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Complete List of Authors:	Mekala, Shekar; Rensselaer Polytechnic Institute, Center for Biotechnology and Interdisciplinary Studies (CBIS) and New York State Center for Polymer Synthesis Peters, Kyle; Case Western Reserve University College of Arts and Sciences, Department of Physics Singer, Kenneth; Case Western Reserve University, Department of Physics Gross, Richard; Rensselaer Polytechnic Institute , Chemistry and Biology

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Biosurfactant-functionalized porphyrin chromophore that forms *J*-aggregates

Shekar Mekala,[a] Kyle C. Peters [b] Kenneth D. Singer [b] and Richard A. Gross*[a]

Structurally complex biosynthesized building blocks whose structures can be systematically varied are of great interest for the synthesis of manipulable self-organizing supramolecular systems. Sophorolipids (SLs) are an important class of glycolipid biosurfactants that consists of a sophorose (glucose disaccharide) polar head group that allows structural diversification by full or selective acetylation at the 6'- and 6''-positions. Porphyrins are a group of naturally-occurring heterocyclic macromolecular organic compounds that have efficient charge transfer properties. Herein we describe the synthesis of SL-porphyrin conjugates where the number of sophorolipid arms, availability of hydrogen bonding sophorose hydroxyl groups and rigidity of the lipid chain were systematically varied. SLs differing in 'sophorose acetylation' and 'lipid unsaturation' were conjugated to zinc-porphyrin dyes by copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) 'click' chemistry. Mono-, di-, and tetra-conjugation of SL-arms to the zinc-porphyrin core provided variation in SL-arm steric effects. UV-vis spectra in methanol/water reveal features indicative of supramolecular J-type aggregates. The synthesized compounds were designed to provide a library of unique bio-based molecules with built-in variation in non-covalent interactions, hydrogen-bonding, π-π stacking, metal-ligand coordination, dipole-dipole, van der Walls, and hydrophobic interactions for future interrogation of supramolecular self-assembly into functional materials for electro-optical applications.

Introduction

Chemists are designing a variety of novel supramolecular self-assembled structures by utilizing covalent and noncovalent forces such as π-π stacking, hydrogen bonding, dipole-dipole, cation-π, anion-π, van der Waals forces and hydrophobic interactions [1]. Moreover, molecular self-assembly by precise control of such intermolecular interactions is an important strategy to generate self-organized structures with desirable properties for functional materials including optoelectronics [2].

Natural polymers, such as DNA and proteins, provide inspiration by forming well defined double helical and folded secondary structural elements (α-helix and β-sheet respectively). Use of these and other natural building blocks provide important routes to control self-assembly and introduce chirality. In addition, by building in natural materials with self-assembly properties, the number of synthetic steps required to prepare hybrid natural-synthetic self-assembled functional materials can be reduced which is critical to their potential commercialization [3]. Furthermore, the demand to replace petroleum-derived chemicals with readily renewable building blocks continues to increase.

Sophorolipids (SLs), a member of the natural glycolipid family, are compelling building blocks for use in functional materials for reasons that follow. SLs are available in large quantities (volumetric yields > 200 g/L) from their conversion by *Candida bombicola* in fermentation processes of readily renewable carbohydrates and lipid feedstocks [4]. SLs readily self-assemble forming nano-structures with supramolecular chirality [5]. SLs consist of a unique disaccharide (sophorose, 2-glucose units linked β-1-2) that is β-glycosidically linked to a sub-terminal hydroxylated fatty acid. Even with its structural complexity, SLs are amenable to selective chemical modifications at sophorose (e.g. acetylation at the 6'- and/or 6''- positions) [6] and lipid (e.g. metathesis, hydrogenation) [7] moieties. Thus, due to their unique structural and self-assembly properties along with their facile synthesis by fermentation, SLs were selected herein as components to construct a family of tunable self-assembling materials for potential use in electro-optical applications.

Porphyrins are a group of natural-occurring heterocyclic macromolecular organic compounds composed of four modified pyrrole subunits that are interconnected at their α carbon atoms via methine bridges (=CH−). They play an important role of light-harvesting antennae in photosynthetic systems [8]. The main driving force for porphyrin structures in nature is to promote specific porphyrin-porphyrin non-covalent interactions within the non-covalent framework of proteins that organizes porphyrins into well-defined tertiary structures [9]. Over the years, researchers have synthesized various porphyrin derivatives and investigated their applications in fields such as supramolecular chemistry [10], artificial photosynthetic systems [11], organic photovoltaics (OPVs) [12], molecular rotors [13], catalysis [14], photodynamic therapy [15] etc. The structure-property relationships of synthetic porphyrins can be manipulated by modification of the porphyrin core and attachment of peripheral groups to adjust the properties of supramolecular self-assembled structures [16].

Given the potential complimentary characteristics of SLs and porphyrins, this study describes synthetic methods to prepare a series of well-defined SL-porphyrin

[a] *Center for Biotechnology and Interdisciplinary Studies (CBIS) and New York State Center for Polymer Synthesis, Rensselaer Polytechnic Institute, Troy, NY, United States*
[b] *Department of Physics, Case Western Reserve University, Cleveland, OH, United States.*
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conjugates. The family of compounds was designed with the goal of varying non-covalent interactions, hydrogen-bonding, π-π stacking, metal-ligand coordination, dipole-dipole, van der Walls, and hydrophobic interactions between SL-porphyrin conjugates. The number of hydroxyl groups available for hydrogen-bonding interactions was varied by altering the degree of acetylation. Two extremes were per- and non-acetylation. An intermediate degree of acetylation was obtained by lipase-catalyzed acetylation that targets the two primary hydroxyl groups at carbons 6'- and 6''. Hydrogenation of the C=C double bond of the SL lipids was used to vary lipid chain flexibility. The resulting SLs were conjugated to zinc-porphyrin dyes by copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) 'click' chemistry. The number of SL arms attached to the porphyrin core was varied to enable interrogation of the interplay between SL-arm steric constraints and porphyrin stacking. UV-vis spectroscopic studies in dilute MeOH:water solution reveal the formation of J-type aggregation for hydrogen-bond-bearing compounds that resemble supramolecular self-assembled entities. Thus, the goal of this work was to create a unique family of SL-porphyrin structures, provide evidence that some of the SL-porphyrin designs enable self-assembly, and in future work use these molecules for in-depth analyses to elucidate how structural variables built into the SL-porphyrins can be used to control multi-chromophoric aggregation and corresponding self-assembled supramolecular structures.

Results and Discussion

Synthesis

To realize efficient bioconjugation reactions of SLs, we sought to place an azido functionality at the SL lipid chain terminus. This was achieved by a chemo-enzymatic synthetic approach. First, naturally derived lactonic sophorolipid (LSL) was ring-opened by methanolysis following a published literature protocol to obtain in 90% to quantitative yields the SL-methyl ester (SLOMe, **1**) as a white solid [17]. Following this, reaction of **1** with 3-azido-1-propanamine, [18] under neat reaction conditions, provided access to the key precursor, azido sophorolipid ($[OH]_7SLN_3$, **2**), obtained as a light-yellow solid in >85% yield (**Scheme 1**). This product was fully characterized by ^1H NMR, ^{13}C NMR and HR-MS (See SI-1 and the Experimental Section). In the next step, the 6' and 6'' positions of the sophorose moiety were regioselectively acetylated using Novozyme-435 (N-435) (immobilized *Candida Antarctica* Lipase B, CALB) as the catalyst and vinyl acetate as the acyl acceptor in anhydrous THF at 50 ^oC for 3-4 days under argon atmosphere [19]. The product, diacetylated azido-SL ($[OAc]_2SLN_3$, **3**, **Scheme 1**), was obtained as a white solid in about 80% yield. Peracetylated azido-SL ($[OAc]_7SLN_3$, **4**, **Scheme 1**) was synthesized following a previously reported procedure by reacting **2** with acetic anhydride in dry THF using dimethylamino pyridine (DMAP) as catalyst [17]. The corresponding product **4** was isolated as a white solid in 78% yield. The structures of azido-functionalized SLs **2**, **3** and **4** were confirmed by analysis of their respective ^1H NMR, ^{13}C NMR and HR-MS spectral data (stacked ^1H NMR spectra in **Fig. SI-1**, stacked ^{13}C NMR spectra in **Fig. SI-2**). Synthesis of Compound **13** was performed to interrogate how increasing SL lipid chain rigidity by hydrogenation of the *cis* double bond would influence self-assembly of the corresponding SL-porphyrin conjugate. The *cis* C=C double bond (9*Z*) of SLOMe (**1**) was converted by catalytic hydrogenation in the presence of Pd/C in methanol at 5 mbar of H_2 for about 8 h at room temperature to give the saturated H_2-SLOMe (**5**) in >95% to quantitative yield. Compound **5** was converted to the azido derivative by reaction with 3-azido-1-propanamine as described for compound **2**. TLC monitored the reaction for disappearance of the starting material. The product, H_2-$(OH)_7SLN_3$ (**6**) (**Scheme 2**), was obtained as an insoluble light-yellow solid and was not characterized by NMR since it was insoluble in a wide-range of organic solvents (e.g. MeOD, DMSO-d_6) at room temperature. To improve the solubility of **6**, it was selectively acetylated at sophorose 6' and 6'' positions with vinyl acetate by N-435 catalysis as described above for compound **3**. The product H_2-$(OAc)_2SLN_3$ (**7**) (**Scheme 2**) was obtained as a light-yellow solid in >60% over two steps from **5**. Unlike Compound **6**, it was soluble in methanol and DMSO. The ^1H and ^{13}C NMR spectra (**Fig. SI-1 and SI-2**, respectively) show that the double bond protons and carbons seen for compound **2** disappear and new ^{13}C NMR resonances corresponding to the saturated carbons are observed.

Azido SL derivatives **2**, **3**, **4** and **7** were then conjugated to the Zn-porphyrins **8** (dialkynyl) [20] and **9** (tetraalkynyl) (**Scheme 3**) [21] by Cu(I) catalyzed azide-alkyne cycloaddition (CuAAC) 'click' reaction [22]. First, we conjugated the compound **8** to **3**, to get the di-conjugated compound **11** using Cu (I) 'click' chemistry in THF:H_2O (1:1) at 60 ^oC, overnight [23] (See experimental section) and this procedure was also used to synthesize all other conjugated compounds (**10**, **12**, **13**, **14** and **15**) (See experimental section for click reaction conditions) (**Schemes 4, 5, 6 and 7**). The synthesized compounds were fully characterized by ^1H NMR, ^{13}C NMR and HRMS. All the di- and tetra- conjugated compounds were synthesized with the addition of 1.25 equiv. of azido-sophorolipid derivative per alkyne group (**Scheme 6**). However, for mono conjugated compound **15** (**Scheme 7**), 0.5 equiv. of azido-sophorolipid per alkyne group was used and the 'click' reaction conditions were identical to that for the synthesis of the di- and tetra-conjugated Compounds.

In the ^1H NMR of all di-conjugated Compounds (**10**, **11**, **12** and **13**), a new signal at δ8.84ppm is seen that corresponds to the triazole proton formed by the 'click' reaction [23]. Furthermore, there is an up-field shift of the methylene protons next to the azide from ~3.3ppm to δ4.53 ppm (*t*) that occurs after the 'click' reaction (see stacked ^1H NMR spectra in **Fig. 1**). These changes in spectra are consistent with successful di-conjugation of compounds **2**, **3**, **4** and **7** with **8** to form Compounds **10**, **11**, **12** and **13**, respectively. The ^1H NMR of tetra-conjugated Compound **14** reveals the triazole proton at (δ8.89ppm), amide NH at (δ8.0ppm) and phenyl ring protons (broad, no splitting) at 8.92 and 8.31 ppm. Also, the pyrrole protons of the porphyrin are overlapped with the phenyl ring protons at 8.31 ppm. These assignments were established by ^1H COSY and HMQC spectral acquisition and analysis (See S.I for spectra). For mono-conjugated compound **15**, in addition to the triazole (8.88 ppm) and amide NH (7.99ppm) protons, a free alkynyl proton at (4.46ppm) in the ^1H NMR is consistent with the mono 'click' Compound **15 (**see stacked ^1H NMR spectra in **Fig. SI.3)**. ^{13}C NMR and ESI-MS data for all the di-, tetra- and mono-conjugated compounds are in agreement with ^1H NMR data (see experimental section and **SI**). After successful synthesis of this library of SL-porphyrin compounds, the influence of chiral amphiphilic sophorolipid arms on the assembly of tethered π-conjugated porphyrin rings was assessed by UV-vis absorption measurements.

UV-vis absorption measurements (in solution)

Inspired by SL's unique ability of self-assembly in water, UV-vis absorption measurements were investigated in water-miscible solvents and their mixture with water, due to the insolubility of compounds in pure water. Compound **11** was chosen for initial investigation as it possesses a combination of acetylation at 6' and 6'' hydroxyl positions for improved solubility along with the potential for hydrogen-bonding between neighboring compounds by the SL motif that can contribute to intermolecular interactions. The absorption spectrum of **11** in neat MeOH exhibits a characteristic peak maximum at λ_{max}=424 nm corresponding to the Soret region of non-interacting porphyrin chromophores [24], and it was found that upon addition of water the absorption spectrum displays a substantial spectroscopic change involving a decrease in optical density accompanied by the emergence of a new peak at 442 nm. **Figure 2a** displays room temperature UV-vis absorption spectra of **11** in water-varying mixtures in MeOH (inset, **Figure 2a**) at constant molar concentration (0.65 x 10^{-6} M).

At 50% water content, spectral band splitting consisting of two peaks at 427 nm and 442 nm is observed that results in a split Soret band with energy separation ~790 cm^{-1}. At higher water content (>50%), the prominent band splitting convolutes into a broadened red-shifted band, and for low water contents (<50%) the absorption band returns to the typical monomeric Soret band located at 424 nm. The distinct Soret splitting is, thus, found to be sensitive to water, with defined splitting features occurring near 50% water content. Other water-miscible solvents we also studied, however prominent Soret band splitting was not observed (see Supporting Information, Figure SI-4). In continuation, the interesting water-induced spectral change of **11** in MeOH:water inspired further study into the influence of water on the aggregation of all compounds in MeOH:water (1:1 v/v) mixture, results are shown in **Figure 2b**. The spectra in **Figure 2b** reveals that di-conjugated SL-porphyrin **10** (non-acetylated) and **11** (di-acetylated) exhibit similar spectral features in MeOH:water (1:1) that consists of a distinct split, red-shifted Soret band. Comparatively, the band splitting is the same (~790 cm^{-1}), however the red-shifted band of **10** is more prominent alluding to a higher tendency to aggregate. The spectral features of **10** and **11** suggest the presence of multi-chromophoric exciton-coupling [25] resulting from the formation of J-type aggregates [26]. In addition, the spectral lineshape of fully acetylated SL-porphyrin conjugate **12** exhibits no distinct band splitting, but instead a diminished and broadened red-shifted band. The spectral features of non-acetylated mono-conjugated SL-porphyrin **15** is similar to **12,** and these shifts can be attributed to pi-aggregation between weakly coupled chromophores [27]. Furthermore, the spectral lineshape of di-conjugated hydrogenated di-acetylated SL-porphyrin **13** is interesting, in that the added hydrocarbon-chain flexibility allows for a further J-aggregate peak near λ_{max}=448 nm. However, the origin of the spectral features of **13** is currently unclear and warrants a more detailed investigation. Additionally, the spectral response of bulky tetra-conjugated compound **14** exhibits a small red-shift from the monomeric state (4 nm) with minimal broadening, vibronic-structure loss, and optical density change, thus consistent with a slight increase in order.

Conclusions

In conclusion, efficient chemo-enzymatic routes provided a rationally designed series of SL-porphyrin conjugates. The goal of structural alterations built into the family of synthesized compounds was to vary non-covalent interactions, hydrogen-bonding, π-π stacking, metal-ligand coordination, dipole-dipole, van der Walls, and hydrophobic interactions between SL-porphyrin conjugates. Variation in hydrogen between sophorose moieties of SL-peripheral arms was achieved by peracetylation and non-acetylation that eliminates and maximizes, respectively, opportunities for hydrogen bonding. To synthesize SLs with an intermediate degree of acetylation, lipase-catalyzed acetylation was used since it targets the two primary hydroxyl groups at carbons 6'- and 6''. Hydrogenation of the C=C double bond of SL lipids increased the rigidity of lipid chains. Conjugation of SLs and zinc-porphyrins was accomplished in moderate to high yields by the Cu (I) catalyzed 'azide-alkyne' cycloaddition (CuAAC) 'click' reaction. In addition, SL-porphyrin conjugates with one, two and four SL peripheral arms were prepared to understand the interplay between steric constraints introduced by multiple arms and porphyrin stacking. The hydrogenated, di-acetylated sophorolipid (**7**) has low solubility in methanol when compared to its corresponding unsaturated derivatives (**2**, **3** and **4**). This is presumably due to the more rigid lipid arms of **7**. Hydrogen-bond-bearing diconjugated compounds **10** and **11** were found to exhibit distinct exciton-coupling indicative of J-type aggregation facilitated by a solvent-sensitive mixture of MeOH:water (1:1 v/v). Thus, this provided important evidence that at least some of the SL-porphyrin designs can self-assembled structures. The results of this work sets the stage for a deeper inquiry into how SL-porphyrin structure can be used to modulate the mechanism of cooperative self-assembly, the influence of the chirality introduced by sophorolipids and the nature of supramolecular structures formed.

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Experimental

Materials and Methods

Starting sophorolipids were synthesized by fermentation as previously reported by W. Gao, et al., [29] and the lactonic sophorolipid acetylated at the 6'- and/or 6''-positions (LSL[6'Ac,6''Ac]) was separated from crude SLs by flash column chromatography eluting with chloroform and methanol (10:1), as previously reported by Peng, Y. et al, [30]. All other chemicals and solvents were analytical grade and used as received without further purification unless otherwise noted. Synthesized SLs and SL-porphyrin conjugated compounds were purified by flash chromatography on an automated Biotage SP system (Charlotte, NC, U.S.A.) using Biotage silica Snap Columns (25, 50 and 100 g per packing) by gradient elution with $CHCl_3$ and CH_3OH mixtures. Thin layer chromatography (TLC) was performed on aluminum backed silica gel sheets purchased from Sigma–Aldrich (silica gel 60 matrix, 0.2mm thickness) using methanol/chloroform as eluent. A cerium–ammonium–molybdate solution was used to visualize eluted compounds. ^{1}H and (^{13}C), COSY and HMQC nuclear magnetic resonance (NMR) spectra were recorded in DMSO-d_6 on a Bruker spectrometer (Billerica, MA, U.S.A.) at 800 (201.193) or 600 (150.903) MHz respectively. The LC/MS used for analyses had an electrospray ionization mass spectrometer in positive mode. MALDI-TOF was used to analyze high molecular weight Compound **14 (**tetraconjugate). Melting point measurements were recorded on a Meltemp instrument.

Synthesis of sophorolipids and sophorolipid-porphyrin conjugates

Synthesis of $(OH)_7$SLOMe (sophorolipid methylester) (1). The method for synthesis of the non-acetylated sophorolipid methylester followed exactly a literature procedure by Bisht et al., [6a]. ^{1}H NMR (800 MHz, DMSO-d6): δ (ppm) 1.13 (3H, d, J = 6.1 Hz, CH3), 1.23-1.51 (22H, m,CH2), 1.96-1.99 (4H, m, 8-CH and 11-CH), 2.27 (2H, t, J = 7.4 Hz, 2-CH2), 2.97-3.0 (7H, m, sugar CH), 3.33-3.49 (3H, m, sugar CH), 3.57 (1H, s, OCH3), 3.61-3.67 (3H, m, sugar CH and 17-CH), 4.20 (1H, t, J = 5.7 Hz, -$CH_2O\underline{H}$), 4.30 (1H, d, J = 7.7 Hz, 1'-CH), 4.38(1H, J = 7.7 Hz, 1''-CH), 4.43 (1H, t, J = 5.7 Hz, -$CH_2O\underline{H}$), 4.87 (1H, d, J = 5.1 Hz, OH), 4.94 (1H, d, J = 4.7Hz, OH), 5.02 (1H, d, J = 5.1 Hz, OH), 5.20 (1H, d, J = 3.4 Hz, OH), 5.32 (2H, m, -CH_2=C$\underline{H}$), 5.50 (1H, d, J = 3.6 Hz, OH)

Synthesis of $(OH)_7SLN_3$ (non-acetylated 3-azido-1-propanamide sophorolipid) (2). To a 50 mL (24/40) round bottomed flask with a magnetic stirrer was added 2.4 g (3.77 mmol) of OH_7SLOMe (**1**) and 3.160 g (31.57 mmol, 8.4 equiv.) of 3-azido-1-propanamine. The contents of the reaction flask was heated neat at 80 ^{o}C under Ar while the reaction progress was monitoring by TLC using 20% MeOH in chloroform. After 18 h, TLC showed the consumption of starting material. Thereafter, the reaction flask was cooled to room temperature, ~ 2 mL of MeOH was added to dissolve the brown waxy reaction mixture that was then transferred (dropwise) into a flask containing 100 mL of diethyl ether with vigorous stirring. The contents of the resulting flask in which a precipitate formed was maintained at about 10^{o}C in a refrigerator overnight. The solid was separated by filtration and, to remove excess of 3-azido-1-propanamine, was washed several times on the filter paper with diethyl ether. Precipitated the solid two more times and the resultant product was dried overnight under vacuum and was 2.41g (91%) was obtained as a light yellow solid. (mp 115-120 ^{o}C); ^{1}H NMR (800 MHz, DMSO-d6): δ (ppm) 1.13 (3H, d, J = 6.1 Hz, CH_3), 1.21-1.33 (18H, m,CH_2), 1.44 – 1.48 (m, 2H, -CH_2), 1.61 – 1.64 (m, 2H, -CH_2-C$\underline{H}_2$-CH_2-N_3), 1.96-1.99 (4H, m, 8-CH and 11-CH), 2.03 (2H, t, J = 7.4 Hz, 2-CH2), 2.97-3.21 (9H, m, sugar CH, -C$\underline{H}_2$-CH_2-CH_2-N_3), 3.32-3.49 (5H, m, sugar CH, CH_2-CH_2-C$\underline{H}_2$-N_3), 3.61-3.67 (3H, m, sugar CH and 17-CH), 4.21 (1H, t, J = 5.7 Hz, -$CH_2O\underline{H}$), 4.30 (1H, d, J = 7.7 Hz, 1'-CH), 4.38(1H, J = 7.8 Hz, 1''-CH), 4.43 (1H, t, J = 5.7 Hz, -$CH_2O\underline{H}$), 4.88 (1H, d, J = 5.2 Hz, OH), 4.95 (1H, d, J = 4.4Hz, OH), 5.04 (1H, d, J = 5.0 Hz, OH), 5.21 (1H, d, J = 3.3 Hz, OH), 5.32 (2H, m, -

CH_2=CH), 5.51 (1H, d, *J* = 3.5 Hz, OH) and 7.80 (1H, t, *J* = 5.4 Hz, -CONH). ^{13}C NMR (201 MHz, DMSO-d_6): δ (ppm) 21.76, 25.03, 25.73, 27.07, 27.11, 28.94, 29.01, 29.11 (2C), 29.15, 29.59, 29.63, 29.66, 35.86, 36.19, 36.61, 48.89, 61.42, 61.49, 70.37, 70.41, 75.50, 76.34, 76.50, 76.68, 76.94, 77.46, 82.53, 101.52, 104.48, 130.07, 130.15 and 172.63. HRMS (ESI): m/z calcd for $C_{33}H_{61}N_4O_{12}$ $[M + H]^+$ 705.43, found 705.427.

Synthesis of $(OAc)_2SLN_3$ (diacetylated [6'- and 6''-positions] 3-azido-1-propanamide sophorolipid) (3). To an oven dried, 100 mL round bottomed flask (24/40) equipped with a magnetic stirrer was added 1.85 g (2.59 mmol) of OH_7SLN_3 (**2**), 25 mL of dry THF, 2.38 mL (25.9 mmol, 10.0 equiv.) of vinyl acetate and 0.6 g of the immobilized enzyme catalyst Novozyme-435. The reaction contents were stirred at 50 °C under Ar (g) for 4-5 days while monitoring by TLC (eluent: 20% MeOH in $CHCl_3$). Then, the enzyme was separated by filtration and THF was evaporated. The crude product was purified by flash column chromatography with gradient elution (MeOH and Chloroform) to get 1.65 g (80%) of product as a white solid. (mp 94-99 °C); ^{1}H NMR (800 MHz, DMSO-d6): δ (ppm) 1.10 (3H, d, *J* = 6.1 Hz, CH_3), 1.19 -1.31 (18H, m,CH_2), 1.44 – 1.49 (m, 2H, -CH_2), 1.61 – 1.64 (m, 2H, -CH_2-CH_2-CH_2-N_3), 1.96-1.99 (4H, m, 8-CH and 11-CH), 2.0 (s, 6H,-COCH3), 2.03 (2H, t, *J* = 8.0 Hz, 2-CH2), 2.99 -3.20 (7H, m, sugar CH, 17-CH), 3.32-3.39 (5H, m, sugar CH, CH_2-CH_2-CH_2-N_3), 3.59 -3.62 (1H, m, sugar CH), 3.99-4.05 (2H, m, 6'a-H and 6''a-H), 4.20-4.24 (2H, m, 6'b-H and 6''b-H), 4.35 (1H, d, *J* = 8.0 Hz, 1'-H), 4.40 (1H, d, *J* = 8.0 Hz, 1''-H), 5.08 (1H, d, *J* = 4.8 Hz, OH), 5.15 (1H, d, J = 5.6 Hz, OH), 5.29 (1H, d, J = 5.6 Hz, OH), 5.31-5.32 (2H, m, -CH_2=CH), 5.44 (1H, d, *J* = 3.2 Hz, OH), 5.60 (1H, d, *J* = 4.8 Hz, OH) and 7.80 (1H, t, *J* = 5.6 Hz, -CONH). ^{13}C NMR (201 MHz, DMSO-d_6): δ (ppm) 21.59, 22.85, 25.43, 26.19, 27.54, 29.40, 29.48, 29.57, 29.61, 30.00, 30.05, 30.10, 36.32, 36.65, 37.17, 49.35, 64.43, 64.75, 70.65, 70.73, 74.05, 74.73, 75.85, 76.75, 76.98, 77.07, 83.86, 101.77, 105.38, 130.55, 130.58, 171.14 (2C) and 173.08. HRMS (ESI): m/z calcd for $C_{37}H_{65}N_4O_{14}$ $[M + H]^+$ 789.45, found 789.449.

Synthesis of $(OAc)_7SLN_3$ (peracetylated 3-azido-1-propanamide sophorolipid) (4). To an oven dried, 100 mL round bottomed flask (24/40) equipped with a magnetic stirrer was added 2.0 g (2.83 mmol) of OH_7SLN_3 (**2**), 50 mL of anhydrous THF, 10 mL of acetic anhydride (excess) and 350 mg of DMAP (excess). Contents were stirred under Ar (g) at room temperature overnight and monitored by TLC (eluent: 0.5% MeOH in EtOAc, R_f 0.68). Then, THF was removed by rotoevaporation, the crude product was extracted into EtOAc, washed with aq.$NaHCO_3$ (3x10 mL) and then dried over anhydrous $NaSO_4$. The solvents were evaporated and the crude product was purified by flash column chromatography with gradient elution (MeOH and EtOAc) to give 2.2 g (78%) of product as thick transparent oil. ^{1}H NMR (800 MHz, DMSO-d_6): δ (ppm) 1.10 (3H, d, *J* = 6.2 Hz, CH_3), 1.20 -1.47 (22H, m, lipid chain CH2, -CH2-N3), 1.61 – 1.64 (2H, m, -CH_2-CH_2-CH_2-N_3), 1.89-2.06 (27H, m, 8-CH and 11-CH, -$COCH_2$-, 2-CH_2, -$COCH_3$),3.07 (2H, q, *J* = 6.6 Hz, -NH-CH_2-CH_2-CH_2N_3), 3.53 (1H, dd, *J* = 9.6, 8.0 Hz, sugar CH), 3.62 (1H, m, 17-CH), 3.90 (1H, m, 5'-H), 3.94 (1H, dd, *J* = 12.8, 2.4 Hz, 6'a-H), 3.99 (1H, dd, *J* = 12.0, 2.4 Hz, 6''a-H), 4.03 (1H, m, 5''-H), 4.14-4.17 (2H, m, 6'b-H and 6''b-H), 4.64 (1H, d, *J* = 7.2 Hz, 1'-H), 4.68 (1H, dd, *J* = 9.6, 8.0 Hz, sugar CH), 4.74 (1H, t, *J* = 9.6 Hz, sugar CH), 4.85-4.88 (2H, m, sugar CH and 1''-H), 5.146 (1H, t, *J* = 9.6 Hz, sugar CH), 5.27 (1H, t, *J* = 9.6 Hz, sugar CH), 5.30-5.36 (2H, m, 9-CH and 10-CH) and 7.80 (1H, t, *J* = 5.6 Hz, -CONH). ^{13}C NMR (201 MHz, DMSO-d_6): δ (ppm) 21.07, 21.13, 21.27, 21.32, 21.40, 21.42, 21.48, 22.34, 25.20, 26.30, 27.52, 27.62, 29.40, 29.50, 29.59, 29.61, 30.04, 30.09, 30.14, 30.27, 36.32, 36.64, 37.20, 49.36, 62.75, 62.84, 69.16, 69.43, 70.99, 71.08, 72.24, 73.29, 74.77, 77.71, 78.01, 100.18, 101.18, 130.43, 130.68, 169.94, 170.10, 170.32, 170.46, 170.70, 170.83, 170.89 and 173.06. HRMS (ESI): m/z calcd for $C_{47}H_{75}N_4O_{19}$ $[M + H]^+$ 999.50, found 999.502.

Synthesis of H_2-$(OAc)_2SLN_3$ – (diacetylated [6'- and 6''-positions], hydrogenated 3-azido-1-propanamide sophorolipid) (7). Into a 200 mL high-pressure glass vessel, 1.0 g (1.57 mmol) of SLOMe (**1**) was added, dissolved in 75 mL of methanol under N_2 (g) flow, and 0.1 g (10% w/w) of Pd/C was added portion wise (~10 min duration). The reaction vessel contents were stirred at 3 bar H_2 atmosphere overnight, Pd/C was removed by filtration and methanol was stripped by roto-evaporation to give 0.98 g (>98%) of hydrogenated Compound **5** as a white solid that was used without purification for conjugation of 3-azido-1-propanamide as described below. (mp 136-139 °C); ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) 1.13 (3H, d, *J* = 6.0 Hz, CH_3), 1.23-1.51 (28H, m,CH_2), 2.27 (2H, t, *J* = 7.8 Hz, 2-CH_2), 2.98-3.21 (7H, m, sugar CH), 3.33-3.50 (3H, m, sugar CH), 3.57 (1H, s, OCH3), 3.61-3.66 (3H, m, sugar CH and 17-CH), 4.20 (1H, t, *J* = 5.4 Hz, -CH_2OH), 4.30 (1H, d, J = 7.8 Hz, 1'-CH), 4.38(1H, *J* = 7.8 Hz, 1''-CH), 4.43 (1H, t, *J* = 5.4 Hz, -CH_2OH), 4.87 (1H, d, *J* = 4.8 Hz, OH), 4.94 (1H, d, *J* = 4.2 Hz, OH), 5.02 (1H, d, *J* = 4.8 Hz, OH), 5.19 (1H, d, *J* = 3.0 Hz, OH), 5.48 (1H, d, *J* = 3.6 Hz, OH). ^{13}C NMR (201 MHz, DMSO-d_6): δ (ppm) 21.28, 24.41, 24.58, 28.43, 28.65, 28.85, 28.97, 29.06 (6C), 29.30, 33.25, 36.15, 51.14, 60.94, 60.99, 69.89, 69.92, 75.00, 75.87, 76.01, 76.19, 76.45, 76.96, 82.01, 101.04, 103.97 and 173.36. HRMS (ESI): m/z calcd for $C_{31}H_{62}NO_{13}$ $[M + NH_4]^+$ 656.42, found 656.42.

Into a 25 mL round bottomed flask was added 0.75 g of Compound **5** (H_2-SLOMe) and 0.705 g (6.6 equiv) of 3-azido-1-propylamine. The reaction flask content was maintained at 80 °C under N_2 for 24 h after which it was cooled to room temperature. Then, the contents of the reaction flask was washed with diethyl ether several times to remove excess of 3-azido-1-propylamine and dried in vacuum overnight to give the

corresponding amide Compound **6** as a light yellow colored solid. Once again, this product was used as received for the subsequent diacetylation step described below.

Into a 50 mL round bottomed flask were added 0.5 g (.707 mmol) of **6** (H_2-SLN_3), vinyl acetate (0.651 mL, 10.0 equiv.), 300 mg of N-435 and 10 mL of dry THF. It is noteworthy that H_2-SLN_3 was insoluble in THF. Nevertheless, the reaction mixture was maintained at 50 °C under N_2 for 4 days. The reaction progress was monitored by TLC (eluent: 20% MeOH in chloroform). Thereafter, the reaction mixture was cooled to room temperature and the immobilized enzyme along with unreacted H_2-SLN_3 was removed by filtration. The crude (dark brown) product was purified by flash column chromatography by gradient elution with MeOH/chloroform and the solvent was removed to give 260 mg (46% from step 2) of product that appears as a transparent solid. (mp 96-102 °C); ^{1}H NMR (800 MHz, DMSO-d^6): δ (ppm) 1.10 (3H, d, *J* = 6.2 Hz, CH_3), 1.18 -1.48 (30H, m, lipid chain CH_2, -CH_2-N_3), 1.61 – 1.64 (2H, m, -CH_2-CH_2-CH_2-N_3), 1.99-2.04 (8H, m, 2-CH_2, -$COCH_3$), 2.99-3.20 (7H, m, sugar CH, -CONH-CH_2-CH_2-CH_2N_3), 3.33-3.40 (3H, m, sugar CH), 3.60-3.62 (1H, m, 17-CH), 3.99 (1H, dd, *J* = 12.0, 5.6 Hz, 6'a-H), 4.04 (1H, dd, *J* = 12.0, 7.2 Hz, 6''a-H), 4.19-4.24 (2H, m, 6'b-H and 6''b-H), 4.35 (1H, d, *J* = 8.0 Hz, 1'-H), 4.39 (1H, d, *J* = 7.2 Hz, 1''-H), 5.08 (1H, d, *J* = 9.6 Hz, sugar CH), 5.15 (1H, d, *J* = 5.6 Hz, sugar CH), 5.29 (1H, d, *J* = 5.6 Hz, sugar CH), 5.44 (1H, d, *J* = 2.4 Hz, sugar CH), 5.61 (1H, d, *J* = 3.2 Hz, sugar CH) and 7.80 (1H, t, *J* = 5.6 Hz, -CONH). ^{13}C NMR (201 MHz, DMSO-d_6): δ (ppm) 21.59, 22.18, 25.46, 26.19, 29.40, 29.57, 29.70, 29.89, 30.01(7C), 30.16, 31.35, 36.32, 36.64, 37.17, 49.35, 64.43, 64.73, 70.65, 70.74, 74.04, 74.73, 75.85, 76.75, 76.97, 77.07, 83.84, 101.76, 105.38, 171.14 (2C) and 173.08. HRMS (ESI): m/z calcd for $C_{37}H_{67}N_4O_{14}$ $[M + H]^+$ 791.47, found 791.47.and $C_{37}H_{66}N_4O_{14}Na$ $[M + Na]^+$ 814.45.

General 'click reaction conditions' for 'sophorolipid-porphyrin' conjugation (example given is the synthesis of Compound 11, OAc_2SLN_3-Por-OAc_2SLN_3). To a 25 mL round bottomed flask (24/40) equipped with a magnetic stirrer was added 51.3 mg (0.065 mmol, 2.11 equiv. (>1 equiv. per alkyne group)) of Compound **3** ($[OAc]_2SLN_3$) dissolved in 3.0 mL of THF followed by 25 mg of Compound **8** ('dialkynyl-Zn-Porphyrin', 0.0308 mmol, 1.0 equiv.) and 3.0 mL of D.I. water. Subsequently, 18.30 mg (0.0924 mmol, 3.0 equiv.) of sodium ascorbate and 15.38 mg (0.0616 mmol, 2.0 equiv.) of $CuSO_4 \bullet 5H_2O$ were also added. The reflux condenser was attached and the contents of the reaction flask was maintained at 60 °C under Ar (g) for about 8-12 h. TLC analysis with 20% MeOH in chloroform showed complete conversion (R_f 0.48 for diconjugated

compound). Then, THF was removed by roto-evaporation to give the crude product. Water (20 or 50 mL varying size of centrifuge tube) was then added to the crude product that was transferred into a centrifuge tube. The contents of the tube were centrifuged 3 times while decanting water each time and replacing it with fresh water. Residual water was removed by lyophilization for 24 h and the resulting crude product was passed through a flash column using gradient elution (MeOH and chloroform) to give a thick, purple, waxy compound that was further dried overnight in-vacuo (40 ^{o}C) giving a dark purple-red colored compound (60.41 mg, 82%). (mp 140-151 ^{o}C); ^{1}H NMR (800 MHz, DMSO-d6): δ (ppm) 1.08 (6H, d, J = 5.6 Hz, CH_3), 1.21-1.53 (58H, m, CH_2 of lipid chain), 1.70 (12H, s, Ar-CH_3), 1.94-2.00 (20H, m, COC$\underline{H}_3$ and –$\underline{CH}_2$-CH=CH-), 2.09-2.12 (8H, m, -$\underline{CH}_2$-CO- and –CH_2-$\underline{CH}_2$-CH_2NHCO-), 2.57 (6H, s, -Ar-CH_3), 3.00-3.02 (2H, m, sugar CH), 3.06-3.20 (16H, m, sugar CH and –CONH-C$\underline{H}_2$-), 3.34-3.60 (10H, m, sugar CH and 17-CH), 3.99 (2H, dd, J = 12. 0, 6.4 Hz, 6'a-H), 4.03 (2H, dd, J = 12.0, 7.2 Hz, 6''a-H), 4.20-4.23 (4H, m, 6'b and 6''b-H), 4.35 (4H, m, 1'C-H and sugar CH), 4.39 (2H, d, J = 8.0 Hz, 1''C-H), 4.529 (4H, t, J = 6.4 Hz, -C$\underline{H}_2$-N_3-), 5.08 (2H, d, J = 4.8 Hz, OH), 5.15 (2H, d, J = 5.6 Hz, OH), 5.28 (2H, d, J = 5.6 Hz, OH), 5.30-5.33 (4H, m, -C$\underline{H}$=C$\underline{H}$-), 5.43 (2H, d, J = 4.0 Hz, OH), 5.59 (2H, d, J = 4.0 Hz, OH), 7.34 (4h, S, Ph-H), 7.94 (24, t, J = 5.6 Hz, -CONH), 8.23 (4H, d, J = 8.0 Hz, Ph-H), 8.26 (4H, d, J = 8.0 Hz, Ph-H), 8.58 (4H, d, J = 4.0 Hz, pyrrole-H), 8.79 (4H, d, J = 4.8 Hz, pyrrole-H) and 8.85 (2H, s, triazole-CH). ^{13}C NMR (201 MHz, DMSO-d6): δ (ppm) 19.49, 21.59(2C), 21.98, 22.18, 22.30, 25.24, 26.24, 27.55, 29.52, 29.61, 29.66, 29.99, 30.09, 30.87, 36.40, 36.73, 37.16, 48.56, 56.95, 64.42, 64.74, 70.66, 70.73, 74.05, 74.73, 75.84, 76.76, 76.98, 77.06, 83.86, 101.76, 105.38, 119.03, 120.134, 122.87(2C),124.27, 128.48, 130.55, 130.84, 131.09, 132.84, 135.65, 137.80, 139.30, 140.07, 143.07, 147.22, 149.94, 150.07, 171.13(2C) and 173.29. HRMS (ESI): m/z calcd for $C_{128}H_{169}N_{12}O_{28}Zn$ $[M + H]^{2+}$ 1193.575, found 1193.5818.

Synthesis of Compound 10 ([OH]$_7$SLN$_3$-Por-[OH]$_7$SLN$_3$). The protocol followed exactly that given above for Compound **11** except that, in place of Compound **3** OAc_2SLN_3, Compound **2** $[OH]_7SLN_3$ (0.154 mmol, 2.5 eq.) and compound **8** (50 mg, 0.617 mmol) was used. The isolated product was 106.86 mg, 78%. (mp 170-181 ^{o}C); ^{1}H NMR (800 MHz, DMSO-d6): δ (ppm) 1.11 (6H, d, J = 6.2 Hz, CH_3), 1.14-1.53 (50H, m, lipid chain CH_2, -C$\underline{H}_2$-N3)1.10 (3H, d, J = 6.2 Hz, CH_3), 1.79 (12H, S, CH_3), 1.95-2.11 (16H, m, -C$\underline{H}_2$CONH-, -NCH_2-$\underline{CH}_2$-CH_2-N_3, -C$\underline{H}_2$-CH=CH-), 2.58 (6H, S, CH3), 2.98-3.65 (m30H, m, 2'-5' and 2''-5''H, 6'ab and 6''ab, -NH-C$\underline{H}_2$-CH_2-CH_2-N_3), 4.23 (2H, t, J = 5.6 Hz, 6'-OH), 4.29 (2H, d, J = 7.2 Hz, 1'-H), 4.37 (2H, d, J = 8.0 Hz, 1''-H), 4.44 (2H, t, J = 5.6 Hz, 6''-OH), 4.53 (4H, t, J = 6.4 Hz, -C$\underline{H}_2$$N_3$), 4.90 (2H, br s, OH), 4.99 (2H, br s, OH), 5.09 (2H, br s, OH), 5.25 (2H, br s, OH), 5.31(4H, m, -C$\underline{H}$=C$\underline{H}$-), 5.58 (2H, br s, OH), 7.31 (4H, s, Ph-H), 7.95 (2H, t, J = 5.6 Hz, -CONH), 8.23 (4H, d, J = 7.2 Hz, Ph-H), 8.27 (4H, d, J = 8.0 Hz, Ph-H), 8.58 (4H, d, J = 4.0 Hz, pyrrole-H), 8.79 (4H, d, J = 4.8 Hz, pyrrole-H) and 8.85 (2H, s, triazole-H). ^{13}C NMR (201 MHz, DMSO-d6): δ (ppm) 21.97, 22.22, 22.36, 25.48, 26.24, 27.56, 29.52, 29.59, 29.65(2C), 30.08(3C), 30.87, 36.39, 36.73, 37.06, 48.56, 61.88, 61.94, 70.82, 70.86, 75.98, 76.80, 76.95, 77.13, 77.40, 77.92, 83.02, 101.99, 104.97, 119.03, 120.13, 122.87 (2C), 124.27, 128.48, 130.53, 130.60, 130.85, 131.09, 132.85, 135.65, 137.79, 139.31, 140.08, 143.08, 147.22, 149.94, 150.07 and 173.30. HRMS (ESI): m/z calcd for $C_{120}H_{161}N_{12}O_{24}Zn$ $[M + H]^{+}$ 2218.10, found 2218.0999.

Synthesis of Compound 12 ([OAc]$_7$SLN$_3$-Por-[OAc]$_7$SLN$_3$). The protocol followed exactly the method described above for Compound **11** except that, in place of Compound **3** OAc_2SLN_3, Compound **4** OAc_7SLN_3 (2.25 equiv.) was used. The isolated product was 69.33 mg, 80% (mp 116-124 ^{o}C); ^{1}H NMR (800 MHz, DMSO-d6): δ (ppm) 1.07 (6H, d, J = 6.4 Hz, CH_3), 1.27-1.52 (56H, m, CH_2 of lipid), 1.78 (12H, s, Ar-CH_3),1.94-2.00 (66H, m, COC$\underline{H}_3$ and –$\underline{CH}_2$-CH=CH-, -$\underline{CH}_2$-CO- and –CH_2-$\underline{CH}_2$-CH_2NHCO), 2.58 (6H, m, Ar-CH_3), 3.17 (4H, q, J = 6.4 Hz, –CONH-C$\underline{H}_2$-), 3.52 (2H, dd, J = 9.6 and 8.0 Hz, sugar CH), 3.58-3.62 (2H, m, 17-CH), 3.88-3.9 (2H, m, sugar CH), 3.93 (2H, dd, J = 12.0 and 1.6 Hz, 6'a-H), 3.99 (2H, dd, J = 12.0 and 2.4 Hz, 6''a-H), 4.02-4.04 (2H, m, sugar CH), 4.13-4.16 (4H, m,6'b-H and 6''b-H), 4.53 (4H, t, J = 8.8 Hz, -C$\underline{H}_2$-N_3), 4.63 (2H, d, J = 7.2 Hz, 1'-H), 4.68 (2H, dd, J = 9.6 and 8.0 Hz, sugar CH), 4.73 (2H, t, J = 9.6 Hz, sugar CH), 4.85-4.87 (m, 4H, sugar CH and 1''-H), 5.13 (2H, t, J = 10.4 Hz, sugar CH), 5.26 (2H, t, J = 8.8 Hz, sugar CH), 5.29-5.35 (4H, m, -C$\underline{H}$=C$\underline{H}$-), 7.31 (4H, s, Ar-H), 7.94 (2H, t, J = 5.6 Hz, -CON$\underline{H}$-), 8.22 (4H, d, J = 8.0 Hz, Ph-H), 8.27 (4H, d, J = 8.0 Hz, Ph-H), 8.58 (4H, d, J = 4.8 Hz, pyrrole-H) 8.79 (4H, d, J = 4.8 Hz, pyrrole-H) and 8.85 (2H, s, triazole-CH). ^{13}C NMR (201 MHz, DMSO-d6): δ (ppm) 21.06, 21.13, 21.26, 21.31, 21.40(2C), 21.46, 21.97, 22.32, 22.35, 25.18, 26.23, 27.53, 27.62, 29.54, 29.67, 30.03, 30.12, 30.26, 30.87, 36.39, 36.72, 37.18, 48.55, 62.74, 62.82, 69.15, 69.41, 70.98, 71.07, 72.23, 73.28, 74.76, 77.69, 78.01, 80.13(2C), 100.12, 101.16, 119.03, 120.13, 122.86(2C), 124.27, 128.48, 130.43, 130.67, 130.84, 131.10, 132.86, 135.65, 137.80, 139.30, 140.07, 143.07, 147.22, 149.94, 150.07, 169.94, 170.10, 170.31, 170.46, 170.69, 170.82, 170.87 and 173.27. HRMS (ESI): m/z calcd for $C_{149}H_{188}N_{12}O_{38}Zn$ $[M + H]^{+}$ 2806.24, found 2806.2403.

Synthesis of Compound 13 (H_2-$(OAc)_2(OH)_5SLN_3)_2$(Zn)Porphyrin. The protocol followed exactly the method described above for Compound **11** except that, in place of Compound **3** OAc_2SLN_3, Compound **7** H_2-OAc_2SLN_3 (2.5 equiv.) was used. The isolated product was 47.9 mg, 65% (mp 130-140 ^{o}C); ^{1}H NMR (800 MHz, DMSO-d6): δ (ppm) 1.07 (6H, d, J = 5.6 Hz, CH3), 1.18-1.52 (58H, m, lipid CH_2), 1.78 (12H, s, Ar-CH_3), 1.98 (12H, s, -$COCH_3$), 2.09-2.12 (8H, m, -COCH2- and –NHCO-CH_2-$\underline{CH}_2$-CH_2-N_3-), 2.57 (6H, s, Ar-CH_3), 2.99 (2H, m, sugar CH), 3.05-3.12 (5H, m, sugar CH), 3.16-3.15 (9H, m, sugar CH and -C$\underline{H}_2$-NHCO-), 3.53-3.39 (4H, m, sugar CH), 3.57-3.60 (2H, m, 17CH), 3.99 (2H, dd, J = 11.2 and 5.6 Hz, 6'a-H), 4.03 (2H, dd, J = 12.0 and 5.6 Hz, 6''a-H), 4.19-4.23(4H, m, 6'b-H and 6''b-H), 4.33 (2H, d, J = 8.0 Hz, 1'-H), 4.38 (2H, d, J = 8.0 Hz, 1''-H), 4.52 (4H, t, J = 7.2 Hz, -C$\underline{H}_2$-N_3), 5.07 (2H, d, J = 4.8 Hz, sugar CH), 5.14 (2H, d, J = 5.6 Hz, sugar CH), 5.27 (2H, d, J = 5.6 Hz, sugar CH), 5.42 (2H, d, J = 3.2 Hz, sugar CH), 5.59 (2H, d, J = 4.0 Hz, sugar CH), 7.31 (4H, s, Ar-H), 7.95 (2H, t, J = 5.6 Hz, -CON$\underline{H}$), 8.22 (4H, d, J = 8.0 Hz, Ph-H), 8.26 (4H, d, J =8.0 Hz, Ph-H), 8.58 (4H, d, J = 4.0 Hz, pyrrole-H), 8.79 (4H, d, J = 4.8 Hz, pyrrole-H) and 8.85 (2H, s, triazole CH). ^{13}C NMR (201 MHz, DMSO-d6): δ (ppm) 21.57, 21.98, 22.16, 22.35, 25.44, 26.24, 29.67, 29.77, 29.94, 30.01(7C), 30.13, 30.86, 36.40, 36.73, 37.15, 48.56, 64.42, 64.73, 70.65, 70.73, 74.03, 74.72, 75.84, 76.74, 76.97, 77.05, 83.83, 101.74, 105.36, 119.03, 120.13, 122.83 (2C), 124.26, 128.48, 130.84, 131.09, 132.83, 135.65, 137.79, 139.30, 140.07, 143.06, 147.21, 149.94, 150.07, 171.13(2C) and173.29. HRMS (ESI): m/z calcd for $C_{128}H_{173}N_{12}O_{28}Zn$ $[M + H]^{+}$ 2390.18, found $[M + H]^{+}$ 2390.19.

Synthesis of tetra-conjugated sophorolipid-porphyrin (14). This compound was prepared according to the general click reaction conditions described above for Compound **11** with the following modification. The minimum relative molar equivalents of Compound **3** ($[OAc]_2SLN_3$) to Compound **9** (tetraalkynyl-Zn-porphyrin, 25 mg, 0.0323 mmol) is 5.0:1.0. The product isolated was 76.14 mg, 60%. (mp 171-180 ^{o}C); ^{1}H NMR (800 MHz, DMSO-d6): δ (ppm) 1.11 (12H, m,CH_3), 1.20 -1.57 (88H, m, lipid CH_2 and -CH_2-C$\underline{H}_2$-CH_2-N_3) , 2.01-2.02 (32H, m, -$COCH_3$ and –C$\underline{H}_2$CONH-), 2.13-2.15 (16H, m, -C=C-C$\underline{H}_2$-), 3.05-3.21 (32H, sugar CH and –CONH-C$\underline{H}_2$-), 3.61-3.63 (4H, m, -CH), 4.03-4.08 (8H, m, 6'a and 6''a CH), 4.23-4.26 (8H, m, 6'b and 6''b CH), 4.36-4.44 (8H, m, 1' and 1'' CH), 4.57 (8H, t, J = 6.4 Hz, -CH_2-CH_2-C$\underline{H}_2$-N_3), 5.12 (4H, t, J = 4.8 Hz, sugar CH), 5.19 (4H, d, J = 5.6 Hz, sugar CH), 5.32-5.63 (20H, sugar CH and –C$\underline{H}$=C$\underline{H}$-), 7.99 (4H, m, -CONH-), 8.30 (16H, br s, pyrrole CH and Ar-H), 8.89 (4H, s, triazole CH) and 8.92 (8H, s, Ar-H). ^{13}C NMR (201 MHz, DMSO-d6): δ (ppm) 21.56, 22.16, 25.26, 25.41, 25.75, 25.81, 26.23, 27.54, 29.25, 29.43, 29.52, 29.65, 29.87, 30.02, 30.09, 30.91, 32.66, 33.25, 36.40, 36.72, 37.14, 48.54, 64.41, 64.72, 70.65, 70.72, 74.03, 74.72, 75.84, 76.75, 76.97, 77.06, 83.81, 85.87, 101.74, 105.36, 120.98, 122.89, 124.34, 130.57, 130.84, 132.64, 134.57, 135.71, 143.13, 147.21, 150.23, 171.13 AND 173.31. MALDI-TOF: m/z calcd for $C_{200}H_{285}N_{20}O_{56}Zn$ $[M+Na]^{+}$ 3948.92, found 3948.4.

Synthesis of mono-conjugated sophorolipid-porphyrin (15). This compound was prepared according to the general click reaction conditions described above for

Compound **11** with the following modification. The relative molar equivalents of Compound **2** ($[OH]_7SLN_3$, 25.0 mg, 0.0354 mmol) to Compound **8** (dialkynyl) is 0.5:1.0. The isolated product was 25.79 mg, 48%. (mp 175-183 ^oC); ^1H NMR (800 MHz, DMSO-d6): δ (ppm) 1.10 (3H, d, J = 6.4 Hz, CH_3), 1.22-1.52 (20H, m,CH_2), 1.78 (s, 12H, $ArCH_3$), 1.96-1.99 (4H, m, 8-CH and 11-CH), 2.08-2.11 (4H, m,-CH_2CONH-, -CH_2-CH_2-CH_2-N_3), 2.57 (6H, s, Ar-CH_3), 2.98-3.22 (11H, m, alkyne-CH, sugar CH, -CH_2-CH_2-CH_2-N_3), 3.39-3.50 (2H, m, sugar CH), 3.61-3.65 (3H, m, sugar CH and 17-CH), 4.21 (1H, t, J = 5.7 Hz, -CH_2OH), 4.30 (1H, d, J = 7.7 Hz, 1′-CH), 4.38(1H, d, J = 7.8 Hz, 1′′-CH), 4.43 (1H, t, J = 5.7 Hz, -CH_2OH), 4.53 (2H, t, J = 6.4 Hz, -CH_2-CH_2-CH_2-N_3), 4.88 (1H, d, J = 5.2 Hz, OH), 4.94 (1H, d, J = 4.4Hz, OH), 5.02 (1H, d, J = 5.0 Hz, OH), 5.20 (1H, br s, OH), 5.31 (2H, m, -CH_2=CH), 5.49 (1H, br s, OH), 7.31 (4H, s, Ar-H), 7.87 (2H, d, J = 7.2 Hz, Ar-H), 7.95 (1H, t, J = 4.8 Hz, -CONH), 8.20 (2H, d, J = 7.2 Hz, Ar-H), 8.23 (2H, d, J = 7.2 Hz, Ar-H), 8.26 (2H, d, J = 8.0 Hz, Ar-H), 8.58 (4H, m, Pyrrole-CH), 8.71 (2H, d, J = 4.0 Hz, Pyrrole-CH), 8.79 (2H, d, J = 4.0 Hz, Pyrrole-CH), 8.85 and (1H, s, triazole CH). ^{13}C NMR (201 MHz, DMSO-d6): δ (ppm) 21.97, 22.21, 22.35, 25.47, 26.23, 27.55, 29.51, 29.59, 29.65, 30.08, 30.86, 36.39, 36.73, 37.02, 48.55, 48.55, 61.87, 61.94, 70.85, 75.95, 76.79, 76.96, 77.14, 77.39, 77.91, 82.98, 101.98, 104.93, 119.13, 124.28, 128.50, 130.53, 130.60, 130.85, 131.13, 131.24, 132.68, 132.91, 135.65, 137.82, 139.30, 140.02, 147.21, 149.76, 149.93, 150.01, 150.08 and 173.30. HRMS (ESI): m/z calcd for $C_{87}H_{101}N_8O_{12}Zn$ $[M + H]^+$, 1513.68, found 1513.68.

Solution preparation and UV-vis measurements

The solvents selected for this study are 1,4-dioxane, DMSO, methanol and their corresponding 1:1 v/v mixtures of each with D.I. water. Solutions were prepared by first dissolving sophorolipid-porphyrin conjugates in pure 1,4-dioxane, DMSO or methanol followed by sonication for 10 minutes at room temperature. In the case of mixed solvent systems, water was added after sonication to further dilute the solution to the target concentration. Thereafter, mixed solvent solutions were sonicated again for 10 minutes at room temperature. Afterwards, the solutions were removed from the sonicating bath and allowed to equilibrate for a minimum of 2 hours at room temperature. UV-vis absorption spectra were recorded at room temperature using a Varian Cary 300 UV-vis spectrophotometer operating in double beam mode over the spectral range of 350-800 nm at a scan rate of 300 nm/min and in increments of 1 or 0.5 nm. Solution spectra of sophorolipid-porphyrin conjugates were acquired in a clean quartz cuvette (1cm optical path length) while a solution of the same solvent and cuvette was used for the reference sample.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

1. (a) F. J. M. Hoeben., P. L. Jonkheijm., E. W. Meijer and A. P. H. J. Schenning, *Chem. Rev.,* 2005**, 105**, 1491; (b) Lehn, J.M, *Pure Appl. Chem.,* 1978*,* **50**, 871; (c) J.W. Steed and J.L. Atwood, Supramolecular Chemistry., John Wiley and Sons Ltd.: London, UK. 2009.

2. (a) G. M. Whitesides and M. Boncheva, *Proc. Natl. Acad. Sci., U.S.A*. 2002, **99**, 4769; (b) G. M. Whitesides and B. Grzybowski, *Science*., 2002, **295**, 2418. (c) Y. He, Y. Yamamoto, W. Jin, T. Fukushima, A. Sacki, S. Seki, N. Ishii and T. Aida, *Adv. Mater.,* 2010*,* 20, 829. (d) Y. Huang, Y. Yan, B. M. Smarsly, Z. Wei and C. F. J. Faul, *J. Mater. Chem.,* 2009*,* **19**, 2356; (e) A. Jain and S. J. George, *Mater. Today.,* 2015*,* **18***,* 206; (f) S. Ogi, V. Stepanenko, J. Thein and F. Würthner, *J. Am. Chem. Soc.* 2016*,* **138**, 670.

3. Edts: H. Langeveld, J. Sanders and M. Meeusen, The Biobased Economy: Biofuels, Materials and Chemicals in the Post-oil Era. Earthscan Ltd, UK. 2010.

4. (a) H.-J. Asmer, S. Lang, F. Wagner and V. Wray, *J. Am. Oil Chem. Soc*. 1988, **65**, 1460; (b) U. Rau, S. Hammen, R. Heckmann., V. Wray and S. Lang, *Ind. Crops Prod*. 2001, **13**, 85; (c) A. P. Tulloch, A. Hill and J. F. T. Spencer, *Can. J. Chem.,* 1968*,* **46**, 3337.

5. (a) S. Q. Zhou, C. Xu, J. Wang, W. Gao, R. Akhverdiyeva, V. Shah and R. Gross, *Langmuir.,* 2004*,* **20**, 7926; (b) N. Baccile, F. Babonneau, J. Jestin, G. Pehau-Arnaudet and I. Van-Bogaert. Unusual, pH-Induced, Self-Assembly of Sophorolipid Biosurfactants. *ACS Nano.* **2012***, 6*, 4763–4776; (c) A. Koh, K. Todd, E. Sherbourne and R. A. Gross, *Langmuir.,* 2017*,* **33**, 5760.

6. (a) K. S. Bisht, R. A. Gross and D. L. Kaplan, *J. Org. Chem.* 1999, **64***,* 780; (b) S. K. Singh, A. P. Felse, A. Nunez, T. A. Foglia and R. A. Gross, *J. Org. Chem.* 2003*,* **68**, 5466.

7. Y. Peng, F. Totsingan, M. A. R. Meier, M. Steinmann, F. Wurm, A. Koh and R. A. Gross, *Eur. J. Lipid Sci. Technol.,* 2015*,* **117**, 217.

8. Edited by K. M. Kadish, K. M. Smith and R. Guilard, *Handbook of porphyrin science with applications to chemistry, physics, materials science, engineering, biology and medicine*., Singapore: World Scientific. 2012.

9. S. Ganapathy, S. Sengupta, P. K. Wawrzyniak, V. Huber, F. Buda, U. Baumeister, F. Werthner and H. J. M. de Groot, *Proc. Natl. Acad. Sci., USA,* 2009*,* **106**, 11472.

10. I. Beletskaya, V. S. Tyurin, A. Y. Tsivadze, R. Guilard and C. Stern, *Chem. Rev.,* 2009, **109**, 1659.

11. (a) G. Steinberg-Yfrach, P. A. Liddell, S.-C. Hung, A. L. Moore, D. Gust and T. A. Moore, *Nature*., 1997, **385**, 239; (b) A. Satake and Y. Kobuke, *Org. Biomol. Chem*., 2007, **5**, 1679.

12. (a) L.-L. Li and E. W.-G, Diau, *Chem. Soc. Rev.,* 2013*,* **42**, 291; (b) S. Mathew, A. Yella, P. Gao, R. Humphry-Baker, B. F. E. Curchod, N. Ashari-Astani, I. Tavernelli, U. Rothlisberger, Md. K. Nazeeruddin and M. Gratzel, *Nature Chemistry.,* 2014*,* **6**, 242.

13. (a) G. S. Kottas, L. I. Clarke, D. Horinek and J. Michl, *Chem. Rev*., 2005*,* **105**, 1281; (b) K. P. Ghiggino, J. A. Hutchison, S. J. Langford, M. J. Latter, M. A. P. Lee, P. R. Lowenstern, C. Scholes, M. Takezaki and B. E. Wilman, *Adv. Funct. Mater.,* **2007***, 17*, 805-813.

14. (a) B. Meunier, *Chem. Rev.* 1992*,* **92**, 1411; (b) P. Thordarson, E. J. A. Bijsterveld, A. E. Rowan and R. J. M. Nolte, *Nature*. 2003, **424**, 915.

15. (a) R. Bonnett, *Chem. Soc. Rev*., 1995*,* **24**, 19; (b) A. Naik, R. Rubbiani, G. Gasser and B. Spingler, *Angew. Chem. Int. Ed.,* 2014*,* **53**, 6938.

16. (a) P. Rothemund, *J. Am. Chem. Soc.,* 1936*,* **58**, 625; (b) J. S. Lindsey, I. C. Schreiman, H. C. Hsu, P. C. Kearney and A. M. Marguerettaz, *J. Org. Chem.* 1987, **52**, 827; (c) M. O. Senge, *Chem. Commun.,* 2011*,* **47**, 1943.

17. J. A. Carr and K. S. Bisht, *Tetharedron*., 2003, **59**, 7713.

18. Y. Shi and J. G. Pierce, *Org. Lett.,* 2015*,* **17**, 968.

19. S. K. Singh, A. P. Felse, A. Nunez, T. A. Foglia and R. A. Gross, *J. Org. Chem.,* 2003*,* **68**, 5466.

20. (a) J. Li, A. Ambroise, S. I. Yang, J. R. Diers, J. Seth, C. R. Wack, D. F. Bocian, D. Holten and J. S. Lindsey, *J. Am. Chem. Soc.,* 1999*,* **121***,* 8927; (b) B. J. Littler, Y. Ciringh and J. S. Lindsey, *J. Org. Chem.* 1999*,* **64***,* 2864; (c) L. Yu and J. S. Lindsey, *J. Org. Chem.,* 2001**, 66***,* 7402.

21. B. Vonhoeren, S. Dalgleish, L. Hu, M. M. Matsushita, K. Awaga and B. J. Ravoo, *ACS Appl. Mater. Interfaces.,* 2015*,* **7**, 7049.

22. (a) V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem. Int. Ed.,* 2002*,* **41**, 2596; (b) F. Himo, T. Lovell, R. Hilgraf, V. V. Rostovtsev, L. Noodleman, K. B. Sharpless and V. V. Fokin, *J. Am. Chem. Soc.* 2005*,* **127**, 210; (c) M. Meldal and C. W. Tornøe, *Chem. Rev*., 2008, **108**, 2952.

23. Y. M. Chabre, P. P. Brisebois, L. Abbassi, S. C. Kerr, J. V. Fahy, I. Marcotte and R. Roy, *J. Org. Chem.,* 2011*,* **76**, 724.

24. G. H. Barnett, M. F. Hudson and K. M. Smith, *J. Chem. Soc. Perkin Trans. I.*, 1975, 1401.

25. (a) M. Kasha, H. R. Rawls and M. A. El-Bayoumi, *Pure and Applied Chemistry.,* 1965, **11**, 371; (b) C. A. Hunter, J. K.M. Sanders and A. J. Stone, *Chemical Physics.* 1989, **133**, 395.

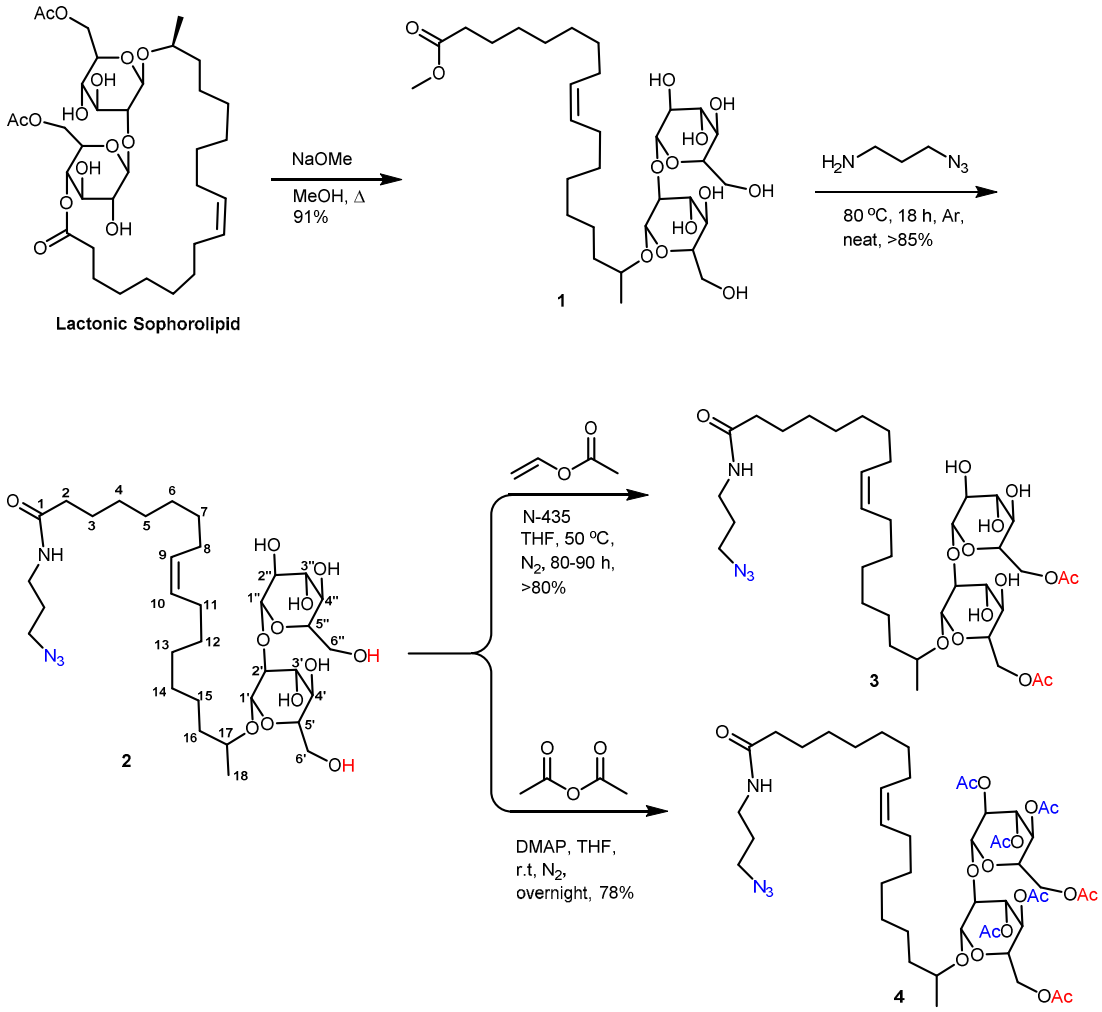
26. A. Eisfeld and J. S. Briggs, *Chemical Physics.,* 2006, **324**, 376.

27. B. Heyne, *Photochemical & Photobiological Sciences.,* 2016, **15**, 1103.

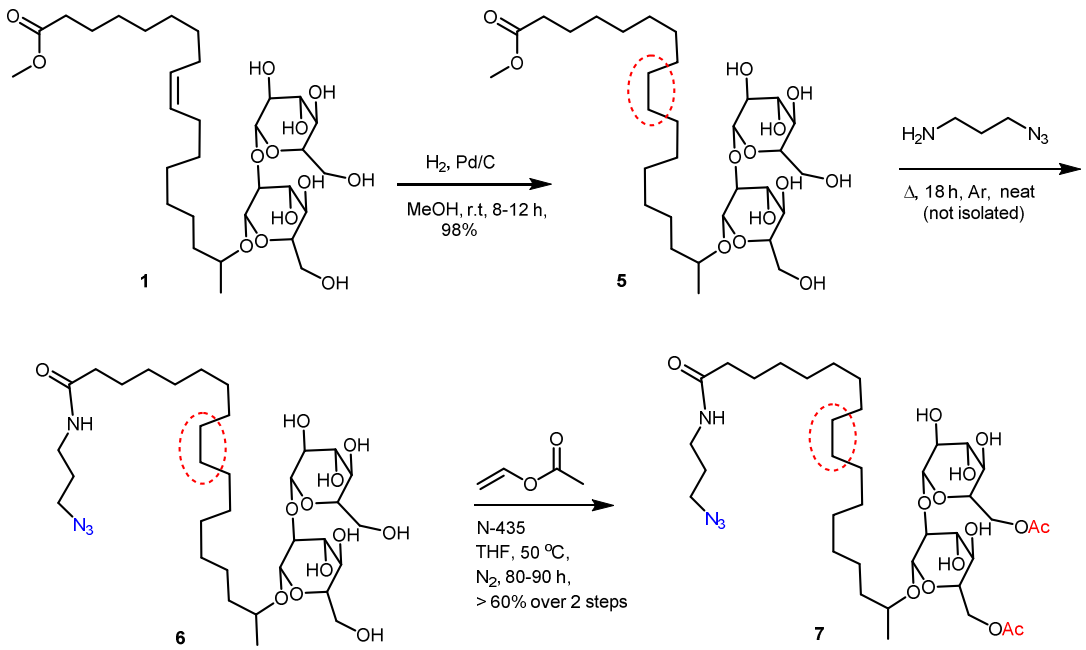
28. F. J. M. Hoeben, P. Jonkheijm, E. W. Meijer and A. P. H. J. Schenning, *Chemical Reviews.,* 2005, **105**, 1491.

29. W. Gao, R. Hagver, V. Shah, W. Xie, R. A. Gross, M. F. Ilker, C. Bell, K. A. Burke and E. B. Coughlin, *Macromolecules.,* 2007, **40**, 145.

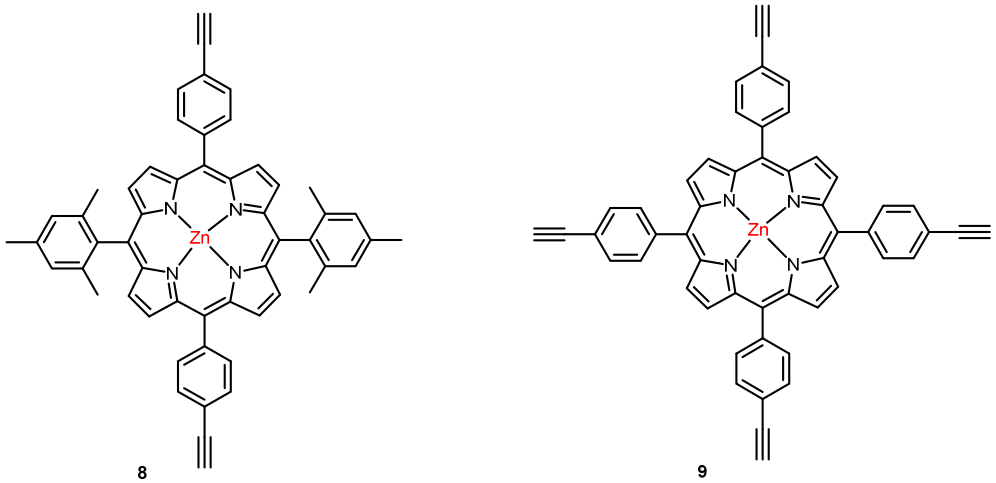
30. Y. Peng, J. Decatur, M. A. R. Meier, R. A. Gross, *Macromolecules*. 2013, **46**, 3293.



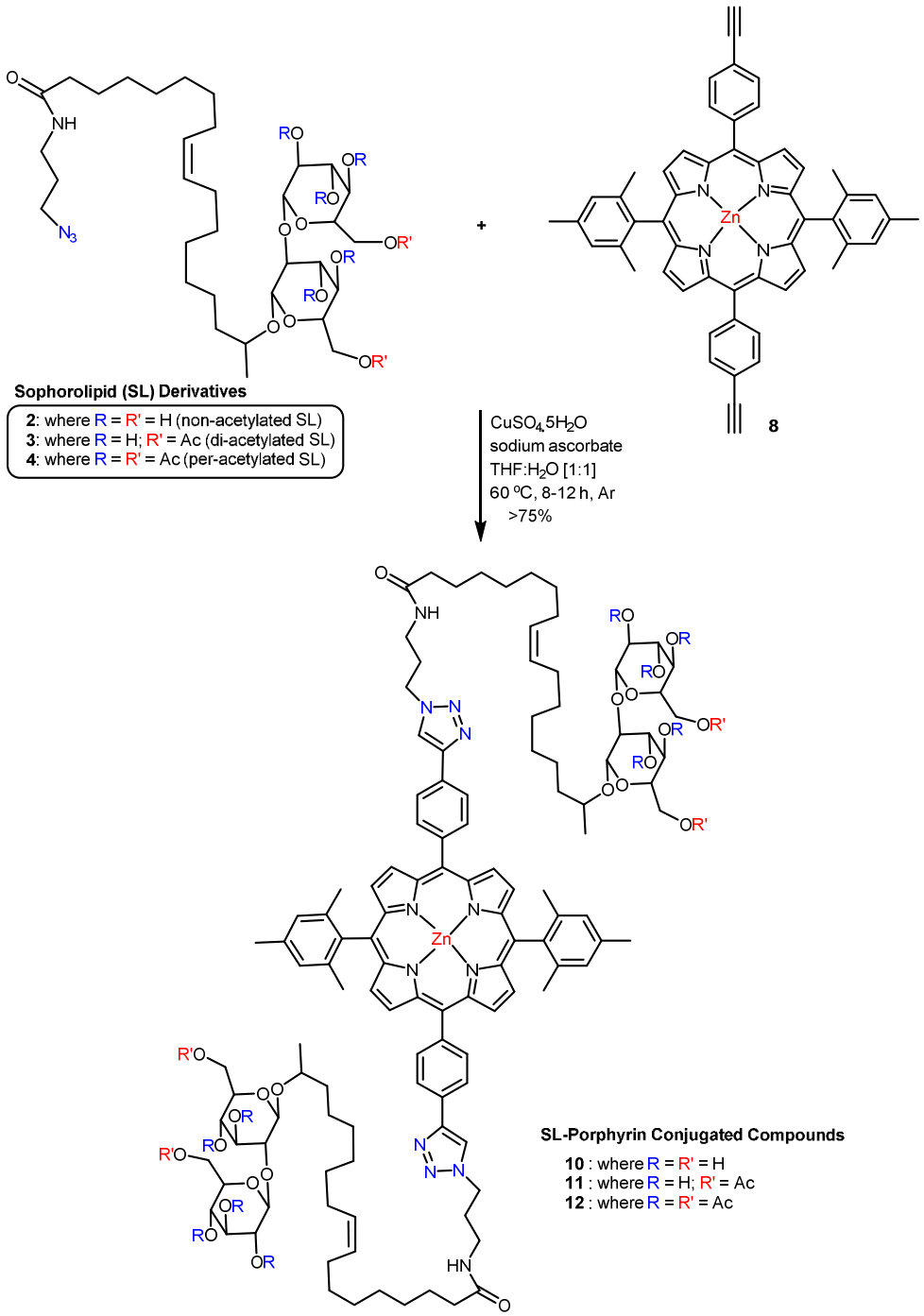
Scheme 1. Synthesis of sophorolipid derivatives **1**, **2**, **3** and **4**.



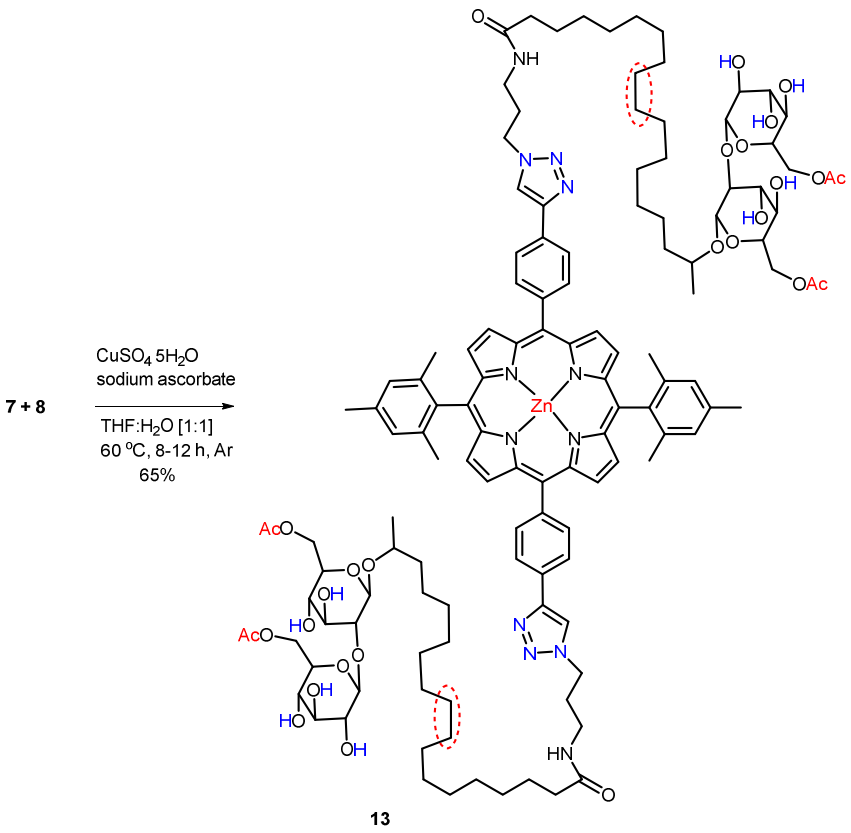
Scheme 2. Synthesis of hydrogenated sophorolipid derivatives **5**, **6** and **7**.



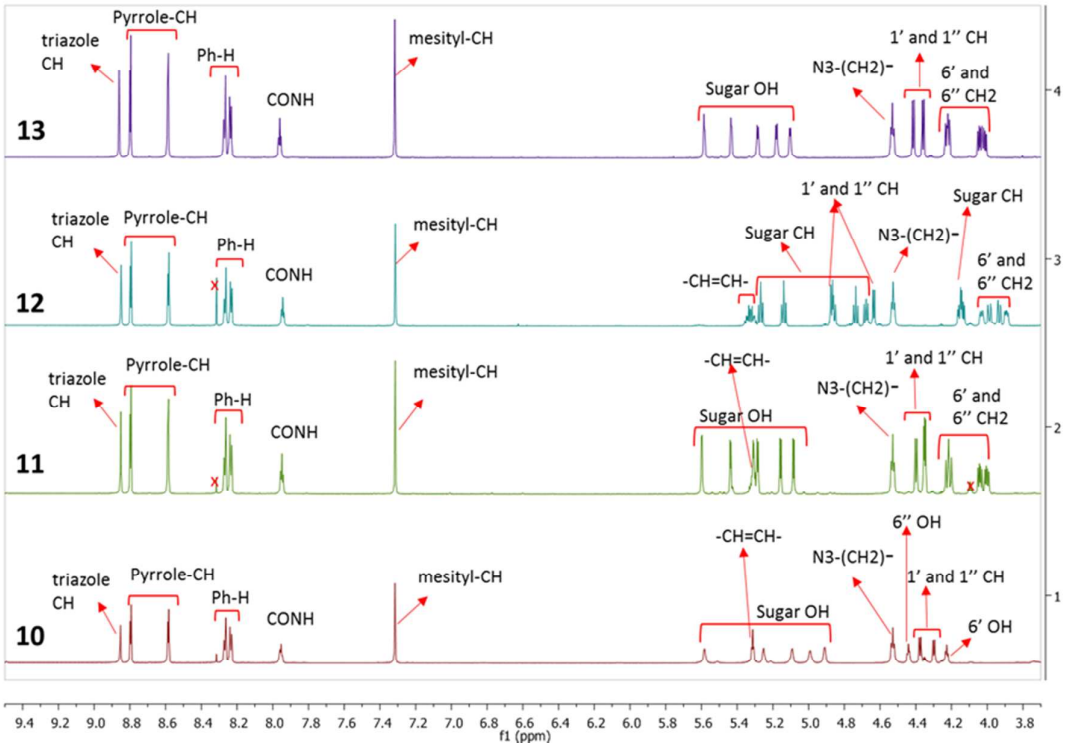
Scheme 3. Structures of Zn-porphyrin derivatives **8** and **9**.

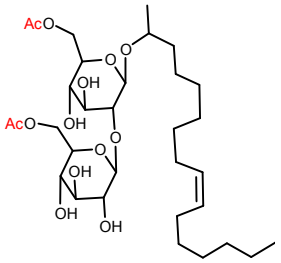


Scheme 4. Structures of sophorolipid-porphyrin conjugated compounds **10**, **11** and **12**.

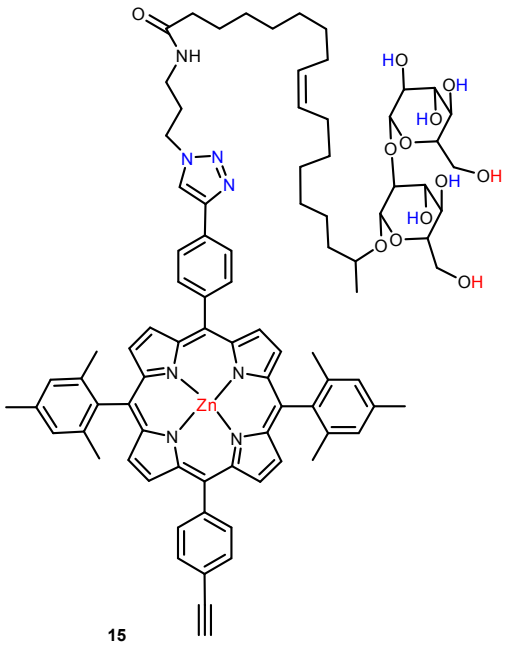


Scheme 5. Sophorolipid-porphyrin conjugated compound **13** (site of hydrogenation showed in a dotted red circle)





Scheme 6. Structure of sophorolipid-porphyrin conjugated compound **14**.



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Scheme 7. Structure of sophorolipid-porphyrin conjugated compound **15**.

Figure 1. Stacked ^{1}H NMR spectra (800 MHz, in DMSO-d_6 solvent) (partial) of diconjugated compounds **10**, **11**, **12** and **13** (bottom to top).

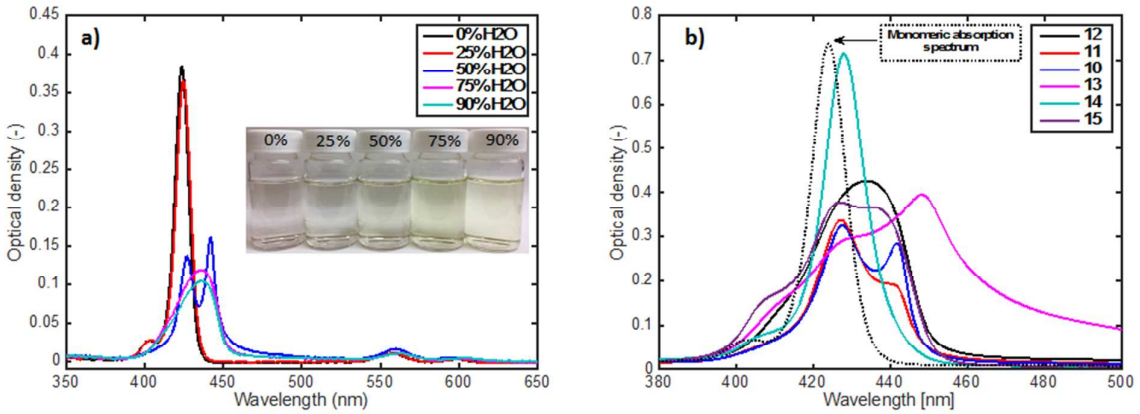
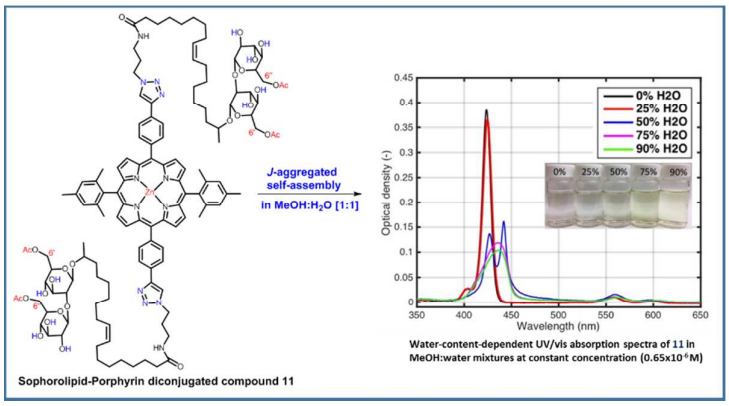


Figure 2: **(a)** Water-content-dependent UV-vis absorption spectra of **11** in MeOH:water mixtures at constant concentration (0.65x10^{-6} M) the inset are pictures of the solutions used for measurements. **(b)** UV-vis absorption spectra of compounds (**10**, **11**, **12**, **13**, **14** and **15**) in MeOH:water (1:1 by vol.) at room temperature (2x10^{-6} M).

Graphical Abstract



GRAPHICAL ABSTRACT

<u>Highlight of the importance of the work</u> - Synthesis of sophorolipid-porphyrin conjugates with built-in variations in non-covalent interactions, H-bonding, π-π stacking, and hydrophobic interactions for supramolecular self-assembly.