Supporting Information

Trehalose-conjugation enhances toxicity of photosensitizers against mycobacteria

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### Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| DCC          | N,N'-Dicyclohexylcarbodiimide |
| DIPEA        | N,N-Diisopropylethylamine |
| DMF          | Dimethylformamide |
| DMSO         | Dimethylsulfoxide |
| ESI-MS       | Electrospray ionization – mass spectrometry |
| HCl          | Hydrochloric acid |
| HOBt         | 1-Hydroxybenzotriazol |
| LB           | Lysogeny Broth |
| LT-ELSD      | Low Temperature - Evaporative light scattering detector |
| RP-HPLC      | Reverse phase high-performance liquid chromatography |
| RP-MPLC      | Reverse phase medium pressure liquid chromatography |
| TFA          | Trifluoroacetic acid |
| TLC          | Thin layer chromatography |
Experimental Details

**Biology**

**Strains, plasmids and growth conditions.** Strains, plasmids and primers used in this study are listed in table S1. Routinely, *M. smegmatis* mc²155, *M. tuberculosis* H37Ra, *M. abscessus* subsp. abscessus or derived strains were cultured in 7H9 broth supplemented with 10 % (v/v) ADC (2% dextrose, 5% albumin, 0.85 % NaCl) and 0.05 % (v/v) Tween 80. For cloning procedures, *E. coli* XL1-Blue was grown in LB medium or LB-agar. For selection purposes kanamycin and/or hygromycin B were used at final concentrations of 100 or 50 µg/mL, respectively. Stock solutions of the synthesized compounds were prepared in DMSO at 10 mM and stored at -20°C.

**Singlet Oxygen Sensor Green Assay.** Detection of singlet oxygen was essentially performed as described by the manufacturers’ protocol (Thermo Fisher Scientific). Briefly, photosensitizers from 10 mM DMSO stocks were diluted in MeOH to a concentration of 1 mM. Sensor green compound was dissolved in MeOH at a concentration of 5 mM (stock solution kept at -20°C under light protection) and further diluted in MeOH to a final concentration of 100 µM. Photosensitizer (2 µL) and sensor green (2 µL) were mixed in a 96-well flat black bottom plate in 200 µL assay buffer (50 mM Tris buffer pH 7.5/D₂O (1:1)) to yield final concentrations of sensor green of 1 µM and photosensitizer 10 µM. Plates were irradiated with light (high pressure sodium lamp, Philips IP65 SON T 150W, 10 mW/cm²) for 30 min on ice (or kept in the dark as control) and in solution fluorescence intensity of sensor green was assessed on a Tecan Sparks platereader (Excitation = 504/525 nm). Controls were assay buffer, each photosensitizer and sensor green alone, respectively. The obtained control values were subtracted as background fluorescence. The experiment was done in triplicates (see Figure S2).

**Minimal inhibitory concentration (MIC).** MIC was determined using the resazurin reduction method as described elsewhere.1 Briefly, pre-cultures of cells (5 mL) were grown in 7H9 broth supplemented with 10 % (v/v) ADC and 0.05 % (v/v) Tween 80 from a single colony and harvested at mid-log phase (OD₆₀₀ ~ 1.0) by centrifugation (10 min, 3000 x g). Cells were diluted in culture broth to an OD₆₀₀ of 0.01. Photosensitizers and control compounds were diluted from 10 mM DMSO stocks in culture broth (300 µM or 50 µM, ciprofloxacin starting concentration 2.5 µM) and subsequently diluted log2 fold in 96-well plates in a final volume of 100 µL. Subsequently, bacterial cells were added (50 µL) and plates were incubated at 37°C in the dark with shaking. After 24 hours, plates were irradiated with light (high pressure sodium lamp, Philips IP65 SON T 150W, 10 mW/cm²) for 30 min on ice or left in the dark, and subsequently 20 µL resazurin (0.15 mg/mL) were added and color change of the wells from blue (resazurin – “no cells, or non-viable cells”) to pink (resorufin – “viable cells”) was observed after 24 h at 37°C. Visual MIC was defined as the lowest concentration of drug that prevented a color change. To generate cell viability curves in respect to compound concentration, absorbance was monitored at 570 nm (resorufin) and 602 nm (background absorbance) using an absorbance microplate reader (Biorad). As a positive control rifampicin, SQ109 or ciprofloxacin were used. Analysis was done in duplicates and repeated at least two times.

**Cell viability in HeLa cells (MTT-assay).** Early passage HeLa cells were grown in T-25 flasks in DMEM medium till they reached ~80% confluency (37°C humidified incubator, 5% CO₂). Cells were trypsinized, washed in DMEM and seeded in 96-well plates (10 000 cells/well) in a volume of 200 µL. Cells were grown over night and compounds 14, 16, control or vehicle were added subsequently in a volume of 150 µL (diluted from DMSO stocks in DMEM, log2 fold dilutions from 200 µM starting concentration). Cells were incubated for 24 hours, then the medium was removed and cells were washed once with phosphate buffered saline (PBS). MTT reagent was added (0.5 mg/mL in DMEM, 120 µL per well) and plates were incubated for 2 hours. MTT reagent was removed carefully and the remaining crystals were dissolved in DMSO (120 µL per well). Absorbance was quantified at 595 nm. Analysis was done in triplicates (see Figure S6).
For micrograph images, early passage HeLa cells were grown in T-25 flasks in DMEM medium till they reached ~80% confluency (37°C humidified incubator, 5% CO₂). Cells were trypsinized, washed in DMEM and seeded in 12-well plates (0.1 x 10⁶ cells/well) in a volume of 1 mL. Cells were grown over night and compounds 14, 16, control or vehicle were added subsequently in a volume of 1 mL at a final concentration of 20 μM. Cells were incubated for 24 hours and subsequently investigated under a microscope and photographed (Evos FL Imaging System, Thermo Fisher Scientific).

**Lipid analysis by thin-layer chromatography (TLC).** Pre-cultures (5 mL) of *M. smegmatis* mc²155 were grown for two days and harvested by centrifugation (10 min, 3000 x g). Subsequently, cells were diluted to an OD₆₀₀ of 0.1 in 10 mL 7H9 medium complemented with Tween 80 and ADC enrichment and incubated with the indicated compound at 10 μM concentration for 6 hours at 37°C, 180 rpm. Cells were harvested by centrifugation (10 min, 3000 x g) and washed twice with ddH₂O (2x5 mL). The remaining pellet was used for lipid extraction with a modified Folch method.² Briefly, cell pellets were weighed and re-suspended in chloroform/methanol (1:2) at 1 g/mL and extracted for 6 hours with shaking at room temperature. After centrifugation (5 min, 14 000 x g), the supernatant was stored and subsequently the pellet was extracted twice with chloroform/methanol (2:1) for 2 hours at room temperature. Supernatants of all three extractions were pooled and extracted with 1 % NaCl solution (5 mL). The organic layer was collected and the solvent was evaporated under reduced pressure. Lipids were re-dissolved in 50 μL chloroform/methanol (2:1) and resolved by thin layer chromatography (solvent system chloroform/methanol/H₂O 20:4:0.5). Lipids were visualized with CuSO₄ (10% CuSO₄ in 8% phosphoric acid solution) and heating, by fluorescence scanning on a gel imager (Vilber Lourmat, Fusion SL) or inspected visually and photographed using a Lumix digital camera (Panasonic).

**Incorporation of ¹⁴C acetate and lipid analysis by TLC and autoradiography.** *M. smegmatis* mc²155 was grown in Middlebrook 7H9 broth (BD biosciences), supplemented with albumin-dextrose-catalase (10%), and 0.05% Tween 80 at 37°C, shaking at 120 rpm. When the culture reached OD₆₀₀ ~ 0.5 the culture was divided into 1 mL aliquots and compound 16 (6AT-I-BODIPY) dissolved in 20 μL DMSO was added at final concentrations of 20, 50 and 100 μM. After 16 hours of cultivation ¹⁴C-acetate (ARC, specific activity 106 mCi/mmol) at a final concentration 0.5 μCi/mL was added and the cells were cultivated another 3 hours at 37°C. Then the cells were harvested and washed twice with 50 mM TrisHCl, pH = 7.5. Two independent experiments were performed in two independent runs.

*M. tuberculosis* H37Ra was grown in Middlebrook 7H9 broth (BD biosciences), supplemented with albumin-dextrose-catalase (10%), and 0.05% Tween 80 at 37°C, shaking at 120 rpm until OD₆₀₀ reached ~ 0.5. Then the culture was divided into 1 mL aliquots and compound 16 (6AT-I-BODIPY) dissolved in 20 μL DMSO was added at a final concentration of 0, 20, 50 and 100 μM. Alternatively, to the cultures treated with 50 and 100 μM compound 16, also isoniazid (INH) was added at final concentrations of 4 and 8 μg/mL. This was followed by the addition of ¹⁴C-acetate (ARC, specific activity 106 mCi/mmol) at a final concentration 0.5 μCi/mL. After the next 24 hours of cultivation, the cells were harvested and washed twice with 50 mM TrisHCl, pH = 7.5. Two independent experiments were performed in two independent runs.

Lipids were isolated by chloroform/methanol extraction in 3 mL CHCl₃:MeOH (1:2); 56°C; 1.5 h followed by 3 mL CHCl₃:MeOH (2:1); 56°C; 1.5 h. The extracts were combined, dried under N₂ and the lipids were subjected to biphasic washing in CHCl₃:MeOH:H₂O (4:2:1).² The bottom organic phase was dried under N₂ and dissolved in 50 μL of chloroform: methanol (2:1). 5 μL of the lipid extracts were quantified for dpm by scintillation spectrometry. 5 μL were loaded on thin-layer chromatography (TLC) silica gel plates F254 (Merck) and the lipids were separated in the mixture of CHCl₃:MeOH:H₂O (20:4:0.5) and visualized by autoradiography.
Preparation and analysis of MAME of isolated lipids. TMM, TDM and lipid 16-A were isolated by preparative TLC. 40 µL of 14C lipid sample extracted from cells treated with 100 µM compound 16 prepared as described above were loaded on silica gel plates F254 (Merck) and the lipids were separated in CHCl₃:MeOH:H₂O (20:4:0.5) and visualized by autoradiography. The silica corresponding to migration of TMM, TDM and lipid 16-A was scraped off the plates and extracted twice with 6 ml of CHCl₃:MeOH (2:1). The extracts were combined together, dried under N₂ and dissolved in 50 µL of CHCl₃:MeOH (2:1). 5 µL of each sample were analyzed by TLC and the remaining 45 µL were dried and subjected to saponification in 1 mL of 15% tetrabutylammonium hydroxide (TBAH); 16 hours at 100°C. Hydrolyzed lipids were methylated with 1.5 mL of dichlormethane and 150 µL of iodomethane for 4 hours, rotating at room temperature, washed with water, extracted with diethyl ether and dried. Dried extracts were dissolved in 20 µL of CHCl₃:MeOH (2:1) and loaded on silica gel plates F254 (Merck). Different forms of mycolic acids methyl esters were separated in three runs in n-hexane: ethyl acetate (95:5) and visualized by autoradiography.

*M. smegmatis* mc²155::dCas9::pGrna_lpqY knockdown construction. Silencing of the gene encoding the ABC-transporter substrate binding protein LpqY (MSMEG_5061) was achieved following the CRISPRi protocol as essentially described by Choudhary et al.³ Briefly, oligonucleotides lpqY_crispr_up and lpqY_crispr_down (see Table S1) were annealed and ligated into SpIHI sites of pGrna. The resulting plasmid pGrna_lpqY was transformed into *M. smegmatis* mc²155::dCas9. Successful downregulation of the gene of interest upon induction with 50 ng/mL Anhydrotetracycline (Atc) for 24 hours was validated by RT-PCR using the primer pair Pr368/Pr369 (see Figure S5).

Analysis of the labeling efficiency in I) *M. smegmatis* mc²155::dCas9::pGrna_lpqY or II) of *M. smegmatis* mc²155 in the presence of the Ag85 complex inhibitor Ebselen.

I) Pre-cultures (5 mL) of *M. smegmatis* mc²155::dCas9::pGrna (empty plasmid) and *M. smegmatis* mc²155::dCas9::pGrna_lpqY were grown in 7H9 medium complemented with Tween 80, ADC and the respective antibiotics for two days and harvested by centrifugation (10 min, 3000 x g). For assessing the dependency of labeling on the trehalose uptake transporter LpqYSugABC, *M. smegmatis* mc²155::dCas9::pGrna_lpqY and *M. smegmatis* mc²155::dCas9::pGrna cells were diluted to OD₆₀₀ 0.05 (10 mL culture volume) and treated with 50 ng/mL Atc for 24 hours to induce downregulation of *lpqY*. Subsequently, cells were diluted at an OD₆₀₀ of 0.3 and incubated with the indicated compound at 10 µM concentration for 6 hours.

II) Pre-cultures (5 mL) of *M. smegmatis* mc²155 were grown in 7H9 medium complemented with Tween 80 and ADC for two days and harvested by centrifugation (10 min, 3000 x g). For assessing the dependency of labeling on the Ag85 complex, we followed the protocol of Kamariza et al.⁴ *M. smegmatis* mc²155 wild-type cells were pre-treated with 100 µg/mL Ebselen for 3 hours (10 mL culture volume, OD₆₀₀ of 0.4). Subsequently, cells were re-diluted at an OD₆₀₀ of 0.4 and incubated with the indicated compound at 10 µM concentration for 6 hours.

Cells were harvested by centrifugation (10 min, 3000 x g). Cells were washed twice with ddH₂O (2x5 mL). The remaining pellet was used for lipid extraction using a modified Folch method.³ Briefly, cell pellets were weighed and re-suspended in chloroform/methanol (1:2) at 1 g/mL and extracted for 6 hours with shaking at room temperature. After centrifugation (5 min, 14 000 x g), the supernatant was stored and subsequently the pellet was extracted twice with chloroform/methanol (2:1) for 2 hours at room temperature. Supernatants of all three extractions were pooled and extracted with 1% NaCl solution (5 mL). The organic layer was collected and the solvent was evaporated under reduced pressure. Lipids were dissolved in 200 µL chloroform/methanol (2:1) and in solution fluorescence intensity was assessed on a Tecan Spark® platereader (Ex/Em: 525/600 nm) in a black 96-well flat bottom plate.
Table S1: Strains, primers and plasmids used in this study.

| Strains | Source |
|---------|--------|
| *M. smegmatis* mc²155 | Gift from Laboratory of Dr. William R. Jacobs Jr., Albert Einstein College Medicine, England |
| *M. abscessus* subsp. abscessus | DSMZ strain collection, # 44196, Type strain |
| *M. tuberculosis* H37Ra | ATCC strain collection, # 25177 |
| *M. smegmatis* mc²155::dCas9 | *M. smegmatis* mc²155 transformed with pTetInt-dcas9 |

| Plasmids | Source |
|----------|--------|
| pGrna | Choudahary et al. |
| pTetInt-dcas9 | Choudahary et al. |
| pGrna::lpqY | this study |

| Primers/Oligos | Sequence |
|----------------|----------|
| Pr368 | 5′-CTGGCAGGATGAGCTCTACG-3′ |
| Pr369 | 5′-ATGCCTTCGTACTGCTTGCC-3′ |
| Pr360 „lpqY_crispr_up“ | 5′-GTCGGCCTCGCGAGACCAG-3′ |
| Pr361 „lpqY_crispr_down“ | 5′-CGCTGGTCTCGCGAGGCGCGAGCACCATG-3′ |
| mysA_fw | 5′-TGAGGATGACGGAGATCCTCG-3′ |
| mysA_rev | 5′-ACGCCTTTTCTCCTTTCGGAC-3′ |

Chemistry

**Chemicals and analytics and general remarks**

Reactions were carried out in an open flask equipped with a magnetic stirrer at room temperature, unless otherwise noted.

Reagents were purchased from commercial suppliers (Acros, Aldrich, Fluka, TCI) and used as received, unless noted otherwise. Photosensitizer 1, 6 (Sigma-Aldrich) and 3 (TCI) were commercially obtained (> 95% purity).

Solvents were obtained in analytical grade and used as received.

HPLC solvents were used as obtained for preparative HPLC and MPLC purifications.

Deuterated solvents for NMR were obtained from Euriso-Top, Germany, in the indicated purity grade and used as received for NMR spectroscopy.

Liquid chromatography was performed using 1) a preparative RP-HPLC equipped with an aQ.C₁₈ column coupled to a LT-ELSD and 2) an automated RP-MPLC, equipped with an aQ.C₁₈ column and coupled to a LT-ELSD.

HPLC analyses were performed using a Thermo Scientific (Dionex, Ultimate 300) analytical HPLC equipped with 1) a Hypersil GOLD aQ.C₁₈ column (3 μ, 3 × 150 mm) coupled to a UV and ESI-MS detector and 2) a Hypersil GOLD C₄ column (3 μ, 3 × 150 mm) coupled to a UV and ESI-MS detector. The methods are as follows:
AD_10000-0595_30_ESI (without buffer)_BODIPY$^a$:

| Time (min) | Flow (mL/min) | % H$_2$O | % CH$_3$CN |
|------------|---------------|----------|------------|
| 0.0        | 0.5           | 95.0     | 5.0        |
| 15.0       | 0.5           | 60.0     | 40.0       |
| 19.0       | 0.5           | 0.0      | 100.0      |
| 24.0       | 0.5           | 0.0      | 100.0      |
| 30.0       | 0.5           | 95.0     | 5.0        |
| 35.0       | Stop run      | Stop run | Stop run   |

$^a$: UV detection at 500 nm and 260 nm
### AD_10000-0595_30_ESI (without buffer)_PPIX002:

| Time (min) | Flow (mL/min) | % A (H₂O:TFA = 99.7:0.3 (v/v)) | % B (CH₃CN:TFA = 99.7:0.3 (v/v)) |
|-----------|---------------|-------------------------------|----------------------------------|
| 0.0       | 0.5           | 95.0                          | 5.0                              |
| 10.0      | 0.5           | 60.0                          | 40.0                             |
| 20.0      | 0.5           | 30.0                          | 70.0                             |
| 25.0      | 0.5           | 30.0                          | 70.0                             |
| 27.0      | 0.5           | 0.0                           | 100.0                            |
| 30.0      | 0.5           | 0.0                           | 100.0                            |
| 33.0      | 0.5           | 95.0                          | 5.0                              |
| 43.0      | Stop run      | Stop run                      | Stop run                         |

*b*: UV detection at 400 nm and 260 nm

### AD_PPIX002_TFA:

| Time (min) | Flow (mL/min) | % H₂O | % CH₃CN | %C (H₂O:TFA = 99.0:1.0 (v/v)) |
|-----------|---------------|-------|---------|-------------------------------|
| 0.0       | 0.5           | 65.0  | 5.0     | 30.0                          |
| 23.0      | 0.5           | 0.0   | 70.0    | 30.0                          |
| 28.0      | 0.5           | 0.0   | 70.0    | 30.0                          |
| 29.0      | 0.5           | 65.0  | 5.0     | 30.0                          |
| 36.0      | Stop run      | Stop run | Stop run | Stop run                     |

### AD_10000-0595_30_ESI (without buffer)_MB003:

| Time (min) | Flow (mL/min) | % H₂O | % CH₃CN |
|-----------|---------------|-------|---------|
| 0.0       | 0.5           | 95.0  | 5.0     |
| 30.0      | 0.5           | 50.0  | 50.0    |
| 33.0      | 0.5           | 10.0  | 90.0    |
| 35.0      | 0.5           | 95.0  | 5.0     |
| 43.0      | Stop run      | Stop run | Stop run | Stop run                     |

*c*: UV detection at 658 nm and 260 nm

**¹H-NMR spectra** were recorded on Bruker 300 MHz spectrometers, Bruker 400 MHz and Bruker 500 MHz spectrometers in the indicated deuterated solvent. Data are reported as follows: chemical shift (δ, ppm), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad signal), coupling constant(s) (J, Hz), integration. All signals were referenced to the internal solvent signal as standard (CD₃OD, δ 3.31; (CD₃)₂SO, δ 2.50).
$^{13}$C- NMR spectra were recorded with $^1$H-decoupling on Bruker 101 MHz (with cryoprobe) spectrometers at 298K in the indicated deuterated solvent. All signals were referenced to the internal solvent signal as standard (CD$_3$OD, δ 49.0; (CD$_3$)$_2$SO, δ 39.52).

Mass spectra were recorded at the mass spectrometry service at the University of Freiburg on Finnigan TSQ 700 MS and Thermo Scientific EXACTIVE spectrometers with Orbitrap analyzer.

**Synthetic procedures**

3-(5,5-difluoro-2,8-diiodo-1,3,7,9-tetramethyl-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)propanoic acid 3

![Chemical Structure](image)

Compound 3 was synthesized as described in the literature.$^5$ The analytical data were identical with the reported literature values.

3-(5,5-difluoro-1,3,7,9-tetramethyl-2,8-di(thiophen-2-yl)-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)propanoic acid 4

![Chemical Structure](image)

15.0 mg (26.0 µmol, 1.0 eq.) 3, 17.1 mg (0.2 mmol, 5.6 eq.) thiophene boronic acid, 1.90 mg (10 mol%, 0.1 eq) Pd(dppf)Cl$_2$ and 22.5 mg (0.1 mmol, 4.1 eq.) K$_3$PO$_4$ were charged in a flask under argon atmosphere. A freshly degassed (30 min with an argon balloon) solvent mixture of 1,4-dioxane and water (10:1) was added and the resulting reaction mixture was refluxed for 17 h under argon atmosphere. The mixture was then cooled down to room temperature, diluted with dichloromethane and filtered through a celite plug. The solvent was concentrated under reduced pressure and the crude product was subjected to KNAUER AZURA preparative RP-HPLC (Method: 0-6 min isocratic H$_2$O/CH$_3$CN 95:5 v/v, followed by 6-42 min H$_2$O/CH$_3$CN gradient till 60:40 v/v, then 42-53 min H$_2$O/CH$_3$CN gradient to 0:100 v/v, and finally 53-70 min H$_2$O/CH$_3$CN isocratic 0:100 v/v; flow rate: 15 mL/min; crude dissolved in ~ 3.0 mL of MeOH) equipped with an aQ. C$_{18}$ column and coupled to a SEDERE SEDEX (model LC) ELSD to obtain pure 4. Isolated yield: 90% (11.4 mg, 23.5 µmol). $R_t = 55$ min.

The analytical data were identical with the literature.$^6$

(2$S$,3$R$,4$R$,5$S$,6$S$)-2-(aminomethyl)-6-(((2$S$,3$S$,4$R$,5$R$,6$S$)-3,4,5-trihydroxy-6(hydroxymethyl) tetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-3,4,5-triol 7
Compound 7 was synthesized as described in the literature. The analytical data were identical with the reported literature values.

\[(2R,3R,4S,5S,6R)-2-(((2R,3R,4R,5S,6R)-3-amino-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-6-(hydroxymethyl)-2-methyltetrahydro-2H-pyran-3,4,5-triol 8\]

Compound 8 was synthesized as described in the literature. The analytical data were identical with the reported literature values.

**General procedure for the synthesis of trehalose-BODIPY conjugates (GP-1)**

All four solids, photosensitizer, amino trehalose, HOBt and DCC were dissolved into DMF in an open flask, equipped with a magnetic stirrer. Subsequently, DIPEA was added; the resulting reaction mixture was stirred in dark, at room temperature for 16 h. Afterwards, it was concentrated under reduced pressure and the residue was subjected to purification by preparative RP-HPLC (Purification method: 0-6 min isocratic H₂O/CH₃CN 95:5 v/v, followed by 6-42 min H₂O/CH₃CN gradient till 60:40 v/v, and finally 42-53 min H₂O/CH₃CN gradient to 0:100 v/v; flow rate: 15 mL/min; crude dissolved in 1-2 mL of MeOH) equipped with a Hypersil GOLD aQ. C₁₈ column (5 µ, 21.2 × 250 mm) column and coupled to a SEDER SEDEX (model LC) ELSD detector.
General procedure for the synthesis of trehalose-porphyrin conjugates (GP-2)

All four solids, photosensitizer, amino trehalose, HOBt and DCC were dissolved into DMF in an open flask, equipped with a magnetic stirrer. Subsequently, DIPEA was added; the resulting reaction mixture was stirred in dark, at room temperature for 16 h. Afterwards, it was concentrated under reduced pressure and the residue was re-dissolved in MeOH to prepare a reverse phase C$_{18}$ silica slurry which was then dry-loaded on a RP-MPLC (Mobile phase: Solvent A: 99:1 v/v H$_2$O:TFA, Solvent B: 99:1 v/v CH$_3$CN:TFA; purification method: 0-6 min isocratic A/B 90:10 v/v, followed by 3-28 min A/B gradient till 20:80 v/v; flow rate: 15 mL/min) equipped with an aQ. C$_{18}$ column and coupled to a SEDERE SEDEX (model LC) ELSD detector for purification.

ds$_3$-(2,8,12,17-tetramethyl-3-(3-oxo-3-(((2S,3S,4R,5S,6S)-3,4,5-trihydroxy-6-(((2S,3S,4R,5R,6S)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-2-yl)methyl)amino)propyl)-13,18-divinylporphyrin-7-yl)propanoic acid 9

Compound 9 was synthesized and isolated according to the general procedure GP-2. Both regioisomers were obtained and not further separated.

16.5 mg (29.3 µmol, 1.0 eq.) 1, 10.0 mg (29.3 µmol, 1.0 eq.) 7, 4.0 mg (29.3 µmol, 1.0 eq) HOBt, 6.0 mg (29.3 µmol, 1.0 eq) DCC, 30.6 µL (175.8 µmol, 6.0 eq.) DIPEA, 2.5 mL DMF. Isolated yield 9: 42% (10.9 mg, 12.3 µmol). R$_t$ = 16 min.

$^1$H NMR (400 MHz, DMSO-d$_6$, mixtures of regioisomers) δ 9.71 (s, 2H), 9.45 (s, 1H), 9.41 (s, 2H), 9.36 (s, 1H), 9.03 (s, 2H), 8.10 – 8.02 (m, 2H), 7.91 – 7.83 (m, 4H), 6.25 – 6.09 (m, 8H), 4.85 – 4.84 (m, 2H), 4.79 – 4.77 (m, 2H), 4.23 (br m, 8H), 3.72 – 3.70 (m, 2H), 3.61 – 3.59 (m, 2H), 3.56 – 3.50 (m, 7H), 3.46 (s, 6H), 3.43 (s, 6H), 3.34 – 3.33 (m, 8H), 3.25 – 3.24 (m, 2H), 3.22 (m, 2H), 3.17 (s, 6H), 3.14 – 3.08 (m, 8H), 2.98 – 2.91 (m, 7H), – 6.42 (br s, 4H); $^{13}$C NMR (101 MHz, DMSO-d$_6$) δ 172.5, 171.0, 157.6, 157.2, 156.8, 156.4, 153.4, 153.2, 133.8, 127.7, 127.5, 119.7, 119.6, 118.0, 116.4, 115.2, 112.3, 95.0, 94.7, 91.9, 91.7, 71.3, 70.9, 70.8, 70.1, 69.9, 69.7, 68.9, 68.6, 59.2, 36.3, 34.9, 20.4, 19.7, 10.7, 10.6, 9.6 (2×C); HRMS (ESI) [M–H]$^-$ calcd. for C$_{46}$H$_{54}$N$_5$O$_{13}$: 884.3724, observed: 884.3724.
3-(18-((2R,3R,4S,5R,6R)-4,5-dihydroxy-6-(hydroxymethyl)-2-((2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)-2-methyltetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-3-yl)amino)-3-oxopropyl)-3,8,13,17-tetramethyl-7,12-divinylporphyrin-2-yl)propanoic acid 10

Compound 10 was synthesized according to the general procedure GP-2. Both regioisomers were obtained and not further separated.

15.9 mg (28.2 µmol, 1.0 eq.) 1, 10.0 mg (28.2 µmol, 1.0 eq.) 8, 3.8 mg (28.2 µmol, 1.0 eq) HOBt, 5.8 mg (28.2 µmol, 1.0 eq) DCC, 29.5 µL (169.0 µmol, 6.0 eq) DIPEA, 2.5 mL DMF. Isolated yield 10: 74% (18.8 mg, 3.4 µmol). \( R_t = 16 \text{ min} \)

\(^1H\) NMR (400 MHz, DMSO-\( d_6 \), mixtures of regioisomers) \( \delta \) 9.79 (s, 2H), 9.30 (s, 2H), 9.22 (s, 2H), 8.59 (s, 2H), 7.95 – 7.84 (m, 4H), 7.63 – 7.56 (m, 2H), 6.16 – 6.00 (m, 8H), 5.29 (m, 2H), 4.26 (br m, 8H), 3.88 – 3.84 (m, 2H), 3.78 – 3.74 (m, 4H), 3.72 – 3.65 (m, 3H), 3.63 – 3.56 (m, 8H), 3.49 (s, 3H), 3.46 (s, 3H), 3.41 (s, 3H), 3.39 (s, 3H), 3.30 – 3.26 (m, 3H), 3.23 (s, 3H), 3.21 (s, 3H), 3.15 – 3.07 (m, 6H), 2.97 – 2.90 (m, 12H), 1.43 (s, 6H), − 5.5 (br s, 4H); \(^13C\) NMR (101 MHz, DMSO-\( d_6 \)) \( \delta \) 174.7, 172.7, 159.3, 158.9, 158.6, 141.1, 140.4, 137.6, 137.5, 137.4, 136.1, 135.6, 135.5, 129.2, 128.9, 122.0, 121.9, 120.1, 117.2, 114.4, 111.5, 101.1, 96.9, 96.6, 90.4, 77.3, 73.7, 73.4, 72.7, 71.3, 70.6, 70.4, 61.2 (2xC), 55.13, 37.9, 36.9, 23.6, 21.6, 12.5 (2xC), 12.2, 11.7, 11.6; HRMS (ESI) [M+H]^+ calcd. for C_{47}H_{58}N_{13}O_{13}: 900.4026, observed: 900.4020.

3,3′-(3,8,13,17-tetramethyl-7,12-divinylporphyrin-2,18-diyl)bis(N-((2R,3R,4S,5S,6R)-4,5-dihydroxy-6-(hydroxymethyl)-2-((2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)-2-methyltetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-3-yl)propanamide) 11

Compound 11 was synthesized and isolated according to the general procedure GP-2.

10.8 mg (19.2 µmol, 1.0 eq.) 1, 17.1 mg (47.9 µmol, 2.5 eq.) 8, 6.5 mg (47.9 µmol, 2.5 eq) HOBt, 10.0 mg (47.9 µmol, 2.5 eq) DCC, 20.0 µL (115.0 µmol, 6.0 eq) DIPEA, 2.5 mL DMF. Isolated yield 11: 22% (5.2 mg, 4.2 µmol). \( R_t = 13 \text{ min} \).
**1H NMR** (400 MHz, DMSO-\textit{d}_6) δ 10.30 – 10.04 (m, 4H), 8.52 – 8.34 (m, 2H), 7.93 – 7.69 (m, 2H), 6.42 (dd, \( J = 17.9, 6.8 \) Hz, 2H), 6.22 (dd, \( J = 11.7, 6.0 \) Hz, 2H), 5.26 (m, 2H), 4.37 (br m, 4H), 4.11 – 4.01 (m, 1H), 3.94 – 3.80 (m, 2H), 3.79 – 3.44 (m, 24H), 3.35 – 3.20 (m, 3H), 2.91 (m, 2H), 1.41 (s, 6H), − 4.34 (br s, 2H);

**13C NMR** (101 MHz, DMSO-\textit{d}_6) δ 170.7, 138.6, 138.5, 135.8, 134.6, 134.1, 127.6, 127.4, 120.2, 116.6, 115.4, 99.1, 95.4, 95.1, 88.4, 75.3, 74.2, 72.5, 71.7, 71.4, 70.8, 69.3, 69.2, 68.5, 68.4, 59.7, 59.2, 59.1, 53.1, 24.2, 23.5, 22.8, 21.7, 20.6, 19.9, 10.8, 10.6, 9.9;

**HRMS** (ESI) [M+H]^+ calcd. for C\textsubscript{60}H\textsubscript{81}N\textsubscript{6}O\textsubscript{22}: 1237.5398, observed: 1237.5385.

**Methyl 3-(2,8,12,17-tetramethyl-3-oxo-3-(((2S,3R,4R,5S,6S)-3,4,5-trihydroxy-6-(((2S,3S,4R,5R,6S)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-2-yl)methyl)amino)propyl)-13,18-divinylporphyrin-7-yl)propanoate 12**

10.9 mg (12.3 µmol, 1.0 eq.) 9 were dissolved in 5 mL of MeOH and 0.1 mL of conc. HCl (37%) were added. Upon refluxing this solution for 15 min, a complete conversion of 9 was observed. The solution was then evaporated to dryness under reduced pressure to isolate compound 12, which was obtained pure (both regioisomers, which were not separated further). Isolated yield 12: 98% (10.8 mg, 12.0 µmol).

**1H NMR** (400 MHz, DMSO-\textit{d}_6, mixtures of regioisomers) δ 10.81 – 10.57 (br m, 8H), 8.46 – 8.32 (m, 4H), 8.19 – 8.15 (m, 2H), 6.48 – 6.42 (m, 4H), 6.33 – 6.27 (m, 4H), 4.92 – 4.89 (m, 2H), 4.80 – 4.77 (m, 2H), 4.39 (br m, 8H), 3.75 (br m, 2H), 3.65 – 3.48 (m, 32H), 3.43 – 3.32 (m, 5H), 3.30 – 3.17 (m, 14H), 3.14 – 3.09 (m, 6H), 3.00 – 2.93 (m, 3H), −2.17 (br s, 4H);

**13C NMR** (101 MHz, DMSO-\textit{d}_6) δ 174.3, 173.5, 173.2, 172.7, 172.6, 142.5, 141.1, 140.6, 138.7, 138.6, 137.1, 136.1, 136.0, 129.2 (2×C), 98.9, 93.7, 93.5, 73.1, 72.9, 72.8, 72.1, 71.9, 71.9, 70.7, 70.6, 61.2, 52.0, 49.1, 37.8, 36.7, 36.2, 22.4, 21.6, 13.5, 13.4, 12.2, 12.1; **HRMS** (ESI) [M+H]^+ calcd. for C\textsubscript{47}H\textsubscript{58}N\textsubscript{6}O\textsubscript{13}: 900.4031, observed: 900.4027.
Compound 13 was synthesized according to the general procedure GP-2 and purified by preparative RP-HPLC (Mobile phase: Solvent A: 99.7:0.3 v/v H₂O:TFA, Solvent B: 99.7:0.3 v/v CH₃CN:TFA; purification method: 0-28 min A/B gradient starting from 95:5 v/v to 60:40 v/v, followed by 28-56 min A/B gradient to 30:70 v/v, and finally 56-70 min A/B isocratic 30:70 v/v; flow rate: 15 mL/min; crude dissolved in 3 mL of MeOH and 2 drops of TFA) equipped with Hypersil GOLD C₄ column (5 µ, 21.2 × 250 mm) column and coupled to a SEDERE SEDEX (model LC) ELSD detector.

20.0 mg (30.4 µmol, 1.0 eq.) 3, 20.7 mg (60.7 µmol, 2.0 eq.) 7, 8.2 mg (60.7 µmol, 2.0 eq) HOBt, 12.5 mg (60.7 µmol, 2.0 eq) DCC, 32.0 µL (182.4 µmol, 6.0 eq) DIPEA, 2.5 mL DMF. Isolated yield 13: 44% (13.2 mg, 13.4 µmol). Rₜ = 55 min.

^1H NMR (500 MHz, DMSO-d₆) δ 8.84 – 8.82 (m, 8H), 8.69 (t, J = 5.6 Hz, 1H), 8.32 – 8.28 (m, 4H), 8.21 – 8.20 (m, 6H), 7.85 – 7.79 (m, 9H), 5.02 (d, J = 3.5 Hz, 1H), 4.99 (d, J = 3.6 Hz, 1H), 4.06 – 4.01 (m, 1H), 3.71 – 3.69 (m, 2H), 3.68 – 3.62 (m, 2H), 3.60 – 3.57 (m, 1H), 3.49 (dd, J = 11.7, 4.9 Hz, 1H), 3.38 (dd, J = 9.5, 3.7 Hz, 1H), 3.33 (dd, J = 9.6, 3.6 Hz, 1H), 3.21 – 3.15 (m, 2H), 3.09 – 3.04 (m, 1H), − 2.92 (br s, 2H); ^13C NMR (126 MHz, DMSO-d₆) δ 167.1, 158.4, 158.1, 141.1, 134.2, 133.9, 128.1, 127.0, 126.0, 120.3, 120.2, 119.1, 116.9, 114.6, 93.6, 93.5, 72.9, 72.6, 72.5, 71.9, 71.7 (2×C), 70.6, 70.1, 60.8, 47.5, 45.7, 33.3, 25.3, 24.5, 8.6; HRMS (ESI) [M+H]^+ calcd. for C₅₇H₅₂N₅O₁₁: 982.3658, observed: 982.3655.

3-(5,5-difluoro-1,3,7,9-tetramethyl-2,8-di(thiophen-2-yl)-5H-4,5i,4i-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)-N-(((2S,3R,4R,5S,6S)-3,4,5-trihydroxy-6-((2S,3S,4R,5R,6S)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-2-yl)methyl)propanamide 14
Compound 14 was synthesized and isolated according to the general procedure GP-1. 5.0 mg (10.3 µmol, 1.0 eq.) 4, 8.8 mg (25.8 µmol, 2.5 eq.) 7, 3.5 mg (25.8 µmol, 2.5 eq.) HOBt, 5.3 mg (25.8 µmol, 2.5 eq.) DCC, 11.0 µL (61.8 µmol, 6.0 eq.) DIPEA. Isolated yield 14: 72% (6.0 mg, 7.4 µmol). R_t = 52 min.

1H NMR (500 MHz, DMSO-d_6) δ 7.94 (t, J = 5.6 Hz, 1H), 7.69 (dd, J = 5.2, 1.2 Hz, 2H), 7.21 (d, J = 3.5 Hz, 1H), 7.20 (d, J = 3.5 Hz, 1H), 7.08 (dd, J = 5.2, 1.2 Hz, 2H), 2×4.86 (d, J = 4.0 Hz, 1H), 3.75 – 3.70 (m, 1H), 3.65 – 3.62 (m, 1H), 3.57 – 3.49 (m, 5H), 3.45 (d, J = 5.1 Hz, 1H), 3.42 (d, J = 5.1 Hz, 1H), 3.23 (dd, J = 9.6, 3.5 Hz, 2H), 3.19 (dd, J = 9.6, 3.5 Hz, 2H), 3.11 – 3.05 (m, 2H), 2.98 – 2.93 (m, 1H), 2.47 (s, 6H), 2.44 (s, 6H); 13C NMR (126 MHz, DMSO-d_6) δ 170.3, 152.9, 138.7, 133.1, 130.6, 128.7, 127.2, 125.9, 93.5, 93.3, 72.8, 72.5 (2×C), 71.6, 71.5, 70.7, 70.1, 60.8, 36.0, 33.3, 24.7, 14.3, 13.2; 11B NMR (160 MHz, DMSO-d_6) δ 0.50 (t, J = 31.6 Hz, 1B); HRMS (ESI) [M]_+ calcd. for C_{36}H_{44}BF_2N_3O_11S_2: 806.2478, observed: 806.2407.

N-((Z)-7-(dimethylamino)-3H-phenothiazin-3-ylidene)-N-methyl-4-oxo-4-(((2S,3R,4R,5S,6S)-3,4,5-trihydroxy-6-(((2S,3S,4R,5R,6S)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-2-yl)methyl)amino)butan-1-aminiumchloride 15

Atto-MB2 NHS ester 6 (1.0 mg, 1.8 µmol, 1.0 eq.) was stirred with 7 (1.2 mg, 3.6 µmol, 2.0 eq.) in 1.0 mL anhydrous DMF under an argon atmosphere for 3 h at room temperature. The solvent was evaporated under reduced pressure. Afterwards, the crude was redissolved in H_2O/MeOH (1 mL, 1:1 v/v) and injected and purified by preparative RP-HPLC (Purification method: 0-139 min H_2O/CH_3CN gradient from 95:5 to 70:30 v/v; flow rate: 15 mL/min; crude dissolved in 1-2 mL of MeOH) equipped with a Hypersil GOLD aQ. C_4 column (5 µ, 21.2 x 250 mm) column and coupled to a SEDERE SEDEX (model LC) ELSD detector to obtain pure 15 (62%, 0.8 mg, 1.1 µmol). R_t = 127 min.

1H NMR (500 MHz, DMSO-d_6) δ 2×7.93 (d, J = 9.5 Hz, 1H), 7.79 (m, 1H), 7.50 (m, 4H), 4.89 (d, J = 3.6 Hz, 1H), 4.87 (d, J = 3.6 Hz, 1H), 3.69 – 3.46 (m, 10H), 3.38 (s, 3H), 3.34 (s, 3H), 3.27 – 3.20 (m, 2H), 3.19 – 3.07 (m, 1H), 2.98 (t, J = 9.3 Hz, 1H), 2.26 (m, 2H), 1.9 – 1.8 (m, 2H) 1.22 (s, 3H); 13C NMR (101 MHz, DMSO-d_6) δ 172.5, 158.8, 158.5, 158.2, 154.4, 153.9, 138.4, 138.3, 135.5, 119.6, 118.9, 115.9, 107.3, 93.9, 93.8, 73.3, 72.9, 72.1, 72.1, 72.0, 71.0, 70.6, 61.2, 52.9, 41.6, 37.5, 31.9, 31.7, 29.5, 29.2, 22.6; HRMS (ESI) [M]^+ calcd. for C_{31}H_{43}N_4O_{11}S: 679.2644, found: 679.2639.
3-(5,5-difluoro-2,8-diido-1,3,7,9-tetramethyl-5H-4i,4j,5j-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)-N-(((2S,3R,4R,5S,6S)-3,4,5-trihydroxy-6-((2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-2-yl)methyl)propanamide 16

Compound 16 was synthesized and isolated according to the general procedure GP-1.

5.0 mg (8.7 µmol, 1.0 eq.) 3, 7.5 mg (21.9 µmol, 2.5 eq.) 7, 3.0 mg (21.9 µmol, 2.5 eq) HOBt, 4.5 mg (21.9 µmol, 2.5 eq) DCC, 9.2 µL (52.5 µmol, 6.0 eq) DIPEA. Isolated yield 16: 43% (3.4 mg, 3.8 µmol). Rf = 52 min.

1H NMR (400 MHz, Methanol-d4) δ 5.09 (d, J = 3.8 Hz, 1H), 5.06 (d, J = 3.7 Hz, 1H), 3.97 – 3.72 (m, 6H), 3.68 (d, J = 5.8 Hz, 1H), 3.65 (d, J = 5.6 Hz, 1H), 3.55 (dd, J = 14.1, 2.9 Hz, 1H), 3.48 – 3.40 (m, 6H), 3.13 (dd, J = 9.9, 8.9 Hz, 1H), 2.57 (s, 6H), 2.55 (s, 6H) 13C NMR (101 MHz, Methanol-d4) δ 174.0, 157.2, 147.1, 144.8, 132.9, 95.9, 95.8, 75.2, 74.7, 74.4, 73.8, 73.8, 73.7, 72.6, 72.5, 63.2, 41.9, 37.9, 26.7, 19.9, 16.8; 11B NMR (128 MHz, Methanol-d4) δ 0.32 (t, J = 31.6 Hz, 1B); HRMS (ESI) [M+Na]+ calcd. for C28H38BF2I2N3O11Na: 918.0555, observed: 918.0549.
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Supporting Figures

Figure S1: Cell killing efficiency of compound 16 is depended on the applied irradiation time. M. smegmatis mc²155 cells were grown in the presence of compound 16 and irradiated after 24 hours with a high pressure sodium lamp for 0, 5, 15 or 30 min (10 mW/cm²). Subsequently cell viability was determined using the resazurin reduction assay. Data represents means of triplicate measurements, error bars indicate +/- SEM.
Figure S2: Fluorescence based assay, in which the probe *Singlet Oxygen Sensor Green* (SOSG) is incubated with the respective photosensitizers during irradiation (high pressure sodium lamp, 30 min, 10 mW/cm²). SOSG selectively reacts with singlet oxygen producing a fluorescent product, which can be quantified following excitation/emission at 504/525 nm. Data represents means of triplicate measurements, error bars indicate +/- SEM.
Figure S3: TLC analysis of lipids isolated from *M. smegmatis* mc²155 treated with 6AT-I-BODIPY (16) at different concentrations. $^{14}$C acetate was added as metabolic label 16 h after addition of 16 and the cells were cultivated for further 3 h. PE: phosphatidylethanolamine, CL: cardiolipin. Two independent experiments were performed, yielding similar results.
Figure S4: Isolation of lipids after incubation of *M. tuberculosis* H37Ra with 6AT-I-BODIPY (16). A) Analysis of lipids extracted from *M. tuberculosis* H37Ra treated with 16 and INH. B) Isolated TMM, 16-A and TDM by preparative TLC and subsequent MAME analysis after saponification. TLC in *n*-hexane/ethyl acetate 3 times run. PE: phosphatidylethanolamine, CL: Cardiolipin. Two independent experiments were performed, yielding similar results.
Figure S5: Silencing of *lpqY* in *M. smegmatis*. To achieve the repression of *MSMEG_5061* (*lpqY*), a pair of complementary oligonucleotides specific to the target ORF near 5’-end (Pr360: 5’-GTGGCGGCTCGCCGAGACCAG-3’ and Pr361: 5’-CGCTGGTCTCGCGGAGCCGACCATG-3’) were synthesized, annealed and cloned in pGrna, as previously described (Choudhary et al. 2015). The recombinant pGrna plasmid containing *MSMEG_5061* specific small guide RNA was transformed into dCas9-expressing *M. smegmatis mc^2^155 to generate knockdown strain namely 5061(-). Simultaneously, *M. smegmatis* harboring dCas9 was transformed with empty plasmid pGrna and used as control. Suppression was achieved by treatment of bacterial cultures at OD\(_{600}\) of ~0.05 with 50ng/ml anhydrotetracycline (ATc) for 24hrs. Shown in (A) is the representative image of the three RT-PCR experiments to evaluate the status of *MSMEG_5061* in the control and 5061(-) strains after ATc treatment. Level of an unrelated gene, *mysA* was simultaneously analysed to validate the specificity of *MSMEG_5061* suppression in 5061(-). B) Relative quantitation of *MSMEG_5061* and *mysA* levels in 5061(-) compared to control. Quantitation was performed by densitometric scanning of the band intensities shown in (A). Mean ± S.D. values from three experiments are shown. Statistical significance is determined by paired Student’s t-test.
Figure S6: Viability of Hela cells treated with compound 16, 14, and control compound (Blasticidin S) for 24 hours. Cell viability was assayed by MTT reduction assay (see SI text). Analysis was done in triplicates. Error bars represent +/- SEM.
Figure S7: Overall appearance of Hela cells treated for 24 hours at 20 μM concentration with compound 16, 14, vehicle or control compound (Blasticidin S). Cells are visually not affected after treatment with compound 16 and 14. Pictures show representative 20 x micrographs.
Attachment: NMR Files and LC chromatograms
Chromatogram and Results

Injection Details

| Injection Name: | AD-153(3)-fr27-repeat02 | Run Time (min): | 23.48 |
|-----------------|-------------------------|-----------------|-------|
| Vial Number:    | GD1                     | Injection Volume: | 20.00 |
| Injection Type: | Unknown                 | Channel:        | UV_VIS_3 |
| Calibration Level: |                         | Wavelength: | 400 |
| Instrument Method: | AD_10000-0595_30_ESI (without buffer)_PIX002 | Bandwidth: | 4 |
| Processing Method: | MS standard             | Dilution Factor: | 1.0000 |
| Injection Date/Time: | 12/Apr/18 12:24         | Sample Weight: | 1.0000 |

Chromatogram

Integration Results

| No. | Peak Name | Retention Time min | Area mAU*min | Height mAU | Relative Area % | Relative Height % | Amount n.a. |
|-----|-----------|--------------------|--------------|------------|-----------------|-------------------|-------------|
| 1   |           | 16.890             | 13.715       | 95.024     | 100.00          | 100.00            | n.a.        |
| Total: |          |                    | 13.715       | 95.024     | 100.00          | 100.00            |             |

HPLC traces of Compound 9 (mixture of regioisomers; 400 nm)
HPLC traces of Compound 9 (mixture of regioisomers; 260 nm)

Injection Details

Injection Name: AD-153(3)-fr27-repeat02  Run Time (min): 23.48
Vial Number: GD1  Injection Volume: 20.00
Injection Type: Unknown  Channel: UV_VIS_1
Calibration Level: Wavelength: 260
Instrument Method: AD_10000-0595_30_ESI (without buffer)_PPIX002  Bandwidth: 2
Processing Method: MS standard  Dilution Factor: 1.0000
Injection Date/Time: 12/Apr/18 12:24  Sample Weight: 1.0000

Chromatogram

Integration Results

| No. | Peak Name | Retention Time min | Area mAU*min | Height mAU | Relative Area % | Relative Height % | Amount n.a. |
|-----|-----------|--------------------|--------------|------------|-----------------|-------------------|-------------|
| 1   |           | 16.890             | 1.032        | 7.664      | 100.00          | 100.00           | n.a.        |
| Total: |          |                    | 1.032        | 7.664      | 100.00          | 100.00           |             |
Chromatogram and Results

**Injection Details**

- **Injection Name:** AD-168-fr42
- **Vial Number:** BE1
- **Injection Type:** Unknown
- **Calibration Level:** Wavelength: 400
- **Instrument Method:** AD_10000-0595_30_ESI (without buffer)_PPIX002
- **Processing Method:** MS standard
- **Injection Date/Time:** 28/May/18 19:36

**Integration Results**

| No. | Peak Name  | Retention Time min | Area mAU*min | Height mAU | Relative Area % | Relative Height % | Amount n.a. |
|-----|------------|--------------------|--------------|------------|-----------------|-------------------|-------------|
| 1   |            | 16.283             | 18.650       | 121.718    | 100.00          | 100.00            | n.a.        |
| Total |           |                    | 18.650       | 121.718    | 100.00          | 100.00            |             |
HPLC traces of Compound 10 (mixture of regioisomers; 260 nm)

**Chromatogram and Results**

**Injection Details**
- **Injection Name:** AD-168-fr42
- **Run Time (min):** 43.00
- **Vial Number:** BE1
- **Injection Volume:** 20.00
- **Injection Type:** Unknown
- **Channel:** UV_VIS_1
- **Calibration Level:** Wavelength: 260
- **Instrument Method:** AD_10000-0595_30_ESI (without buffer)_PPIX002
- **Bandwidth:** 2
- **Processing Method:** MS standard
- **Dilution Factor:** 1.0000
- **Injection Date/Time:** 28/May/18 19:36
- **Sample Weight:** 1.0000

**Chromatogram**

**Integration Results**

| No. | Peak Name | Retention Time min | Area mAU*min | Height mAU | Relative Area % | Relative Height % | Amount n.a. |
|-----|-----------|--------------------|--------------|------------|----------------|-------------------|-------------|
| 1   |           | 16.283             | 1.478        | 9.894      | 100.00         | 100.00           | n.a.       |
| Total: |           |                    | 1.478        | 9.894      | 100.00         | 100.00           |             |
HPLC traces of Compound 11 (400 nm)

Chromatogram and Results

In the provided chromatogram, the following details are recorded:

**Injection Details**
- **Injection Name:** AD-168-fr7
- **Run Time (min):** 36.00
- **Vial Number:** BD1
- **Injection Volume:** 20.00 µL
- **Injection Type:** Unknown
- **Channel:** UV_VIS_3
- **Calibration Level:** Wavelength: 400 nm
- **Instrument Method:** AD_PPIX002_TFA
- **Bandwidth:** 4
- **Processing Method:** MS standard
- **Dilution Factor:** 1.0000
- **Injection Date/Time:** 15/May/18 14:01
- **Sample Weight:** 1.0000 g

**Integration Results**

| No. | Peak Name | Retention Time (min) | Area (mAU*min) | Height (mAU) | Relative Area (%) | Relative Height (%) | Amount (n.a.) |
|-----|-----------|----------------------|----------------|-------------|-------------------|--------------------|--------------|
| 1   |           | 11.730               | 254.926        | 1494.144    | 100.00            | 100.00             | n.a.         |

**Total:**
- Area: 254.926 mAU
- Height: 1494.144 mAU
- Relative Area: 100.00%
- Relative Height: 100.00%

**Chromatogram**

The chromatogram shows the integrated peak at 11.730 minutes with a peak area of 254.926 mAU and a peak height of 1494.144 mAU.
Chromatogram and Results

Injection Details

Injection Name: AD-168-fr7  Run Time (min): 36.00
Vial Number: BD1 Injection Volume: 20.00
Injection Type: Unknown  Channel: UV_VIS_1
Calibration Level: AD_PPIX002_TFA Wavelength: 260
Instrument Method: AD_PPIX002_TFA Bandwidth: 2
Processing Method: MS standard Dilution Factor: 1.0000
Injection Date/Time: 15/May/18 14:01 Sample Weight: 1.0000

Chromatogram

Integration Results

| No. | Peak Name | Retention Time min | Area mAU*min | Height mAU | Relative Area % | Relative Height % | Amount n.a. |
|-----|-----------|--------------------|--------------|-----------|-----------------|-------------------|-------------|
| 1   |           | 11.730             | 12.347       | 76.392    | 100.00          | 100.00           | n.a.        |
| Total: |          |                    | 12.347       | 76.392    | 100.00          | 100.00           |             |

HPLC traces of Compound 11 (260 nm)
**Chromatogram and Results**

**Injection Details**

| Parameter                  | Value                        |
|----------------------------|------------------------------|
| Injection Name             | AD-210-final_HCl             |
| Vial Number                | BE1                          |
| Injection Type             | Unknown                      |
| Calibration Level          |                               |
| Instrument Method          | AD_10000-0595_30_ESI (without buffer)_PPIX002 |
| Processing Method          | MS standard                  |
| Injection Date/Time        | 13/Dec/18 16:04              |
| Run Time (min)             | 43.00                        |
| Injection Volume           | 4.00                         |
| Channel                    | UV_VIS_1                     |
| Wavelength                 | 260                          |
| Bandwidth                  | 2                            |
| Dilution Factor            | 1.0000                       |
| Sample Weight              | 1.0000                       |
| Amount                      | n.a.                         |

**Chromatogram**

**Integration Results**

| No. | Peak Name | Retention Time min | Area mAU*min | Height mAU | Relative Area % | Relative Height % | Amount n.a. |
|-----|-----------|--------------------|--------------|-----------|-----------------|-------------------|-------------|
| 1   | 1 - 18.440| 18.440             | 8.335        | 33.457    | 100.00          | 100.00            | n.a.        |
| Total|           |                    | 8.335        | 33.457    | 100.00          | 100.00            |             |

HPLC traces of Compound 12 (mixture of regioisomers; 260 nm)
HPLC traces of Compound 13 (400 nm)

**Chromatogram and Results**

**Injection Details**

| Injection Name:            | AD-169-fr-30       |
|----------------------------|--------------------|
| Vial Number:               | BC1                |
| Injection Type:            | Unknown            |
| Calibration Level:         |                    |
| Instrument Method:         | AD_10000-0595_30_ESI (without buffer)_PPIX002 |
| Processing Method:         | MS standard        |
| Injection Date/Time:       | 29/May/18 20:49    |

**Integration Results**

| No. | Peak Name | Retention Time (min) | Area (mAU*min) | Height (mAU) | Relative Area (%) | Relative Height (%) | Amount (n.a.) |
|-----|-----------|----------------------|----------------|--------------|-------------------|---------------------|---------------|
| 1   |           | 20.813               | 24.331         | 152.037      | 100.00            | 100.00              | n.a.          |

**Total:**

|                   | 24.331 | 152.037 | 100.00 | 100.00 | n.a. |

**Chromatogram**

[C4_Amit #423 [manually integrated] AD-169-fr-30 UV_VIS_3 WVL 400 nm]

**Integration Results**

| No. | Peak Name | Retention Time (min) | Area (mAU*min) | Height (mAU) | Relative Area (%) | Relative Height (%) | Amount (n.a.) |
|-----|-----------|----------------------|----------------|--------------|-------------------|---------------------|---------------|
| 1   |           | 20.813               | 24.331         | 152.037      | 100.00            | 100.00              | n.a.          |

**Total:**

|                   | 24.331 | 152.037 | 100.00 | 100.00 | n.a. |
**Chromatogram and Results**

**Injection Details**

| Parameter                      | Details                                      |
|-------------------------------|----------------------------------------------|
| Injection Name                | AD-169-fr-30                                 |
| Vial Number                   | BC1                                          |
| Injection Type                | Unknown                                      |
| Calibration Level             | Wavelength: 260                              |
| Instrument Method             | AD_10000-0595_30_ESI (without buffer)_PPIX002|
| Processing Method             | MS standard                                  |
| Injection Date/Time           | 29/May/18 20:49                              |

**Chromatogram**

- **Retention Time:** 20.813 min
- **Area:** 3.393 mAU*min
- **Height:** 21.610 mAU
- **Relative Area:** 100.00%
- **Relative Height:** 100.00%

**Integration Results**

| No. | Peak Name      | Retention Time | Area  | Height | Relative Area | Relative Height | Amount |
|-----|----------------|----------------|-------|--------|---------------|-----------------|--------|
| 1   |                | 20.813         | 3.393 | 21.610 | 100.00        | 100.00         | n.a.   |

**Total:**

|                | 3.393 | 21.610 | 100.00 | 100.00 |
### Injection Details

| Parameter                        | Value                          |
|----------------------------------|--------------------------------|
| Injection Name                   | AD-178-fr.16                   |
| Vial Number                      | BB2                            |
| Injection Type                   | Unknown                        |
| Calibration Level                | UV_VIS_3                       |
| Instrument Method                | AD_10000-0595_30_ESI (without buffer)_BODIPY |
| Processing Method                | MS standard                    |
| Injection Date/Time              | 18/Jul/18 13:39                |
| Run Time (min)                   | 35.00                          |
| Injection Volume                 | 7.00                           |
| Channel                          | UV_VIS_3                       |
| Wavelength                       | 500                            |
| Bandwidth                        | 4                              |
| Dilution Factor                  | 1.0000                         |
| Sample Weight                    | 1.0000                         |

### Chromatogram

![Chromatogram Image]

### Integration Results

| No. | Peak Name | Retention Time min | Area mAU*min | Height mAU | Relative Area % | Relative Height % | Amount n.a. |
|-----|-----------|--------------------|--------------|------------|-----------------|-------------------|-------------|
| 1   |           | 19.920             | 88.103       | 1364.543   | 100.00          | 100.00           | n.a.        |

**Total:**

88.103, 1364.543, 100.00, 100.00

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*HPLC traces of Compound 14 (500 nm)*

Instrument: HPLC_MS  Sequence: aQ_Amit

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*Default/Integration*

Chromeleon (c) Dionex

Version 7.2.5.9717

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LC 11
Chromatogram and Results

Injection Details

Injection Name: AD-178-fr.16  Run Time (min): 35.00
Vial Number: BB2  Injection Volume: 7.00
Injection Type: Unknown  Channel: UV_VIS_1
Calibration Level:  Wavelength: 260
Instrument Method: AD_10000-0595_30_ESI (without buffer)_BODIPY  Bandwidth: 2
Processing Method: MS standard  Dilution Factor: 1.0000
Injection Date/Time: 18/Jul/18 13:39  Sample Weight: 1.0000

Chromatogram

Integration Results

| No. | Peak Name | Retention Time min | Area mAU*min | Height mAU | Relative Area % | Relative Height % | Amount n.a. |
|-----|-----------|--------------------|--------------|-----------|----------------|------------------|------------|
| 1   |           | 19.917             | 34.648       | 554.716   | 100.00         | 100.00           | n.a.       |
| Total |          |                    | 34.648       | 554.716   | 100.00         | 100.00           |             |
HPLC traces of Compound 15 (658 nm)

Chromatogram and Results

**Injection Details**

- **Injection Name:** AD-172-fr.15
- **Run Time (min):** 43.00
- **Vial Number:** BE5
- **Injection Volume:** 25.00
- **Injection Type:** Unknown
- **Channel:** UV_VIS_4
- **Calibration Level:** Wavelength: 658
- **Instrument Method:** AD_10000-0595_30_ESI (without buffer)_MB003
- **Bandwidth:** 4
- **Processing Method:** MS standard
- **Dilution Factor:** 1.0000
- **Injection Date/Time:** 05/Jun/18 09:43
- **Sample Weight:** 1.0000

**Integration Results**

| No. | Peak Name | Retention Time min | Area mAU*min | Height mAU | Relative Area % | Relative Height % | Amount n.a. |
|-----|-----------|--------------------|--------------|------------|-----------------|-------------------|-------------|
| 1   |           | 18.430             | 6.086        | 18.184     | 100.00          | 100.00           | n.a.        |
| Total|           |                    | 6.086        | 18.184     | 100.00          | 100.00           | n.a.        |
## Chromatogram and Results

### Injection Details

| Parameter                      | Value                  |
|-------------------------------|------------------------|
| Injection Name                | AD-172-fr.15           |
| Vial Number                   | BE5                    |
| Injection Type                | Unknown                |
| Calibration Level             | Wavelength: 260        |
| Instrument Method             | AD_10000-0595_30_ESI (without buffer)_MB003 |
| Processing Method             | MS standard            |
| Injection Date/Time           | 05/Jun/18 09:43        |

### Chromatogram

![Chromatogram](image)

### Integration Results

| No. | Peak Name  | Retention Time min | Area mAU*min | Height mAU | Relative Area % | Relative Height % | Amount n.a. |
|-----|------------|--------------------|--------------|------------|-----------------|-------------------|-------------|
| 1   |            | 18.430             | 0.588        | 2.091      | 100.00          | 100.00            | n.a.        |

Total: 0.588 2.091 100.00 100.00
**Injection Details**

| Parameter                  | Value                        |
|----------------------------|------------------------------|
| Injection Name:            | AD-180-fr15                  |
| Vial Number:               | BA2                          |
| Injection Type:            | Unknown                      |
| Calibration Level:         | UV_VIS_3                     |
| Instrument Method:         | AD_10000-0595_30_ESI (without buffer)_BODIPY |
| Processing Method:         | MS standard                  |
| Injection Date/Time:       | 06/Aug/18 17:56              |
| Run Time (min):            | 35.00                        |
| Injection Volume:          | 10.00                        |
| Channel:                   | UV_VIS_3                     |
| Wavelength:                | 500                          |
| Bandwidth:                 | 4                            |
| Dilution Factor:           | 1.0000                       |
| Sample Weight:             | 1.0000                       |

**Chromatogram**

**Integration Results**

| No. | Peak Name | Retention Time (min) | Area (mAU*min) | Height (mAU) | Relative Area (%) | Relative Height (%) | Amount (n.a.) |
|-----|-----------|----------------------|----------------|-------------|-------------------|--------------------|---------------|
| 1   | aQ_Amit   | 19.467               | 126.001        | 1787.914    | 100.00            | 100.00             | n.a.          |
|     | Total     |                      | 126.001        | 1787.914    | 100.00            | 100.00             |               |
Chromatogram and Results

Injection Details

| Injection Name         | AD-180-fr15          | Run Time (min): 35.00 |
|------------------------|----------------------|-----------------------|
| Vial Number            | BA2                  | Injection Volume: 10.00 |
| Injection Type         | Unknown              | Channel: UV_VIS_1     |
| Calibration Level      |                      | Wavelength: 260       |
| Instrument Method      | AD_10000-0595_30_ESI(without buffer)_BODIPY | Bandwidth: 2 |
| Processing Method      | MS standard          | Dilution Factor: 1.0000 |
| Injection Date/Time    | 06/Aug/18 17:56      | Sample Weight: 1.0000 |

Chromatogram

Integration Results

| No. | Peak Name | Retention Time (min) | Area (mAU*min) | Height (mAU) | Relative Area (%) | Relative Height (%) | Amount (n.a.) |
|-----|-----------|----------------------|----------------|--------------|-------------------|--------------------|---------------|
| 1   |           | 19.463               | 21.197         | 301.513      | 100.00            | 100.00             | n.a.          |

Total: 21.197 301.513 100.00 100.00
**Chromatogram and Results**

### Injection Details

| Parameter                  | Value                  |
|----------------------------|------------------------|
| Injection Name             | blank 101              |
| Vial Number                | BA1                    |
| Injection Type             | Unknown                |
| Calibration Level          |                        |
| Instrument Method          | AD_10000-0595_30_ESI (without buffer)_PPIX002 |
| Processing Method          | MS standard            |
| Injection Date/Time        | 29/May/18 06:35        |
| Instrument Method          | AD_10000-0595_30_ESI (without buffer)_PPIX002 |
| Channel                    | UV_VIS_3               |
| Wavelength                 | 400                    |
| Bandwidth                  | 4                      |
| Dilution Factor            | 1.0000                 |
| Sample Weight              | 1.0000                 |
| Run Time (min)             | 43.00                  |
| Injection Volume           | 20.00                  |
| Injection Type             | Unknown                |
| Channel                    | UV_VIS_3               |
| Calibr. Level              |                        |

### Chromatogram

![Chromatogram](image)

### Integration Results

| No. | Peak Name | Retention Time (min) | Area [mAU*min] | Height [mAU] | Relative Area [%] | Relative Height [%] | Amount [n.a.] |
|-----|-----------|----------------------|----------------|--------------|------------------|--------------------|---------------|
| Total: |           |                      | 0.000          | 0.000        | 0.00             | 0.00               |               |

**HPLC traces of blank sample (400 nm)**
HPLC traces of blank sample (260 nm)

Injection Details

Injection Name: blank 101  
Vial Number: BA1  
Injection Type: Unknown  
Calibration Level:  
Instrument Method: AD_10000-0595_30_ESI (without buffer)_PPIX002  
Processing Method: MS standard  
Injection Date/Time: 29/May/18 06:35  
Run Time (min): 43.00  
Injection Volume: 20.00  
Channel: UV_VIS_1  
Wavelength: 260  
Bandwidth: 2  
Dilution Factor: 1.0000  
Sample Weight: 1.0000

Chromatogram

Integration Results

| No. | Peak Name     | Retention Time | Area  | Height | Relative Area | Relative Height | Amount |
|-----|---------------|----------------|-------|--------|---------------|-----------------|--------|
|     |               | min            | mAU*min | mAU    | %             | %            | n.a.   |
| Total|               |                | 0.000  | 0.000  | 0.00          | 0.00           |        |

Instrument:HPLC_MS   Sequence:C4_Amit
$^1$H of Compound 9 (mixture of regioisomers; DMSO-$d_6$)
$^{13}$C of Compound 9 (mixture of regioisomers; DMSO-$d_6$)
2D NMR analysis of Compound 9

Trehalose unit

- $H_d = \text{Trehalose anomeric C-H}$
- $H_g = \text{Trehalose CH}_2$
- $H_h = \text{Trehalose C-H (except } H_d)$

Porphyran unit

- $H_a = \text{Porphyran ring C-H}$
- $H_e = \text{Porphyran CH}_2$
- $H_f = \text{Porphyran CH}_3$
$^1$H-$^{13}$C HSQC of Compound 9 (mixture of regioisomers; DMSO-$d_6$)
$^1$H-$^{13}$C HMBC of Compound 9 (mixture of regioisomers; DMSO-$d_6$)

$H_a$, $H_b$, $H_c$, $H_d$, $H_e$

$^1J_{C-H}$

NMR 5
$^1$H of Compound 10 (mixture of regioisomers; DMSO-$d_6$)
$^{13}$C of Compound 10 (mixture of regioisomers; DMSO-$d_6$)
2D NMR analysis of Compound 10

**Trehalose unit**
- $H_d =$ Trehalose anomeric C-H
- $H_g =$ Trehalose CH$_2$
- $H_h =$ Trehalose C-H (except $H_d$)
- $H_i =$ Trehalose CH$_3$

**Porphyrin unit**
- $H_a =$ Porphyrin ring C-H
- $H_e =$ Porphyrin CH$_2$
- $H_f =$ Porphyrin CH$_3$
DQF-COSY of Compound 10 (mixture of regioisomers; DMSO-d$_6$)

DuJeFe05-411402,Dutta,AD-205-MC,DMSO,DQF-COSY
$^{1}$$H^{13}$C HSQC of Compound 10 (mixture of regioisomers; DMSO-$d_6$)
$^1$H of Compound 11 (DMSO-$d_6$)
$^1$H of Compound 12 (mixture of regioisomers; DMSO-$d_6$)

![Chemical Structure](image)

Porphyrid NH
$^{13}$C of Compound 12 (mixture of regioisomers; DMSO-$d_6$)
2D NMR analysis of Compound 12

Trehalose unit

H_d = Trehalose anomeric C-H

Porphyrin unit

H_e = Porphyrin CH_2
DQF-COSY of Compound 12 (mixture of regioisomers; DMSO-$d_6$)
$^1$H of Compound 13 (DMSO-$d_6$)

Porphyrin NH

N

N

H

N

H

O

O

H

O

H

O

H

O

H

N

H

Porphyrin NH

f1 (ppm)

9.0 8.8 8.6 8.4 8.2 8.0 7.8 7.6

f1 (ppm)

11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 0.0
$^{13}$C of Compound 13 (DMSO-$d_6$)
$^1$H of Compound 14 (DMSO-$d_6$)
$^{13}$C of Compound 14 (DMSO-$d_6$)
$^{11}$B of Compound 14 (DMSO-$d_6$)
$^1$H of Compound 15 (DMSO-$d_6$)

![Chemical Structure of Compound 15]

NMR Spectra:
- $f_1$ (ppm) values: 1.96, 1.16, 4.01
- $f_1$ (ppm) values: 8.0, 7.9, 7.8, 7.7, 7.6, 7.5
- $f_1$ (ppm) values: 4.90, 4.88, 4.86, 4.84
- $f_1$ (ppm) values: 2.95, 3.00, 3.05, 3.10, 3.15, 3.20, 3.25

Other NMR Parameters:
- $f_1$ (ppm) values: 3.26, 2.13, 2.26, 1.01, 1.02, 2.08, 3.36, 3.55, 10.49
- $f_1$ (ppm) values: 4.01, 1.16, 1.96
- $f_1$ (ppm) values: 2.08, 1.02, 1.01
- $f_1$ (ppm) values: 3.25, 3.20, 3.15, 3.10, 3.05, 3.00, 2.95
- $f_1$ (ppm) values: 2.36, 2.2, 2.1, 2.0, 1.9, 1.8
$^{13}$C of Compound 15 (DMSO-$d_6$)
2D NMR analysis of Compound 15

$H_a$ = Aromatic C-H
$H_b$ = Trehalose anomeric C-H
$H_h$ = Trehalose C-H (except $H_b$)
$H_i$ = Trehalose CH$_2$
DQF-COSY of Compound 15 (mixture of regioisomers; DMSO-$d_6$)
$^1$H-$^{13}$C HSQC of Compound 15 (DMSO-$d_6$)
$^{1}H^{13}C$ HMBC of Compound 15 (DMSO-$d_6$)
$^1$H of Compound 16 (MeOH-d$_4$)
$^{13}$C of Compound 16 (MeOH-$d_4$)
$^{11}$B of Compound 16 (MeOH-$d_4$)