Photoswitchable diacylglycerols enable optical control of protein kinase C

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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 μM 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 DAGs.

(a) PhoDAG-1 was synthesized in four steps and 57% overall yield. (b) A representative NMR spectrum of PhoDAG-1 (in CDCl₃), displaying the aromatic region. Both the trans- and cis-azobenzene protons give signals under ambient lighting conditions, where PhoDAG-1 exists as ≈10% the cis-isomer. (c) The chemical syntheses of the short-chain photoswitchable DAGs, PhoDAG-2 and PhoDAG-3.
Supplementary Note 3 | Synthetic Procedures

NMR multiplicities in the following experimental procedures are abbreviated as follows: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; sext, sextet; hept, heptet; br, broad; m, multiplet.

Synthesis of S-2,3-O-isopropylidene-1-O-stearoyl-sn-glycerol (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.\(^1\) Spectral characteristics matched those previously reported\(^2\).

R(-)-2,3-O-Isopropylidene-sn-glycerol (5, 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\(_2\)Cl\(_2\) (75 mL) under an argon atmosphere. To this solution was added dry NEt\(_3\) (6.16 mL, 4.48 g, 44.0 mmol, 2.0 equiv.) and the reaction was cooled to 0 \(^{\circ}\)C. Stearoyl chloride was dissolved in dry CH\(_2\)Cl\(_2\) (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\(_2\)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\(_3\) (2x50 mL) and brine (2x50 mL) solutions. The organic phase was then dried over anhydrous Na\(_2\)SO\(_4\) and filtered. The filtrate was then concentrated and purified by flash silica gel chromatography (150 g SiO\(_2\), 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): \(R_f = 0.25\).

\(^1\)H NMR (CDCl\(_3\), 400 MHz, 25 \(^{\circ}\)C): \(\delta\) 4.35–4.28 (m, 1 H, H\(_2\)G), 4.17 (dd, 1 H, HG\(_1\)\(_a\), J = 11.7, 4.7 Hz), 4.12–4.05 (m, 2 H, HG\(_1\)\(_b\), HG\(_3\)\(_a\)), 3.75 (dd, 1H, H\(_3\)\(_b\), J = 8.4, 6.2 Hz) 2.34 (t, 2 H, H\(_2\)L\(_a,b\)), 1.67–1.58 (m, 2 H, H\(_3\)L\(_a,b\)), 1.43 (s, 3 H, H\(_6\)), 1.37 (s, 3 H, H\(_{CH3}\)), 1.33–1.21 (m, 28 H, H\(_{Alk}\)), 0.87 (t, 3 H, H\(_8\)L\(_a,b,c\)), J = 6.5 Hz).

\(^{13}\)C NMR (CDCl\(_3\), 100 MHz, 25 \(^{\circ}\)C): \(\delta\) 173.1 (C\(_1\)S), 109.9 (C), 73.8 (C\(_{Glycerol}\)), 66.5 (C\(_{Glycerol}\)), 64.6 (C\(_{Glycerol}\)), 34.2 (C\(_2\)S), 32.1 (C\(_3\)S), 29.9–29.8 (m, C\(_{Alk}\)), 2xCH\(_3\)), 29.7 (C\(_{Alk}\)), 29.6 (C\(_{Alk}\)), 29.5 (C\(_{Alk}\)), 29.4 (C\(_{Alk}\)), 29.3 (C\(_{Alk}\)), 26.8 (C\(_{Alk}\)), 25.5 (C\(_{Alk}\)), 25.0 (C\(_{Alk}\)), 22.8 (C\(_{Alk}\)), 14.3 (C\(_{18}\)S).

HRMS (EI\(^+\)): \(m/z\) calcd. for [C\(_{23}\)H\(_{43}\)O\(_4\)]\(^+\): 383.3156, found: 383.3172 ([M–CH\(_3\)]\(^+\)).
Synthesis of 1-O-stearoyl-3-O-triethilsilyl-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. 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, 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 Na₂CO₃ solution (10%, 10 mL) followed by I₂ (610 mg, 2.4 mmol, 1.95 equiv.). The solution was stirred rapidly for 2.5 h at room temperature. A further portion of I₂ 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 Na₂S₂O₃ (50 mL), H₂O (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 SiO₂, 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^H NMR (CDCl₃, 400 MHz, 25 °C): δ 4.18 (m, 2 H, H1G_a,b), 3.92–3.82 (m, 1 H, H2G), 3.67 (dd, 1 H, H3G_a, J = 10.3 Hz, 4.5 Hz), 3.60 (dd, 1 H, H3G_b, J = 10.0 Hz, 5.7 Hz), 2.55 (d, 1 H, OH, J = 5.3 Hz), 2.33 (t, 2 H, H2S_a,b, J = 7.6 Hz), 1.66–1.58 (m, 2 H, H3S_a,b), 1.34–1.20 (m, 28 H, 14xCH₂(stearoyl)), 0.96 (t, 9 H, 3xCH₃(TES)), J = 7.8 Hz), 0.88 (t, 3 H, H18S_a,b,c, J = 6.9 Hz), 0.61 (q, 6 H, 3xCH₂(TES)), J = 7.9 Hz).

^1^3^C NMR (CDCl₃, 100 MHz, 25 °C): δ 174.1 (C1S), 70.2 (C2G), 65.1 (C1G), 63.5 (C3G), 34.3 (C2S), 32.1 (C_alk), 29.9–29.7 (m, C_alk), 29.6 (C_alk), 29.5 (C_alk), 29.4 (C_alk), 29.3 (C_alk), 25.1 (C3S), 22.9 (C_alk), 14.3 (C18S), 6.8 (3C, 3xCH₃(TES)), 4.4 (3C, 3xCH₂(TES)).

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

\[
\begin{align*}
\text{FAAzo-4}^4 (1.08 \text{ g, 3.3 mmol, 2.0 equiv.}), N-(3\text{-dimethylaminopropyl})-N'\text{-ethylcarbodiimide (EDC, 773 mg, 4.38 mmol, 3.0 equiv.) and DMAP (20.2 mg, 166 \mu\text{mol, 0.1 equiv.) were dissolved in dry 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 °C, a solution of 1-\(O\)-stearoyl-3-\(O\)-triethylsilyl-\(sn\)-glycerol (7, 787 mg, 1.66 mmol, 1.0 equiv.) in dry CH\(_2\)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 CH\(_2\)Cl\(_2\) (200 mL) and washed with H\(_2\)O (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 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.}
\end{align*}
\]
TLC (10:1 hexanes:EtOAc): \( R_f = 0.50 \).

\(^1\)H NMR (CDCl\(_3\), 400 MHz, 25 °C): \( \delta \) 7.85–7.80 (m, 4 H, H7A\(_{a,b}\), H12A\(_{a,b}\)), 7.31 (d, 4 H, H6A\(_{a,b}\), H13A\(_{a,b}\), J = 8.2 Hz), 5.13–5.05 (m, 1 H, H2G), 4.38 (dd, 1 H, H1G\(_a\), J = 12.1 Hz, 3.6 Hz), 4.17 (dd, 1 H, H1G\(_b\), J = 12.1 Hz, 6.1 Hz), 3.73 (d, 2 H, H3G\(_{a,b}\), J = 5.3 Hz), 2.73 (t, 2 H, H4A\(_{a,b}\), J = 7.7 Hz), 2.69 (t, 2 H, H15A\(_{a,b}\), J = 7.8 Hz), 2.37 (t, 2 H, H2A\(_{a,b}\), J = 7.5 Hz), 2.29 (t, 2 H, H2S\(_{a,b}\), J = 7.6 Hz), 2.00 (quin, 2 H, H3A\(_{a,b}\), J = 7.4 Hz), 1.69–1.53 (m, 4 H, H3S\(_{a,b}\), H16A\(_{a,b}\)), 1.43–1.34 (m, 2 H, H17A\(_{a,b}\)), 1.33–1.21 (m, 28 H, H17S\(_{a,b}\), 13xCH\(_2\)(alk)), 0.97–0.85 (m, 15 H, H18A\(_{a,b,c}\), H17S\(_{a,b,c}\), 3xCH\(_3\)(TES)), 0.59 (q, 6 H, 3xCH\(_2\)(TES), J = 8.0 Hz).

\(^{13}\)C NMR (CDCl\(_3\), 100 MHz, 25 °C): \( \delta \) 173.7 (C1S), 172.8 (C1A), 151.4 (C_{azo}), 151.1 (C\(_{azo}\)), 146.5 (C\(_{azo}\)), 144.6 (C\(_{azo}\)), 129.3 (2 C, C\(_{azo}\)), 129.2 (2 C, C\(_{azo}\)), 123.0 (2 C, C\(_{azo}\)), 122.9 (2 C, C\(_{azo}\)), 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\(_{Alk}\)), 29.6 (C\(_{Alk}\)), 29.5 (C\(_{Alk}\)), 29.4 (C\(_{Alk}\)), 29.3 (C\(_{Alk}\)), 26.5 (C3A), 25.1 (C16A), 22.9 (C17S), 22.5 (C17A), 14.3 (C18S), 14.1 (C18A), 6.8 (3 C, 3xCH\(_3\)(TES)), 4.4 (3 C, 3xCH\(_2\)(TES)).

HRMS (EI\(^+\)): \( m/z \) calcd. for [C\(_{20}H\(_{24}N\(_2\)O\(_2\))]\(^+\): 778.5680, found: 778.5675 ([M–e \(^+\)]\(^+\)).
Synthesis of \(2\text{-O-}(4\text{-}(4\text{-}((4\text{-butylphenyl)diazenyl})phenyl)butanoyl)\text{-1-O-stearoyl-sn-glycerol (PhoDAG-1, 1)}\)

\[ \text{PhoDAG-1} \]

\(2\text{-O-}(4\text{-}(4\text{-}((4\text{-butylphenyl)diazenyl})phenyl)butanoyl)\text{-1-O-stearoyl-3-O-triethylsilyl-sn-glycerol}\)

(8, 500 mg, 0.641 mmol, 1.0 equiv.) was first dissolved in \(\text{CH}_2\text{Cl}_2\) (5 mL) and added to a solution of \(\text{FeCl}_2\text{-6H}_2\text{O}\) (5 mM in 25 mL 3:1 \(\text{MeOH:CH}_2\text{Cl}_2\)). This solution was stirred at room temperature for 30 min. The solution was then diluted with \(\text{EtOAc}\) (200 mL) and washed with \(\text{H}_2\text{O}\) (2x200 mL). The organic phase was then dried over \(\text{Na}_2\text{SO}_4\). 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 \(2\text{-O-}(4\text{-}(4\text{-}((4\text{-butylphenyl)diazenyl})phenyl)butanoyl)\text{-1-O-stearoyl-sn-glycerol (PhoDAG-1, 0.425 mg, quant.) as an orange solid.}\)

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).

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

$^{13}$C NMR (CDCl$_3$, 100 MHz, 25 °C): $\delta$ 174.0 (C1S), 173.1 (C1A), 151.5 (C$_{Azo}$), 151.1 (C$_{Azo}$), 146.5 (C$_{Azo}$), 144.4 (C$_{Azo}$), 129.3 (2 C, C$_{Azo}$), 129.2 (2 C, C$_{Azo}$), 123.0 (2 C, C$_{Azo}$), 122.9 (2 C, C$_{Azo}$), 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$_{Alk}$), 29.6 (C$_{Alk}$), 29.5 (C$_{Alk}$), 29.4 (C$_{Alk}$), 29.3 (C$_{Alk}$), 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, 1499, 1472, 1460, 1414, 1379, 1256, 1235, 1214, 1188, 1173, 1138, 1096, 1068, 1052, 1031, 1012, 962, 888, 832, 718, 679.

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

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

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 CH\textsubscript{2}Cl\textsubscript{2} (10 mL) under an argon atmosphere and then NEt\textsubscript{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\textquotesingle-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 \textit{R}(-)-2,3-O-Isopropylidene-sn-glycerol (5, 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 CH\textsubscript{2}Cl\textsubscript{2} and washed once with a saturated aqueous NaHCO\textsubscript{3} solution, and twice with H\textsubscript{2}O. The organic phase was then dried over Na\textsubscript{2}SO\textsubscript{4} and filtered. The filtrate was concentrated under reduced pressure and the resulting oil was purified by flash silica gel chromatography (30 g SiO\textsubscript{2}, pre-adsorbed on 1.5 g of SiO\textsubscript{2}, 40:1 to 10:1 hexanes:EtOAc) to yield \textit{S}-2,3-O-isopropylidene-1-O-octanoyl-sn-glycerol (9, 537.3 mg, 92%) as a colorless oil.

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

\textit{\textsuperscript{1}H NMR} (CDCl\textsubscript{3}, 400 MHz, 25 °C): \(\delta\ 4.32–4.24\ \text{(m, 1 H, H2G)}, 4.17–4.09\ \text{(m, 1 H, HG\textsubscript{1a})}, 4.09–4.00\ \text{(m, 2 H, HG\textsubscript{1b}, HG\textsubscript{3a})}, 3.73–3.68\ \text{(m, 1H, H3\textsubscript{b})} 2.31\ \text{(t, 2 H, H2L\textsubscript{a,b}, J = 7.3 Hz)}, 1.65–1.54\ \text{(m, 2 H, H3L\textsubscript{a,b})}, 1.40–1.33\ \text{(s, 6 H, 2xH\textsubscript{CH3})}, 1.31–1.17\ \text{(m, 8 H, H\textsubscript{alk})}, 0.84\ \text{(t, 3 H, H8L\textsubscript{a,b,c}, J = 6.7 Hz}).

\textit{\textsuperscript{13}C NMR} (CDCl\textsubscript{3}, 100 MHz, 25 °C): \(\delta\ 173.7\ \text{(C1L)}, 109.9\ \text{(C2)}, 73.7\ \text{(C\text{Glycerol})}, 66.4\ \text{(C\text{Glycerol})}, 66.6\ \text{(C\text{Glycerol})}, 34.2\ \text{(C2S)}, 31.7\ \text{(C3S)}, 29.2\ \text{(C\text{CH3})}, 29.0\ \text{(C\text{CH3})}, 26.8\ \text{(C\text{alk})}, 25.5\ \text{(C\text{alk})}, 25.0\ \text{(C\text{alk})}, 22.7\ \text{(C\text{alk})}, 14.5\ \text{(C18L})

\text{HRMS (EI\textsuperscript{*}}): \(m/z\ \text{calcd. for [C\textsubscript{13}H\textsubscript{23}O\textsubscript{4}]\textsuperscript{+}: 243.1591, found: 243.1595 ([M–CH\textsubscript{3}]\textsuperscript{+}).
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 DCE (6 mL) under an argon atmosphere and then dry 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 H2O (2x60 mL). The organic phase was then dried over Na2SO4 and filtered. The filtrate was concentrated under reduced pressure. The resulting oil was dissolved in THF (8 mL) and then aqueous Na2CO3 (4 mL, 10% w/w) and I2 (1.1 g, 4.4 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 Na2S2O3 (70 mL) and H2O (2x70 mL). The organic phase was dried over Na2SO4 and filtered. The filtrate was concentrated under reduced pressure and the resulting oil was purified by flash silica gel chromatography (60 g SiO2, 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): Rf = 0.30.

1H NMR (CDCl3, 400 MHz, 25 °C): δ 4.19–4.08 (m, 2 H, H1Ga,b), 3.98–3.87 (m, 1 H, H2G), 3.63 (dd, 1 H, H3Ga, J1 = 10.5 Hz, J2 = 5.3 Hz), 3.56 (dd, 1 H, H3Gb, J1 = 10.3 Hz, J2 = 5.4 Hz), 2.69 (d, 1 H, OH, J = 5.4 Hz), 2.34 (t, 2 H, H2a,b, J = 7.5 Hz), 1.67–1.58 (m, 2 H, H3a,b), 1.34–1.20 (m, 8 H, Halk), 0.96 (t, 9 H, 3xCH3(TES), J = 7.9 Hz), 0.87 (t, 3 H, H8a,b,c, J = 6.9 Hz), 0.61 (q, 6 H, 3xCH2(TES)), J = 7.9 Hz).

13C NMR (CDCl3, 100 MHz, 25 °C): δ 174.1 (C1L), 70.2 (CXG), 65.1 (CXG), 63.5 (CXG), 34.3 (C2), 31.8 (Calk), 29.2 (Calk), 29.1 (Calk), 25.1 (Calk), 21.8 (C7L), 14.2 (C8L), 6.8 (3C, 3xCH3(TES)), 4.4 (3C, 3xCH2(TES)).

IR (neat, ATR): ν = 3489, 2955, 2929, 2876, 1740, 1458, 1416, 1380, 1239, 1166, 1100, 1006, 976, 842, 807, 744, 728, 676, 619, 588, 601, 564.

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

FAAzo-4 (136.3 mg, 0.42 mmol, 2.0 equiv.) was dissolved in dry CH$_2$Cl$_2$ (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$_2$Cl$_2$ and then washed three times with H$_2$O. The organic phase was dried over Na$_2$SO$_4$, filtered, and the filtrate was concentrated under reduced pressure. The resulting dark orange oil was purified by flash silica gel chromatography (15 g SiO$_2$, 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).

\(^1\)H NMR (CDCl\(_3\), 400 MHz, 25°C): \( \delta \) 7.87–7.79 (m, 4 H, H7A\(_{a,b}\), H12A\(_{a,b}\)), 7.31 (d, 4 H, H6A\(_{a,b}\), H13A\(_{a,b}\), \( J = 8.7 \) Hz), 5.14–5.05 (m, 1 H, H2G), 4.42–4.33 (m, 1 H, H1G\(_a\)), 4.23–4.13 (m, 1 H, H1G\(_b\)), 3.77–3.69 (m, 2 H, H3G\(_{a,b}\)), 2.78–2.67 (m, 4 H, H4A\(_{a,b}\), H15A\(_{a,b}\)), 2.37 (t, 2 H, H1A\(_{a,b}\), \( J = 7.1 \) Hz), 2.30 (t, 2 H, H2L\(_{a,b}\), \( J = 7.3 \) Hz), 2.07–1.95 (m, 2 H, H3A\(_{a,b}\)), 1.69–1.52 (m, 4 H, H3L\(_{a,b}\), H16A\(_{a,b}\)), 1.44–1.33 (m, 2 H, H17A\(_{a,b}\)), 1.33–1.19 (m, 8 H, H\(_{alk}\)), 0.99–0.90 (m, 12 H, H18A\(_{a,b,c}\), 3xCH\(_3\)(TES)), 0.86 (t, 3 H, H8L\(_{a,b,c}\)), 0.59 (q, 6 H, 3xCH\(_2\)(TES)), \( J = 8.0 \) Hz).

\(^{13}\)C NMR (CDCl\(_3\), 100 MHz, 25°C): \( \delta \) 173.6 (C1S), 172.8 (C1A), 151.4 (C\(_{azo}\)), 151.9 (C\(_{azo}\)), 146.4 (C\(_{azo}\)), 144.6 (C\(_{azo}\)), 129.3 (2 C, C\(_{azo}\)), 129.2 (2 C, C\(_{azo}\)), 123.0 (2 C, C\(_{azo}\)), 122.9 (2 C, C\(_{azo}\)), 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\(_{alk}\)), 29.1 (C\(_{alk}\)), 26.5 (C\(_{alk}\)), 25.0 (C\(_{alk}\)), 22.7 (C\(_{alk}\)), 22.5 (C\(_{alk}\)), 14.2 (C18L), 14.1 (C18A), 6.8 (3 C, 3xCH\(_3\)(TES)), 4.4 (3 C, 3xCH\(_2\)(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\(^+\)): \( m/z \) calcd. for [C\(_{37}H_{59}N_2O_5Si]\(^+\): 639.4193, found: 639.4142 ([M+H\(^+\)]\(^+\)).
Synthesis of 2-\(O-(4-(4-(4\text{-butylphenyl})\text{diazenyl})\text{phenyl})\text{butanoyl})\)-1-\(O\text{-octanoyl-sn-glycerol}\) (PhoDAG-2, 2)

2-\(O-(4-(4-(4\text{-butylphenyl})\text{diazenyl})\text{phenyl})\text{butanoyl})\)-1-\(O\text{-octanoyl-sn-glycerol}\) (PhoDAG-2) was prepared from 2-\(O-(4-(4-(4\text{-butylphenyl})\text{diazenyl})\text{phenyl})\text{butanoyl})\)-1-\(O\text{-octanoyl-3-O\text{-triethylsilyl-sn-glycerol}}\) (11, 28.0 mg, 0.044 mmol, 1.0 equiv.) as described above in the synthesis of 2-\(O-(4-(4-(4\text{-butylphenyl})\text{diazenyl})\text{phenyl})\text{butanoyl})\)-1-\(O\text{-stearoyl-sn-glycerol}\) (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).

\(^1\)H NMR (CDCl\(_3\), 400 MHz, \(25^\circ\)C): \(\delta 7.86–7.79\) (m, 4 H, \(H7_{a,b}, H12_{a,b}\)), \(7.31\) (d, 4 H, \(H6_{a,b}, H13_{a,b}\), \(J = 8.7\) Hz), \(5.15–5.05\) (m, 1 H, \(H2G\)), \(4.37–4.30\) (m, 1 H, \(H3G_{a}\)), \(4.27–4.20\) (m, 1 H, \(H3G_{b}\), \(J = 7.5\) Hz), \(2.74\) (t, 2 H, \(H4_{a,b}\), \(J = 7.5\) Hz), \(2.69\) (t, 2 H, \(H15_{a,b}\), \(J = 7.8\) Hz), \(2.40\) (t, 2 H, \(H2_{a,b}\), \(J = 7.5\) Hz), \(2.36–2.29\) (m, 2 H, \(H2_{a,b}\)), \(2.07–1.97\) (m, 3 H, \(H3_{a,b}\), \(J = 7.0\) Hz), \(1.70–1.50\) (m, 4 H, \(H16_{a,b}, HL3_{a,b}\), \(J = 7.6\) Hz), \(1.44–1.18\) (m, 10 H, \(H_{\text{alk}}\), \(J = 7.3\) Hz), \(0.90–0.81\) (t, 3 H, \(H8L_{a,b,c}\), \(J = 7.6\) Hz).

\(^{13}\)C NMR (CDCl\(_3\), 100 MHz, \(25^\circ\)C): \(\delta 174.0\) (C1L), \(173.0\) (C1A), \(152.8\) (C\(_{\text{Azo}}\)), \(151.3\) (C\(_{\text{Azo}}\)), \(146.5\) (C\(_{\text{Azo}}\)), \(144.4\) (C\(_{\text{Azo}}\)), \(129.3\) (2 C, C\(_{\text{Azo}}\)), \(129.2\) (2 C, C\(_{\text{Azo}}\)), \(123.9\) (2 C, C\(_{\text{Azo}}\)), \(122.9\) (2 C, C\(_{\text{Azo}}\)), \(72.4\) (C2G), \(62.1\) (C3G), \(61.6\) (C1G), \(35.7\) (C15A), \(35.0\) (C4A), \(34.2\) (C2L), \(33.61\) (C\(_{\text{Alk}}\)), \(33.58\) (C\(_{\text{Alk}}\)), \(31.8\) (C\(_{\text{Alk}}\)), \(29.2\) (C\(_{\text{Alk}}\)), \(29.1\) (C\(_{\text{Alk}}\)), \(26.4\) (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\(^+\)): \(m/z\) calcd. for \([\text{C}_{31}\text{H}_{44}\text{N}_{2}\text{O}_{5}]^+\): 524.3250, found: 524.3245 (\([\text{M–e}^-]\)).

UV-Vis (50 \(\mu\)M in DMSO): \(\lambda_{\text{max}}(\pi–\pi^\ast) = 340\) nm. \(\lambda_{\text{max}}(n–\pi^\ast) = 440\) nm.
Synthesis of 4-(Phenyl diazenyl)phenyl butanoic acid (FAAzo-9, 4)

4-((4-aminophenyl)butyric acid (200 mg, 1.12 mmol, 1.0 equiv.) was first dissolved in CH$_2$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 SiO$_2$, 99:1 CH$_2$Cl$_2$:AcOH) to yield 4-(phenyl diazenyl)phenyl butanoic acid (FAAzo-9, 4, 320.8 mg, quant.) as an orange solid.

TLC (99:1 CH$_2$Cl$_2$:AcOH): $R_f = 0.17$.

$^1$H NMR (CDCl$_3$, 400 MHz, 25 °C): $\delta$ 7.94–7.82 (m, 4 H, H$_7$$_{a,b}$, H$_{11}$$_{a,b}$), 7.55–7.43 (m, 3 H, H$_{13}$$_{a,b}$, H14), 7.34 (d, 2 H, H$_6$$_{a,b}$, $J = 4.0$ Hz), 7.26 (t, 2 H, H$_4$$_{a,b}$, $J = 7.8$ Hz), 2.42 (t, 2 H, H$_2$$_{a,b}$, $J = 7.3$ Hz), 2.02 (dd, 2 H, H$_3$$_{a,b}$, $J = 7.8, 7.3$ Hz).

$^{13}$C NMR (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, C$_{6,a,b}$, C$_{13,a,b}$), 123.2-122.9 (4 C, C$_{7,a,b}$, C$_{12,a,b}$), 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$^+$): $m/z$ calcd. for [C$_{16}$H$_{16}$N$_2$O$_2$]$^+$: 268.1212, found: 268.1206 ([M–e$^-$]$^+$).

UV-Vis (50 μM in DMSO): $\lambda_{\text{max}}$(π–π*) = 330 nm. $\lambda_{\text{max}}$(n–π*) = 425 nm.

Melting point (°C): 135.5–137.5.
Synthesis of 2-\(\text{O}(4-(4\text{-(phenyldiazenyl)phenyl})\text{butanoyl})-1\text{-O-octanoyl-3-O}\text{-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\(_2\)Cl\(_2\) (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-\(\text{O-octanoyl-3-O}\text{-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\(_2\)Cl\(_2\) (100 mL) and washed with H\(_2\)O (3x50 mL). The organic phase was dried over Na\(_2\)SO\(_4\) and then filtered. The filtrate was concentrated under reduced pressure. The residue was purified by flash silica gel chromatography (20 g SiO\(_2\), hexanes:ethyl acetate 20:1→10:1) to yield 2-\(\text{O}(4-(4\text{-(phenyldiazenyl)phenyl})\text{butanoyl})-1\text{-O-octanoyl-3-O}\text{-triethylsilyl-sn-glycerol (12, 100 mg, 61%) as an orange oil.}\\

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

^1^H NMR (CDCl_3, 400 MHz, 25 °C): δ 7.94–7.83 (m, 4 H, H7_{A,b}, H12_{A,b}), 7.55–7.43 (m, 3 H, H14A, H13_{A,b}), 7.33 (d, 2 H, H6_{A,b}, J = 8.2 Hz), 5.15–5.05 (m, 1 H, H2G), 4.41–4.33 (m, 1H, H1G_{b}), 4.22–4.13 (m, 1 H, H1G_{b}), 3.78–3.69 (m, 2 H, H3G_{a,b}), 2.74 (t, 2 H, H4_{a,b}, J = 7.2 Hz), 2.38 (t, 2 H, H2A, J = 7.6 Hz), 2.30 (t, 2 H, H2L_{a,b}, J = 7.5 Hz), 2.08–1.96 (m, 2 H, H3A_{a,b}), 1.64–1.54 (m, 2 H, H3L_{a,b}), 1.34–1.19 (m, 8 H, H_{Alk}), 0.94 (t, 9 H, 3xCH_3(TES), J = 8.3 Hz), 0.90–0.82 (t, 3 H, H8L_{a,b,c}, J = 6.9 Hz), 0.59 (m, 6 H, 3xCH_2(TES), J = 8.2 Hz).

^1^3^C NMR (CDCl_3, 100 MHz, 25 °C): δ 173.6 (C1L), 172.8 (C1A), 152.8 (C_{Azo}), 151.3 (C_{Azo}), 145.0 (C_{Azo}), 131.0 (C_{Azo}), 129.3 (2C, C_{Azo}), 129.2 (2 C, C_{Azo}), 123.1 (2 C, C_{Azo}), 122.9 (2 C, C_{Azo}), 72.1 (C2G), 62.6 (C3G), 61.3 (C1G), 35.0 (C4A), 34.3 (C2L), 33.6 (C2A), 31.8 (C_{Alk}), 29.2 (C_{Alk}), 29.1 (C_{Alk}), 26.5 (C_{Alk}), 25.0 (C_{Alk}), 22.7 (C7L), 14.2 (C8L), 6.8 (3 C, 3xCH_3(TES)), 4.4 (3 C, 3xCH_2(TES)).

IR (neat, ATR): ν = 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^+): m/z calcd. for [C_{33}H_{51}N_2O_5Si]^+: 583.3567, found: 583.3567 ([M+H]^+).
Synthesis of 2-O-(4-(4-(phenyldiazenyl)phenyl)butanoyl)-1-O-octanoyl-sn-glycerol (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 (12, 22.0 mg, 0.038 mmol, 1 equiv.) as described above in the synthesis of 2-O-(4-(4-(4-butylyphenyl)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).

\(^1\)H NMR (CDCl\textsubscript{3}, 400 MHz, 25 °C): \( \delta \) 7.93–7.83 (m, 4 H, H\textsubscript{7a,b}, H\textsubscript{11a,b}), 7.55–7.43 (m, 3 H, H\textsubscript{14A}, H\textsubscript{13a,b}), 7.33 (d, 2 H, H\textsubscript{6a,b}, J = 8.3 Hz), 5.14–5.04 (q, 1 H, H\textsubscript{2G}, J = 4.9 Hz), 4.38–4.29 (m, 1 H, H\textsubscript{3G}), 2.32 (t, 2 H, H\textsubscript{2L}, J = 7.7 Hz), 2.07–1.96 (m, 2 H, H\textsubscript{3A}), 1.65–1.55 (m, 2 H, H\textsubscript{3L}), 1.34–1.20 (m, 8 H, H\textsubscript{alk}), 0.90–0.82 (t, 3 H, H\textsubscript{8L}, J = 7.1 Hz).

\(^{13}\)C NMR (CDCl\textsubscript{3}, 100 MHz, 25 °C): \( \delta \) 173.4 (C1L), 173.0 (C1A), 152.8 (C\textsubscript{Azo}), 151.3 (C\textsubscript{Azo}), 144.8 (C\textsubscript{Azo}), 130.9 (C\textsubscript{Azo}), 129.3 (2 C, C\textsubscript{Azo}), 129.2 (2 C, C\textsubscript{Azo}), 123.2 (2 C, C\textsubscript{Azo}), 122.9 (2 C, C\textsubscript{Azo}), 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\textsubscript{Alk}), 29.0 (C\textsubscript{Alk}), 26.4 (C\textsubscript{Alk}), 25.0 (C\textsubscript{Alk}), 22.7 (C7A), 14.2 (C8A).

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

HRMS (EI\textsuperscript{+}): \( \text{m/z calcd. for [C}_{27}\text{H}_{36}\text{N}_{2}\text{O}_{5}]^{+}: 468.2624 \), found: 468.2622 ([M–e\textsuperscript–])

UV-Vis (50 \( \mu \)M in DMSO): \( \lambda_{\text{max}}(\pi\rightarrow\pi^*) = 325 \text{ nm} \). \( \lambda_{\text{max}}(n\rightarrow\pi^*) = 440 \text{ nm} \).
Supplementary Note 4 | NMR SPECTRA

S-2,3-\(\text{O}\)-isopropylidene-1-\(\text{O}\)-stearoyl-sn-glycerol (6)
1-O-stearoyl-3-O-triethylsilyl-sn-glycerol (7)
2-O-(4-(4-((4-butylphenyl)diazenyl)phenyl)butanoyl)-1-O-stearoyl-3-O-triethylsilyl-sn-glycerol (8)
2-O-(4-(4-((4-butylyphenyl)diazenyl)phenyl)butanoyl)-1-O-stearoyl-sn-glycerol (PhoDAG-1, 1)
4-(phenyldiazenyl)phenyl butanoic acid (FAazo-9, 4)
S-2,3-\textit{O}-isopropylidene-1-\textit{O}-octanoyl-\textit{sn}-glycerol (9)
1-O-octanoyl-3-O-triethylsilyl-sn-glycerol (10)
2-Ο-(4-(4-(4-buty1phenyl)diazenyl)phenyl)butanoyl)-1-Ο-octanoyl-3-Ο-triethylsilyl-sn-glycerol (11)
2-O-(4-(4-((4-butylnphenyl)diazenyl)phenyl)butanoyl)-1-O-octanoyl-sn-glycerol (PhoDAG-2, 2)
2-O-(4-(phenyldiazenyl)phenyl)butanoyl)-1-O-octanoyl-3-O-triethylsilyl-sn-glycerol (12).
2-O-(4-(phenyldiazenyl)phenyl)butanoyl)-1-O-octanoyl-sn-glycerol (PhoDAG-3, 3)
REFERENCES


