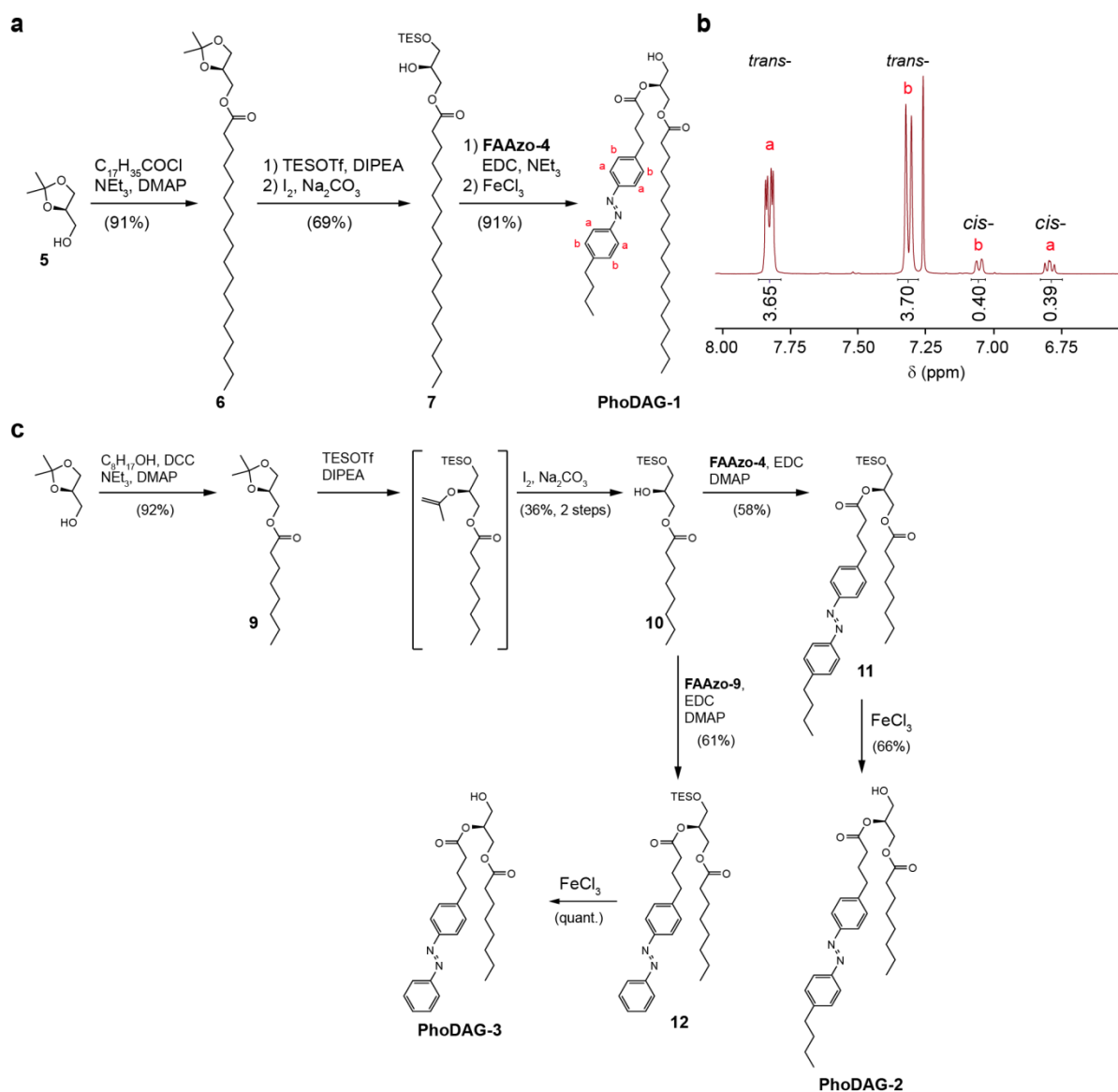
**a**



**b**



**Supplementary Note 1 | Synthesis and characterization of a short-chain photoswitchable fatty acid.** (a) The chemical synthesis of **FAAzo-9 (4)**. (b) UV-Vis spectroscopy showed that **FAAzo-9** (50  $\mu$ M in DMSO) could be isomerized between its *cis*- and *trans*-configurations with UV-A and blue light, respectively. Absorption spectra are shown for the *dark*-adapted (black), *UV*-adapted (grey) and *blue*-adapted (blue) photostationary states.

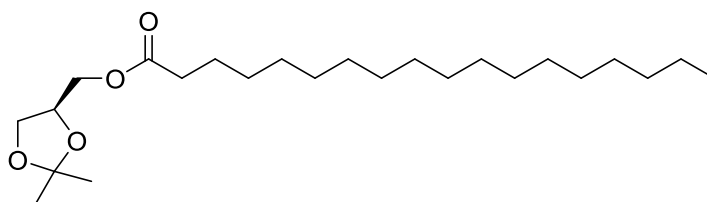


### Supplementary Note 2 | Synthesis and characterization of the photoswitchable diacylglycerols.

(a) **PhoDAG-1** was synthesized in four steps and 57% overall yield. (b) A representative NMR spectrum of **PhoDAG-1** (in  $\text{CDCl}_3$ ), displaying the aromatic region. Both the *trans*- and *cis*- azobenzene protons give signals under ambient lighting conditions, where **PhoDAG-1** exists as  $\approx 10\%$  the *cis*-isomer. (c) The chemical syntheses of the short-chain photoswitchable DAGs, **PhoDAG-2** and **PhoDAG-3**.

## Supplementary Note 3 | Synthetic Procedures

### Synthesis of **S-2,3-O-isopropylidene-1-O-stearoyl-*sn*-glycerol (6)**



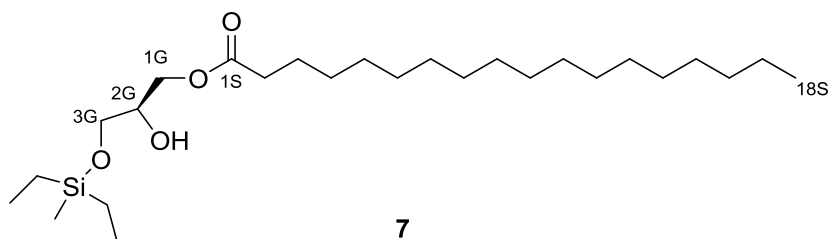
6

**S-2,3-O-Isopropylidene-1-O-stearoyl-*sn*-glycerol (6)** was prepared using a modified procedure as previously described by Gaffney *et al.*<sup>67</sup> Spectral characteristics matched those previously reported<sup>68</sup>. **R(-)-2,3-O-Isopropylidene-*sn*-glycerol** (2.85 g, 22.0 mmol, 1.0 equiv.) and 4-(dimethylamino)pyridine (DMAP, 268 mg, 2.20 mmol, 0.1 equiv.) were dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (75 mL) under an argon atmosphere. To this solution was added dry NEt<sub>3</sub> (6.16 mL, 4.48 g, 44.0 mmol, 2.0 equiv.) and the reaction was cooled to 0 °C. Stearoyl chloride was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (25 mL) and slowly added to the previously prepared solution. The reaction was allowed to slowly warm to room temperature and stirred for 2 h. H<sub>2</sub>O (28 mL) was slowly added and the biphasic solution was stirred rapidly for 10 min. The phases were then separated, and the organic phase was washed with aqueous HCl (2 M, 50 mL), followed by saturated aqueous NaHCO<sub>3</sub> (2x50 mL) and brine (2x50 mL) solutions. The organic phase was then dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and filtered. The filtrate was then concentrated and purified by flash silica gel chromatography (150 g SiO<sub>2</sub>, 20:1 hexane:EtOAc) to yield **S-2,3-O-isopropylidene-1-O-stearoyl-*sn*-glycerol (6)**, 6.99 g, 81%) as an off-white solid.

**TLC (20:1 hexanes:EtOAc):** R<sub>f</sub> = 0.25.

**<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz, 25 °C):** δ 4.35–4.28 (m, 1 H, H<sub>2G</sub>), 4.17 (dd, 1 H, HG<sub>1a</sub>, J = 11.7, 4.7 Hz), 4.12–4.05 (m, 2 H, HG<sub>1b</sub>, HG<sub>3a</sub>), 3.75 (dd, 1H, H<sub>3b</sub>, J = 8.4, 6.2 Hz) 2.34 (t, 2 H, H<sub>2L<sub>a,b</sub></sub>, J = 7.4 Hz), 1.67–1.58 (m, 2 H, H<sub>3L<sub>a,b</sub></sub>), 1.43 (s, 3 H, H<sub>CH<sub>3</sub></sub>), 1.37 (s, 3 H, H<sub>CH<sub>3</sub></sub>), 1.33–1.21 (m, 28 H, H<sub>alk</sub>), 0.87 (t, 3 H, H<sub>8L<sub>a,b,c</sub></sub>, J = 6.5 Hz).

### Synthesis of 1-O-stearoyl-3-O-triethylsilyl-*sn*-glycerol (7)



**1-O-stearoyl-3-O-triethylsilyl-*sn*-glycerol (7)** was prepared using a modified procedure as previously described by Nadler *et al.*<sup>15</sup> Spectral characteristics matched those previously reported.

**S-2,3-O-Isopropylidene-1-O-stearoyl-*sn*-glycerol (6)**, 500 mg, 1.25 mmol, 1.0 equiv.) was dissolved in dry 1,2-dichloroethane (DCE, 20 mL) under an argon atmosphere. *N,N*-Diisopropylethylamine (DIPEA, 1.20 mL, 6.88 mmol), followed by triethylsilyl trifluoromethanesulfonate (TESOTf, 496 mg, 0.425 mL, 1.88 mmol, 1.5 equiv.) were added at room temperature and the reaction was stirred at 90 °C. After 1 h, a second portion of TESOTf (169 mg, 0.145 mL, 0.5 equiv.) was added and the reaction was stirred at 90 °C for 2.5 h. Upon consumption of the starting material as determined by TLC analysis, the solution was cooled to room temperature, diluted with EtOAc, and then washed with aqueous hydrochloric acid (0.1 M, 50 mL) and brine (50% saturated, 2x50 mL). The organic phase was concentrated under reduced pressure and the resulting oil was dissolved in THF (20 mL). To this solution was added an aqueous Na<sub>2</sub>CO<sub>3</sub> solution (10%, 10 mL) followed by I<sub>2</sub> (610 mg, 2.4 mmol, 1.95 equiv.). The solution was stirred rapidly for 2.5 h at room temperature. A further portion of I<sub>2</sub> was added (300 mg, 1.2 mmol, 1.0 equiv.) and the solution was stirred at room temperature for 1 h. The solution was diluted with EtOAc (50 mL) and washed with saturated aqueous Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (50 mL), water (2x50 mL) and brine (50 mL) solutions. The organic phase was then dried over anhydrous sodium sulfate and filtered. The filtrate was concentrated under reduced pressure and the resulting oil was purified by flash column chromatography (50 g SiO<sub>2</sub>, 20:1 hexanes:EtOAc) to yield **1-O-stearoyl-3-O-triethylsilyl-*sn*-glycerol (7)**, 412 mg, 69%) as a colorless liquid.

**TLC (10:1 hexanes:EtOAc):**  $R_f = 0.23$ .

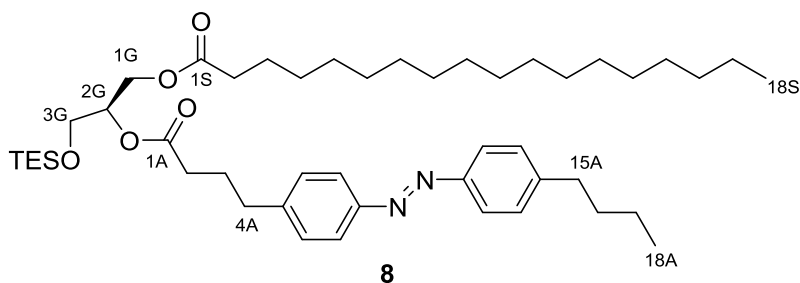
**$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz, 25 °C):**  $\delta$  4.18 (m, 2 H, H1G<sub>a,b</sub>), 3.92–3.82 (m, 1 H, H2G), 3.67 (dd, 1 H, H3G<sub>a</sub>,  $J = 10.3$  Hz, 4.5 Hz), 3.60 (dd, 1 H, H3G<sub>b</sub>,  $J = 10.0$  Hz, 5.7 Hz), 2.55 (d, 1 H, OH,  $J = 5.3$  Hz), 2.33 (t, 2 H, H2S<sub>a,b</sub>,  $J = 7.6$  Hz), 1.66–1.58 (m, 2 H, H3S<sub>a,b</sub>), 1.34–1.20 (m, 28 H, 14xCH<sub>2</sub>(stearyl)), 0.96 (t, 9 H, 3xCH<sub>3</sub>(TES),  $J = 7.8$  Hz), 0.88 (t, 3 H, H18S<sub>a,b,c</sub>,  $J = 6.9$  Hz), 0.61 (q, 6 H, 3xCH<sub>2</sub>(TES),  $J = 7.9$  Hz).

**$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz, 25 °C):**  $\delta$  174.1 (C1S), 70.2 (C2G), 65.1 (C1G), 63.5 (C3G), 34.3 (C2S), 32.1 (C<sub>alk</sub>), 29.9-29.7 (m, C<sub>alk</sub>), 29.6 (C<sub>alk</sub>), 29.5 (C<sub>alk</sub>), 29.4 (C<sub>alk</sub>), 29.3 (C<sub>alk</sub>), 25.1 (C3S), 22.9 (C<sub>alk</sub>), 14.3 (C18S), 6.8 (3C, 3xCH<sub>3</sub>(TES)), 4.4 (3C, 3xCH<sub>2</sub>(TES)).

**HRMS (ESI<sup>+</sup>):**  $m/z$  calcd. for  $[\text{C}_{27}\text{H}_{56}\text{NaO}_4\text{Si}]^+$ : 495.3846, found: 495.3850 ( $[\text{M}+\text{Na}]^+$ ).



## Synthesis of 2-O-(4-(4-((4-butylphenyl)diazenyl)phenyl)butanoyl)-1-O-stearoyl-3-O-triethylsilyl-*sn*-glycerol (**8**)



**FAAzo-4**<sup>19</sup> (1.08 g, 3.3 mmol, 2.0 equiv.), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide (EDC, 773 mg, 4.38 mmol, 3.0 equiv.) and DMAP (20.2 mg, 166  $\mu$ mol, 0.1 equiv.) were dissolved in dry  $\text{CH}_2\text{Cl}_2$  (50 mL) under an argon atmosphere. This solution was stirred at room temperature for 15 min. After cooling to 0  $^\circ\text{C}$ , a solution of **1-O-stearoyl-3-O-triethylsilyl-*sn*-glycerol** (**7**, 787 mg, 1.66 mmol, 1.0 equiv.) in dry  $\text{CH}_2\text{Cl}_2$  (30 mL) was slowly added. The solution was warmed to room temperature and stirred overnight under an argon atmosphere. The solution was then diluted with  $\text{CH}_2\text{Cl}_2$  (200 mL) and washed with water (2x100 mL) and brine (100 mL). The solution was then filtered and the filtrate was concentrated under reduced pressure. The resulting red oil was purified by flash silica gel chromatography (170 g  $\text{SiO}_2$ , 30:1 hexanes:EtOAc) to yield **2-O-(4-(4-((4-butylphenyl)diazenyl)phenyl)butanoyl)-1-O-stearoyl-3-O-triethylsilyl-*sn*-glycerol** (**8**, 944 mg, 72%) as a red oil.

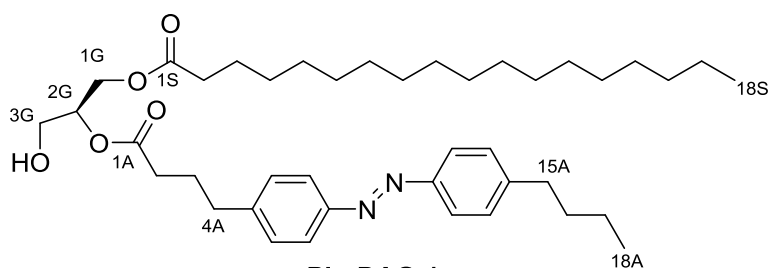
**TLC (10:1 hexanes:EtOAc):**  $R_f = 0.50$ .

**$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz, 25 °C):**  $\delta$  7.85–7.80 (m, 4 H, H7A<sub>a,b</sub>, H12A<sub>a,b</sub>), 7.31 (d, 4 H, H6A<sub>a,b</sub>, H13A<sub>a,b</sub>,  $J = 8.2$  Hz), 5.13–5.05 (m, 1 H, H2G), 4.38 (dd, 1 H, H1G<sub>a</sub>,  $J = 12.1$  Hz, 3.6 Hz), 4.17 (dd, 1 H, H1G<sub>b</sub>,  $J = 12.1$  Hz, 6.1 Hz), 3.73 (d, 2 H, H3G<sub>a,b</sub>,  $J = 5.3$  Hz), 2.73 (t, 2 H, H4A<sub>a,b</sub>,  $J = 7.7$  Hz), 2.69 (t, 2 H, H15A<sub>a,b</sub>,  $J = 7.8$  Hz), 2.37 (t, 2 H, H2A<sub>a,b</sub>,  $J = 7.5$  Hz), 2.29 (t, 2 H, H2S<sub>a,b</sub>,  $J = 7.6$  Hz), 2.00 (quin, 2 H, H3A<sub>a,b</sub>,  $J = 7.4$  Hz), 1.69–1.53 (m, 4 H, H3S<sub>a,b</sub>, H16A<sub>a,b</sub>), 1.43–1.34 (m, 2 H, H17A<sub>a,b</sub>), 1.33–1.21 (m, 28 H, H17S<sub>a,b</sub>, 13xCH<sub>2</sub>(Stear)), 0.97–0.85 (m, 15 H, H18A<sub>a,b,c</sub>, H17S<sub>a,b,c</sub>, 3xCH<sub>3</sub>(TES)), 0.59 (q, 6 H, 3xCH<sub>2</sub>(TES),  $J = 8.0$  Hz).

**$^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz, 25 °C):**  $\delta$  173.7 (C1S), 172.8 (C1A), 151.4 (C<sub>Azo</sub>), 151.1 (C<sub>Azo</sub>), 146.5 (C<sub>Azo</sub>), 144.6 (C<sub>Azo</sub>), 129.3 (2 C, C<sub>Azo</sub>), 129.2 (2 C, C<sub>Azo</sub>), 123.0 (2 C, C<sub>Azo</sub>), 122.9 (2 C, C<sub>Azo</sub>), 72.1 (C2G), 62.6 (C3G), 61.3 (C1G), 35.7 (C15A), 35.0 (C4A), 34.3 (C2S), 33.7 (C2A), 33.6 (C2S), 32.1 (C3S), 29.9–29.7 (m, C<sub>Alk</sub>), 29.6 (C<sub>Alk</sub>), 29.5 (C<sub>Alk</sub>), 29.4 (C<sub>Alk</sub>), 29.3 (C<sub>Alk</sub>), 26.5 (C3A), 25.1 (C16A), 22.9 (C17S), 22.5 (C17A), 14.3 (C18S), 14.1 (C18A), 6.8 (3 C, 3xCH<sub>3</sub>(TES)), 4.4 (3 C, 3xCH<sub>2</sub>(TES)).

**HRMS (EI<sup>+</sup>):**  $m/z$  calcd. for [C<sub>20</sub>H<sub>24</sub>N<sub>2</sub>O<sub>2</sub>]<sup>+</sup>: 778.5680, found: 778.5675 ([M-e]<sup>+</sup>).

**Synthesis of 2-O-(4-(4-((4-butylphenyl)diazenyl)phenyl)butanoyl)-1-O-stearoyl-*sn*-glycerol (PhoDAG-1, 1)**



**PhoDAG-1**

**1**

**2-O-(4-(4-((4-Butylphenyl)diazenyl)phenyl)butanoyl)-1-O-stearoyl-3-O-triethylsilyl-*sn*-glycerol**

(**8**, 500 mg, 0.641 mmol, 1.0 equiv.) was first dissolved in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and added to a solution of FeCl<sub>2</sub>·6H<sub>2</sub>O (5 mM in 25 mL 3:1 MeOH:CH<sub>2</sub>Cl<sub>2</sub>). This solution was stirred at room temperature for 30 min. The solution was then diluted with EtOAc (200 mL) and washed with water (2x200 mL). The organic phase was then dried over Na<sub>2</sub>SO<sub>4</sub>. The mixture was filtered and the filtrate was concentrated under reduced pressure. The resulting oil was purified by flash silica gel chromatography (3:1 hexanes:EtOAc) to yield **2-O-(4-(4-((4-butylphenyl)diazenyl)phenyl)butanoyl)-1-O-stearoyl-*sn*-glycerol (PhoDAG-1**, 0.425 mg, quant.) as an orange solid. *Note: Short reaction times and quick chromatography are essential to avoid acyl chain migration.*

**TLC (3:1 hexanes:EtOAc):**  $R_f = 0.45$  (*trans*), 0.35 (*cis*).

**$^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz, 25 °C):**  $\delta$  7.85–7.80 (m, 4 H, H7A<sub>a,b</sub>, H12A<sub>a,b</sub>), 7.31 (d, 4 H, H6A<sub>a,b</sub>, H13A<sub>a,b</sub>,  $J = 8.1$  Hz), 5.10 (quin, 1 H, H2G,  $J = 5.1$  Hz), 4.34 (dd, 1 H, H3G<sub>a</sub>,  $J = 11.9$  Hz, 4.5 Hz), 4.24 (dd, 1 H, H3G<sub>b</sub>,  $J = 12.0$  Hz, 5.8 Hz), 3.73 (t, 2 H, H1G<sub>a,b</sub>,  $J = 5.2$  Hz), 2.74 (t, 2 H, H4A<sub>a,b</sub>,  $J = 7.6$  Hz), 2.69 (t, 2 H, H15A<sub>a,b</sub>,  $J = 7.6$  Hz), 2.40 (t, 2 H, H2A<sub>a,b</sub>,  $J = 7.3$  Hz), 2.32 (t, 2 H, H2S<sub>a,b</sub>,  $J = 7.5$  Hz), 2.06–1.97 (m, 2 H, H3A<sub>a,b</sub>), 1.69–1.51 (m, 4 H, H16A<sub>a,b</sub>, H3S<sub>a,b</sub>), 1.44–1.32 (m, 2 H, H17A<sub>a,b</sub>), 1.34–1.18 (m, 28 H, H17S<sub>a,b</sub>, 28xHS<sub>Alk</sub>), 0.94 (t, 3 H, H18A<sub>a,b,c</sub>,  $J = 7.1$  Hz), 0.88 (t, 3 H, H18S<sub>a,b,c</sub>,  $J = 7.1$  Hz).

**$^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 100 MHz, 25 °C):**  $\delta$  174.0 (C1S), 173.1 (C1A), 151.5 (C<sub>Azo</sub>), 151.1 (C<sub>Azo</sub>), 146.5 (C<sub>Azo</sub>), 144.4 (C<sub>Azo</sub>), 129.3 (2 C, C<sub>Azo</sub>), 129.2 (2 C, C<sub>Azo</sub>), 123.0 (2 C, C<sub>Azo</sub>), 122.9 (2 C, C<sub>Azo</sub>), 72.4 (C2G), 62.1 (C3G), 61.6 (C1G), 35.7 (C15A), 35.0 (C4A), 34.2 (C2S), 33.6 (C2A), 33.6 (C3S), 29.9–29.7 (m, C<sub>Alk</sub>), 29.6 (C<sub>Alk</sub>), 29.5 (C<sub>Alk</sub>), 29.4 (C<sub>Alk</sub>), 29.3 (C<sub>Alk</sub>), 26.4 (C3A), 25.0 (C16A), 22.9 (C17S), 22.5 (C17A), 14.3 (C18S), 14.1 (C18A).

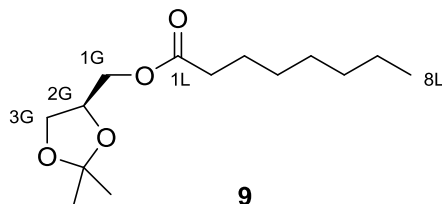
**IR (neat, ATR):**  $\tilde{\nu} = 3483, 2955, 2917, 2850, 1728, 1711, 1601, 1499, 1472, 1460, 1414, 1379, 1256, 1235, 1214, 1188, 1173, 1138, 1096, 1068, 1052, 1031, 1012, 962, 888, 832, 718, 679.$

**HRMS (EI<sup>+</sup>):**  $m/z$  calcd. for  $[\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_2]^+$ : 324.1838, found: 324.1834 ( $[\text{M}-\text{e}]^+$ ).

**UV-Vis (25  $\mu\text{M}$  in DMSO):**  $\lambda_{\text{max}}(\pi-\pi^*) = 340$  nm.  $\lambda_{\text{max}}(n-\pi^*) = 442$  nm (**Fig. 1e**).

**Melting point (°C):** 66.5–67.2.

## Synthesis of **S-2,3-O-isopropylidene-1-O-octanoyl-*sn*-glycerol (9)**

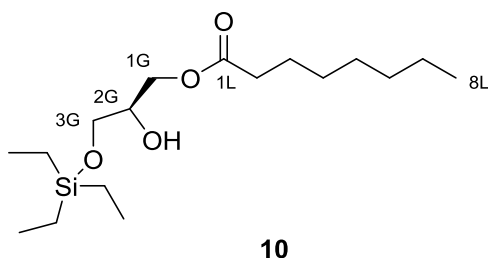


Octanoic acid (490 mg, 3.4 mmol, 1.5 equiv.) was first dissolved in dry  $\text{CH}_2\text{Cl}_2$  (10 mL) under an argon atmosphere and then  $\text{NEt}_3$  (0.310 mL, 2.27 mmol, 1 equiv.) and DMAP (27.7 mg, 0.227 mmol, 0.1 equiv.) were added. The solution was then cooled to 0 °C and then *N,N'*-dicyclohexylcarbodiimide (DCC, 1.171 mg, 5.68 mmol, 2.5 equiv.) was added. This mixture was stirred at 0 °C for 30 min and then the **R(-)-2,3-O-Isopropylidene-*sn*-glycerol** (0.280 mL, 2.27 mmol, 1.0 equiv.) was added. The solution was stirred for a further 2 h, and then was diluted with  $\text{CH}_2\text{Cl}_2$  and washed once with a saturated aqueous  $\text{NaHCO}_3$  solution, and twice with  $\text{H}_2\text{O}$ . The organic phase was then dried over  $\text{Na}_2\text{SO}_4$  and filtered. The filtrate was concentrated under reduced pressure and the resulting oil was purified by flash silica gel chromatography (30 g  $\text{SiO}_2$ , pre-adsorbed on 1.5 g of  $\text{SiO}_2$ , 40:1 to 10:1 hexanes:EtOAc) to yield **S-2,3-O-isopropylidene-1-O-octanoyl-*sn*-glycerol (9)**, 537.3 mg, 92%) as a colorless oil.

**TLC (20:1 hexanes:EtOAc):**  $R_f = 0.09$ .

**$^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz, 25 °C):**  $\delta$  4.32–4.24 (m, 1 H, H<sub>2G</sub>), 4.17–4.09 (m, 1 H, HG<sub>1a</sub>), 4.09–4.00 (m, 2 H, HG<sub>1b</sub>, HG<sub>3a</sub>), 3.73–3.68 (m, 1 H, H<sub>3b</sub>), 2.31 (t, 2 H, H<sub>2L<sub>a,b</sub></sub>,  $J = 7.3$  Hz), 1.65–1.54 (m, 2 H, H<sub>3L<sub>a,b</sub></sub>), 1.40–1.33 (s, 6 H, 2xH<sub>CH<sub>3</sub></sub>), 1.31–1.17 (m, 8 H, H<sub>alk</sub>), 0.84 (t, 3 H, H<sub>8L<sub>a,b,c</sub></sub>,  $J = 6.7$  Hz).

### Synthesis of 1-O-octanoyl-3-O-triethylsilyl-*sn*-glycerol (10)



**S-2,3-O-isopropylidene-1-O-octanoyl-*sn*-glycerol (9)**, 453.2 mg, 1.75 mmol, 1.0 equiv.) was first dissolved in dry 1,2-dichloroethane (6 mL) under an argon atmosphere and then dry *N,N*-diisopropylethylamine (DIPEA, 1.5 mL, 9.65 mmol, 5.5 equiv.) was added to the solution. The solution was then warmed to 90 °C and TESOTf (1.4 mL, 6.13 mmol, 3.5 equiv.) was added and the reaction stirred for 2 h. The mixture was then diluted with EtOAc (60 mL) and washed with aqueous HCl (0.1 M, 60 mL) and then H<sub>2</sub>O (2x60 mL). The organic phase was then dried over Na<sub>2</sub>SO<sub>4</sub> and filtered. The filtrate was concentrated under reduced pressure. The resulting oil was dissolved in THF (8 mL) and then aqueous Na<sub>2</sub>CO<sub>3</sub> (4 mL, 10% w/w) and I<sub>2</sub> (1.110 g, 4.38 mmol, 2.5 equiv.) were added to the solution. The reaction was stirred for 75 min at room temperature, and was then diluted with EtOAc (70 mL) and washed once with saturated aqueous Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (70 mL) and H<sub>2</sub>O (2x70 mL). The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub> and filtered. The filtrate was concentrated under reduced pressure and the resulting oil was purified by flash silica gel chromatography (60 g SiO<sub>2</sub>, 20:1→15:1→10:1 hexanes:EtOAc) to yield **1-O-octanoyl-3-O-triethylsilyl-*sn*-glycerol (10)**, 211 mg, 36% as a colourless oil.

**TLC (9:1 hexanes:EtOAc):**  $R_f = 0.30$ .

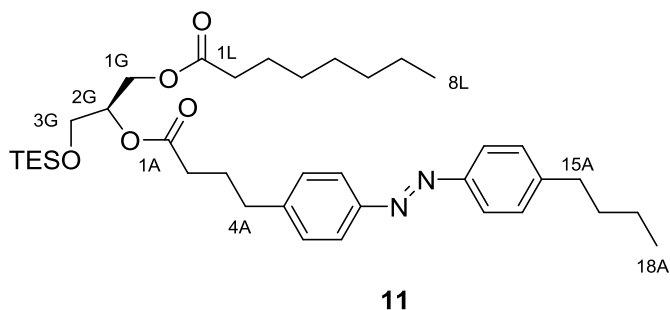
**$^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz, 25 °C):**  $\delta$  4.19–4.08 (m, 2 H, H1G<sub>a,b</sub>), 3.98–3.87 (m, 1 H, H2G), 3.63 (dd, 1 H, H3G<sub>a</sub>,  $J_1 = 10.5$  Hz,  $J_2 = 5.3$  Hz), 3.56 (dd, 1 H, H3G<sub>b</sub>,  $J_1 = 10.3$  Hz,  $J_2 = 5.4$  Hz), 2.69 (d, 1 H, OH,  $J = 5.4$  Hz), 2.34 (t, 2 H, H2<sub>a,b</sub>,  $J = 7.5$  Hz), 1.67–1.58 (m, 2 H, H3<sub>a,b</sub>), 1.34–1.20 (m, 8 H, H<sub>alk</sub>), 0.96 (t, 9 H, 3xCH<sub>3</sub>(TES),  $J = 7.9$  Hz), 0.87 (t, 3 H, H8<sub>a,b,c</sub>,  $J = 6.9$  Hz), 0.61 (q, 6 H, 3xCH<sub>2</sub>(TES),  $J = 7.9$  Hz).

**$^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 100 MHz, 25 °C):**  $\delta$  174.1 (C1L), 70.2 (CXG), 65.1 (CXG), 63.5 (CXG), 34.3 (C2), 31.8 (C<sub>alk</sub>), 29.2 (C<sub>alk</sub>), 29.1 (C<sub>alk</sub>), 25.1 (C<sub>alk</sub>), 22.8 (C7L), 14.2 (C8L), 6.8 (3C, 3xCH<sub>3</sub>(TES)), 4.4 (3C, 3xCH<sub>2</sub>(TES)).

**IR (neat, ATR):**  $\tilde{\nu} = 3489, 2955, 2929, 2876, 1740, 1458, 1416, 1380, 1239, 1166, 1100, 1006, 976, 842, 807, 744, 728, 676, 619, 588, 601, 564$ .

**HRMS (ESI<sup>+</sup>):**  $m/z$  calcd. for  $[\text{C}_{17}\text{H}_{37}\text{O}_4\text{Si}]^+$ : 333.2461, found: 333.2460 ( $[\text{M}+\text{H}^+]^+$ ).

**Synthesis of 2-O-(4-(4-((4-butylphenyl)diazenyl)phenyl)butanoyl)-1-O-octanoyl-3-O-triethylsilyl-*sn*-glycerol (11)**



**FAAzo-4**<sup>19</sup> (136.3 mg, 0.42 mmol, 2.0 equiv.) was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (12 mL) under an argon atmosphere, and then DMAP (5.13 mg, 0.042 mmol, 0.1 equiv.) and EDC (0.11 mL, 0.63 mmol, 3.0 equiv.) were added. The solution was stirred at room temperature for 20 min and then **1-O-octanoyl-3-O-triethylsilyl-*sn*-glycerol (10)**, 68.9 mg, 0.21 mmol, 1.0 equiv.) was added. The solution was stirred overnight at room temperature, diluted with CH<sub>2</sub>Cl<sub>2</sub> and then washed three times with H<sub>2</sub>O. The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and the filtrate was concentrated under reduced pressure. The resulting dark orange oil was purified by flash silica gel chromatography (15 g SiO<sub>2</sub>, hexanes:EtOAc 20:1→10:1) to yield **2-O-(4-(4-((4-butylphenyl)diazenyl)phenyl)butanoyl)-1-O-octanoyl-3-O-triethylsilyl-*sn*-glycerol (11)**, 77.4 mg, 58%) as an orange oil.



**TLC (3:1 hexanes:EtOAc):**  $R_f = 0.48$  (*trans*), 0.30 (*cis*).

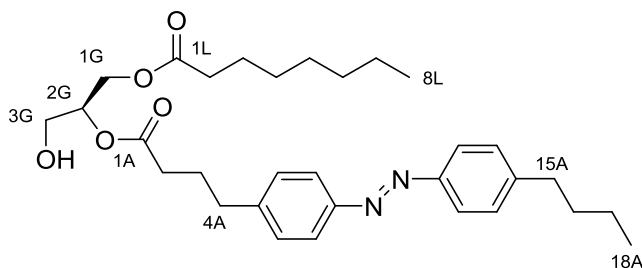
**$^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz, 25 °C):**  $\delta$  7.87–7.79 (m, 4 H, H7A<sub>a,b</sub>, H12A<sub>a,b</sub>), 7.31 (d, 4 H, H6A<sub>a,b</sub>, H13A<sub>a,b</sub>,  $J = 8.7$  Hz), 5.14–5.05 (m, 1 H, H2G), 4.42–4.33 (m, 1H, H1G<sub>a</sub>), 4.23–4.13 (m, 1 H, H1G<sub>b</sub>), 3.77–3.69 (m, 2 H, H3G<sub>a,b</sub>), 2.78–2.67 (m, 4 H, H4A<sub>a,b</sub>, H15A<sub>a,b</sub>), 2.37 (t, 2 H, H1A<sub>a,b</sub>,  $J = 7.1$  Hz), 2.30 (t, 2 H, H2L<sub>a,b</sub>,  $J = 7.3$  Hz), 2.07–1.95 (m, 2 H, H3A<sub>a,b</sub>), 1.69–1.52 (m, 4 H, H3L<sub>a,b</sub>, H16A<sub>a,b</sub>), 1.44–1.33 (m, 2 H, H17A<sub>a,b</sub>), 1.33–1.19 (m, 8 H, H<sub>alk</sub>), 0.99–0.90 (m, 12 H, H18A<sub>a,b,c</sub>, 3xCH<sub>3</sub>(TES)), 0.86 (t, 3 H, H8L<sub>a,b,c</sub>), 0.59 (q, 6 H, 3xCH<sub>2</sub>(TES),  $J = 8.0$  Hz).

**$^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 100 MHz, 25 °C):**  $\delta$  173.6 (C1S), 172.8 (C1A), 151.4 (C<sub>Azo</sub>), 151.9 (C<sub>Azo</sub>), 146.4 (C<sub>Azo</sub>), 144.6 (C<sub>Azo</sub>), 129.3 (2 C, C<sub>Azo</sub>), 129.2 (2 C, C<sub>Azo</sub>), 123.0 (2 C, C<sub>Azo</sub>), 122.9 (2 C, C<sub>Azo</sub>), 72.1 (C2G), 62.6 (C3G), 61.3 (C1G), 35.7 (C15A), 35.0 (C4A), 34.3 (C2A), 33.63 (C2A), 33.59 (C3A), 31.8 (C3L), 29.2 (C<sub>Alk</sub>), 29.1 (C<sub>Alk</sub>), 26.5 (C<sub>Alk</sub>), 25.0 (C<sub>Alk</sub>), 22.7 (C<sub>Alk</sub>), 22.5 (C<sub>Alk</sub>), 14.2 (C18L), 14.1 (C18A), 6.8 (3 C, 3xCH<sub>3</sub>(TES)), 4.4 (3 C, 3xCH<sub>2</sub>(TES)).

**IR (neat, ATR):**  $\tilde{\nu} = 3028, 2955, 2930, 2874, 2859, 1918, 1739, 1602, 1580, 1498, 1458, 1416, 1378, 1302, 1240, 1226, 1156, 1145, 1104, 1013, 977, 844, 800, 744, 728, 674, 642, 618, 601, 571, 564.$

**HRMS (ESI<sup>+</sup>):**  $m/z$  calcd. for [C<sub>37</sub>H<sub>59</sub>N<sub>2</sub>O<sub>5</sub>Si]<sup>+</sup>: 639.4193, found: 639.4142 ([M+H]<sup>+</sup>).

**Synthesis of 2-O-(4-(4-((4-butylphenyl)diazenyl)phenyl)butanoyl)-1-O-octanoyl-*sn*-glycerol (PhoDAG-2, 2)**



**PhoDAG-2**

**2**

**2-O-(4-(4-((4-butylphenyl)diazenyl)phenyl)butanoyl)-1-O-octanoyl-*sn*-glycerol (PhoDAG-2)** was prepared from **2-O-(4-(4-((4-butylphenyl)diazenyl)phenyl)butanoyl)-1-O-octanoyl-3-O-triethylsilyl-*sn*-glycerol (7, 28.0 mg, 0.044 mmol, 1.0 equiv.)** as described above in the synthesis of **2-O-(4-(4-((4-butylphenyl)diazenyl)phenyl)butanoyl)-1-O-stearoyl-*sn*-glycerol (PhoDAG-1)**. **PhoDAG-2 (13.3 mg, 66%)** was isolated as an orange oil. *NOTE: all reactants and reagents were scaled according to molarity.*

**TLC (2:1 hexanes:EtOAc):**  $R_f$  : 0.39 (*trans*), 0.28 (*cis*).

**$^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz, 25 °C):**  $\delta$  7.86–7.79 (m, 4 H, H7A<sub>a,b</sub>, H12A<sub>a,b</sub>), 7.31 (d, 4 H, H6A<sub>a,b</sub>, H13A<sub>a,b</sub>,  $J = 8.7$  Hz), 5.15–5.05 (m, 1 H, H2G), 4.37–4.30 (m, 1H, H3G<sub>a</sub>), 4.27–4.20 (m, 1 H, H3G<sub>b</sub>), 3.76–3.70 (m, 2 H, H1G<sub>a,b</sub>), 2.74 (t, 2 H, H4A<sub>a,b</sub>,  $J = 7.5$  Hz), 2.69 (t, 2 H, H15A<sub>a,b</sub>,  $J = 7.8$  Hz), 2.40 (t, 2 H, H2A<sub>a,b</sub>,  $J = 7.5$  Hz), 2.36–2.29 (m, 2 H, H2L<sub>a,b</sub>), 2.07–1.97 (m, 3 H, H3A<sub>a,b</sub>), 1.70–1.50 (m, 4 H, H16A<sub>a,b</sub>, HL3<sub>a,b</sub>), 1.44–1.18 (m, 10 H, H<sub>alk</sub>), 0.93 (t, 3 H, H18A<sub>a,b,c</sub>,  $J = 7.3$  Hz), 0.90–0.81 (t, 3 H, H8L<sub>a,b,c</sub>,  $J = 7.6$  Hz).

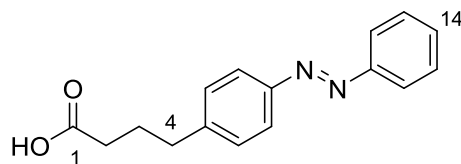
**$^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 100 MHz, 25 °C):**  $\delta$  174.0 (C1L), 173.0 (C1A), 152.8 (C<sub>Azo</sub>), 151.3 (C<sub>Azo</sub>), 146.5 (C<sub>Azo</sub>), 144.4 (C<sub>Azo</sub>), 129.3 (2 C, C<sub>Azo</sub>), 129.2 (2 C, C<sub>Azo</sub>), 123.9 (2 C, C<sub>Azo</sub>), 122.9 (2 C, C<sub>Azo</sub>), 72.4 (C2G), 62.1 (C3G), 61.6 (C1G), 35.7 (C15A), 35.0 (C4A), 34.2 (C2L), 33.61 (C<sub>Alk</sub>), 33.58 (C<sub>Alk</sub>), 31.8 (C<sub>Alk</sub>), 29.2 (C<sub>Alk</sub>), 29.1 (C<sub>Alk</sub>), 26.4 (C3A), 25.0 (C3L), 22.7 (C7L), 22.5 (C17A), 14.2 (C8L), 14.1 (C18A).

**IR (neat, ATR):**  $\tilde{\nu} = 3466, 2956, 2929, 2858, 1739, 1602, 1498, 1458, 1417, 1378, 1225, 1159, 1103, 1051, 1014, 844, 728, 634, 614, 591, 576, 568.$

**HRMS (EI<sup>+</sup>):**  $m/z$  calcd. for  $[\text{C}_{31}\text{H}_{44}\text{N}_2\text{O}_5]^+$ : 524.3250, found: 524.3245 ( $[\text{M}-\text{e}]^+$ ).

**UV-Vis (50  $\mu\text{M}$  in DMSO):**  $\lambda_{\text{max}}(\pi-\pi^*) = 340$  nm.  $\lambda_{\text{max}}(n-\pi^*) = 440$  nm.

### Synthesis of 4-(Phenyldiazenyl)phenyl butanoic acid (FAAzo-9, 4)



FAAzo-9

4

4-(4-aminophenyl)butyric acid (200 mg, 1.12 mmol, 1.0 equiv.) was first dissolved in  $\text{CH}_2\text{Cl}_2$  (20 mL). Nitrosobenzene (143.4 mg, 1.34 mmol, 1.2 equiv.) and AcOH (5 mL) were added, and the solution was then stirred at room temperature overnight. The solvents were then removed under reduced pressure. The resulting crude residue was purified by flash silica gel chromatography (20 g  $\text{SiO}_2$ , 99:1  $\text{CH}_2\text{Cl}_2$ :AcOH) to yield **4-(phenyldiazenyl)phenyl butanoic acid (FAAzo-9, 320.8 mg, quant.)** as an orange solid.

**TLC (99:1  $\text{CH}_2\text{Cl}_2$ :AcOH):**  $R_f = 0.17$ .

**$^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz, 25 °C):**  $\delta$  7.94–7.82 (m, 4 H,  $\text{H}_{7\text{a,b}}$ ,  $\text{H}_{11\text{a,b}}$ ), 7.55–7.43 (m, 3 H,  $\text{H}_{13\text{a,b}}$ ,  $\text{H}_{14}$ ), 7.34 (d, 2 H,  $\text{H}_{6\text{a,b}}$ ,  $J = 4.0$  Hz), 2.76 (t, 2 H,  $\text{H}_{4\text{a,b}}$ ,  $J = 7.8$  Hz), 2.42 (t, 2 H,  $\text{H}_{2\text{a,b}}$ ,  $J = 7.3$  Hz), 2.02 (dd, 2 H,  $\text{H}_{3\text{a,b}}$ ,  $J = 7.8, 7.3$  Hz).

**$^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 100 MHz, 25 °C):**  $\delta$  178.7 (C1), 152.8 (C11), 151.4 (C8), 144.8 (C5), 131.0 (C14), 129.4–129.2 (4 C,  $\text{C}_{6\text{a,b}}$ ,  $\text{C}_{13\text{a,b}}$ ), 123.2–122.9 (4 C,  $\text{C}_{7\text{a,b}}$ ,  $\text{C}_{12\text{a,b}}$ ), 35.0 (C4), 33.2 (C2), 26.2 (C3).

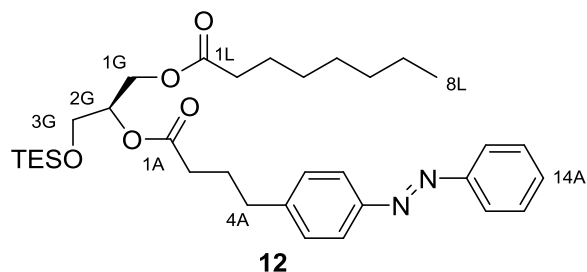
**IR (neat, ATR):**  $\tilde{\nu} = 3041, 2944, 1693, 1601, 1500, 1486, 1462, 1439, 1411, 1339, 1303, 1282, 1252, 1215, 1152, 1106, 1071, 1020, 914, 851, 822, 787, 764, 745, 732, 684, 638, 616, 577, 561$ .

**HRMS (EI<sup>+</sup>):**  $m/z$  calcd. for  $[\text{C}_{16}\text{H}_{16}\text{N}_2\text{O}_2]^+$ : 268.1212, found: 268.1206 ( $[\text{M}-\text{e}]^+$ ).

**UV-Vis (50  $\mu\text{M}$  in DMSO):**  $\lambda_{\text{max}}(\pi-\pi^*) = 330$  nm.  $\lambda_{\text{max}}(n-\pi^*) = 425$  nm.

**Melting point (°C):** 135.5–137.5.

**Synthesis of 2-O-(4-(4-(phenyldiazenyl)phenyl)butanoyl)-1-O-octanoyl-3-O-triethylsilyl-*sn*-glycerol (12)**



**4-(Phenyldiazenyl)phenyl butanoic acid (FAAzo-9, 153.4 mg, 0.57 mmol, 2.0 equiv.)** was dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (15 mL) under an argon atmosphere. DMAP (3.4 mg, 0.028 mmol, 0.1 equiv.) and EDC (0.15 mL, 0.84 mmol, 3.0 equiv.) were then added to the solution. The mixture was stirred at room temperature for 20 min and then **1-O-octanoyl-3-O-triethylsilyl-*sn*-glycerol (10, 95.1 mg, 0.28 mmol, 1.0 equiv.)** was added. The solution was stirred overnight at room temperature, and was then diluted with CH<sub>2</sub>Cl<sub>2</sub> (100 mL) and washed with H<sub>2</sub>O (3x50 mL). The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub> and then filtered. The filtrate was concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (20 g SiO<sub>2</sub>, hexanes:ethyl acetate 20:1→10:1) to yield **2-O-(4-(4-(phenyldiazenyl)phenyl)butanoyl)-1-O-octanoyl-3-O-triethylsilyl-*sn*-glycerol (12, 100 mg, 61%)** as an orange oil.

**TLC (9:1 hexanes:EtOAc):**  $R_f = 0.43$  (*trans*), 0.25 (*cis*).

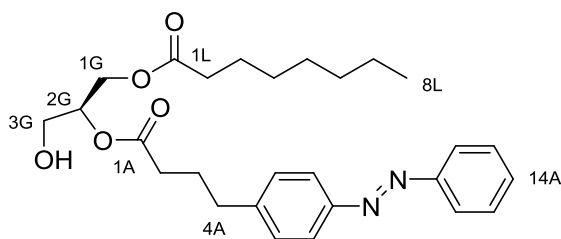
**$^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz, 25 °C):**  $\delta$  7.94–7.83 (m, 4 H, H7A<sub>a,b</sub>, H12A<sub>a,b</sub>), 7.55–7.43 (m, 3 H, H14A, H13A<sub>a,b</sub>), 7.33 (d, 2 H, H6A<sub>a,b</sub>,  $J = 8.2$  Hz), 5.15–5.05 (m, 1 H, H2G), 4.41–4.33 (m, 1H, H1G<sub>a</sub>), 4.22–4.13 (m, 1 H, H1G<sub>b</sub>), 3.78–3.69 (m, 2 H, H3G<sub>a,b</sub>), 2.74 (t, 2 H, H4A<sub>a,b</sub>,  $J = 7.2$  Hz), 2.38 (t, 2 H, H2A,  $J = 7.6$  Hz), 2.30 (t, 2 H, H2L<sub>a,b</sub>,  $J = 7.5$  Hz), 2.08–1.96 (m, 2 H, H3A<sub>a,b</sub>), 1.64–1.54 (m, 2 H, H3L<sub>a,b</sub>), 1.34–1.19 (m, 8 H, H<sub>Alk</sub>), 0.94 (t, 9 H, 3xCH<sub>3</sub>(TES),  $J = 8.3$  Hz), 0.90–0.82 (t, 3 H, H8L<sub>a,b,c</sub>,  $J = 6.9$  Hz), 0.59 (m, 6 H, 3xCH<sub>2</sub>(TES),  $J = 8.2$  Hz).

**$^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 100 MHz, 25 °C):**  $\delta$  173.6 (C1L), 172.8 (C1A), 152.8 (C<sub>Azo</sub>), 151.3 (C<sub>Azo</sub>), 145.0 (C<sub>Azo</sub>), 131.0 (C<sub>Azo</sub>), 129.3 (2C, C<sub>Azo</sub>), 129.2 (2 C, C<sub>Azo</sub>), 123.1 (2 C, C<sub>Azo</sub>), 122.9 (2 C, C<sub>Azo</sub>), 72.1 (C2G), 62.6 (C3G), 61.3 (C1G), 35.0 (C4A), 34.3 (C2L), 33.6 (C2A), 31.8 (C<sub>Alk</sub>), 29.2 (C<sub>Alk</sub>), 29.1 (C<sub>Alk</sub>), 26.5 (C<sub>Alk</sub>), 25.0 (C<sub>Alk</sub>), 22.7 (C7L), 14.2 (C8L), 6.8 (3 C, 3xCH<sub>3</sub>(TES)), 4.4 (3 C, 3xCH<sub>2</sub>(TES)).

**IR (neat, ATR):**  $\tilde{\nu} = 2955, 2930, 2875, 1738, 1603, 1500, 1458, 1415, 1378, 1300, 1240, 1225, 1144, 1103, 1070, 1004, 847, 797, 743, 727, 688, 638, 616, 598, 563.$

**HRMS (ESI<sup>+</sup>):**  $m/z$  calcd. for  $[\text{C}_{33}\text{H}_{51}\text{N}_2\text{O}_5\text{Si}]^+$ : 583.3567, found: 583.3567 ( $[\text{M}+\text{H}]^+$ ).

### Synthesis of 2-O-(4-(4-(phenyldiazenyl)phenyl)butanoyl)-1-O-octanoyl-*sn*-glycerol (PhoDAG-3, 3)



**PhoDAG-3**

**3**

**2-O-(4-(4-(phenyldiazenyl)phenyl)butanoyl)-1-O-octanoyl-*sn*-glycerol (PhoDAG-3)** was prepared from **2-O-(4-(4-(phenyldiazenyl)phenyl)butanoyl)-1-O-octanoyl-3-O-triethylsilyl-*sn*-glycerol (8,** 22.0 mg, 0.038 mmol, 1 equiv.) as described above in the synthesis of **2-O-(4-(4-((4-butylphenyl)diazenyl)phenyl)butanoyl)-1-O-stearoyl-*sn*-glycerol (PhoDAG-1)**. PhoDAG-3 (17.7 mg, quant.) was isolated as an orange oil. *NOTE: all reactants and reagents were scaled according to molarity.*

**TLC (9:1 hexanes:ethyl acetate):**  $R_f$  = 0.20 (*trans*), 0.18 (*cis*).

**$^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz, 25 °C):**  $\delta$  7.93–7.83 (m, 4 H, H7A<sub>a,b</sub>, H11A<sub>a,b</sub>), 7.55–7.43 (m, 3 H, H14A, H13A<sub>a,b</sub>), 7.33 (d, 2 H, H6A<sub>a,b</sub>,  $J$  = 8.3 Hz), 5.14–5.04 (q, 1 H, H2G,  $J$  = 4.9 Hz), 4.38–4.29 (m, 1 H, H3G<sub>a</sub>), 4.27–4.19 (m, 1 H, H3G<sub>b</sub>), 3.78–3.68 (m, 2 H, H1G<sub>a,b</sub>), 2.75 (t, 2 H, H4A<sub>a,b</sub>,  $J$  = 7.7 Hz), 2.40 (t, 2 H, H2A<sub>a,b</sub>,  $J$  = 7.7 Hz), 2.32 (t, 2 H, H2L<sub>a,b</sub>,  $J$  = 7.7 Hz), 2.07–1.96 (m, 2 H, H3A<sub>a,b</sub>), 1.65–1.55 (m, 2 H, H3L<sub>a,b</sub>), 1.34–1.20 (m, 8 H, H<sub>alk</sub>), 0.90–0.82 (t, 3 H, H8L<sub>a,b,c</sub>,  $J$  = 7.1 Hz).

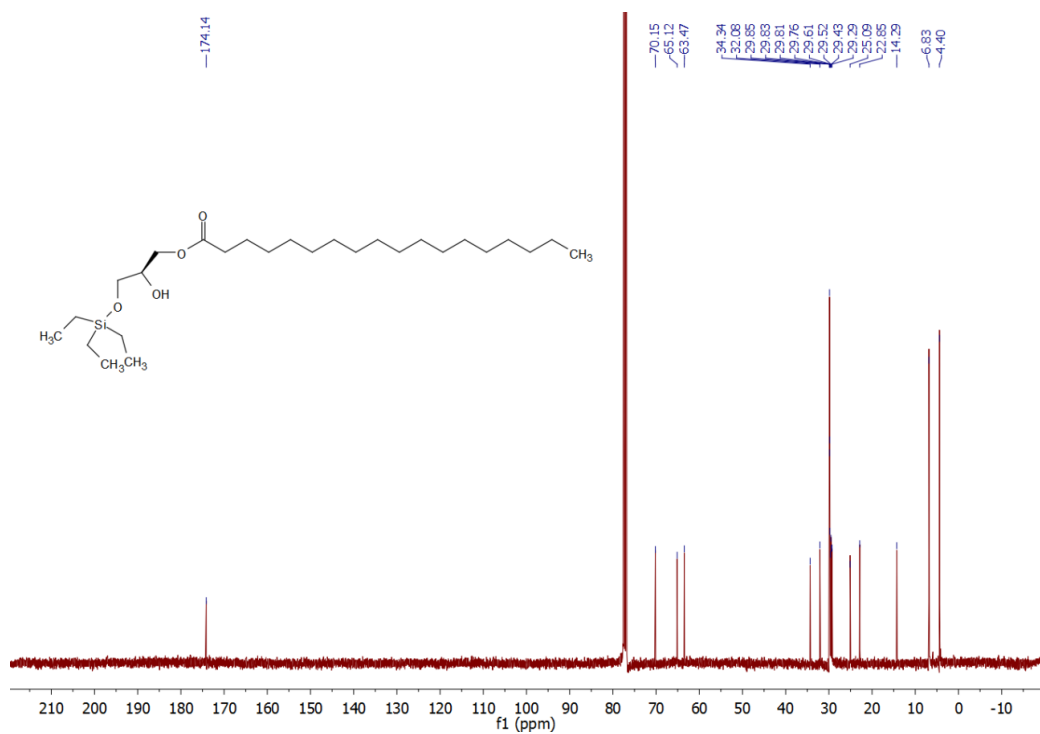
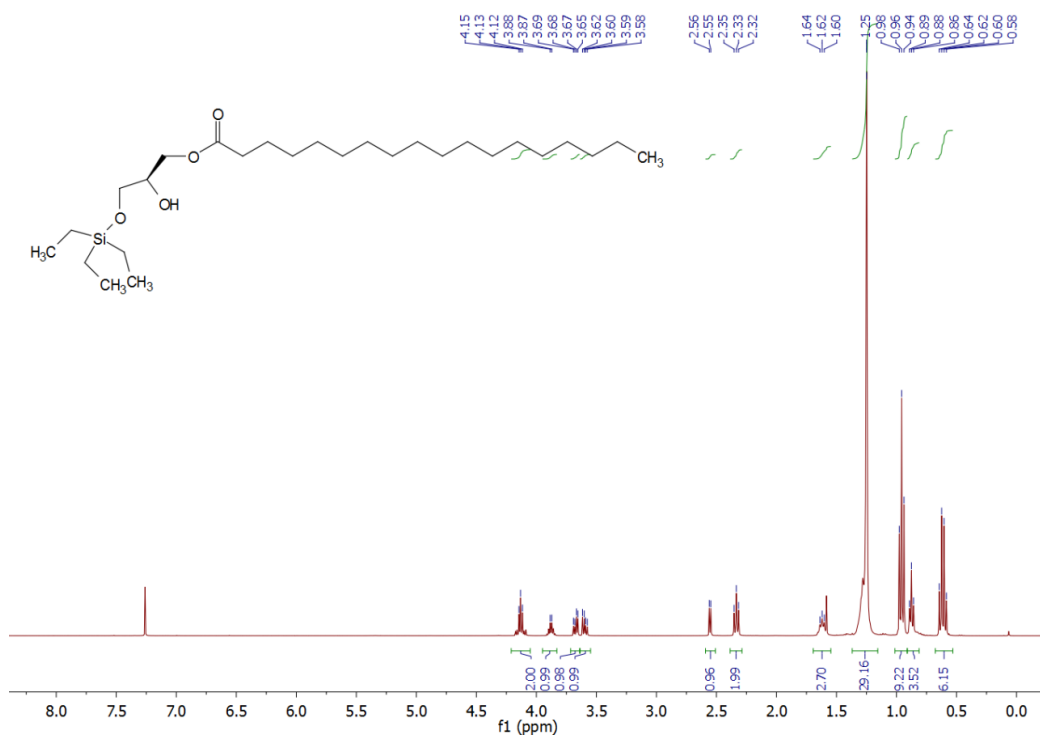
**$^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 100 MHz, 25 °C):**  $\delta$  173.4 (C1L), 173.0 (C1A), 152.8 (C<sub>Azo</sub>), 151.3 (C<sub>Azo</sub>), 144.8 (C<sub>Azo</sub>), 130.9 (C<sub>Azo</sub>), 129.3 (2 C, C<sub>Azo</sub>), 129.2 (2 C, C<sub>Azo</sub>), 123.2 (2 C, C<sub>Azo</sub>), 122.9 (2 C, C<sub>Azo</sub>), 72.4 (C2G), 62.1 (C3G), 61.6 (C1G), 35.0 (C4A), 34.2 (C2L), 33.6 (2 C, C2A, C14A), 31.8 (C6L), 29.2 (C<sub>Alk</sub>), 29.0 (C<sub>Alk</sub>), 26.4 (C<sub>Alk</sub>), 25.0 (C<sub>Alk</sub>), 22.7 (C7A), 14.2 (C8A).

**IR (neat, ATR):**  $\tilde{\nu}$  = 3466, 2928, 2857, 1739, 1603, 1458, 1416, 1377, 1224, 1157, 1104, 1052, 847, 768, 690, 615, 601, 590, 568, 554.

**HRMS (EI<sup>+</sup>):**  $m/z$  calcd. for  $[\text{C}_{27}\text{H}_{36}\text{N}_2\text{O}_5]^+$ : 468.2624, found: 468.2622 ( $[\text{M}-\text{e}]^+$ ).

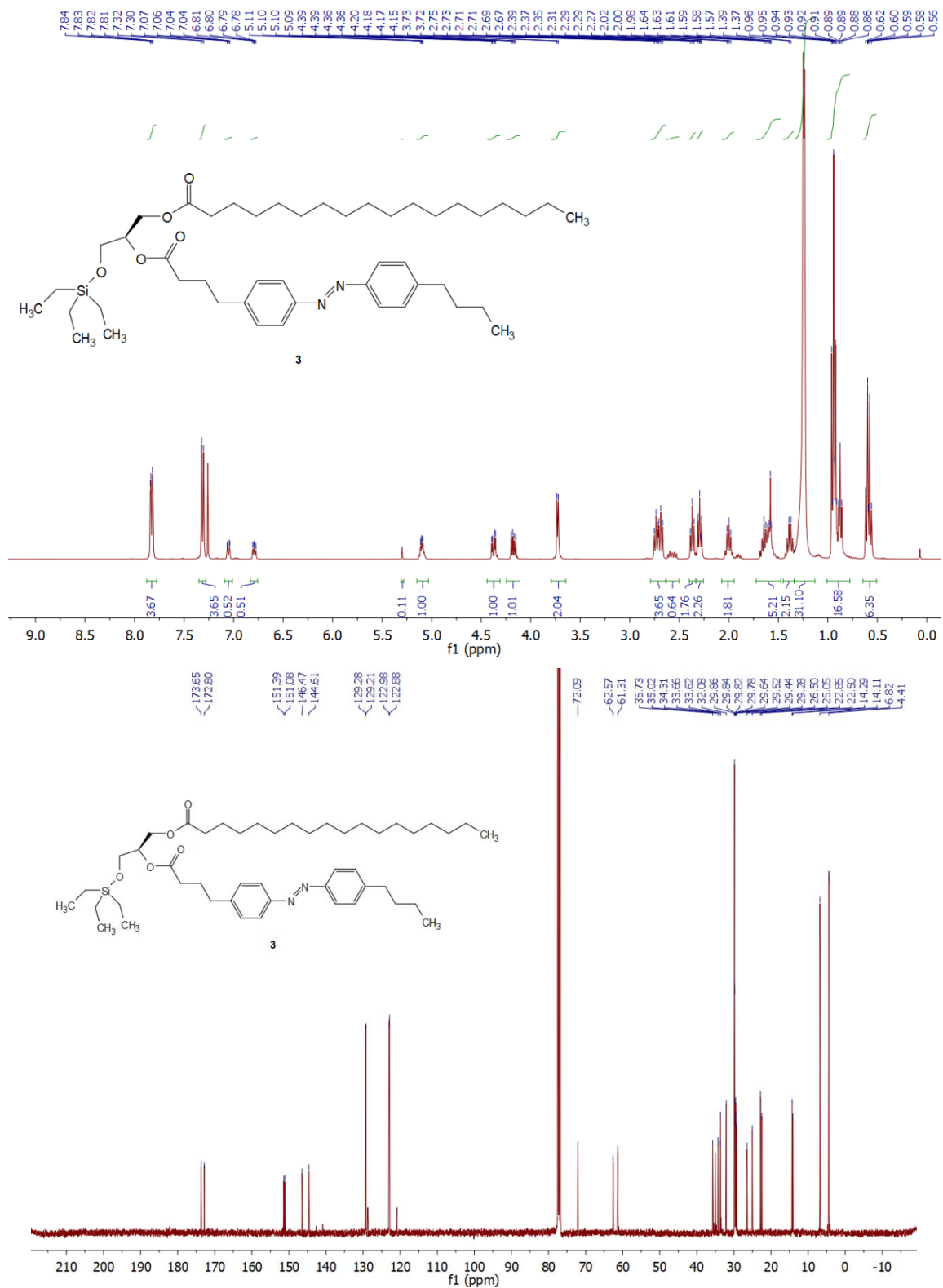
**UV-Vis (50  $\mu\text{M}$  in DMSO):**  $\lambda_{\text{max}}(\pi-\pi^*)$  = 325 nm.  $\lambda_{\text{max}}(n-\pi^*)$  = 440 nm.

# NMR SPECTRA

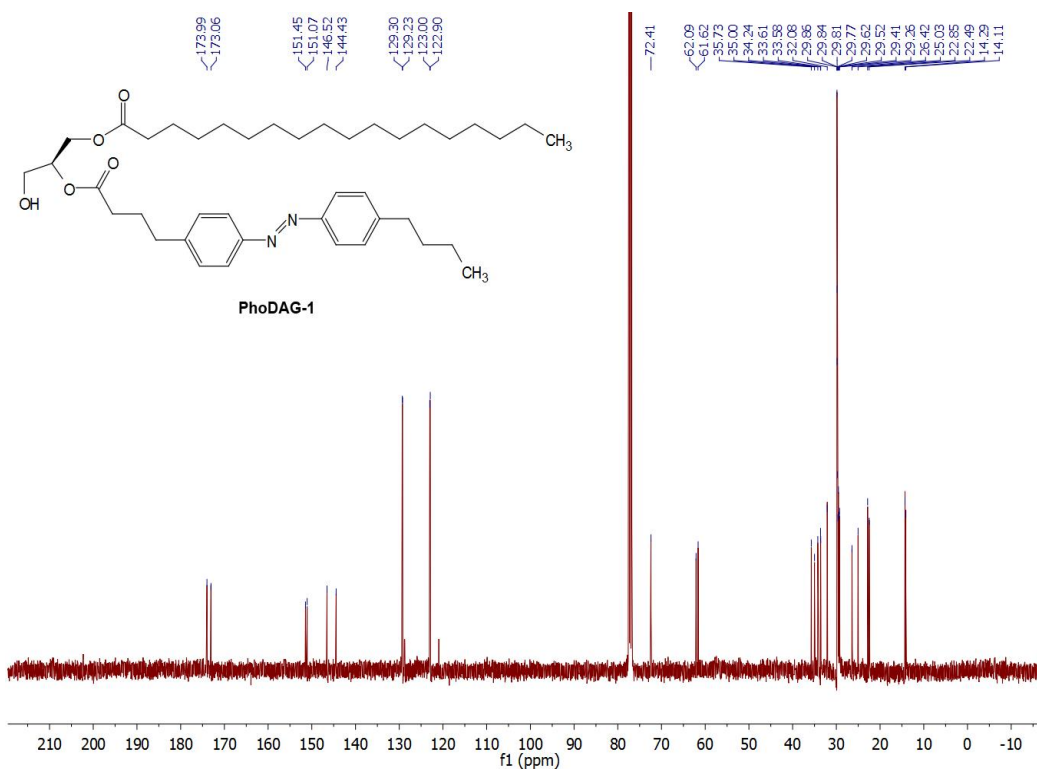
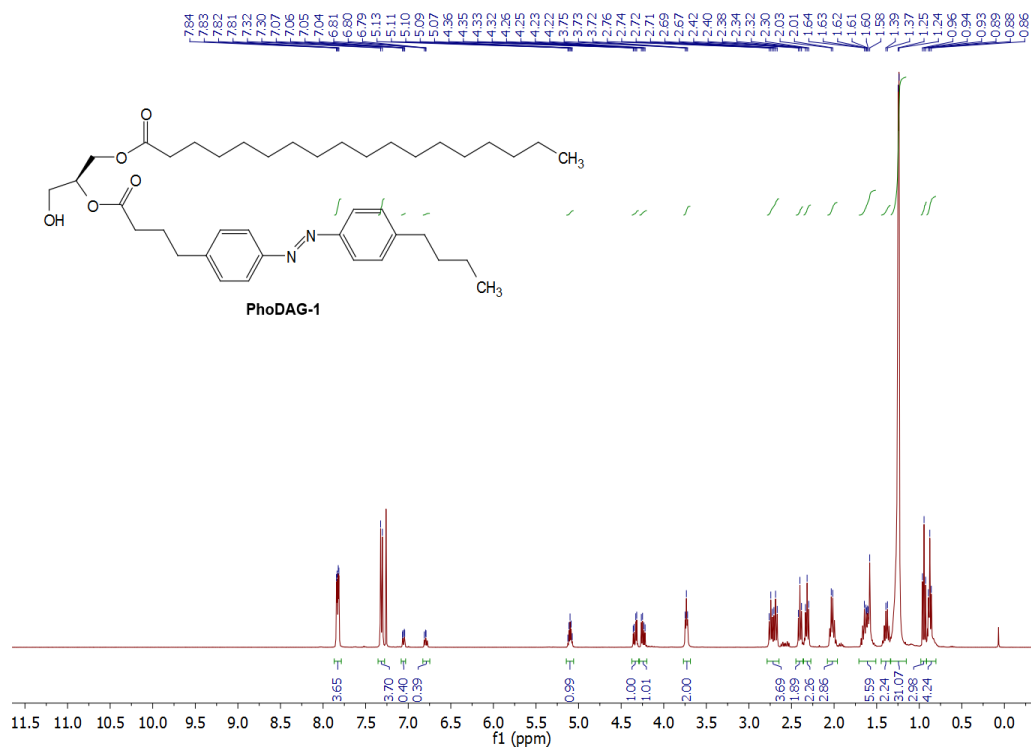


Supplementary Note 4 | <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of 1-O-stearoyl-3-O-triethylsilyl-*sn*-glycerol (7).

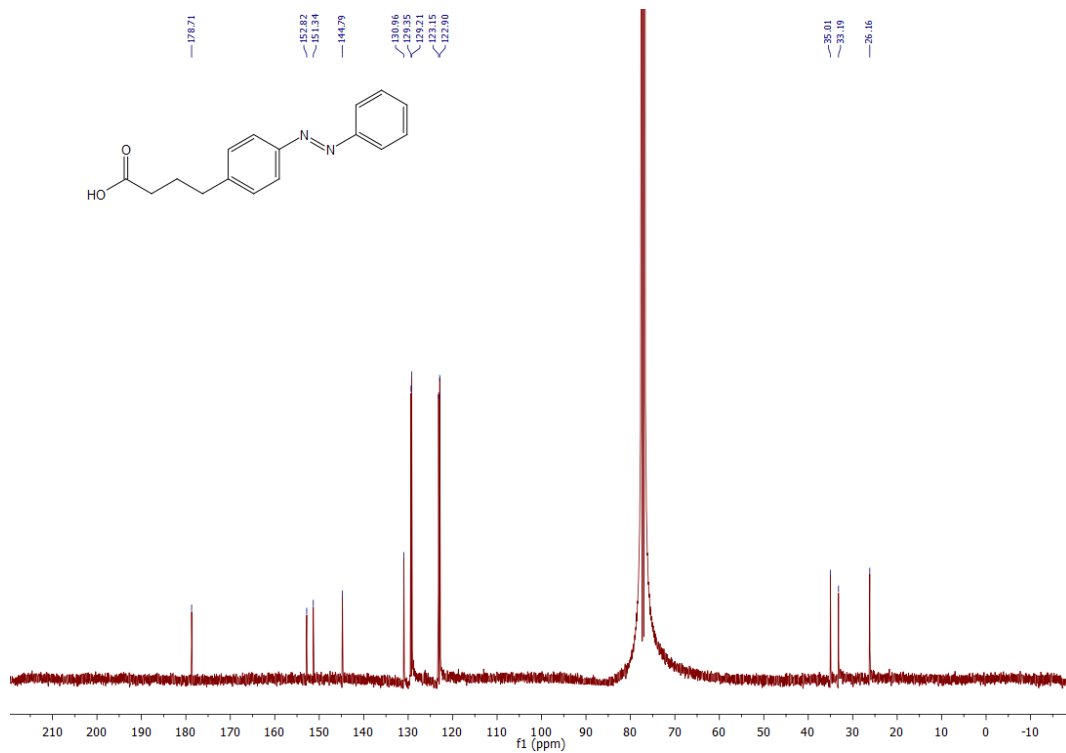
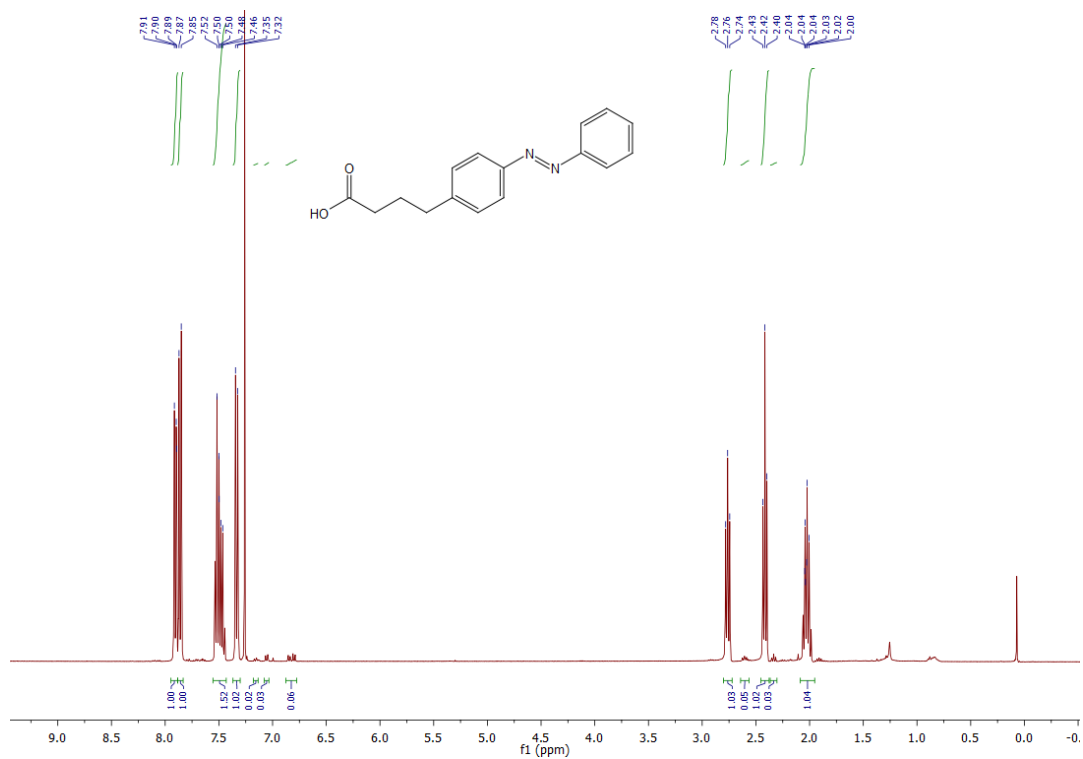




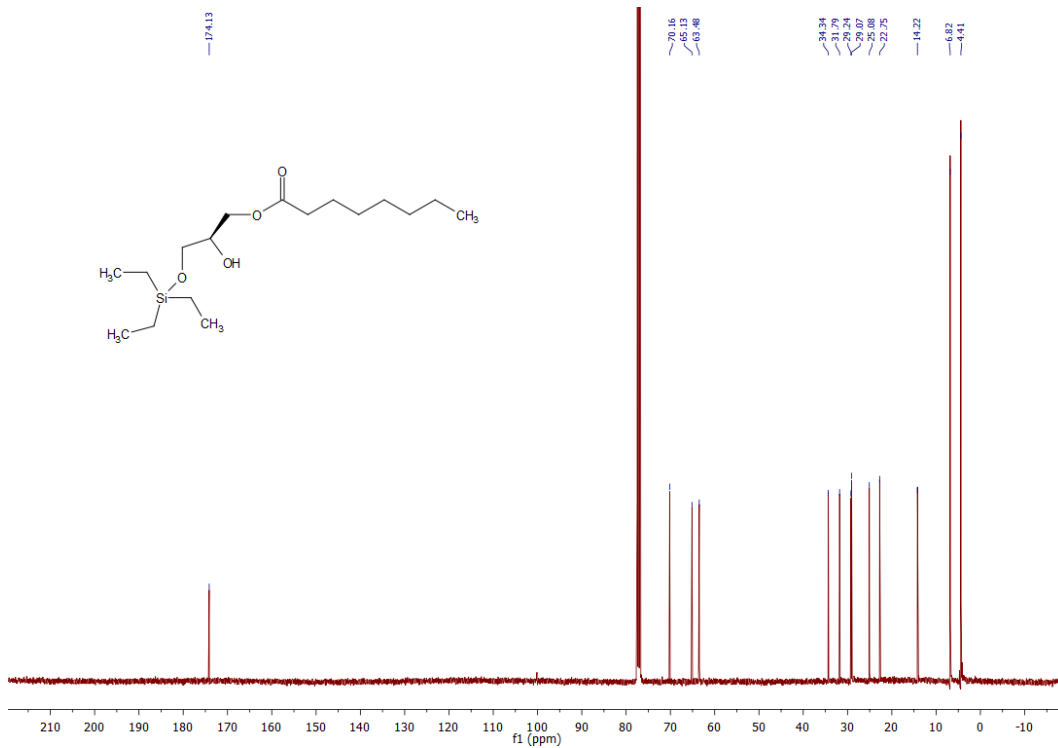
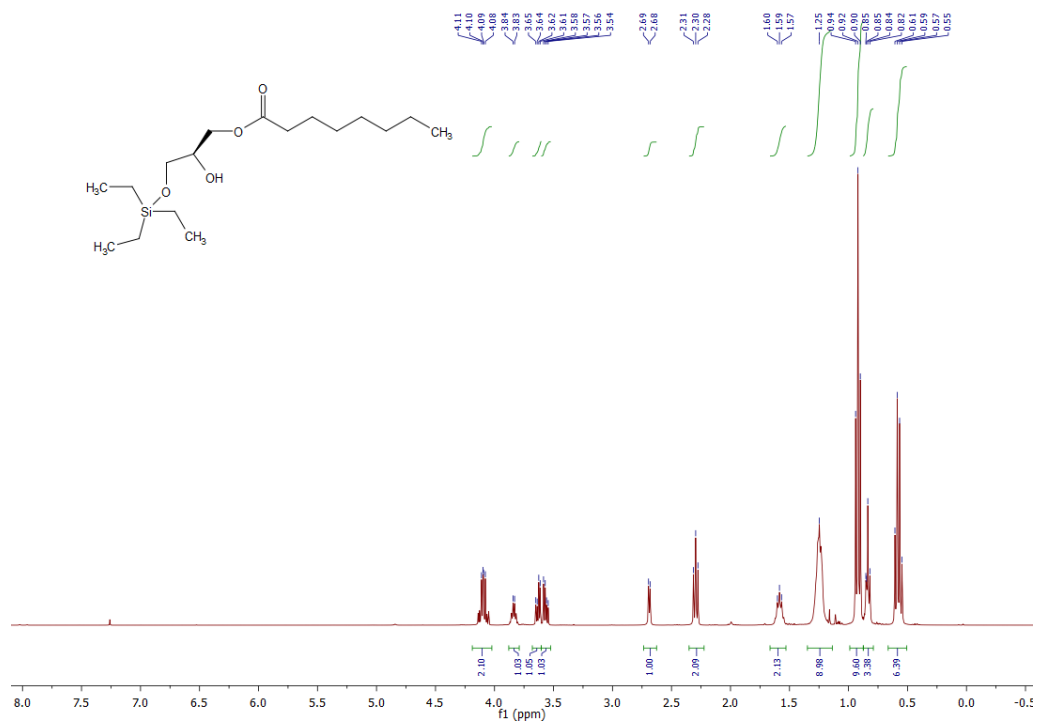
**Supplementary Note 5 | <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of 2-O-(4-(4-((4-butylphenyl)diazenyl)phenyl)butanoyl)-1-O-stearoyl-3-O-triethylsilyl-*sn*-glycerol (**8**).**



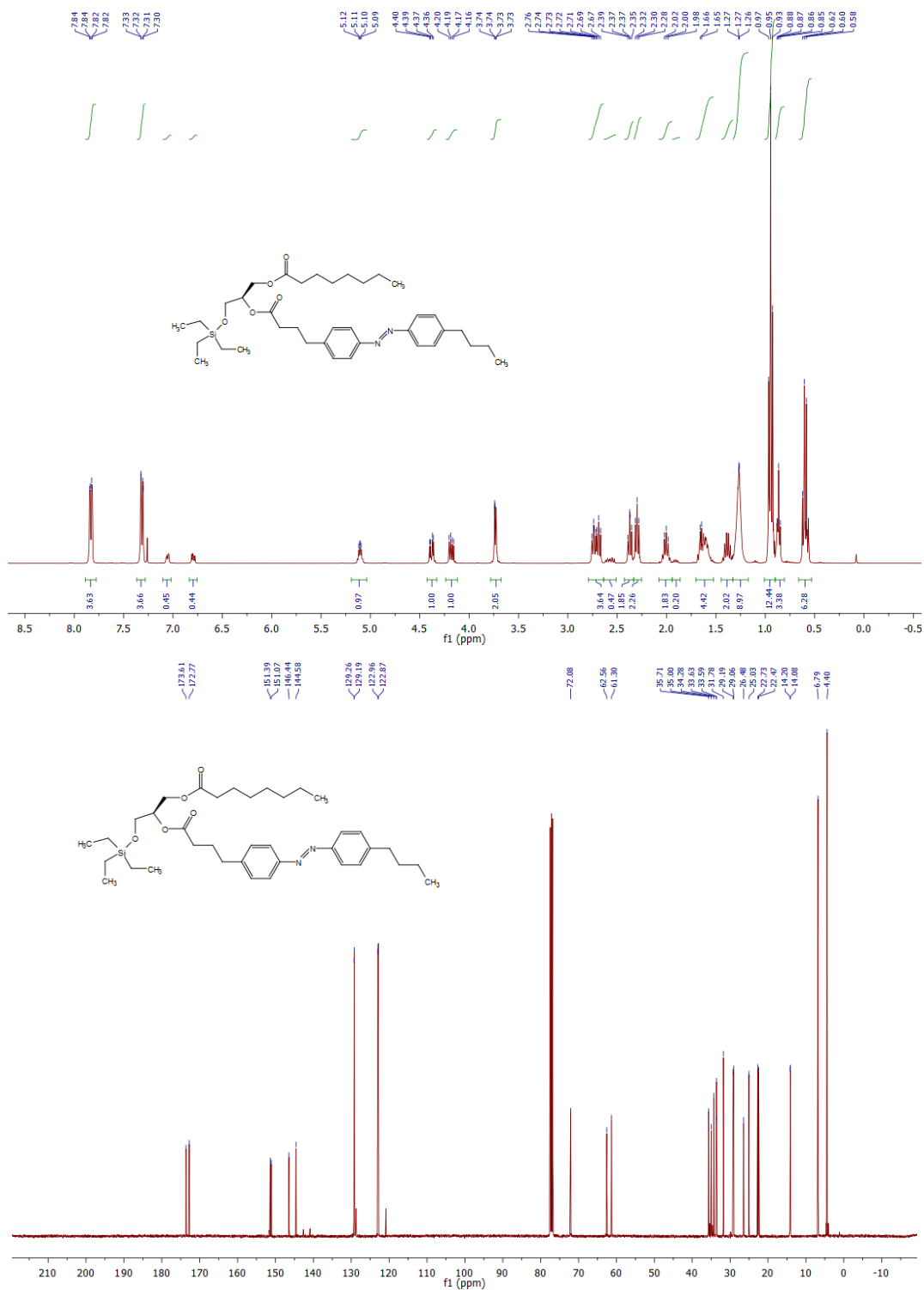
**Supplementary Note 6** |  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra of 2-O-(4-(4-((4-butylphenyl)diazenyl)phenyl)butanoyl)-1-O-stearoyl-*sn*-glycerol (**PhoDAG-1**, **1**).



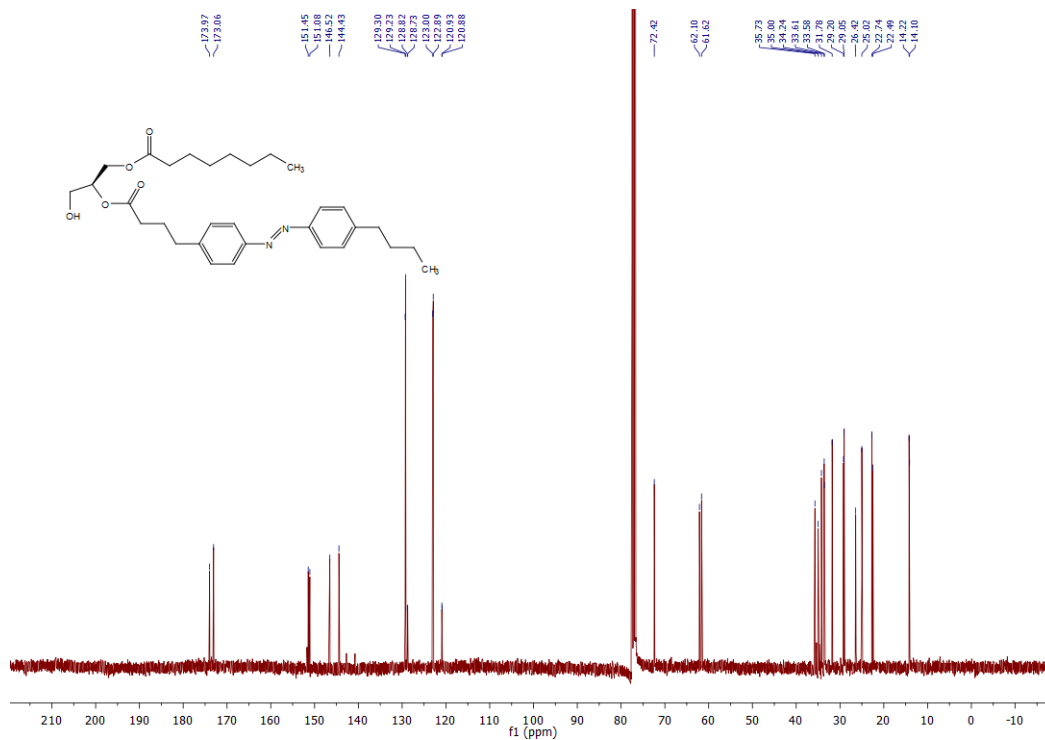
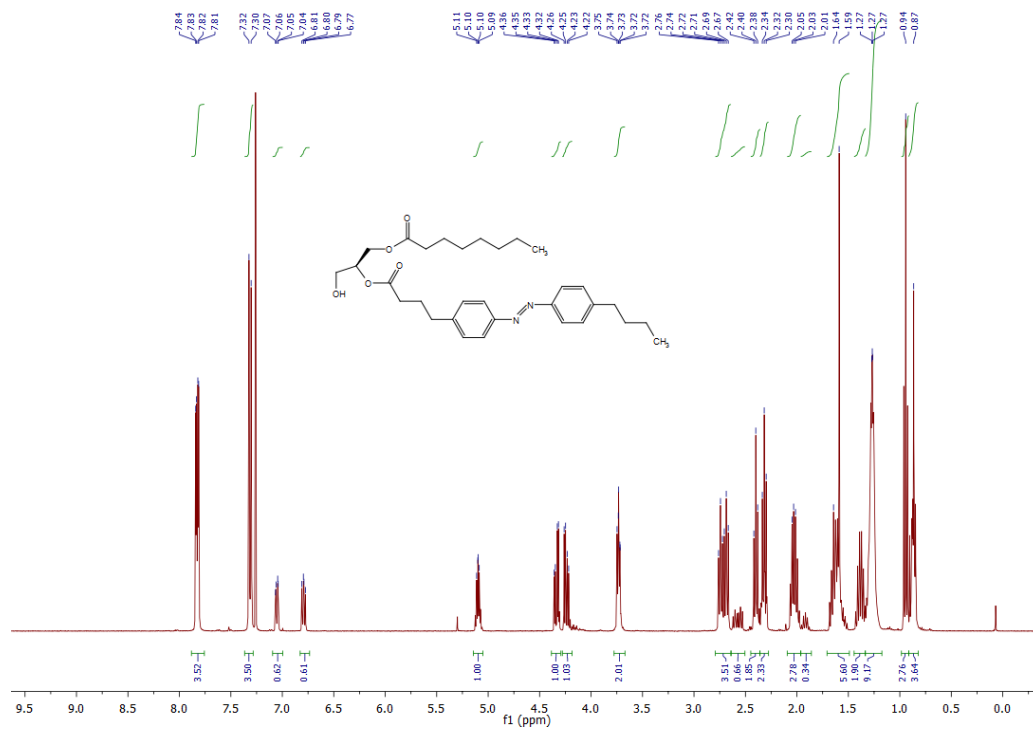
Supplementary Note 7 | <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of 4-(Phenyldiazenyl)phenyl butanoic acid (FAAzo-9, 4).



Supplementary Note 8 | <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of 1-O-octanoyl-3-O-triethylsilyl-*sn*-glycerol (10).



**Supplementary Note 9** | <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of 2-O-(4-(4-((4-butylphenyl)diazenyl)phenyl)butanoyl)-1-O-octanoyl-3-O-triethylsilyl-*sn*-glycerol (**11**).



**Supplementary Note 10 | <sup>1</sup>H- and <sup>13</sup>C-NMR spectra of 2-O-(4-(4-((4-butylphenyl)diazenyl)phenyl)butanoyl)-1-O-octanoyl-*sn*-glycerol (PhoDAG-2, 2).**







**Supplementary Movie 1 | PhoDAG-1 enabled optical control of PKC $\delta$ -RFP translocation.** A representative stack of fluorescence images showing PKC $\delta$ -RFP fluorescence in HeLa cells transiently expressing the reporter. The application of **PhoDAG-1** (red circle, 100  $\mu$ M) did not affect the localization of PKC $\delta$ -RFP. On  $\lambda = 375$  nm irradiation, PKC $\delta$ -RFP translocated to the plasma membrane. After the termination of irradiation, PKC $\delta$ -RFP diffused back to the cytoplasm. This effect could be repeated over multiple cycles. Each frame was acquired in 4 s intervals, and the video is played back at 6 fps.