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Supporting Information

Optical Manipulation of Gb_3 Enriched Lipid Domains: Impact of Isomerization on Gb_3-Shiga Toxin B Interaction

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LIST OF FIGURES, TABLES, AND MOVIES (CLICK ON THE BLUE LINK TO REACH THE DESIRED FIGURE)

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B) Biophysical Part

Movie S1. Domain reorganization of a phase-separated membrane containing 20 mol% C_7AZOC_1. Acquisition: 3 s per frame. Video frame rate: 20 frames per second.

Movie S2. AFM time-lapse of a phase-separated membrane containing 5 mol% C_7AZOC_1 after irradiation. Acquisition: 45 s per frame. Video frame rate: 5 frames per second.

Movie S3. CLSM time-lapse of a phase-separated membrane containing no photo-Gb_3. Acquisition: 5 s per frame. Video frame rate: 25 frames per second.

Movie S4. AFM time-lapse of a phase-separated membrane containing no photo-Gb_3. Acquisition: 40 s per frame. Video frame rate: 2 frames per second.

Movie S5. Fluidification of a phase-separated membrane containing 20 mol% C_1AZOC_7 after 6 minutes of UV irradiation. Acquisition: 3 s per frame. Video frame rate: 20 frames per second.

Movie S6. AFM time-lapse of the interactions of Shiga Toxin B with photo-Gb_3. Acquisition: 40 s per frame. Video frame rate: 2 frames per second.

Movie S7. CLSM time-lapse of the interactions of Shiga Toxin B with photo-Gb_3. Acquisition: 5 s per frame. Video frame rate: 25 frames per second.

A) Preparative Part: Synthesis and Characterization of Photo-switchable Gb_3s

1. General Information

All solvents were purchased as HPLC grade solvents and stored under molecular sieves. Air and moisture sensitive reactions were carried out in oven-dried or flame-dried glassware, septum-capped under atmospheric pressure of argon. Commercially available compounds were used without further purification unless otherwise stated. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on a 300, 400, 500 or 600 MHz instrument using the residual signals from $CHCl_3$, δ = 7.26 ppm, δ = 77.0 ppm and tetramethyl silane (TMS) δ = 0.00 ppm as internal references for ^{1}H and ^{13}C chemical shifts, respectively. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quintet, m = multiplet.

ESI-HRMS mass spectrometry was carried out on a FTICR instrument.

IR spectra were measured on an ATR spectrometer.

UV-vis spectra were measured on a Varian Cary 100 Bio photometer with temperature control

Optical Rotation were measured on a common polarimeter.

Dialysis was performed in deionized water using cellulose ester tubing with a molecular weight cut-off of 100- 500 g/mol.

Gel permeation HPLC (LC-9101) was carried out in recycling chloroform system.

2. General Procedures

General procedure 1 (GP1): Staudinger reduction/acylation

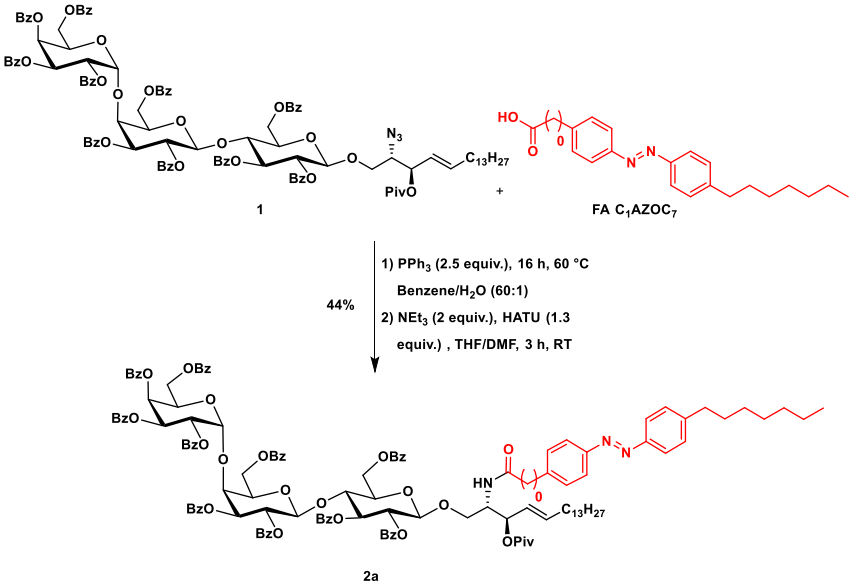
To a solution of the azide in anhydrous benzene were added triphenylphosphine and water. The suspension was heated to 60 °C (oil bath temperature) for 16 h. The solvents were removed under reduced pressure; the residue was azeotroped with toluene (3×) and dried in high vacuum for 2 h. The fatty acid was dissolved in anhydrous THF and DIPEA was added. Afterwards, HATU in anhydrous DMF was added dropwise. The reaction mixture was stirred 20 min at room temperature. Then the amine was dissolved in anhydrous THF and added to the reaction mixture. The reaction mixture was quenched by adding ethyl acetate and brine. The organic layer was washed with brine (2×) and the combined aqueous phases were re-extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. Gel permeation HPLC, followed by column chromatography on silica gel afforded corresponding glycolipid.

General procedure 2 (GP2): Zemplén deprotection

To a solution of the protected photo-switchable Gb_3 in methanol/dichloromethane (3:1) a solution of sodium methoxide (5.4 M in MeOH) was added at ambient temperature until a pH value >12 was reached. The reaction was stirred at 50 °C, then neutralized with Amberlite®, filtered and concentrated under vacuum.

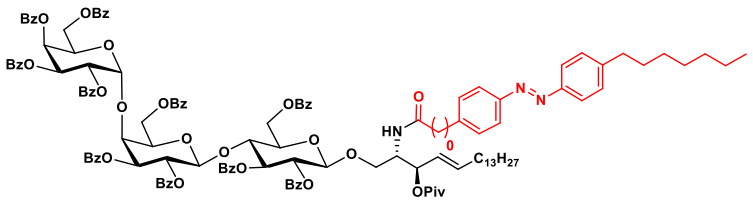
3. Procedures and analytical data for photo-switchable Gb_3s

3.1 Reduction/acylation reaction with **FA C_1AZOC$_7$**



The reduction of azide **1** (24.0 mg, 0.012 mmol, 1.0 equiv.) was performed according to general procedure **GP1** by using triphenylphosphine (7.86 mg, 0.03 mmol, 2.5 equiv.) in anhydrous benzene (2.8 ml) and water (34 µl). In a brown flask, photoswitchable fatty acid **FA C_1AZOC$_7$** (5.83 mg, 0.018 mmol, 2.5 equiv.) was dissolved in anhydrous THF (1.4 mL), then NEt_3 (4.59 µl, 0.036 mmol, 3.0 equiv.) and a solution of TBTU (6.84 mg, 0.018 mmol, 1.5 equiv.) in anhydrous DMF (318 µl) was added. The reaction mixture was stirred at ambient temperature for 15 min. The amine which was prepared in the first step was dried under vacuum at least 2 h and dissolved in anhydrous THF (1.0 mL), then it was added dropwise to the activated acid at ambient temperature. The reaction mixture was stirred at ambient temperature for 3 h. EtOAc was added and the organic phase thoroughly washed with brine (3×). The combined aqueous phases were re-extracted with ethyl acetate. The organic layer was dried with Na_2SO_4, filtered and the solvent removed under reduced pressure. Column chromatography on silica gel (DCM/MeOH, 40:1) followed by gel permeation chromatography afforded the pure photo-switchable Gb_3 **2a** (12 mg, 5.41 µmol, 44%) as an orange oil.

_O_-(2,3,4,6-Tetra-_O_-benzoyl-α-D-galactopyranosyl)-(1→4)-(2,3,6-tri-_O_-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-_O_-benzoyl-β-D-glucopyranosyl-(1→1)-(2S,3_R_,4_E_)-3-_O_-pivaloyl-2-(_N_-(4-((4-heptylphenyl)diazenyl)benzamide) (2a)



TLC (2:3 / EtOAc:*n*-pentane): Rf = 0.68.

^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) 0.88 (dt, J = 11.5, 6.8 Hz, 6 H), 1.05 (s, 9 H), 1.11 – 1.36 (m, 33 H), 1.67 (m_c, 2 H), 1.91 (m_c, 2 H), 2.70 (t, J = 7.7 Hz, 2 H), 3.57 – 3.61 (m, 2 H), 3.87 – 3.97 (m, 2 H), 4.07 (dd, J = 11.2, 5.6 Hz, 1 H), 4.13 (dd, J = 9.6, 2.7 Hz, 1 H), 4.22 (dt, J = 9.3, 7.2 Hz, 1 H), 4.33 (d, J = 2.6 Hz, 1 H), 4.41 – 4.51 (m, 2 H), 4.57 – 4.60 (m, 1 H), 4.66 (d, J = 7.8 Hz, 1 H), 4.89 (d, J = 7.8 Hz, 1 H), 4.94 (t, J = 7.2 Hz, 1 H), 5.16 (dd, J = 10.8, 2.6 Hz, 1 H), 5.30 (t, J = 7.3 Hz, 1 H), 5.35 – 5.39 (m, 2 H), 5.46 (d, J = 3.6 Hz, 1 H), 5.67 (dd, J = 11.0, 3.5 Hz, 1 H), 5.72 – 5.82 (m, 2 H), 6.00 (dd, J = 10.9, 3.4 Hz, 1 H), 6.17 (d, J = 2.6 Hz, 1 H), 6.38 (d, J = 9.1 Hz, 1 H), 7.15-7.25 (m, 12 H), 7.29-7.52 (m, 21 H), 7.60-7.78 (m, 10 H), 7.85-7.98 (m, 13 H), 8.09 (d, J = 7.2 Hz, 2 H).

^{13}C NMR (150 MHz, DMSO-d_6): δ (ppm) 14.10, 14.12, 22.66, 22.69, 26.90, 26.91, 29.01, 29.08, 29.1, 29.2, 29.3, 29.4, 29.5, 29.64, 29.67, 29.7, 31.3, 31.7, 31.9, 32.2, 35.9, 38.6, 50.9, 60.4, 61.5, 62.4, 67.3, 67.7, 67.9, 69.0, 69.6, 69.9, 72.50, 72.55, 72.9, 73.0, 73.2, 73.6, 75.8, 98.8, 100.7, 101.2, 122.6, 123.0, 125.0, 127.7, 128.1, 128.20, 128.24, 128.32, 128.38, 128.44, 128.49, 128.51, 128.53, 128.59, 128.7, 129.0, 129.1, 129.2, 129.3, 129.46, 129.49, 129.5, 129.64, 129.67, 129.70, 129.78, 129.84, 129.88, 132.78, 132.9, 133.10, 133.14, 133.16, 133.23, 133.3, 133.34, 133.6, 135.5, 137.0, 147.2, 150.8, 154.2, 164.8, 164.9, 165.1, 165.4, 165.6, 165.7, 166.13, 166.18, 166.25, 176.9.

IR (ATR): $\tilde{\nu}$ (cm^{-1}) 2927, 2855, 1665, 1293, 1096, 1066, 1028, 709.

HR-MS (ESI): *m/z* calcd for $C_{131}H_{137}N_3O_{29}Na^+$ 2238.9235, found: 2238.9251.

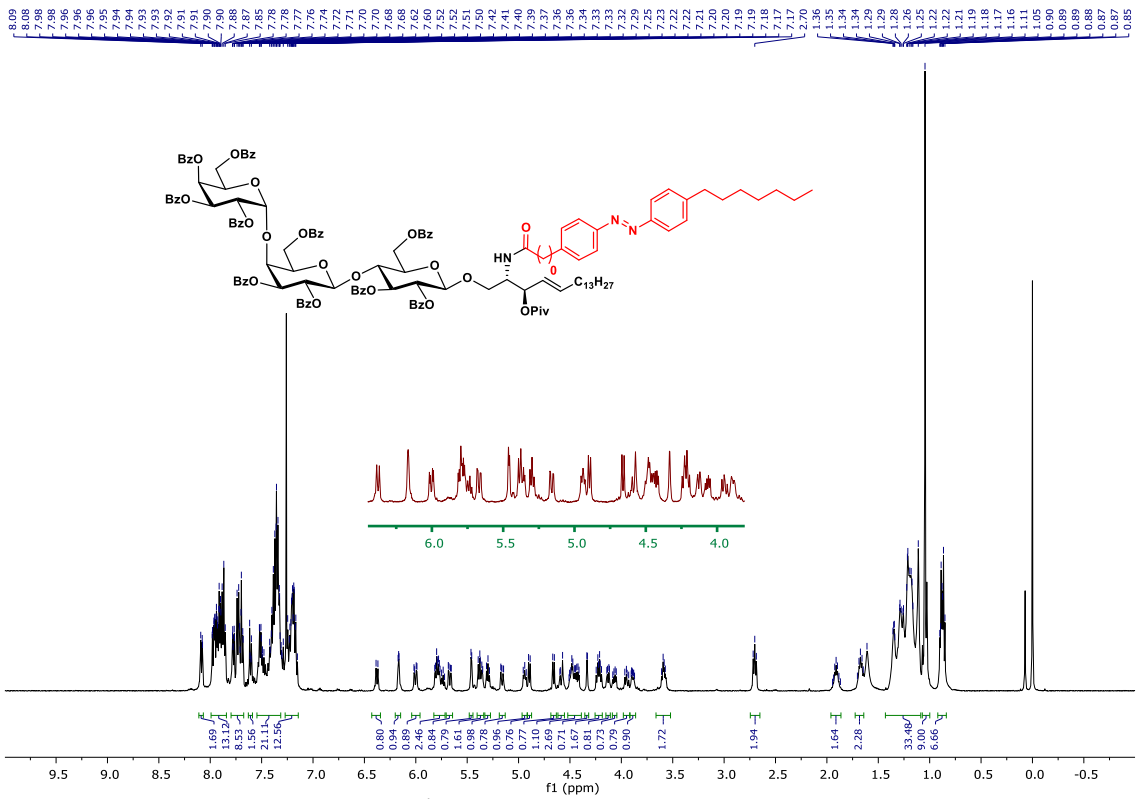


Figure S1. ^{1}H NMR ($CDCl_3$, 600 MHz) spectrum of **2a**

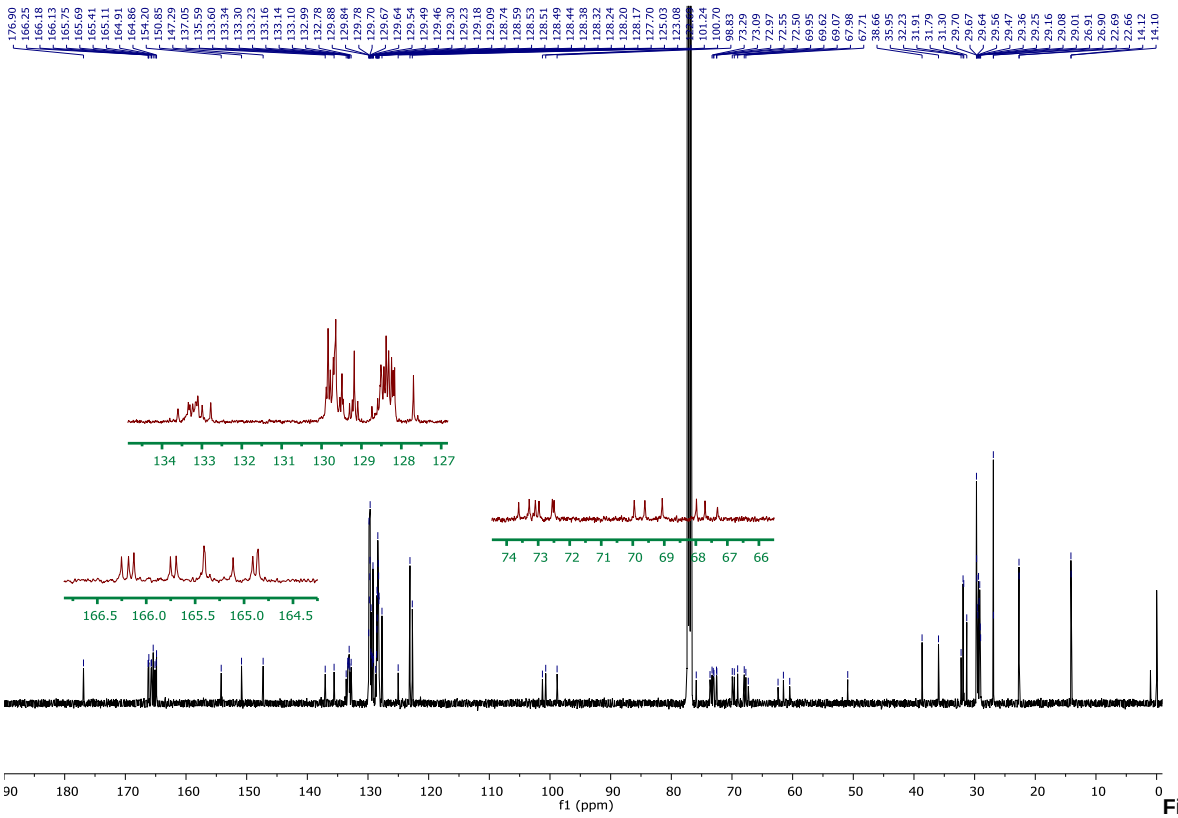
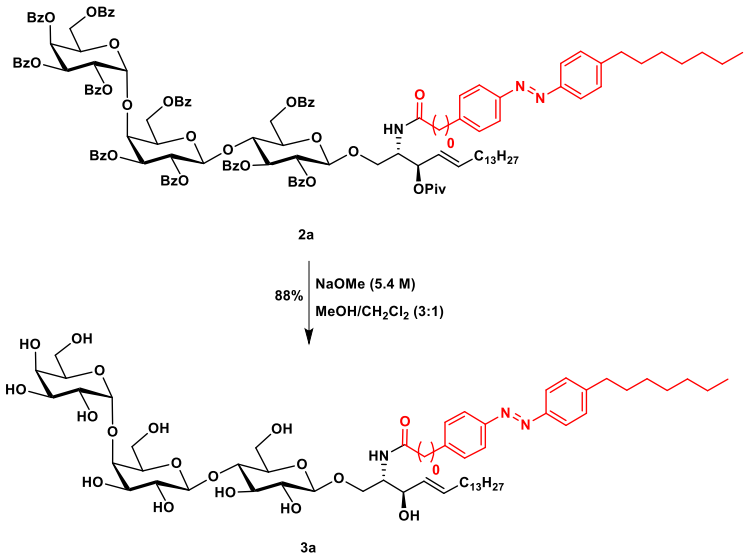


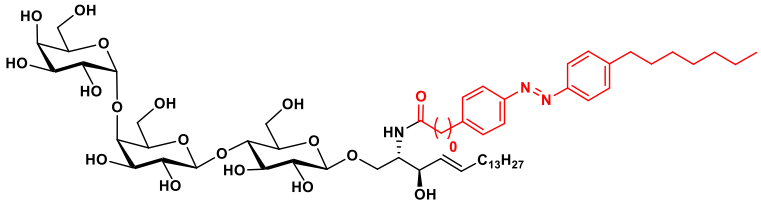
Figure S2. ^{13}C NMR (DMSO-d_6, 150 MHz) spectrum of **2a**

3.2 Deprotection: Synthesis of **3a (Gb$_3$-C$_1$AZOC$_7$)**



The deprotection of glycolipid **2a** (12.0 mg, 5.41 µmol, 1.0 equiv.) was performed according to general procedure **GP2** in methanol (3.6 mL) and dichloromethane (1.2 mL) for 24 h at 50 °C. After a dialysis of 3 d with 5 L of H_2O and subsequent lyophilization glycolipid **3a** (5.2 mg, 4.75 µmol, 88%) was obtained as a yellow solid.

***O*-(α-D-Galactopyranosyl)-(1→4)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-(*N*-(4-((4-heptylphenyl)diazenyl)benzamide)-4-octadecen-1,3-diol (3a)**



^{1}H NMR (500 MHz, DMSO-*d*$_6$/some drops CD_3OD): *δ* (ppm) 0.85 (dt, *J* = 16.5, 6.8 Hz, 6 H), 0.97 – 1.32 (m, 36 H), 1.84 – 2.00 (m$_c$, 2 H), 2.69 (t, *J* = 7.7 Hz, 2 H), 3.09 (m$_c$, 1 H), 3.55 – 3.80 (m, 10 H), 4.17 (m$_c$, 1 H), 4.27 (d, *J* = 6.6 Hz, 2 H), 4.42 (d, *J* = 4.3 Hz, 1 H), 4.45 (t, *J* = 5.6 Hz, 1 H), 4.58 (d, *J* = 6.4 Hz, 1 H), 4.62 – 4.67 (m, 2 H), 4.79 – 4.83 (m, 3 H), 5.07 (d, *J* = 4.5 Hz, 1 H), 5.17 (d, *J* = 4.7 Hz, 1 H), 5.36 (d, *J* = 3.9 Hz, 1 H), 5.42 (dd, *J* = 15.1, 6.4 Hz, 1 H), 5.57 (m$_c$, 1 H), 7.44 (d, *J* = 8.1 Hz, 2 H), 7.86 (d, *J* = 8.0 Hz, 2 H), 7.92 (d, *J* = 8.2 Hz, 2 H), 8.04 (d, *J* = 8.2 Hz, 2 H), 8.21 (d, *J* = 8.2 Hz, 1 H).

^{13}C NMR (125 MHz, DMSO-d_6/some drops CD_3OD): δ (ppm) 13.8, 22.11, 22.13, 28.4, 28.5, 28.16, 28.17, 28.90, 28.96, 29.02, 29.05, 29.06, 29.10, 29.14, 29.17, 29.27, 30.7, 31.2, 31.3, 54.0, 58.7, 59.2, 60.3, 68.6, 68.7, 69.2, 70.8, 71.1, 72.8, 74.8, 75.0, 100.6, 103.6, 103.8, 122.1, 122.8, 128.5, 128.6, 129.3, 131.1, 136.5, 147.1, 147.3, 150.2, 153.3, 165.2.

IR (ATR): $\tilde{\nu}$ (cm^{-1}) 3370, 2922, 2853, 1636, 1532, 1458, 1156, 1037, 856.

HR-MS (ESI): *m/z* calcd for $C_{131}H_{137}N_3O_{29}Na^+$ 1114.6039, found: 1114.6031.

UV-Vis (12.6 μM in CH_3OH): λ_{max} = 335 nm.

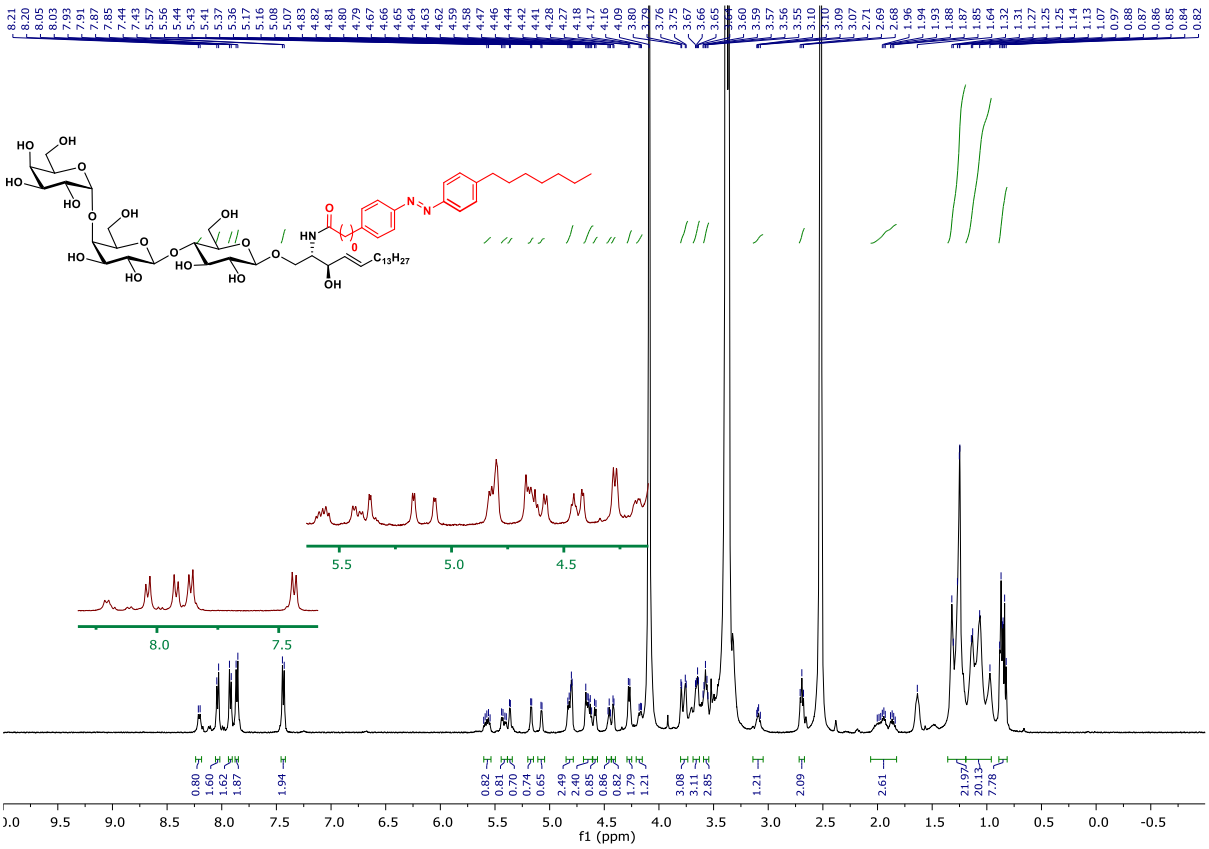


Figure S3. ^{1}H NMR (DMSO-d_6, 600 MHz) spectrum of **3a**

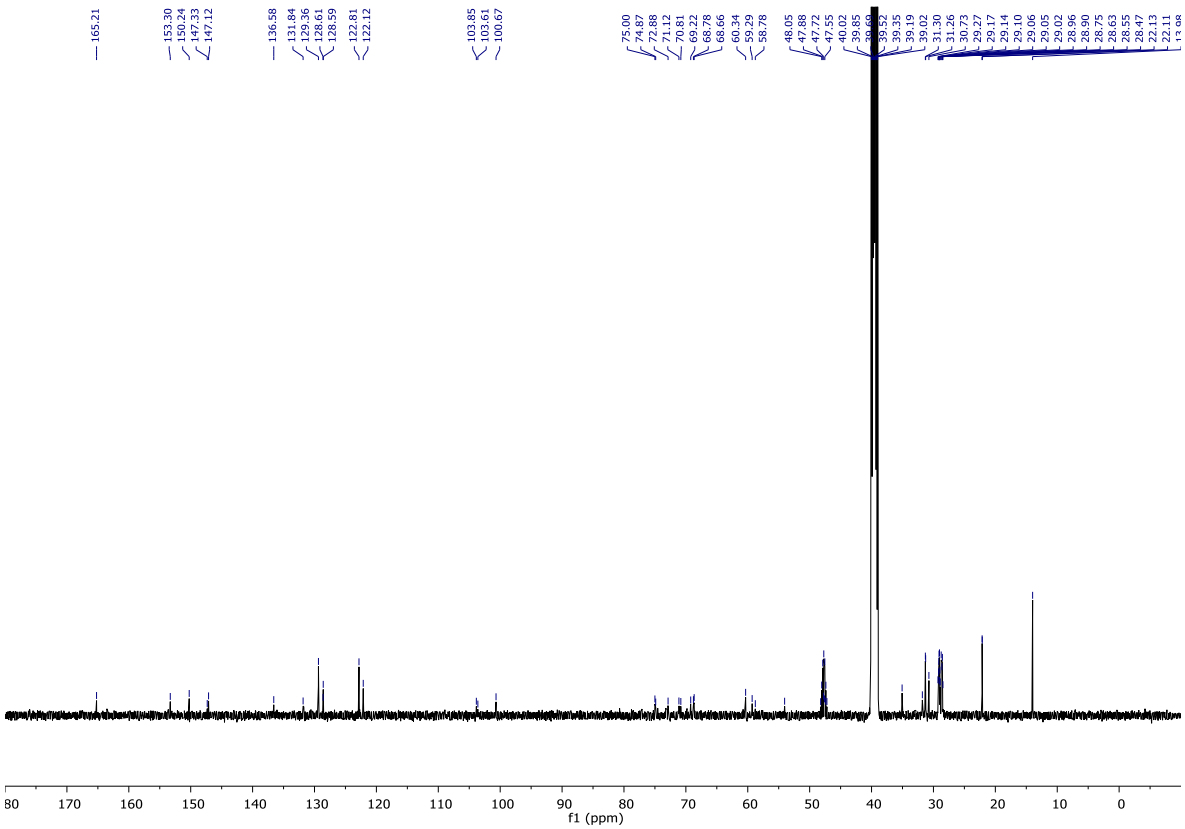
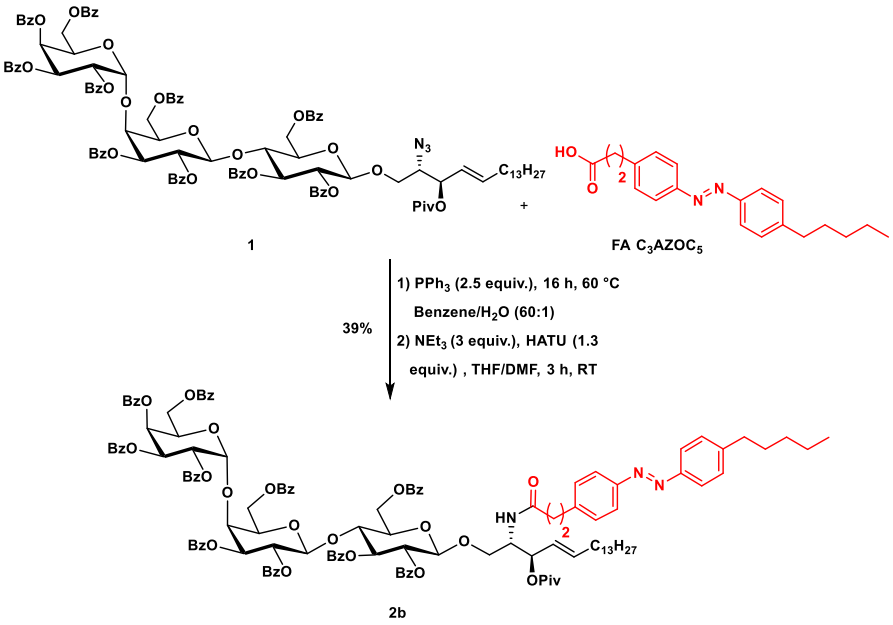


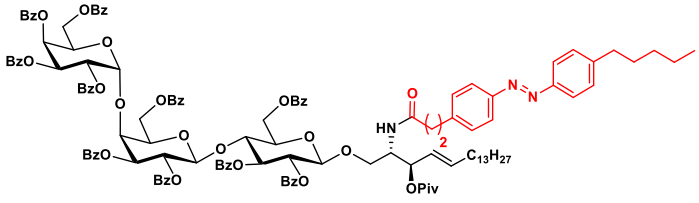
Figure S4. ^{13}C NMR (DMSO-d_6, 150 MHz) spectrum of **3a**

3.3 Reduction/acylation reaction with **FA C_3AZOC_5**



The reduction of azide **1** (40.0 mg, 0.021 mmol, 1.0 equiv.) was performed according to general procedure **GP1** by using triphenylphosphine (14.0 mg, 0.052 mmol, 2.5 equiv.) in anhydrous benzene (3.8 ml) and water (56 µl). In a brown flask, photo-switchable fatty acid **3** (10.0 mg, 0.031 mmol, 2.5 equiv.) was dissolved in anhydrous THF (2 mL), then NEt_3 (8.78 µl, 0.063 mmol, 2.0 equiv.) and a solution of TBTU (10.0 mg, 0.063 mmol, 1.5 equiv.) in anhydrous DMF (530 µl) was added. The reaction mixture was stirred at ambient temperature for 15 min. The amine which was prepared in the first step was dried under vacuum at least 2 h and dissolved in anhydrous THF (1.5 mL), then it was added dropwise to the activated acid at ambient temperature. The reaction mixture was stirred at ambient temperature for 3 h. EtOAc was added and the organic phase thoroughly washed with brine (3×). The combined aqueous phases were re-extracted with ethyl acetate. The organic layer was dried with Na_2SO_4, filtered and the solvent removed under reduced pressure. Column chromatography on silica gel (DCM/MeOH, 40:1) was followed by gel permeation chromatography to afford the pure photo-switchable Gb_3 **2b** (18 mg, 8.12 µmol, 39%) as an orange oil.

***O*-(2,3,4,6-Tetra-*O*-benzoyl-α-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-pivaloyl-2-(*N*-(3-(4-(*p*-pantylphenyldiazenyl)phenyl)propionamide) (2b)**



TLC (2:3 / EtOAc:n-pentane): Rf = 0.66.

^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) 0.86 (t, J = 7.1 Hz, 3 H), 0.90 (t, J = 7.0 Hz, 3 H), 1.02 (s, 9 H), 1.20 – 1.27 (m, 28 H), 1.34 – 1.36 (m, 4 H), 1.65 – 1.68 (m, 2 H), 1.89 – 1.93 (m, 2 H), 1.98 – 2.06 (m, 2 H), 2.17 (s, 2 H), 2.68 (t, J = 7.7 Hz, 2 H), 2.76 (t, J = 8.2 Hz, 1 H), 3.41 (dd, J = 9.7, 3.9 Hz, 1 H), 3.58 (dd, J = 7.9, 5.7 Hz, 1 H), 3.85 – 3.88 (m, 1 H), 3.94 – 4.00 (m,

2 H), 4.07 (dd, *J* = 11.0, 5.5 Hz, 1 H), 4.19 – 4.25 (m, 3 H), 4.34 (d, *J* = 2.4 Hz, 1 H), 4.43 (td, *J* = 11.4, 10.9, 4.8 Hz, 1 H), 4.57 – 4.61 (m, 2 H), 4.90 (d, *J* = 7.7 Hz, 1 H),), 4.93 – 4.95 (m, 1 H), 5.13 – 5.18 (m, 2 H), 5.23 – 5.27 (m, 1 H), 5.34 (dd, *J* = 9.6, 7.7 Hz, 1 H), 5.46 (d, *J* = 3.5 Hz, 1 H), 5.57 (d, *J* = 9.2 Hz, 1 H), 5.64 – 5.69 (m, 2 H), 5.78 – 5.83 (m, 2 H), 6.01 (dd, *J* = 10.9, 3.4 Hz, 1 H), 6.17 (dd, *J* = 3.5, 1.5 Hz, 1 H), 7.13-7.25 (m, 10 H), 7.27-7.42 (m, 20 H), 7.47-7.54 (m, 4 H), 7.68-7.98 (m, 22 H), 8.09-8.10 (m, 2 H).

^{13}C NMR (150 MHz, DMSO-*d*$_6$): *δ* (ppm) 14.1, 14.2, 22.6, 22.8, 27.0, 29.1, 29.3, 29.5, 29.6, 29.7, 29.8, 29.84, 29.85, 31.0, 31.14, 31.16, 31.6, 32.0, 32.3, 35.9, 37.4, 38.7, 50.5, 60.5, 61.6, 62.4, 67.7, 67.8, 68.1, 69.2, 69.7, 70.1, 72.6, 72.7, 73.0, 73.1, 73.2, 73.7, 76.0, 99.0, 101.0, 101.4, 120.82, 120.84, 122.92, 122.96, 125.1, 128.33, 128.37, 128.41, 128.42, 128.48, 128.52, 128.58, 128.60, 128.67, 128.69, 128.7, 129.0, 129.2, 129.3, 129.4, 129.5, 129.6, 129.72, 129.76, 129.80, 129.86, 129.89, 129.94, 129.99, 130.0, 132.9, 133.1, 133.2, 133.3, 133.4, 133.5, 133.71, 133.77, 136.9, 144.0, 146.5, 151.0, 151.3, 165.0, 165.02, 165.1, 165.3, 165.52, 165.58, 165.8, 165.9, 166.2, 166.4, 171.1, 177.0.

IR (ATR): $\tilde{\nu}$ (cm^{-1}) 2928, 2858, 1726, 1598, 1504, 1263, 1096, 1069, 1030, 709.

HR-MS (ESI): *m/z* calcd for $C_{131}H_{137}N_3O_{29}Na^+$ 2239.9235, found: 2239.9232.

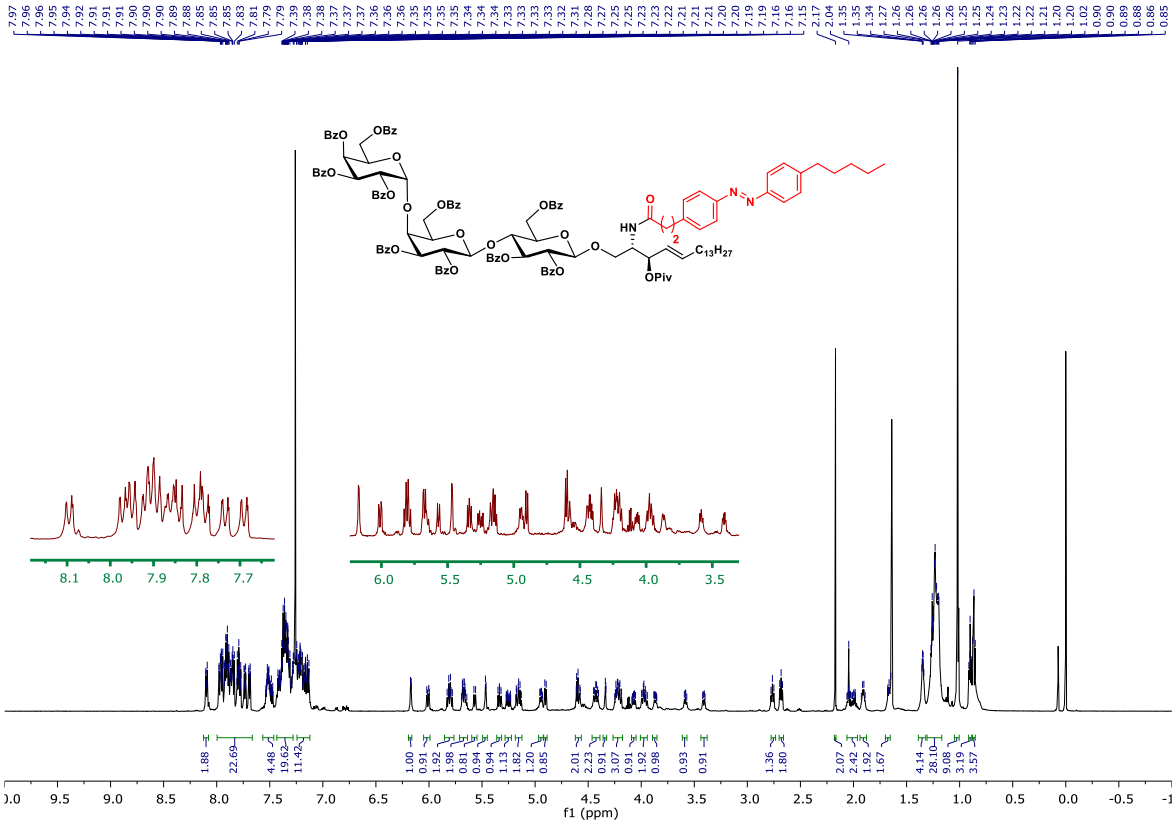


Figure S5. ^{1}H NMR ($CDCl_3$, 600 MHz) spectrum of **2b**

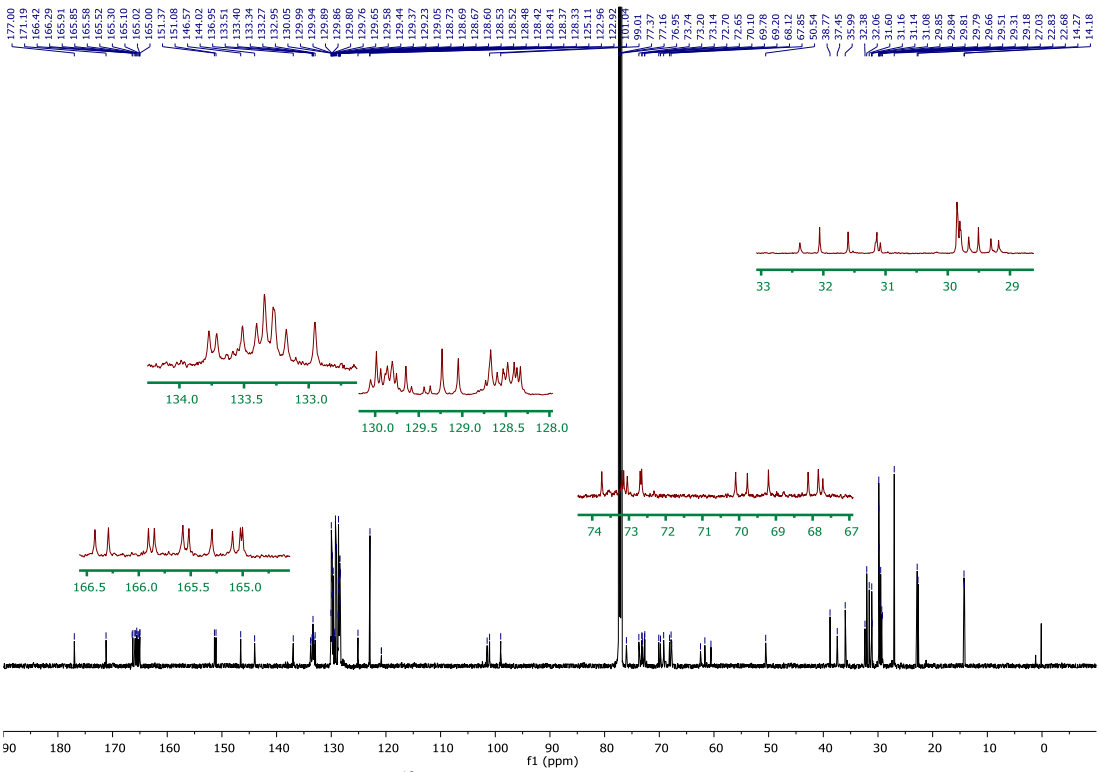
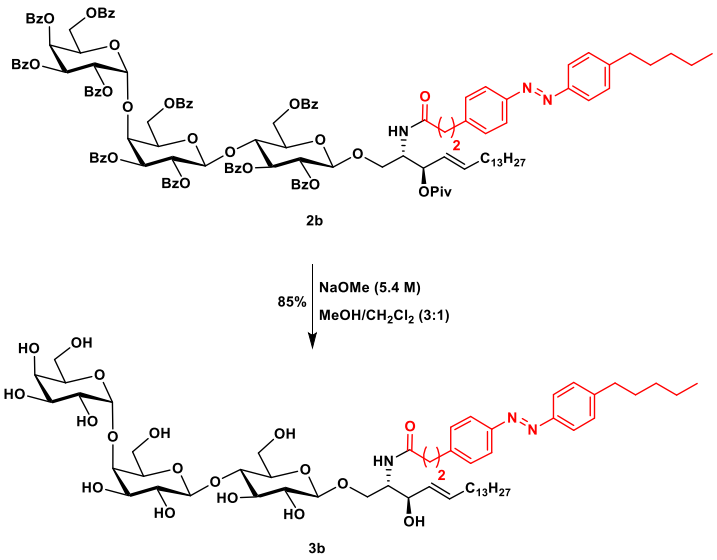


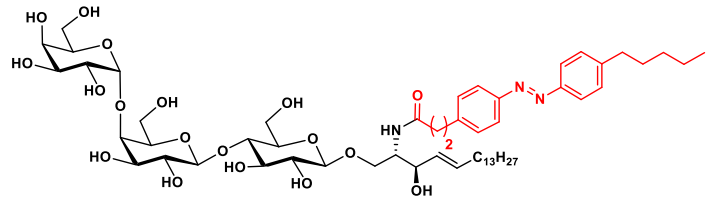
Figure S6. ^{13}C NMR ($CDCl_3$, 150 MHz) spectrum of **2b**

3.4 Deprotection: Synthesis of **3a (Gb$_3$-C$_1$AzoC$_7$)**



The deprotection of glycolipid **2b** (18.0 mg, 8.11 µmol, 1.0 equiv.) was performed according to general procedure **GP2** in methanol (5.0 mL) and dichloromethane (1.7 mL) for 24 h at 50 °C. After a dialysis of 3 d with 5 L of H_2O and subsequent lyophilization glycolipid **3b** (Mixture of *trans/cis-azo*: 10:1, 7.5 mg, 6.86 µmol, 85%) was obtained as a yellow solid.

***O*-(α-D-Galactopyranosyl)-(1→4)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-(*N*-(3-(4-(*p*-pantylphenyldiazenyl)phenyl)propionamide)-4-octadecen-1,3-diol (3b)**



(Note: *trans/cis*: 10:1 was formed)

^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) 0.82 (t, J = 7.1 Hz, 3 H), 0.88 (t, J = 7.0 Hz, 3 H), 1.15 – 1.25 (m, 32 H), 1.29 – 1.33 (m, 6 H), 1.64 (m_c, 2 H), 1.89 (m_c, 2 H), 2.40 – 2.49 (m, 3 H), 2.67 – 2.69 (m, 1 H), 2.91 (m_c, 2 H), 3.08 (m_c, 1 H), 3.29 – 3.37 (m, 10 H), 3.49 – 3.59 (m, 5 H), 3.63 – 3.68 (m, 2 H), 3.71 – 3.81 (m, 5 H), 3.92 (q, J = 69 Hz, 1 H), 3.99 (dd, J = 10.1, 4.8 Hz, 1 H), 4.09 (t, J = 6.4 Hz, 1 H), 4.17 (d, J = 7.8 Hz, 1 H), 4.30 (d, J = 7.7 Hz, 1 H), 4.53 (d, J = 4.2 Hz, 1 H), 4.58 (t, J = 5.6 Hz, 1 H), 4.66 – 4.69 (m, 4 H), 4.81 (d, J = 3.8 Hz, 1 H), 4.89 (t, J = 5.5 Hz, 1 H), 4.91 (d, J = 5.5 Hz, 1 H), 4.96 (d, J = 5.6 Hz, 1 H), 5.22 (d, J = 4.8 Hz, 1 H), 5.30 – 5.34 (m, 2 H), 5.54 (dt, J = 14.5, 9.6 Hz, 1 H), 7.40-7.44 (m, 4 H), 7.79-7.81 (m, 4 H), 7.87 (d, J = 9.1 Hz, 1 H).

^{13}C NMR (150 MHz, DMSO-d_6): δ (ppm) 13.84, 13.87, 21.8, 22.0, 28.5, 28.6, 28.8, 28.95, 28.99, 29.03, 29.07, 30.3, 30.7, 30.9, 31.2, 31.6, 34.8, 36.5, 39.9, 53.1, 59.0, 60.11, 60.17, 68.52, 68.58, 68.95, 69.0, 70.46, 70.72, 71.0, 72.7, 73.0, 74.3, 74.7, 74.8, 76.9, 80.4, 100.5, 103.4, 103.6, 122.3, 122.4, 129.10, 129.15, 131.1, 131.2, 145.0, 146.1, 150.1, 150.2, 170.8.

IR (ATR): $\tilde{\nu}$ (cm^{-1}) 3337, 2922, 2853, 1630, 1542, 1371, 1148, 1039, 693.

HR-MS (ESI): *m/z* calcd for $C_{131}H_{137}N_3O_{29}Na^+$ 1114.6039, found: 1114.6031.

UV-Vis (15.7 μM in CH_3OH): λ_{max} = 333 nm.

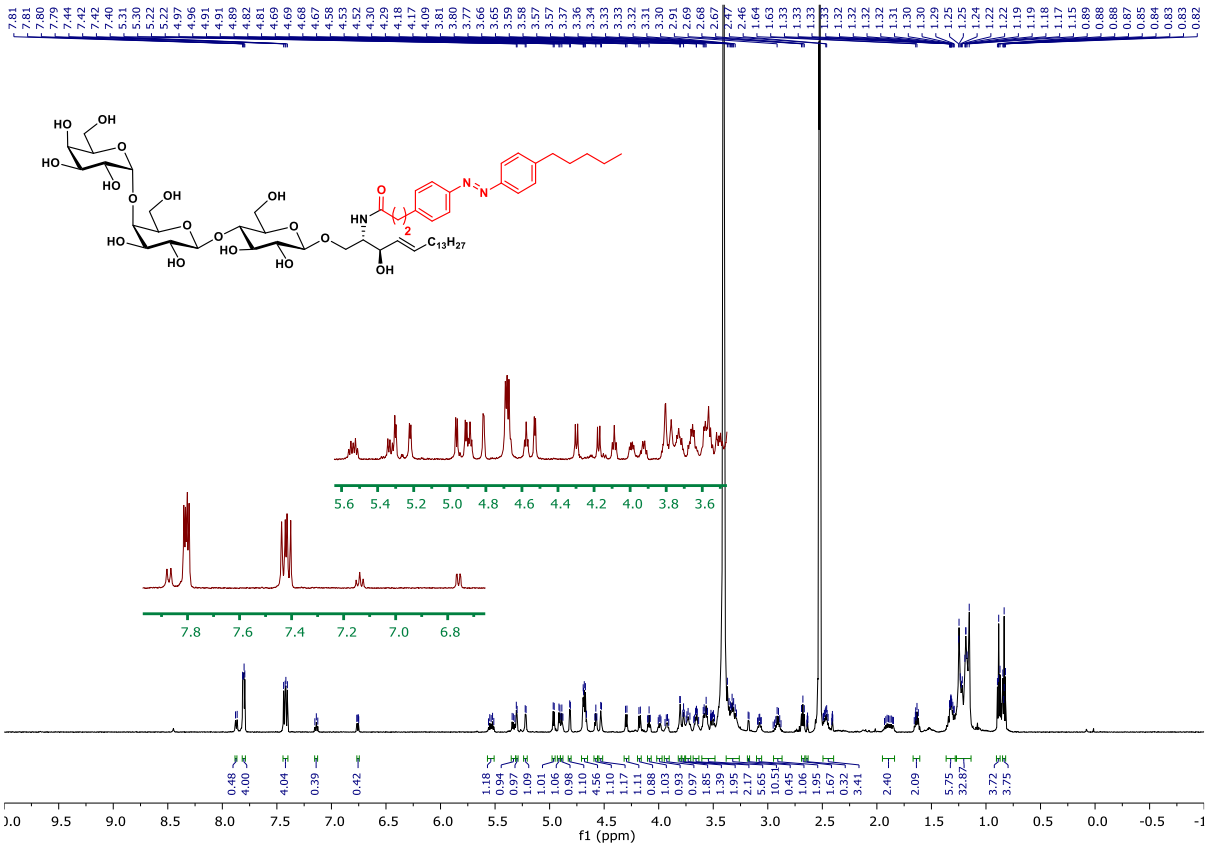


Figure S7. ^{1}H NMR (DMSO-d_6, 600 MHz) spectrum of **3b**

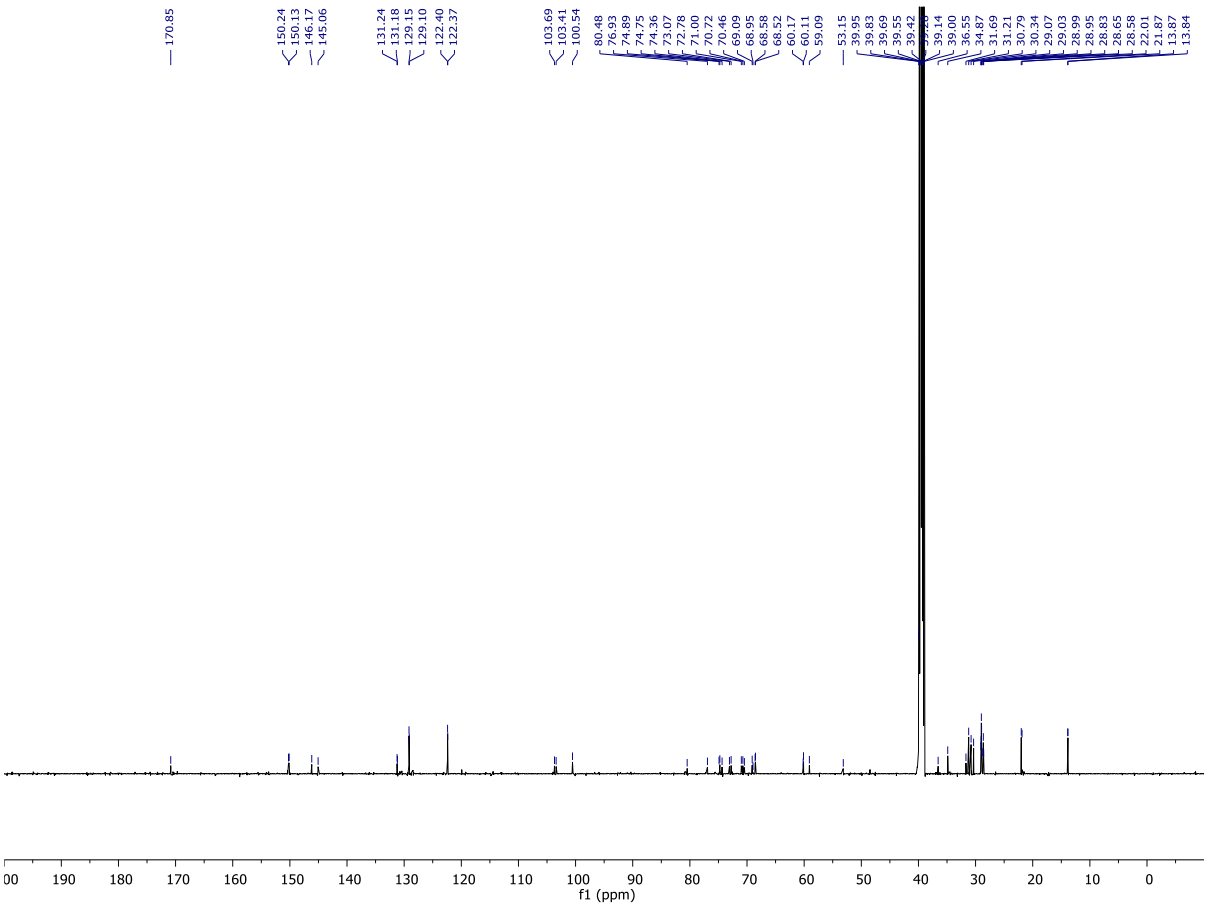
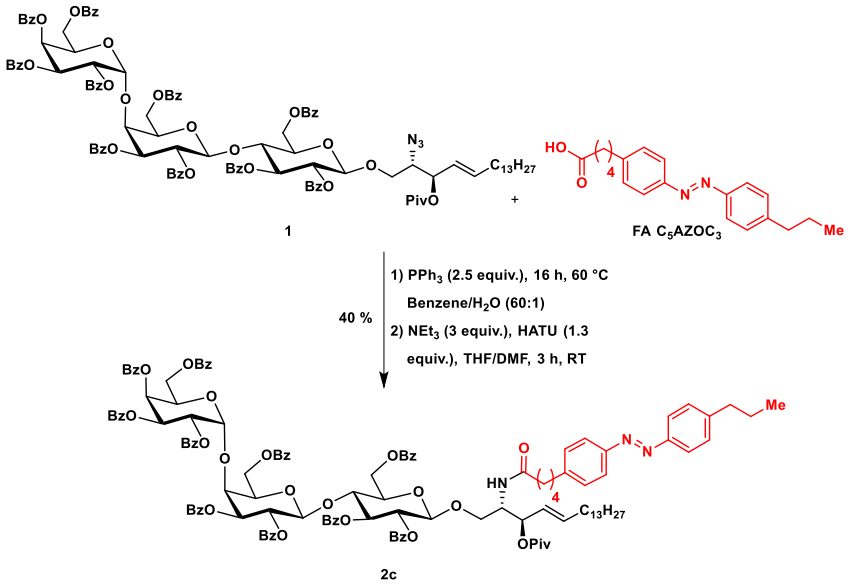


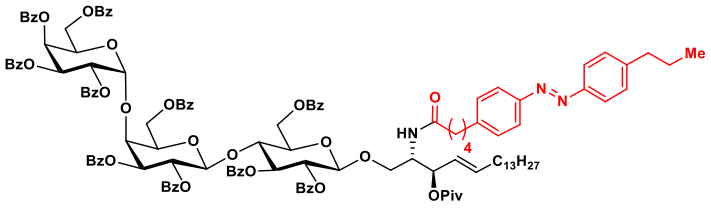
Figure S8. ^{13}C NMR (DMSO-d_6, 150 MHz) spectrum of **3b**

3.5 Reduction/acylation reaction with **FA C_5AZOC_3**



The reduction of azide **1** (35.0 mg, 0.018 mmol, 1.0 equiv.) was performed according to general procedure **GP1** by using triphenylphosphine (12.0 mg, 0.045 mmol, 2.5 equiv.) in anhydrous benzene (3.3 ml) and water (49 µl). In a brown flask, photo-switchable fatty acid **4** (9.00 mg, 0.027 mmol, 2.5 equiv.) was dissolved in anhydrous THF (2 mL), then NEt_3 (7.56 µl, 0.054 mmol, 3.0 equiv.) and a solution of TBTU (10.0 mg, 0.027 mmol, 1.5 equiv.) in anhydrous DMF (463 µl) was added. The reaction mixture was stirred at ambient temperature for 15 min. The amine which was prepared in first step was dried under vacuum at least 2 h and dissolved in anhydrous THF (1.5 mL), then it was added dropwise to the activated acid at ambient temperature. The reaction mixture was stirred at ambient temperature for 3 h. EtOAc was added and the organic phase thoroughly washed with brine (3×). The combined aqueous phases were re-extracted with ethyl acetate. The organic layer was dried with Na_2SO_4, filtered and the solvent removed under reduced pressure. Column chromatography on silica gel (DCM/MeOH, 40:1) was followed by gel permeation chromatography to afford the pure photo-switchable Gb_3 **2c**(12.5 mg, 5.63 µmol, 31%) as an orange solid.

***O*-(2,3,4,6-Tetra-*O*-benzoyl-α-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-pivaloyl-2-(*N*-(5-(4-(*p*-propylphenyldiazenyl)phenyl)pentanamide) (2c)**



TLC (2:3 / EtOAc:n-pentane): Rf = 0.65.

^{1}H NMR (600 MHz, $CDCl_3$): *δ* (ppm) 0.87 (t, *J* = 7.1 Hz, 3 H), 0.97 (t, *J* = 7.3 Hz, 3 H), 1.01 (s, 9 H), 1.17 – 1.26 (m, 28 H), 1.40 – 1.44 (m, 2 H), 1.67 – 1.71 (m, 4 H), 1.88 (q, *J* = 7.0 Hz, 2 H), 2.55 (t, *J* = 7.3 Hz, 2 H), 2.65 – 2.68 (m, 3 H), 3.43 (dd, *J* = 9.7, 3.8 Hz, 1 H), 3.58 (dd, *J* = 7.8, 5.7 Hz, 1 H), 3.87 (ddd, *J* = 9.8, 4.4, 2.1 Hz, 1 H), 3.94 – 3.99 (m, 2 H), 4.07 (dd, *J* =

11.1, 5.6 Hz, 1 H), 4.19 – 4.24 (m, 3 H), 4.33 (d, *J* = 3.5 Hz, 1 H), 4.41 – 4.46 (m, 2 H), 4.55 – 4.60 (m, 2 H), 4.62 (d, *J* = 7.8 Hz, 1 H),), 4.90 (d, *J* = 7.8 Hz, 1 H), 4.93 – 4.96 (m, 1 H), 5.13 – 5.18 (m, 2 H), 5.23 – 5.28 (m, 1 H), 5.35 (dd, *J* = 9.6, 7.8 Hz, 1 H), 5.46 (d, *J* = 3.6 Hz, 1 H), 5.52 (d, *J* = 9.2 Hz, 1 H), 5.64 (t, *J* = 6.9 Hz, 1 H), 5.67 (dd, *J* = 10.9, 3.5 Hz, 1 H), 5.78 – 5.82 (m, 2 H), 6.01 (dd, *J* = 10.9, 3.4 Hz, 1 H), 6.17 (dd, *J* = 3.4, 1.5 Hz, 1 H), 7.15-7.25 (m, 10 H), 7.30-7.39 (m, 2 H), 7.47-7.52 (m, 4 H), 7.68-7.98 (m, 26 H), 8.09-8.11 (m, 10 H).

^{13}C NMR (150 MHz, $CDCl_3$): *δ* (ppm) 13.5, 13.9, 22.4, 24.2, 24.8, 26.6, 28.7, 28.9 29.1, 29.2, 29.44, 29.45, 29.49, 30.4, 31.7, 31.9, 35.2, 35.7, 37.7, 38.4, 50.0, 60.2, 61.3, 62.1, 67.45, 67.49, 67.7, 68.8, 69.4, 69.7, 72.2, 72.3, 72.74, 72.77, 72.8, 73.3, 75.6, 98.6, 100.6, 101.1, 122.50, 122.55, 122.58, 124.7, 127.9, 128.01, 128.04, 128.08, 128.12, 128.16, 128.18, 128.23, 128.24, 128.32, 128.34, 128.36, 128.7, 128.83, 128.85, 128.87, 128.9, 129.0, 129.1, 129.24, 129.29, 129.30, 129.37, 129.40, 129.43, 129.44, 129.46, 129.50, 129.58, 129.61, 129.63, 129.7, 132.5, 132.8, 132.91, 132.98, 133.0, 133.1, 133.3, 133.4, 136.5, 145.1, 145.7, 150.81, 150.88, 164.64, 164.68, 164.70, 164.9, 165.1, 165.2, 165.4, 165.5, 165.9, 166.0, 171.8, 176.6.

IR (ATR): $\tilde{\nu}$ (cm^{-1}) 2926, 2857, 1728, 1505, 1266, 1099, 1030, 754.

HR-MS (ESI): *m/z* calcd for $C_{131}H_{137}N_3O_{29}Na^+$ 2239.9263, found: 2239.9241.

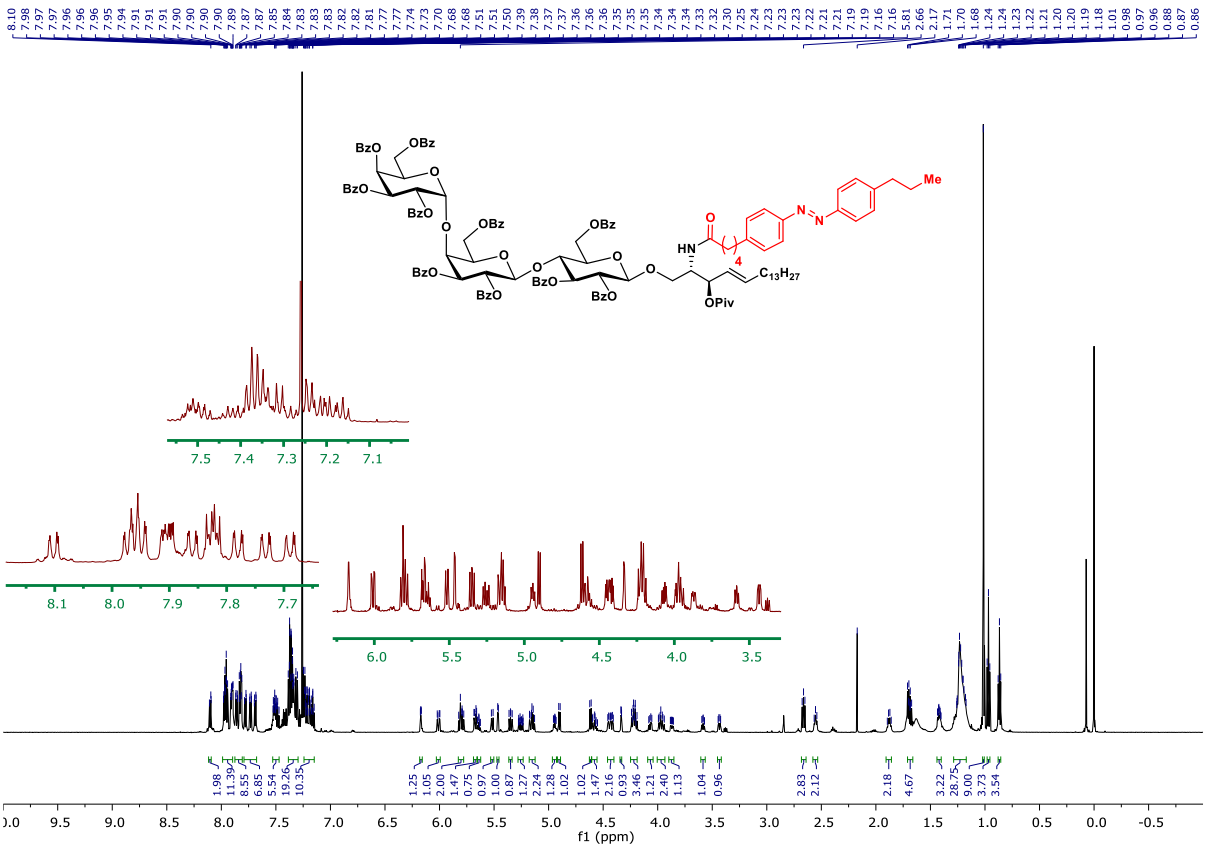


Figure S9. ^{1}H NMR ($CDCl_3$, 600 MHz) spectrum of **2c**

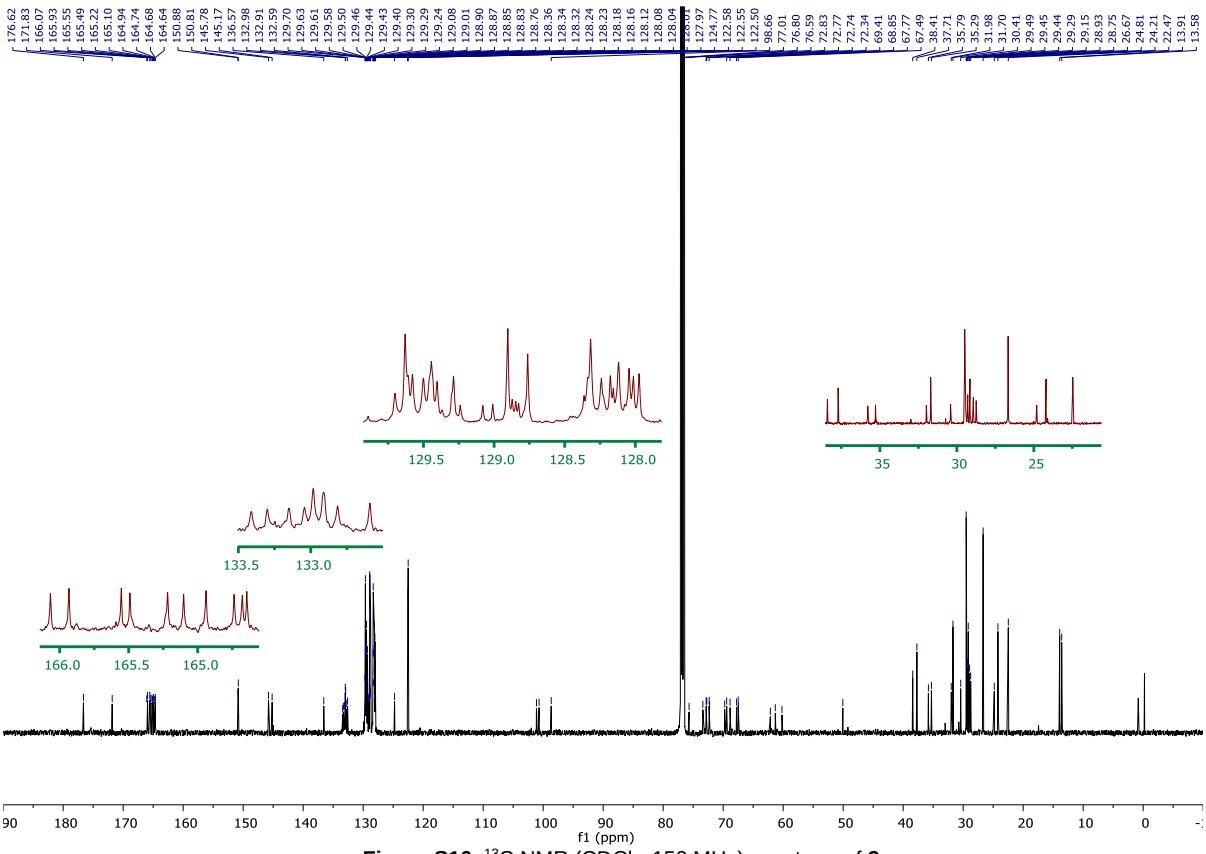
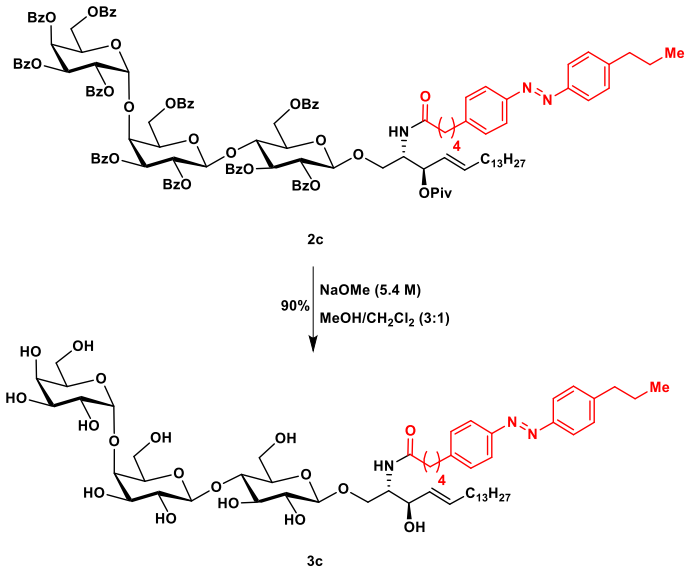


Figure S10. ^{13}C NMR ($CDCl_3$, 150 MHz) spectrum of **2c**

3.6 Deprotection: Synthesis of **3c (Gb_3-C_5AZOC$_3$)**

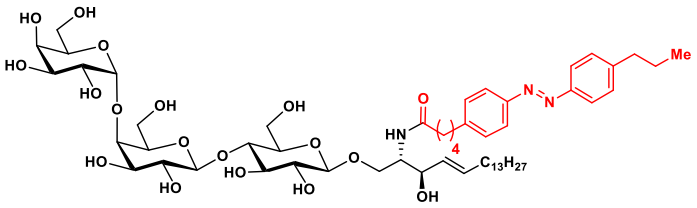


2c

3c

The deprotection of glycolipid **2c** (12.5 mg, 5.63 µmol, 1.0 equiv.) was performed according to general procedure **GP2** in methanol (3.6 mL) and dichloromethane (1.2 mL) for 24 h at 50 °C. After a dialysis of 3 d with 5 L of H_2O and subsequent lyophilization glycolipid **3c** (5.53 mg, 5.07 µmol, 90%) was obtained as a pale yellow solid.

***O*-(α-D-Galactopyranosyl)-(1→4)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-(*N*-(5-(4-(*p*-propylphenyldiazenyl)phenyl)pentanamide)-4-octadecen-1,3-diol (3c)**



^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) 0.82 (t, J = 7.0 Hz, 3 H), 0.90 (t, J = 7.3 Hz, 3 H), 1.15 – 1.23 (m, 27 H), 1.50 – 1.55 (m, 2 H), 1.58 (q, J = 7.2 Hz, 2 H), 1.63 (m_c, 2 H), 1.83 (m_c, 1 H), 2.11 (t, J = 7.2 Hz, 1 H), 2.64 – 2.68 (m, 4 H), 3.05 (td, J = 8.2, 3.8 Hz, 1 H), 3.29 – 3.35 (m, 6 H), 3.48 – 3.51 (m, 2 H), 3.54 – 3.57 (m, 2 H), 3.59 – 3.80 (m, 7 H), 3.88 (q, J = 7 Hz, 1 H), 3.97 (dd, J = 10.2, 4.9 Hz, 1 H), 4.08 (t, J = 6.3 Hz, 1 H), 4.17 (d, J = 7.7 Hz, 1 H), 4.25 (d, J = 7.7 Hz, 1 H), 4.40 (d, J = 4.3 Hz, 1 H), 4.44 (t, J = 5.4 Hz, 1 H), 4.57 (d, J = 6.6 Hz, 1 H), 4.61 (t, J = 6 Hz, 1 H), 4.63 – 4.65 (m, 2 H), 4.78 – 4.82 (m, 2 H), 4.91 (d, J = 5.5 Hz, 1 H), 5.15 (d, J = 4.7 Hz, 1 H), 5.19 (d, J = 3.8 Hz, 1 H), 5.34 (dd, J = 15.4, 7.3 Hz, 1 H), 5.51 (dt, J = 14.2, 6.7 Hz, 1 H), 7.38-7.42 (m, 4 H), 7.60 (d, J = 9.1 Hz, 1 H), 7.79-7.81 (m, 4 H).

^{13}C NMR (150 MHz, DMSO-d_6): δ (ppm) 13.5, 13.9, 22.0, 23.8, 24.9, 28.5, 28.6, 28.7, 28.9, 29.0, 29.03, 29.05, 29.08, 30.2, 31.2, 31.6, 34.7, 35.2, 37.0, 39.96, 52.9, 59.2, 60.2, 68.5, 68.7, 69.1, 70.70, 70.74, 71.04, 72.7, 73.1, 74.4, 74.7, 74.9, 77.0, 80.6, 100.6, 103.4, 103.7, 122.41, 122.43, 122.44, 122.46, 129.1, 129.2, 129.3, 131.2, 131.4, 145.9, 150.2, 171.7.

IR (ATR): $\tilde{\nu}$ (cm^{-1}) 3382, 2924, 2854, 1642, 1554, 1261, 1075, 1036, 805.

HR-MS (ESI): *m/z* calcd for $C_{131}H_{137}N_3O_{29}Na^+$ 2239.9263, found: 2239.9241.

UV-Vis (10.8 μM in CH_3OH): λ_{max} = 334 nm.

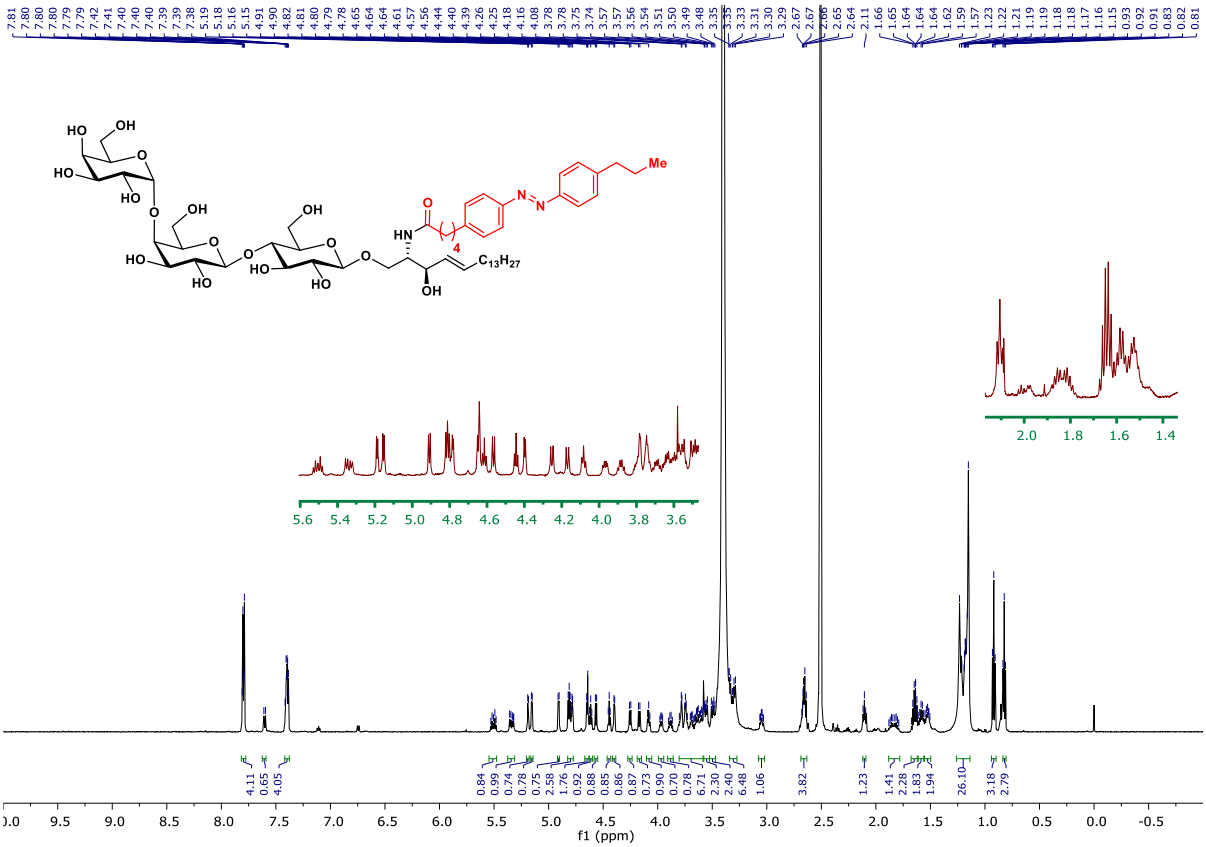


Figure S11. ^{1}H NMR (DMSO-d_6, 600 MHz) spectrum of **3c**

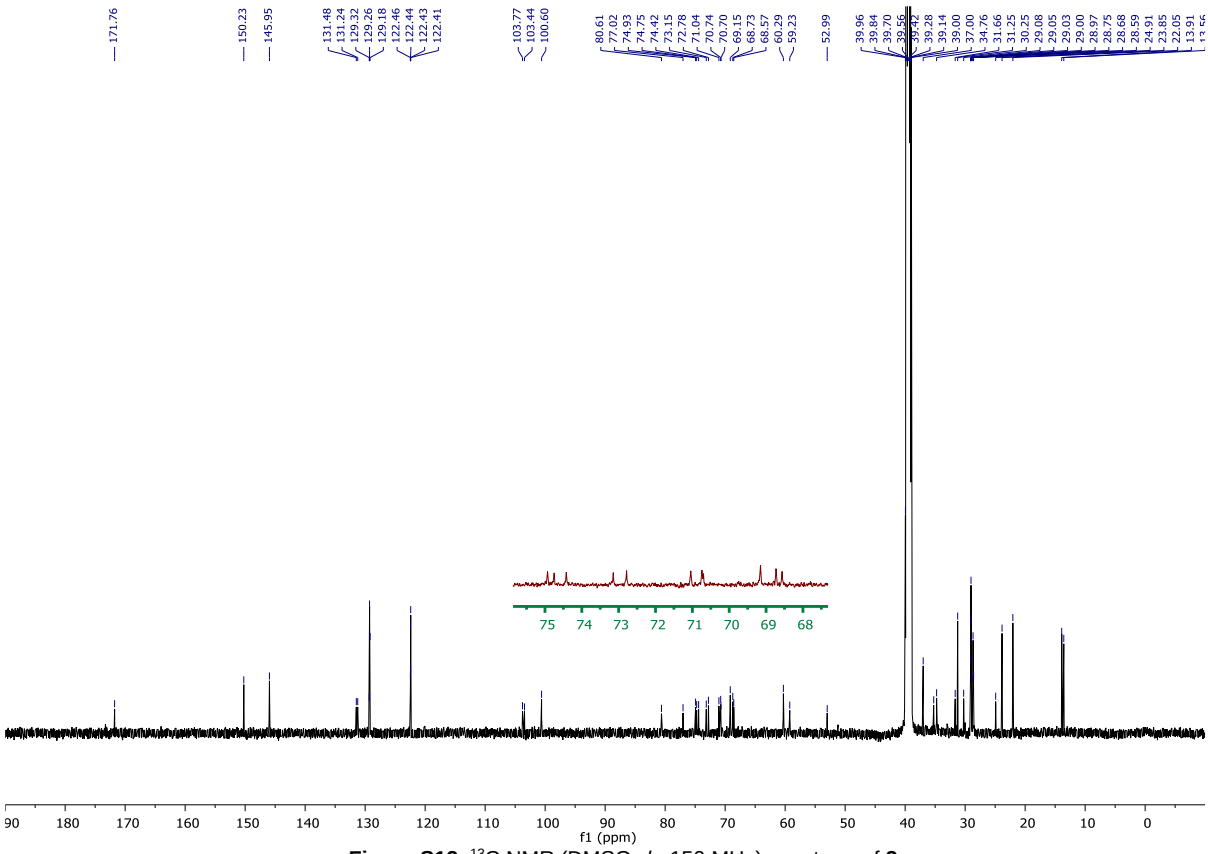
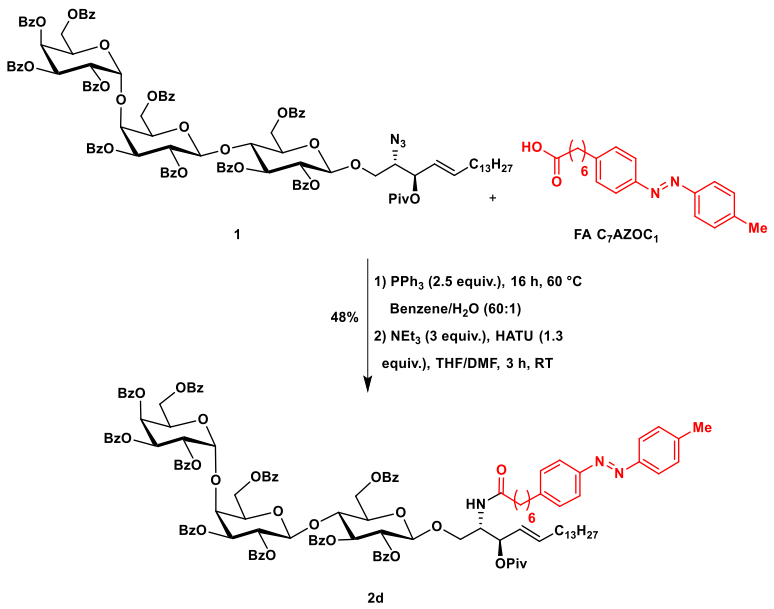


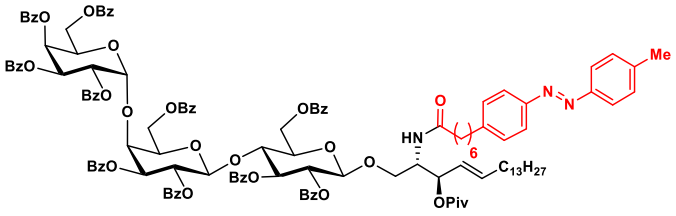
Figure S12. ^{13}C NMR (DMSO-d_6, 150 MHz) spectrum of **3c**

3.7 Reduction/acylation reaction with **FA C_7AZOC$_1$**



The reduction of azide **1** (40.0 mg, 0.021 mmol, 1.0 equiv.) was performed according to general procedure **GP1** by using triphenylphosphine (14.0 mg, 0.052 mmol, 2.5 equiv.) in anhydrous benzene (3.8 ml) and water (56 µl). In a brown flask, photo-switchable fatty acid **7** (10.0 mg, 0.031 mmol, 1.5 equiv.) was dissolved in anhydrous THF (2 mL), then NEt_3 (8.78 µl, 0.063 mmol, 2.0 equiv.) and a solution of TBTU (12.0 mg, 0.031 mmol, 1.5 equiv.) in anhydrous DMF (530 µl) was added. The reaction mixture was stirred at ambient temperature for 15 min. The amine which was prepared in first step was dried under vacuum at least 2 h and dissolved in anhydrous THF (2 mL), then it was added dropwise to the activated acid at ambient temperature. The reaction mixture was stirred at ambient temperature for 3 h. EtOAc was added and the organic phase thoroughly washed with brine (3×). The combined aqueous phases were re-extracted with ethyl acetate. The organic layer was dried with Na_2SO_4, filtered and the solvent removed under reduced pressure. Column chromatography on silica gel (DCM/MeOH, 40:1) was followed by gel permeation chromatography to afford the pure photo-switchable Gb_3 **2d** (22 mg, 9.9 µmol, 48 %) as an orange solid.

***O*-(2,3,4,6-Tetra-*O*-benzoyl-α-D-galactopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-pivaloyl-2-(*N*-(7-(4-(*p*-tolyldiazenyl)phenyl)heptanamide) (2d)**



TLC (2:3 / EtOAc:n-pentane): Rf = 0.70.

^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 0.87 (t, *J* = 7.1 Hz, 3 H), 1.02 (s, 9 H), 1.21 – 1.28 (m, 29 H), 1.56 – 1.61 (m, 2 H), 1.67 (t, *J* = 7.7 Hz, 2 H), 1.91 (q, *J* = 7.2 Hz, 2 H), 2.43 (s, 3 H), 2.64 (t, *J* = 7.7 Hz, 2 H), 3.42 (dd, *J* = 9.8, 3.8 Hz, 1 H), 3.58 (dd, *J*

= 8.0, 5.8 Hz, 1 H), 3.85 – 3.88 (m, 1 H), 3.94 – 4.00 (m, 2 H), 4.07 (dd, *J* = 11.1, 5.4 Hz, 1 H), 4.19 – 4.24 (m, 3 H), 4.34 (d, *J* = 2.7 Hz, 1 H), 4.41 – 4.45 (m, 2 H), 4.57 – 4.62 (m, 2 H), 4.90 (d, *J* = 7.9 Hz, 1 H), 4.93 – 4.95 (m, 1 H), 5.12 – 5.17 (m, 2 H), 5.26 (ddd, *J* = 15.3, 7.4, 1.8 Hz, 1 H), 5.35 (dd, *J* = 9.6, 7.7 Hz, 1 H), 5.46 – 5.49 (m, 2 H), 5.63 – 5.68 (m, 2 H), 5.78 – 5.82 (m, 2 H), 6.01 (dd, *J* = 10.9, 3.3 Hz, 1 H), 6.17 (bs, 1 H), 7.16 – 7.25 (m, 8 H), 7.28 – 7.31 (m, 6 H), 7.34 – 7.43 (m, 16 H), 7.48 – 7.53 (m, 4 H), 7.68 – 7.98 (m, 22 H), 8.10 (d, *J* = 8.0, 2 H).

^{13}C NMR (150 MHz, $CDCl_3$): *δ* (ppm) 13.9, 21.2, 22.4, 25.1, 26.6, 28.72, 28.75, 28.9, 29.1, 29.2, 29.43, 29.45, 29.49, 30.8, 31.7, 32.0, 35.5, 36.0, 38.4, 50.0, 67.4, 67.7, 68.8, 69.4, 69.4, 72.2, 72.3, 72.74, 72.77, 72.8, 75.6, 98.6, 100.6, 101.1, 122.50, 122.52, 124.8, 127.9, 128.01, 128.04, 128.12, 128.17, 128.2, 128.31, 128.37, 128.80, 128.86, 128.88, 129.01, 129.04, 129.08, 129.25, 129.29, 129.32, 129.41, 129.44, 129.46, 129.48, 129.50, 129.58, 129.60, 129.63, 129.7, 132.5, 132.81, 132.89, 132.91, 132.97, 133.0, 133.1, 133.2, 133.4, 136.5, 140.9, 145.7, 150.6, 150.7, 164.64, 164.66, 164.72, 164.9, 165.0, 165.2, 165.4, 165.5, 165.9, 166.0, 172.0, 176.5.

IR (ATR): $\tilde{\nu}$ (cm^{-1}) 2926, 2856, 1725, 1676, 1504, 1317, 1095, 1066, 1027, 755, 707.

HR-MS (ESI): *m/z* calcd for $C_{131}H_{137}N_3O_{29}Na^+$ 2239.9238, found: 2239.9262.

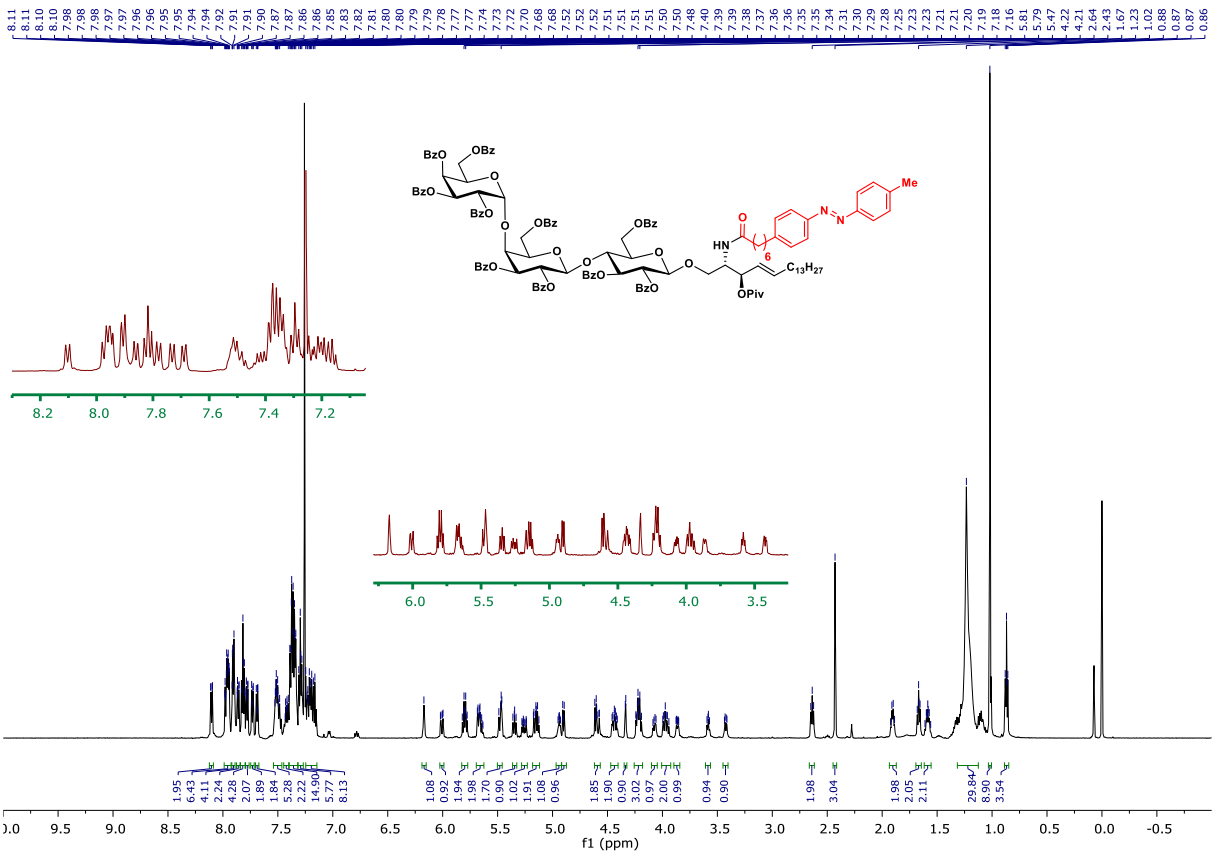


Figure S13. ^{1}H NMR ($CDCl_3$, 600 MHz) spectrum of **2d**

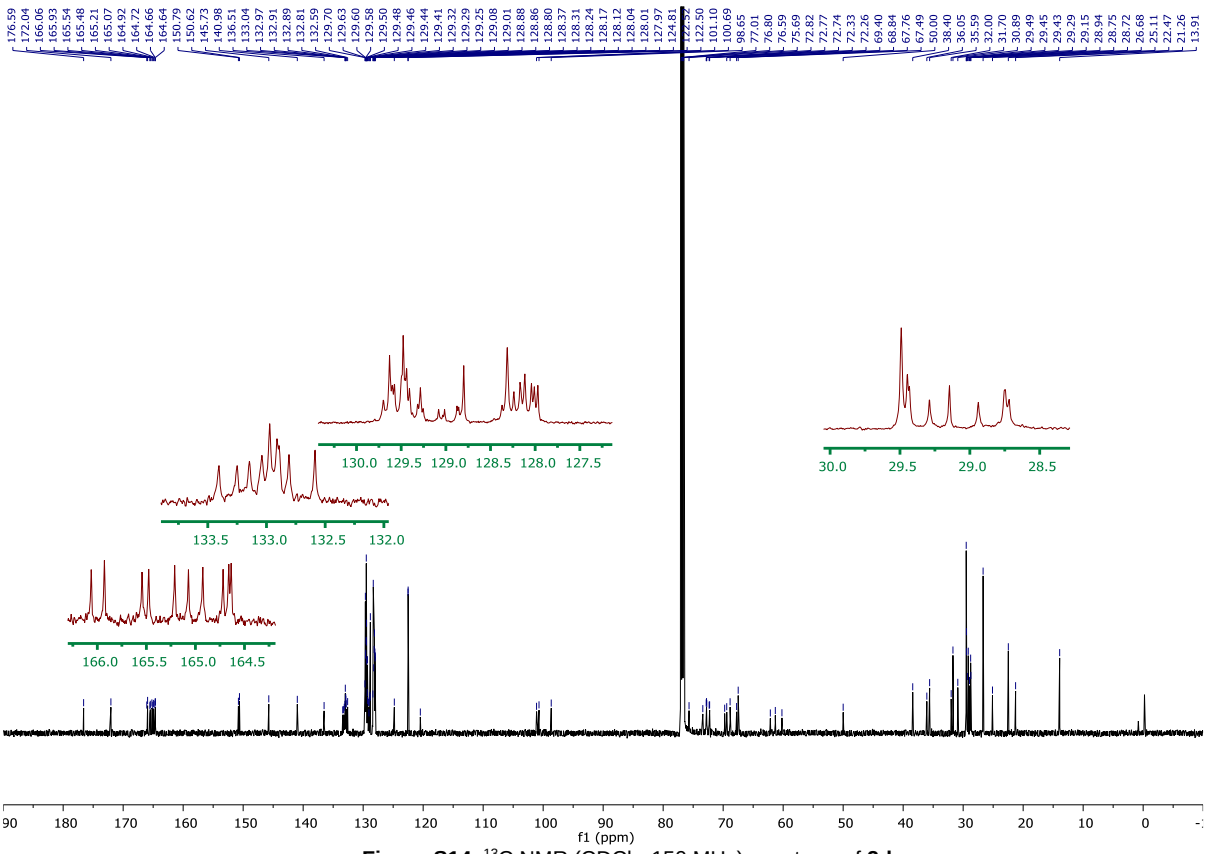
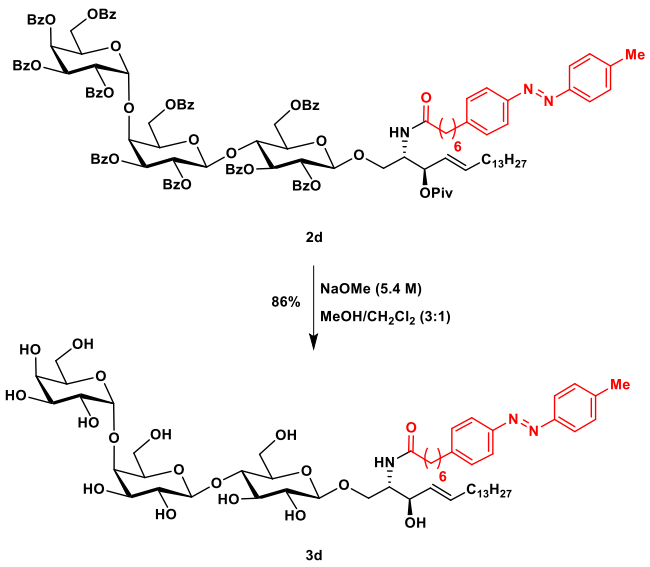


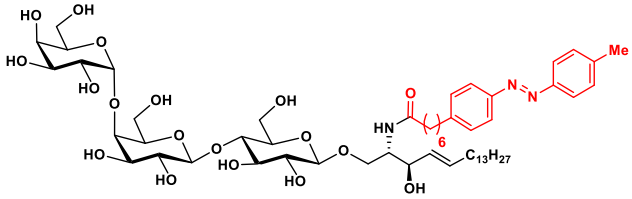
Figure S14. ^{13}C NMR ($CDCl_3$, 150 MHz) spectrum of **2d**

3.8 Deprotection: Synthesis of **3d (Gb$_3$-C$_7$AZOC$_1$)**



The deprotection of glycolipid **2d** (20.0 mg, 9.0 µmol, 1.0 equiv.) was performed according to general procedure **GP2** in anhydrous methanol (6 mL) and anhydrous dichloromethane (2 mL) for 24 h at 50 °C. After a dialysis of 3 d with 5 L of H_2O and subsequent lyophilization glycolipid **3d** (8.50 mg, 7.78 mmol, 86%) was obtained as a yellow solid.

***O*-(α-D-Galactopyranosyl)-(1→4)-(β-D-galactopyranosyl)-(1→4)-β-D-glucopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-(*N*-(7-(4-(*p*-tolyldiazenyl)phenyl)heptanamide)-4-octadecen-1,3-diol (3d)**



^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) 0.84 (t, J = 7.1 Hz, 3 H), 1.19 (bs, 16H), 1.23 – 1.32 (m, 14 H), 1.46 – 1.49 (m, 2 H), 1.59 – 1.64 (m, 2 H), 1.91 – 1.96 (m, 2 H), 2.06 (t, J = 7.4 Hz, 2 H), 2.42 (s, 3 H, CH_3), 2.68 (t, J = 7.7 Hz, 2 H), 3.06 (dt, J = 8.3, 4.1 Hz, 1 H), 3.29 – 3.38 (m, 15 H), 3.42 – 3.83 (m, 13 H), 3.90 (q, J = 7.1 Hz, 1 H), 3.99 (dd, J = 10.1, 4.9 Hz, 1 H), 4.09 – 4.11 (m, 4 H), 4.18 (d, J = 7.8 Hz, 1 H), 4.27 (d, J = 7.7 Hz, 1 H), 4.42 (d, J = 4.3 Hz, 1H), 4.47 (t, J = 5.4 Hz, 1H), 4.59 (d, J = 6.0 Hz, 1H), 4.65 – 4.67 (m, 2H), 4.80 – 4.84 (m, 3H), 4.92 (d, J = 5.5 Hz, 1H), 5.18 (dd, J = 10.1, 4.3 Hz, 2H), 5.34 – 5.39 (m, 1H), 5.55 (dt, J = 14.3, 6.7 Hz, 1H), 7.41 (dd, J = 8.4, 3.1 Hz, 4H), 7.58 (d, J = 9.1 Hz, 1H), 7.78 – 7.82 (m, 1H).

^{13}C NMR (150 MHz, DMSO-d_6): δ (ppm) 13.9, 20.9, 22.0, 25.2, 28.4, 28.5, 28.60, 28.67, 28.7, 28.9, 29.0, 29.03, 29.07, 30.6, 31.2, 31.7, 34.9, 35.5, 39.9, 47.5, 47.6, 47.7, 52.9, 59.2, 60.2, 60.3, 68.5, 68.7, 69.1, 69.7, 70.6, 70.7, 71.0, 72.7, 73.1, 74.4, 74.7, 74.9, 77.1, 80.6, 100.5, 103.4, 103.7, 122.40, 122.43, 129.1, 129.8, 131.3, 131.4, 141.4, 146.1, 150.0, 150.1, 171.8.

IR (ATR): $\tilde{\nu}$ (cm^{-1}) 3334, 2922, 2854, 1643, 1551, 1144, 1025, 589.

HR-MS (ESI): *m/z* calcd for $C_{56}H_{89}N_3O_{18}Na^+$ 1114.6039, found: 1114.6034.

UV-Vis (10.9 μM in CH_3OH): λ_{max} = 332 nm.

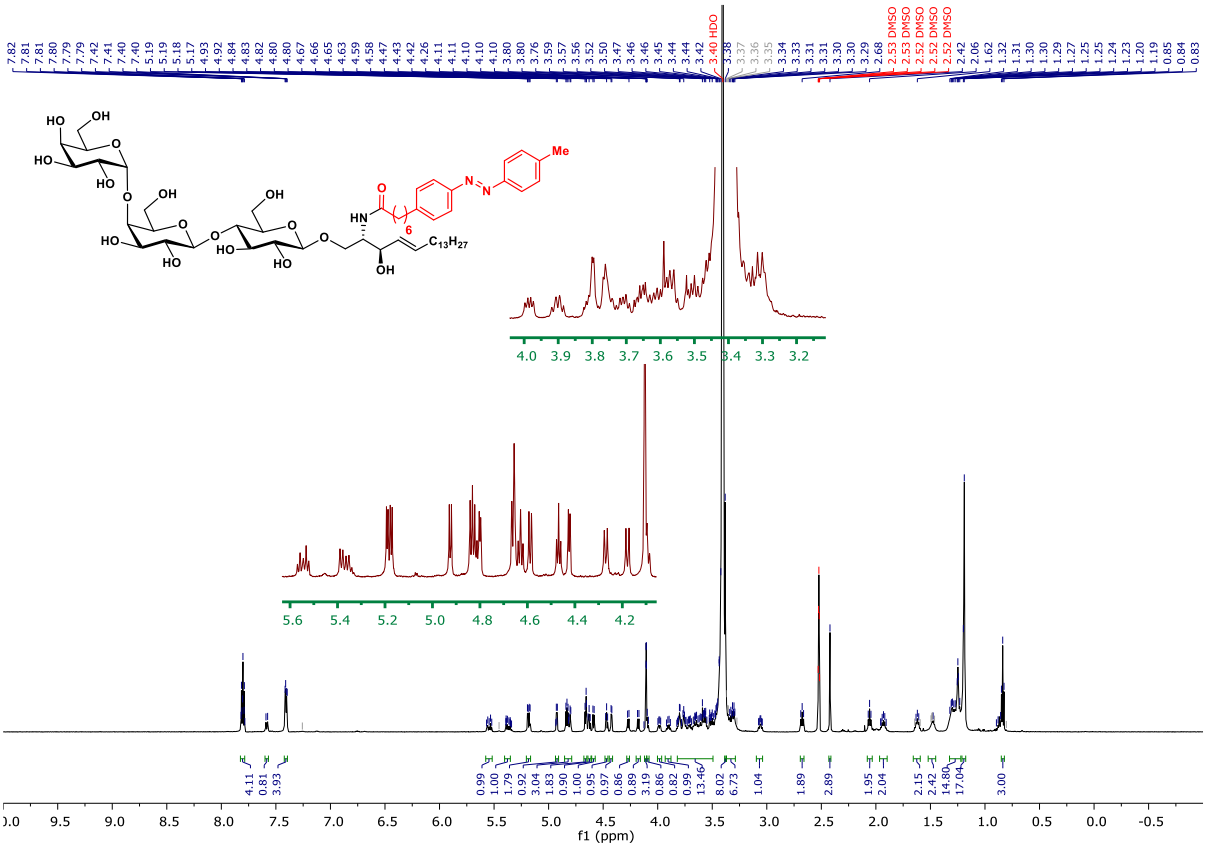


Figure S15. ^{1}H NMR (DMSO-d_6, 600 MHz) spectrum of **3d**

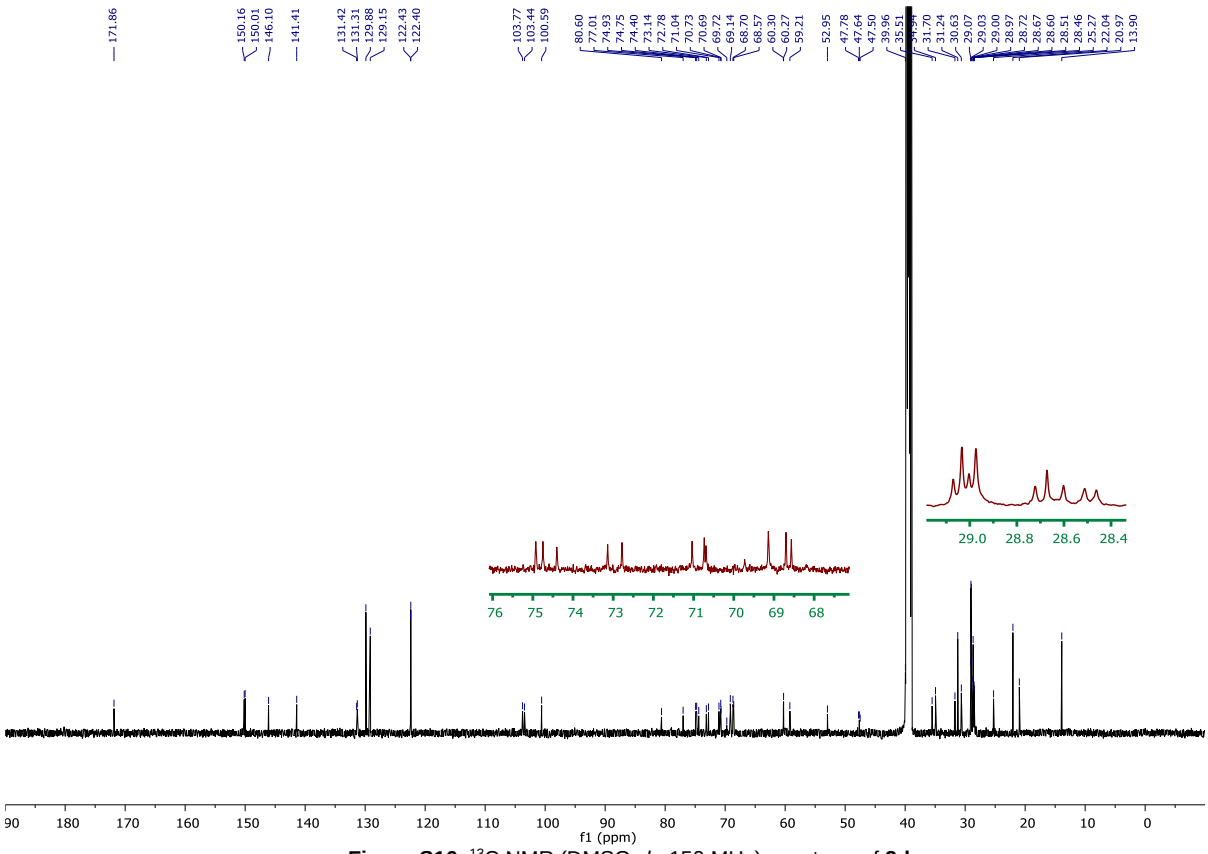


Figure S16. ^{13}C NMR (DMSO-d_6, 150 MHz) spectrum of **3d**

B) Biophysical Part

Kinetics of photo-Gb_3s thermal relaxation in phase-separated vesicles

UV/Vis spectroscopy. A V-650 spectrophotometer from Jasco (Pfungstadt, Hesse, Germany) was used to perform the kinetics experiments. Vesicles containing 20% of the corresponding photo-Gb_3 derivative were irradiated with UV light for 3 min. The thermal relaxation to the *trans*-state was monitored by measuring the absorbance (OD_{340}) at 340 nm for about 13.3 hours. To determine the lifetime of the *cis*-configuration of the photo-Gb_3 molecules, a biexponential function (eq. (S1)) was fit to the data:

$$OD_{340} = OD_{\max 1}\left(1 - e^{t/t_1}\right) + OD_{\max 2}(1 - e^{t/t_2}) \quad \text{(S1)}$$

OD_{max1} and OD_{max2} are the corresponding maximum *OD*-values, t_1 and t_2 the time constants.

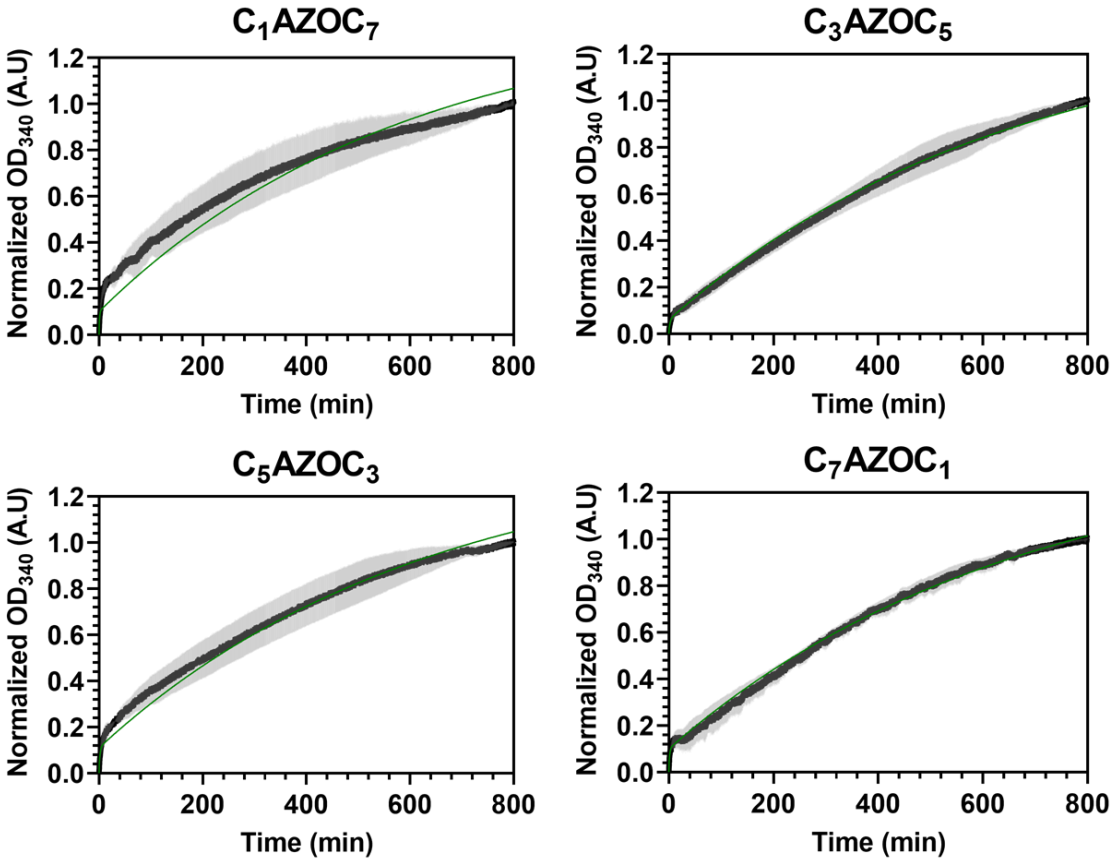


Figure S17. Kinetics of the thermal relaxation of photo-Gb_3 derivatives in phase-separated vesicles composed of DOPC/SM_{18}/cholesterol/photo-Gb_3/ATTO 655 DOPE, 37:20:22:20:1 (*n*/*n*). The absorbance at 340 nm (OD_{340}, mean of three independent experiments) is plotted vs. time (black data points). The data was normalized to the maximum absorbance measured at 800 min. The error bars (grey) are the standard deviation of the mean of three independent experiments. The solid green lines are the results of fitting eq. (S1) to the data with constraints of OD_{max1} (0.1) and OD_{max2} (1.3).

Table S1. Thermal relaxation of photo-Gb_3 derivatives in phase-separated vesicles. The lifetime of the *cis*-configuration is given by the time constant t_2 (slow component).

	C_1AZOC_7	C_3AZOC_5	C_5AZOC_3	C_7AZOC_1
OD_{max1}	0.20 ± 0.003	0.06 ± 0.001	0.17 ± 0.002	0.082 ± 0.001
t_1 / min	3.8 ± 0.9	2.0 ± 0.6	1.7 ± 0.5	1.4 ± 0.2
OD_{max2}	0.95 ± 0.01	1.55 ± 0.01	1.12 ± 0.01	1.55 ± 0.02
t_2 / min	586 ± 5	651 ± 3	626 ± 4	656 ± 2

AFM micrographs of a phase-separated membrane after photo-Gb_3 isomerization

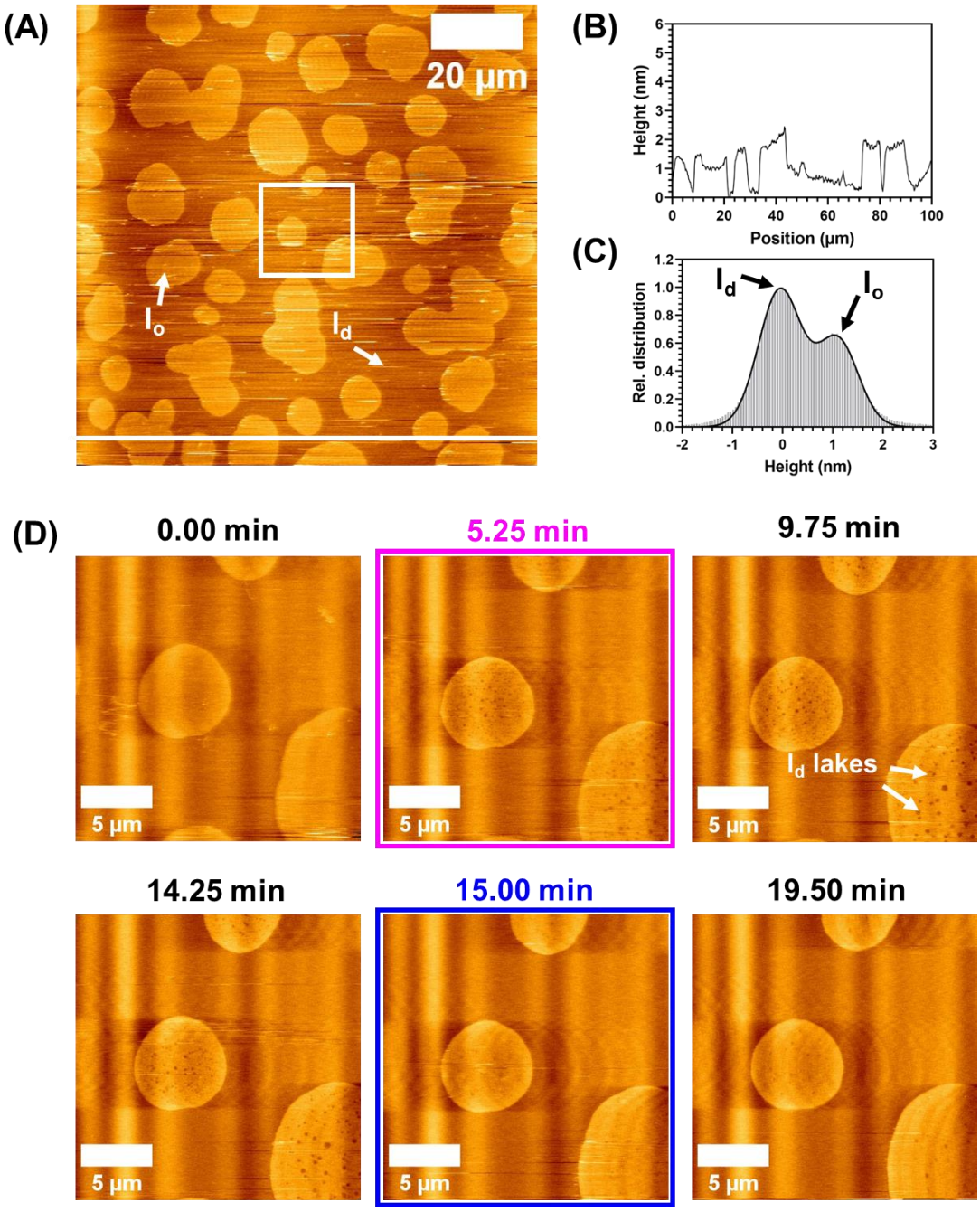


Figure S18. Atomic force micrographs of a phase-separated membrane containing 5 mol% photo-Gb_3 (see also Movie S2). Membrane composition: DOPC/SM_{18}/cholesterol/Gb_3-C_7AZOC_1/ATTO 655 DOPE, 39:35:20:5:1 (*n*/*n*). The l_d phase is lower (darker color) than the l_o phase (brighter). The membrane was illuminated with UV light after 5.25 minutes (magenta box) and blue light after 15 minutes (blue box). (**A**) Overview image of the membrane before isomerization. (**B**) Representative height profile of the membrane (white line) before irradiation. (**C**) Histogram analysis of the height distribution (white square). The average height difference between the l_o and l_d phase was 1.14 ± 0.42 nm. (**D**) Zoom in (white square) and time series of the membrane before and after light irradiation. Upon UV irradiation (magenta), l_d lakes appear within the l_o domains. The l_d lakes shrink immediately upon blue light irradiation (blue).

CLSM micrographs of a phase-separated membrane containing no photo-Gb_3

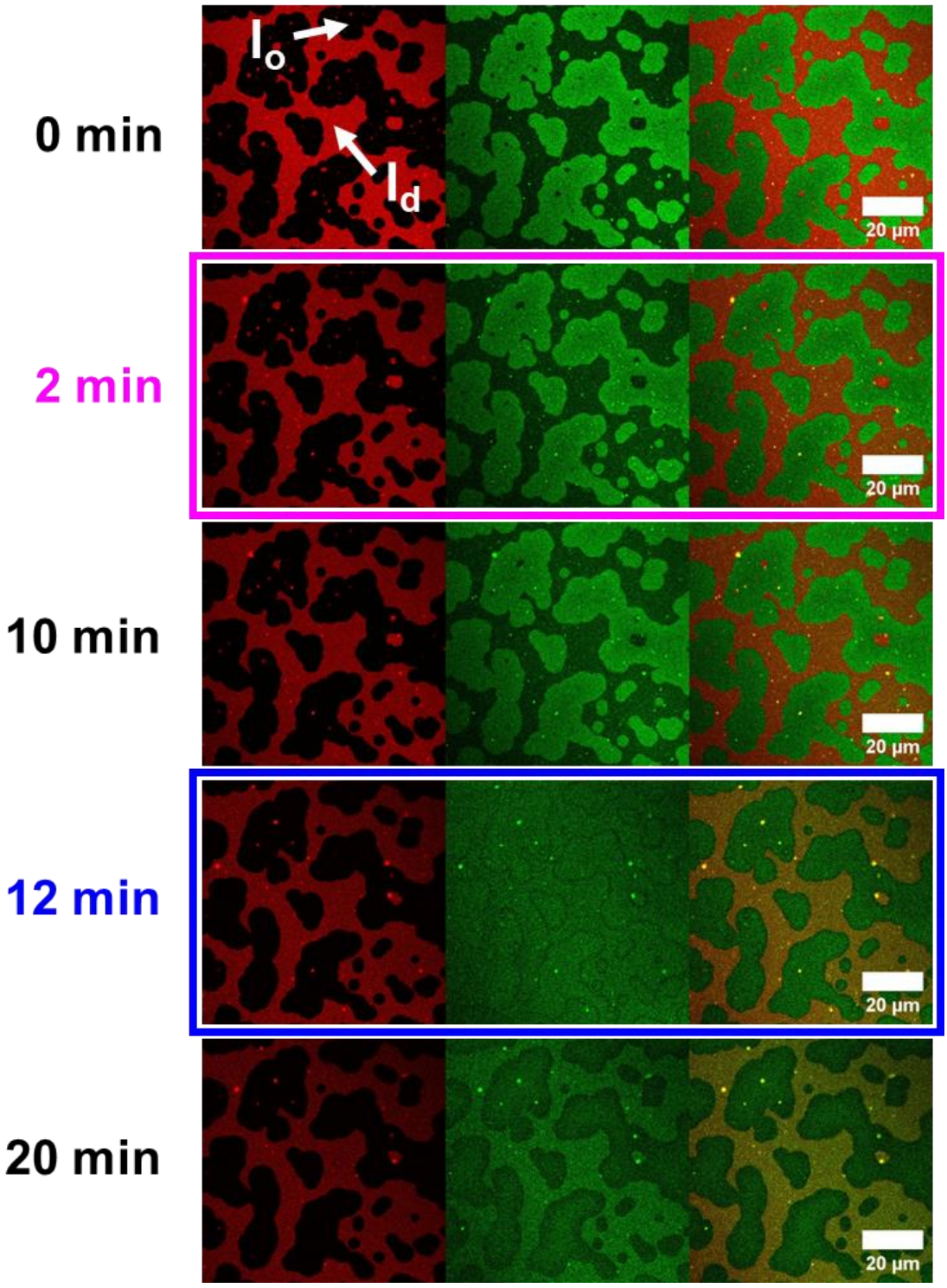


Figure S19. CLSM images of a phase-separated membrane containing no photo-Gb_3 (see also Movie S3). Membrane composition: DOPC/SM_{18}/cholesterol/ATTO 655 DOPE/BODIPY-cholesterol, 39:39:20:1:1 (*n*/*n*). ATTO 655 DOPE (red) labels the l_d phase, whereas BODIPY-cholesterol (green) labels the l_o phase. The membrane was illuminated with UV light after 2 minutes (magenta box) and blue light after 12 minutes (blue box). No l_d or l_o lakes, respectively are observed upon UV and blue light irradiation. Partial bleaching of the BODIPY-cholesterol (λ_{ex} = 495 nm) is observed upon blue light irradiation (λ_{irr} = 435-460 nm).

AFM micrographs of a phase-separated membrane containing no photo-Gb_3

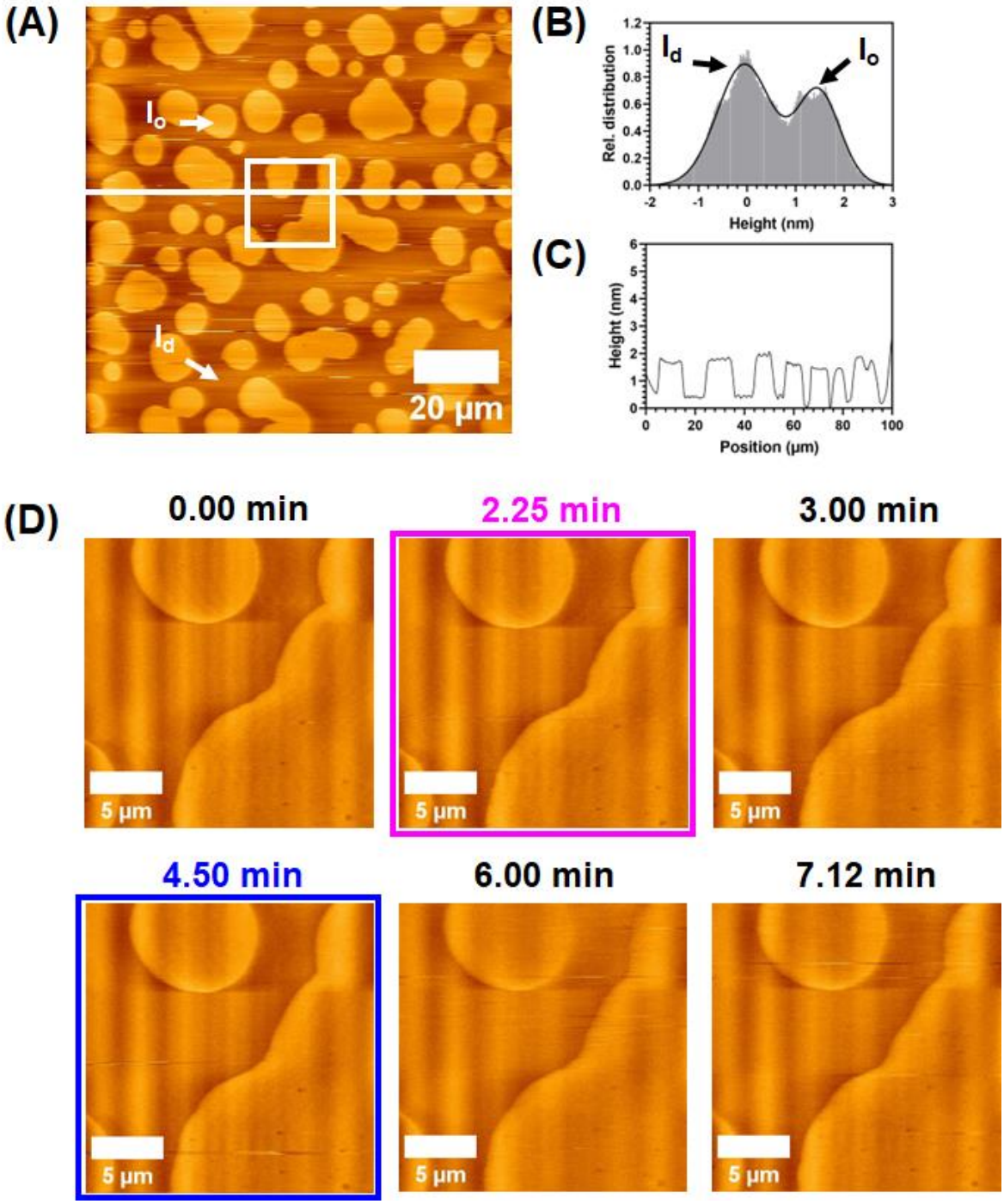


Figure S20. AFM images of a phase-separated membrane containing no photo-Gb_3 (see also Movie S4). Composition: DOPC/SM_{18}/cholesterol/ATTO 655 DOPE/BODIPY-cholesterol, 39:39:20:1:1 (*n*/*n*). The l_d phase is lower (darker color) than the l_o phase (brighter). The membrane was illuminated with UV light after 2.25 minutes (magenta box) and blue light after 4.50 minutes (blue box). (**A**) Overview image of the membrane before irradiation. (**B**) Representative height profile of the membrane (white line) before irradiation. (**C**) Histogram analysis of the height distribution. The average height difference between the l_o and l_d phase was 1.52 ± 0.50 nm. (**D**) Zoom in (white square) and time series before and after light irradiation. The membrane remains constant upon UV (magenta) and blue light (blue) irradiation.

Observation of a phase-separated membrane containing photo-Gb_3 during CLSM imaging

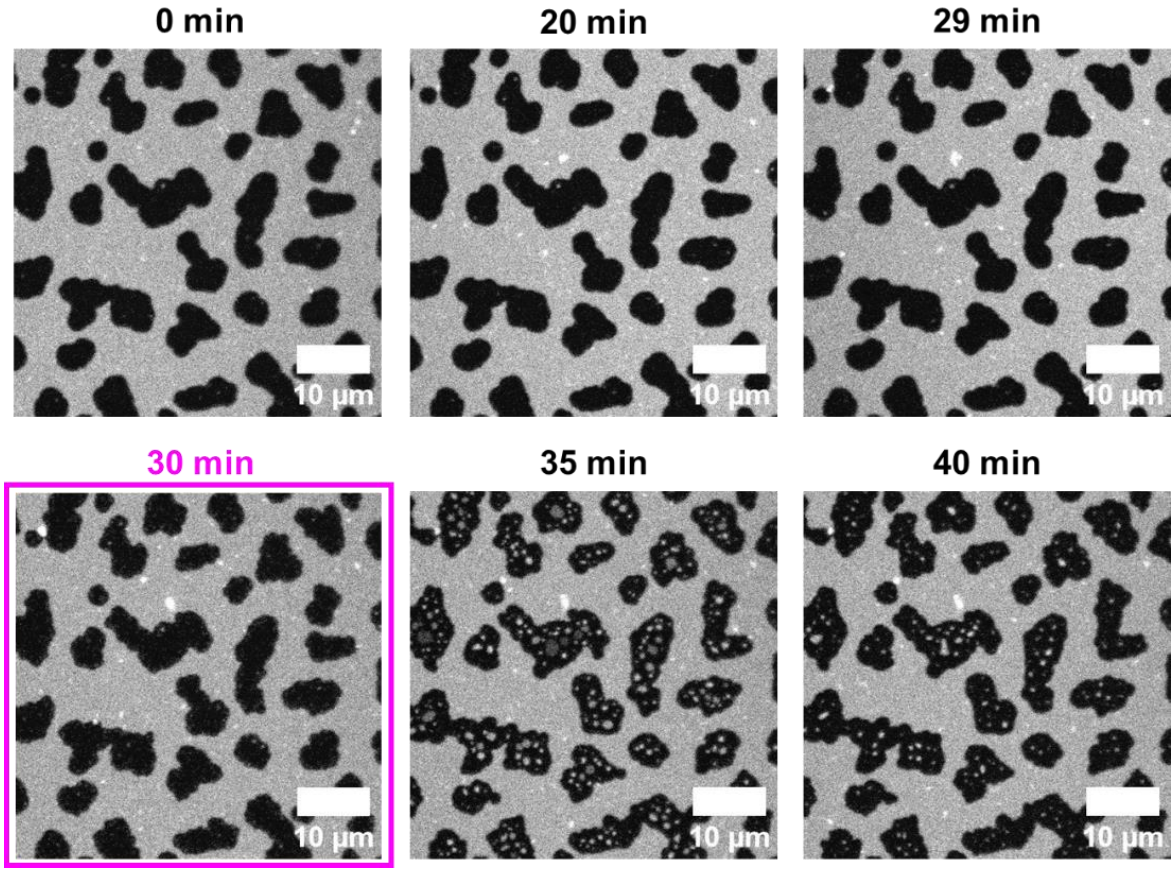


Fig. S21. Time-lapse series (fluorescence micrographs) of a phase-separated membrane containing 20 mol% photo-Gb_3 before and after UV irradiation (magenta box). Membrane composition: DOPC/SM_{18}/cholesterol/Gb_3-C_3AZOC_5/ATTO 655 DOPE, 37:20:22:20:1 (*n*/*n*). The membrane was illuminated with UV light after 30 minutes (magenta box).

Observation of a phase-separated membrane containing photo-Gb_3 during AFM imaging

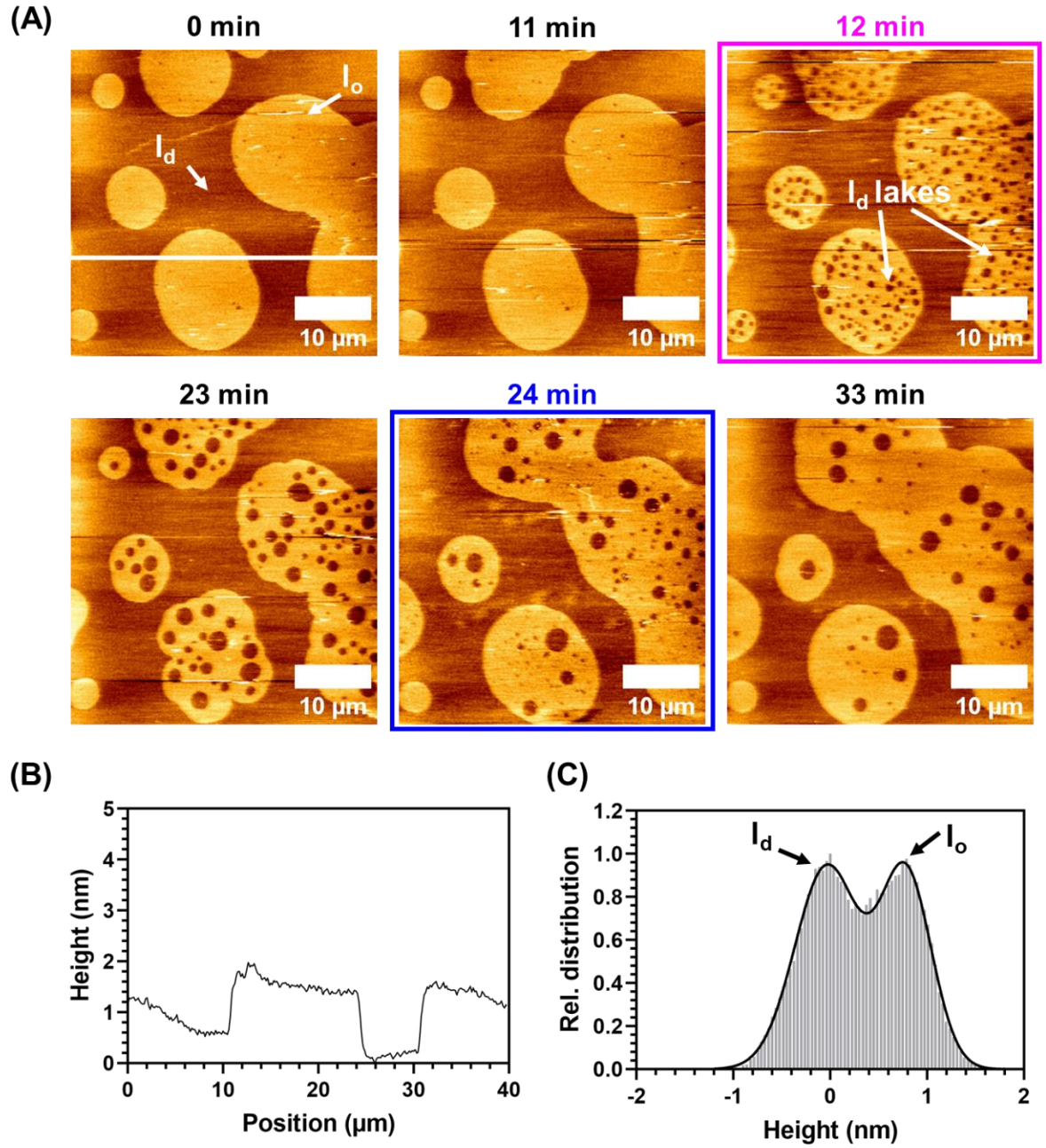


Fig. S22. (A) Time series of the membrane before and after light irradiation. AFM images of a phase-separated membrane containing 20 mol% photo-Gb_3 before and after UV (magenta box) and blue light (blue box) irradiation. Membrane composition: DOPC/SM_{18}/cholesterol/Gb_3-C_5AZOC_3/ATTO 655 DOPE, 37:20:22:20:1 (*n*/*n*). The membrane was illuminated with UV light after 12 minutes (magenta box) and blue light after 24 minutes (blue box). **(B)** Representative height profile (white line) and **(C)** histogram analysis of the height distribution before irradiation. The average height difference between the l_o and l_d phase was 0.81 ± 0.30 nm.

Influence of the position of the azobenzene on membrane ordering

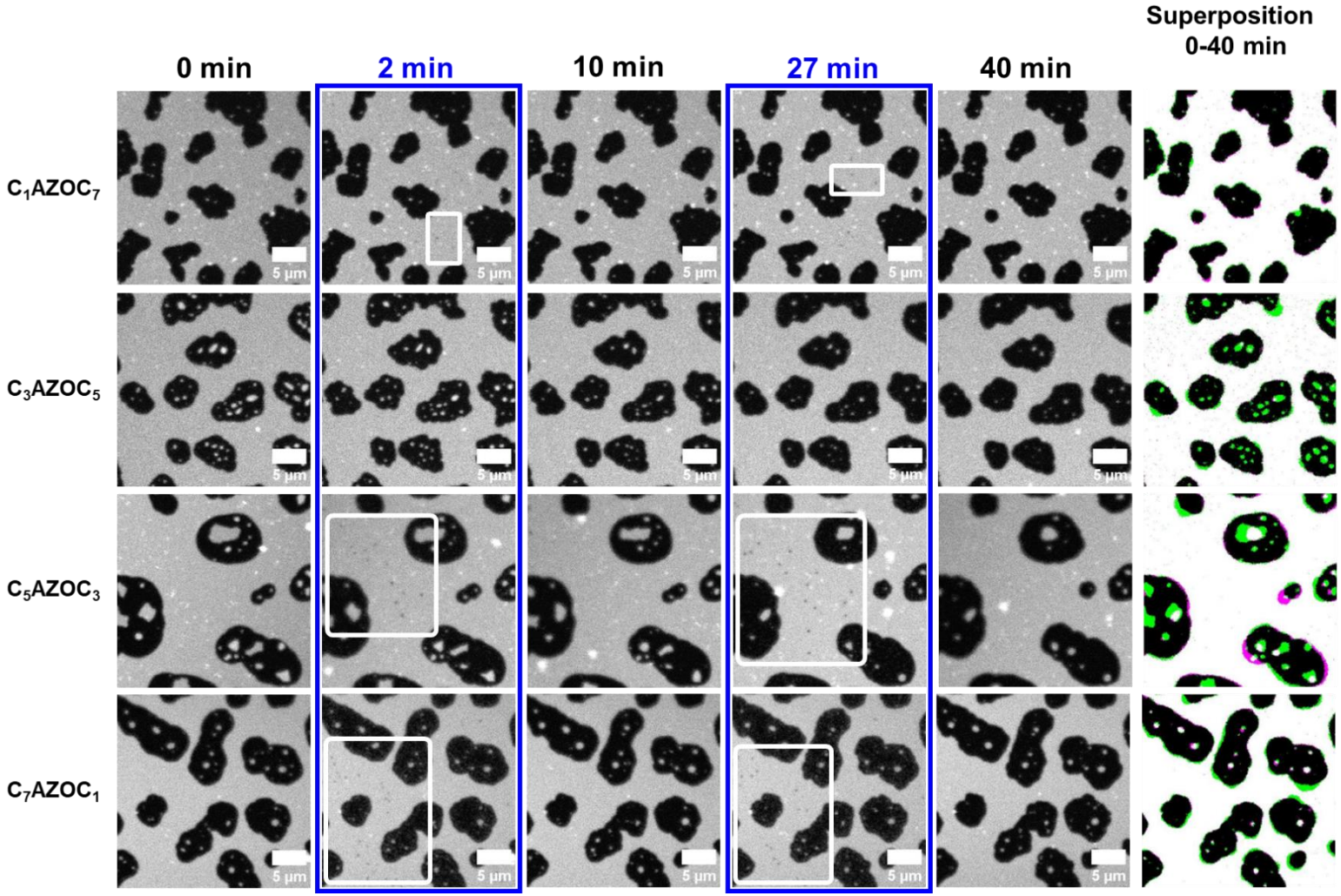


Figure S23. Influence of the position of the azobenzene group on membrane organization after blue light irradiation. Membrane composition: DOPC/SM_{18}/cholesterol/photo-Gb_3/ATTO 655 DOPE, 37:20:22:20:1 (*n*/*n*). In the fluorescence micrographs, the l_o phase appears black while the l_d phase is labeled with ATTO 655 DOPE. l_d lakes are present at t = 0 min indicating the previously induced *cis*-photoisomerization of the photo-Gb_3. The membrane was subsequently illuminated with blue light after 2 and 27 minutes (blue boxes). Blue-light irradiation induced the formation of l_o lakes in the l_d phase (highlighted with white rectangles). Images at t = 0 and t = 40 min were converted to black and white images (last row) and then superimposed to better visualize the variation of the morphology of the domains. The pink color highlights the initial morphology of the domains at t = 0 min while the green color displays the morphology at t = 40 min.

AFM micrographs and height profiles of Shiga toxin B clusters after photo-Gb_3 isomerization

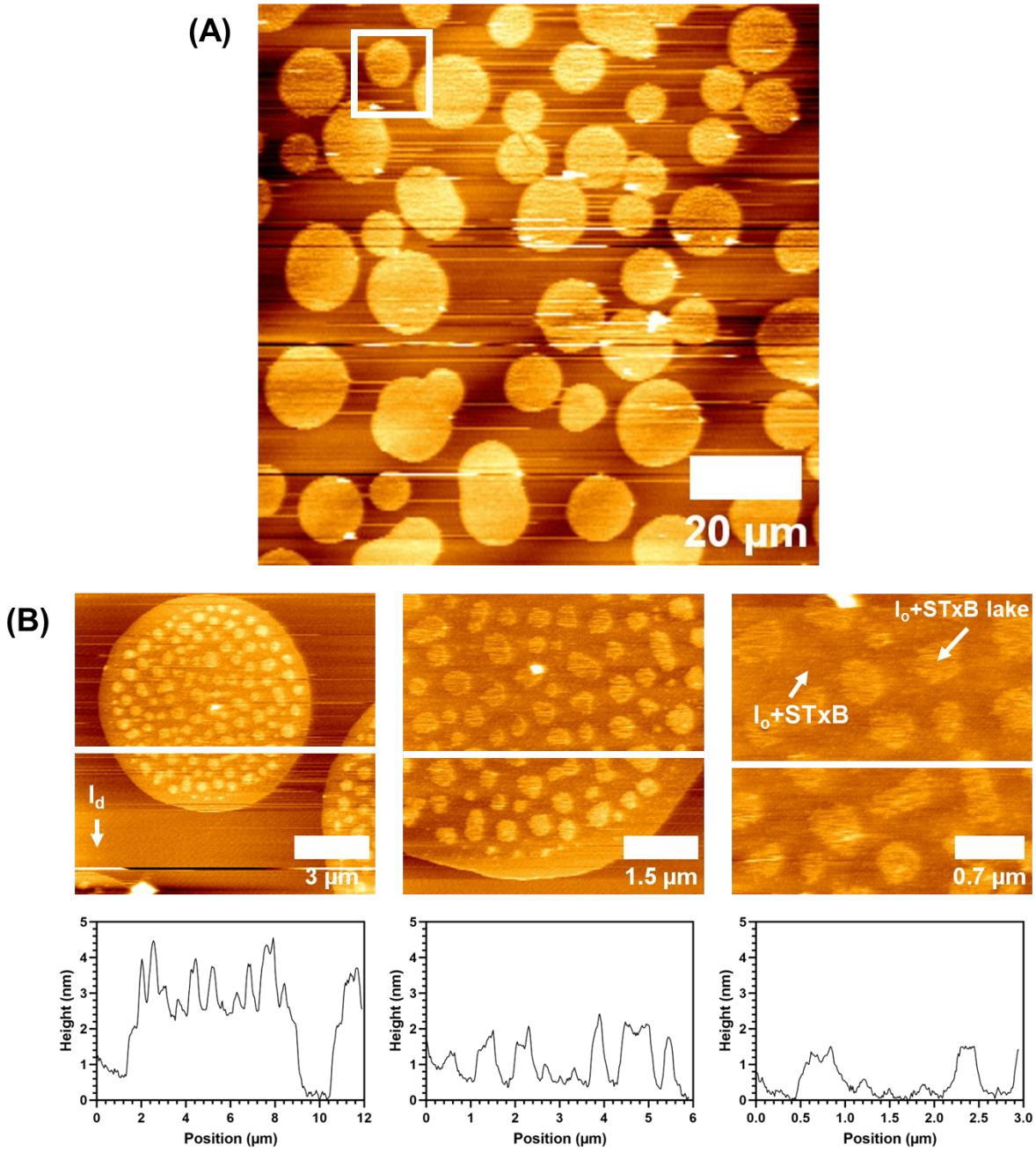


Figure S24. AFM images and height profiles of STxB bound (300 nM) after photo-Gb_3 isomerization (see also Movie S6). Membrane composition: DOPC/SM_{18}/cholesterol/C_7AZOC_1/ATTO 655 DOPE, 37:30:22:10:1 (*n*/*n*). (**A**) Overview image of the membrane after isomerization. (**B**) Zoom in (white square) and corresponding height profiles (white lines) of the protein lakes.

CLSM micrographs (Z-stack) of Shiga toxin B clusters after photo-Gb_3 isomerization

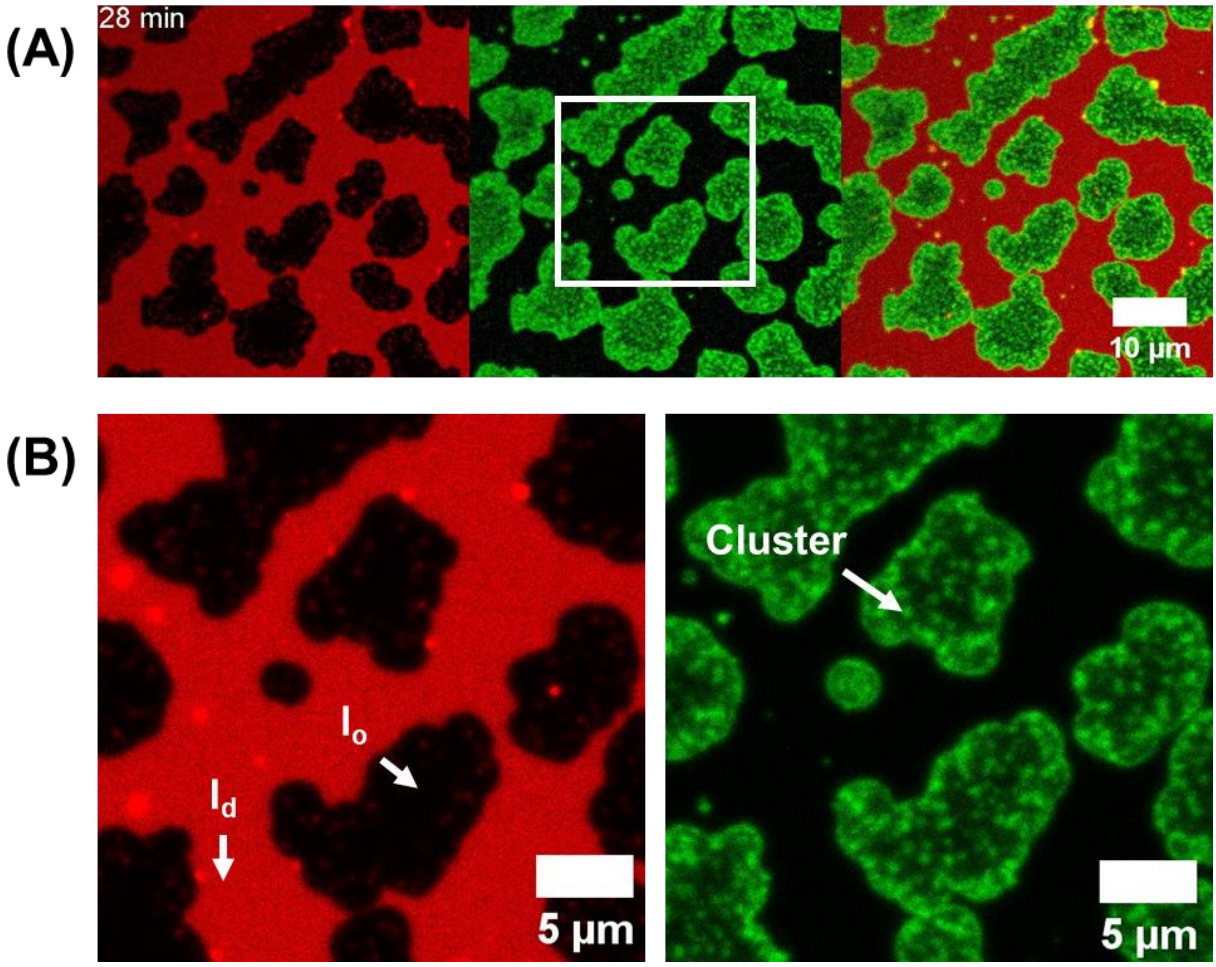


Figure S25. CLSM images of Shiga toxin B clusters after photo-Gb_3 isomerization: (see also Movie S7). Composition: DOPC/SM_{18}/Cholesterol/C_7AZOC_1/ATTO 655 DOPE, 37:30:22:10:1 (*n*/*n*). Cy_3-STxB concentration: 300 nM. The red fluorescence (ATTO 655 DOPE) shows the membrane whereas the green fluorescence (Cy_3) represents the protein. (**A**) Membrane after light irradiation. (**B**) Zoom in (white square) on the protein clusters formed after irradiation. A *z*-stack (10 slices, range: 3.7 µm) was recorded resulting in a *z*-projection (average intensities) to better visualize the protein structures.