Supporting Information

Trehalose-conjugation enhances toxicity of photosensitizers against mycobacteria

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Abbreviations

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* mc^{2}155, *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/D_2O (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, Philipps IP65 SON T 150W, 10 mW/cm^2) 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 (E_x/E_m=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 (OD_{600} ~ 1.0) by centrifugation (10 min, 3000 x g). Cells were diluted in culture broth to an OD_{600} 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, Philipps IP65 SON T 150W, 10 mW/cm^2) 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% CO_2). 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% CO_2). Cells were trypsinized, washed in DMEM and seeded in 12-well plates (0.1 x 10^6 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 Scientfic).

Lipid analysis by thin-layer chromatography (TLC). Pre-cultures (5 mL) of *M. smegmatis* mc^2155 were grown for two days and harvested by centrifugation (10 min, 3000 x g). Subsequently, cells were diluted to an OD_{600} of 0.1 in 10 mL 7H9 medium complemented with Tween 80 and ADC enrichment and incubated with the indicted 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 ddH_2O (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/H_2O 20:4:0.5). Lipids were visualized with $CuSO_4$ (10% $CuSO_4$ 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 ^{14}C acetate and lipid analysis by TLC and autoradiography. *M. smegmatis* mc^2155 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 OD_{600} ~ 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 ^{14}C-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 OD_{600} 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 ^{14}C-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 $CHCl_3$:MeOH (1:2); 56°C; 1.5 h followed by 3 mL $CHCl_3$:MeOH (2:1); 56°C; 1.5 h. The extracts were combined, dried under N_2 and the lipids were subjected to biphasic washing in $CHCl_3$:MeOH:H_2O (4:2:1).[2] The bottom organic phase was dried under N_2 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 $CHCl_3$:MeOH:H_2O (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 ^{14}C 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 $CHCl_3$:MeOH:H_2O (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 $CHCl_3$:MeOH (2:1). The extracts were combined together, dried under N_2 and dissolved in 50 µL of $CHCl_3$: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 $CHCl_3$: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* mc^{2}155::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 SphI and AclI sites of pGrna. The resulting plasmid pGrna_*lpqY* was transformed into *M. smegmatis* mc^{2}155::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* mc^{2}155::dCas9::pGrna_*lpqY* or II) of *M. smegmatis* mc^{2}155 in the presence of the Ag85 complex inhibitor Ebselen.

I) Pre-cultures (5 mL) of *M. smegmatis* mc^{2}155::dCas9::pGrna (empty plasmid) and *M. smegmatis* mc^{2}155::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* mc^{2}155::dCas9::pGrna_*lpqY* and *M. smegmatis* mc^{2}155::dCas9::pGrna cells were diluted to OD_{600} 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 OD_{600} of 0.3 and incubated with the indicated compound at 10 µM concentration for 6 hours.

II) Pre-cultures (5 mL) of *M. smegmatis* mc^{2}155 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* mc^{2}155 wild-type cells were pre-treated with 100 µg/mL Ebselen for 3 hours (10 mL culture volume, OD_{600} of 0.4). Subsequently, cells were re-diluted at an OD_{600} 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 washes twice with ddH_2O (2x5 mL). The remaining pellet was used for lipid extraction using 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 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	
Name	**Source**
M. smegmatis mc^{2}155	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 mc^{2}155::dCas9	*M. smegmatis* mc^{2}155 transformed with pTetInt-*dcas9*
Plasmids	
Name	**Source**
pGrna	Choudahary *et al.*
pTetInt-*dcas9*	Choudahary *et al.*
pGrna::*lpqY*	this study
Primers/Oligos	
Name	**Sequence**
Pr368	5‘-CTGGCAGGATGAGCTCTACG-3‘
Pr369	5‘-ATGCCTTCGTACTGCTTGCC-3‘
Pr360 „lpqY_crispr_up“	5‘-GTCGGCCTCGGCGAGACCAG-3‘
Pr361 „lpqY_crispr_down“	5‘-CGCTGGTCTCGCCGAGGCCGACCATG-3‘
mysA_fw	5‘-TTGAGGTGACCGACGATCTCG-3‘
mysA_rev	5‘-ACGCCTTGTCCTTCTCGGAC-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.C_{18} column coupled to a LT-ELSD and 2) an automated RP-MPLC, equipped with an aQ.C_{18} 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.C_{18} column (3 µ, 3 × 150 mm) coupled to a UV and ESI-MS detector and 2) a Hypersil GOLD C_4 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_2O	% CH_3CN
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[b]:

Time (min)	Flow (mL/min)	% A (H_2O:TFA = 99.7:0.3 (v/v))	% B (CH_3CN: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[b]:

Time (min)	Flow (mL/min)	% H_2O	% CH_3CN	%C (H_2O: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[c]:

Time (min)	Flow (mL/min)	% H_2O	% CH_3CN
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

c: UV detection at 658 nm and 260 nm

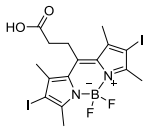
^{1}H-NMR spectra were recorded on Bruker 300 MHz spectrometers, Bruker 400 MHz and Bruker 500 MHz spectrometers in the indicated deuterated solvent. Data are reported as follows: chemical shift (δ, ppm), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad signal), coupling constant(s) (*J*, Hz), integration. All signals were referenced to the internal solvent signal as standard (CD_3OD, δ 3.31; $(CD_3)_2SO$, 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_3OD, δ 49.0; $(CD_3)_2SO$, 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.

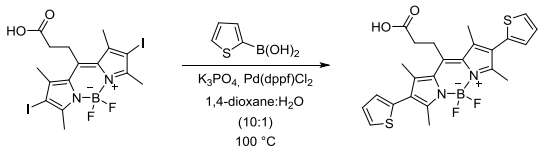
Synthetic procedures

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



Compound **3** was synthesized as described in the literature.[5] The analytical data were identical with the reported literature values.

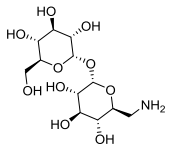
3-(5,5-difluoro-1,3,7,9-tetramethyl-2,8-di(thiophen-2-yl)-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)propanoic acid 4



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_3PO_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_2O/CH_3CN 95:5 v/v, followed by 6-42 min H_2O/CH_3CN gradient till 60:40 v/v, then 42-53 min H_2O/CH_3CN gradient to 0:100 v/v, and finally 53-70 min H_2O/CH_3CN 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.

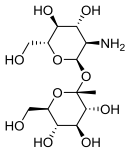
The analytical data were identical with the literature.[6]

(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-2*H*-pyran-2-yl)oxy)tetrahydro-2*H*-pyran-3,4,5-triol 7



Compound **7** was synthesized as described in the literature.[7] The analytical data were identical with the reported literature values.

(2*R*,3*R*,4*S*,5*S*,6*R*)-2-(((2*R*,3*R*,4*R*,5*S*,6*R*)-3-amino-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)-6-(hydroxymethyl)-2-methyltetrahydro-2*H*-pyran-3,4,5-triol <u>8</u>



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

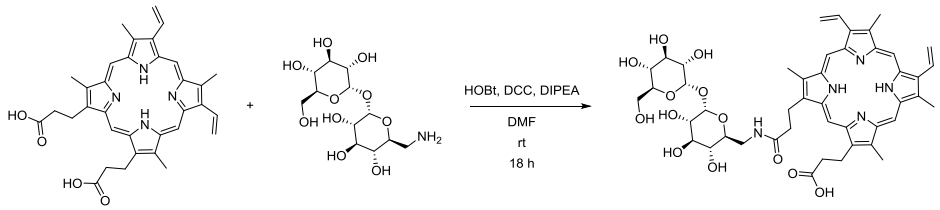
General procedure for the synthesis of trehalose-BODIPY conjugates (<u>GP-1</u>)

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 H_2O/CH_3CN 95:5 v/v, followed by 6-42 min H_2O/CH_3CN gradient till 60:40 v/v, and finally 42-53 min H_2O/CH_3CN 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. C_{18} column (5 µ, 21.2 × 250 mm) column and coupled to a SEDERE 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_2O:TFA, Solvent B: 99:1 v/v CH_3CN: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.

ds3-(2,8,12,17-tetramethyl-3-(3-oxo-3-((((2*S*,3*R*,4*R*,5*S*,6*S*)-3,4,5-trihydroxy-6-(((2*S*,3*S*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)tetrahydro-2*H*-pyran-2-yl)methyl)amino)propyl)-13,18-divinylporphyrin-7-yl)propanoic acid 9

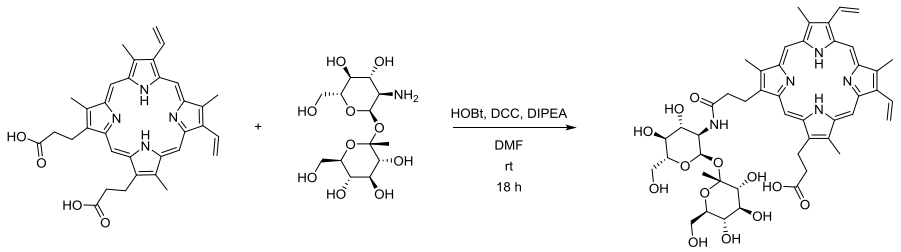


Compound **9** was synthesized and isolated according to the general procedure **GP-2**. Both regioisomers were obtained and not further separated.

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-d6, 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, 135.4, 135.2, 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 $C_{46}H_{54}N_5O_{13}$: 884.3724, observed: 884.3724.

3-(18-(3-(((2*R*,3*R*,4*R*,5*S*,6*R*)-4,5-dihydroxy-6-(hydroxymethyl)-2-(((2*R*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)-2-methyltetrahydro-2*H*-pyran-2-yl)oxy)tetrahydro-2*H*-pyran-3-yl)amino)-3-oxopropyl)-3,8,13,17-tetramethyl-7,12-divinylporphyrin-2-yl)propanoic acid <u>10</u>

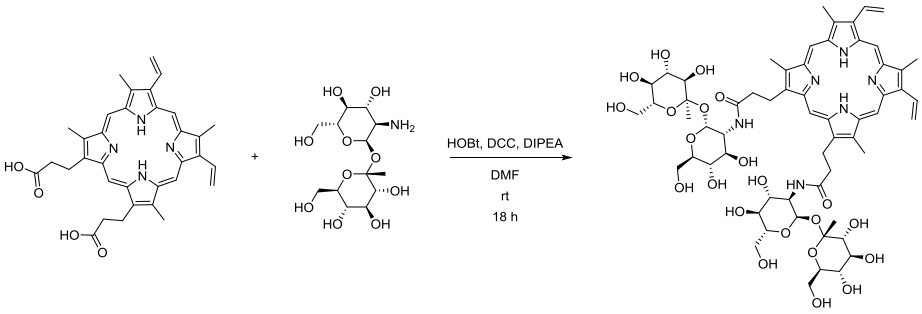


Compound **10** was synthesized according to the general procedure **<u>GP-2</u>**. Both regioisomers were obtained and not further separated.

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 min

^{1}H NMR (400 MHz, DMSO-d_6, mixtures of regioisomers) δ 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); **^{13}C NMR** (101 MHz, DMSO-d_6) δ 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 (2×C), 55.13, 37.9, 36.9, 23.6, 21.6, 12.5 (2×C), 12.2, 11.7, 11.6; **HRMS** (ESI) $[M+H]^+$ calcd. for $C_{47}H_{58}N_5O_{13}$: 900.4026, observed: 900.4020.

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

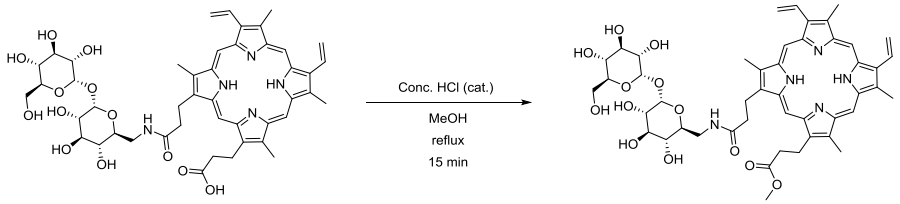


Compound **11** was synthesized and isolated according to the general procedure **<u>GP-2</u>**.

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 min.

^{1}H NMR (400 MHz, DMSO-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 – 3.90 (m, 1H), 3.88 – 3.80 (m, 2H), 3.79 – 3.44 (m, 24H), 3.35 – 3.20 (m, 3H), 3.20 – 2.99 (m, 7H), 2.91 (m, 2H), 1.41 (s, 6H), − 4.34 (br s, 2H); **^{13}C NMR** (101 MHz, DMSO-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, 31.3, 24.2, 23.5, 22.8, 21.7, 20.6, 19.9, 10.8, 10.6, 9.9; **HRMS** (ESI) $[M+H]^+$ calcd. for $C_{60}H_{81}N_6O_{22}$: 1237.5398, observed: 1237.5385.

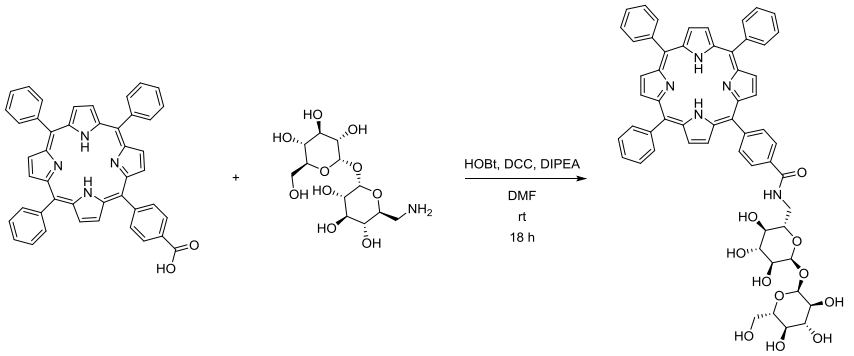
Methyl 3-(2,8,12,17-tetramethyl-3-(3-oxo-3-((((2S,3R,4R,5S,6S)-3,4,5-trihydroxy-6-(((2S,3S,4R,5R,6S)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-2-yl)methyl)amino)propyl)-13,18-divinylporphyrin-7-yl)propanoate 12



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).

^{1}H NMR (400 MHz, DMSO-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); **^{13}C NMR** (101 MHz, DMSO-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 $C_{47}H_{58}N_5O_{13}$: 900.4031, observed: 900.4027.

***N*-(((2*S*,3*R*,4*R*,5*S*,6*S*)-3,4,5-trihydroxy-6-(((2*S*,3*S*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)tetrahydro-2*H*-pyran-2-yl)methyl)-4-(10,15,20-triphenylporphyrin-5-yl)benzamide 13**

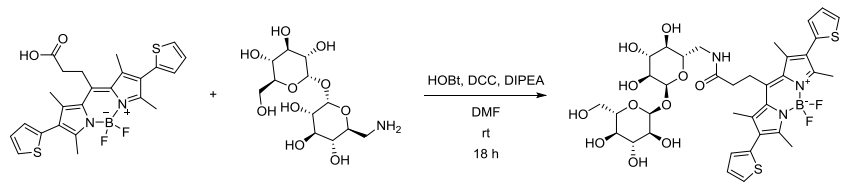


Compound **13** was synthesized according to the general procedure **GP-2** and purified by preparative RP-HPLC (Mobile phase: Solvent A: 99.7:0.3 v/v H_2O:TFA, Solvent B: 99.7:0.3 v/v CH_3CN:TFA; purification method: 0-28 min A/B gradient starting from 95:5 v/v to 60:40 v/v, followed by 28-56 min A/B 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 C_4 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). R_t = 55 min.

^{1}H NMR (500 MHz, DMSO-d_6) 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); **^{13}C NMR** (126 MHz, DMSO-d_6) 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 $C_{57}H_{52}N_5O_{11}$: 982.3658, observed: 982.3655.

3-(5,5-difluoro-1,3,7,9-tetramethyl-2,8-di(thiophen-2-yl)-5*H*-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)-*N*-(((2*S*,3*R*,4*R*,5*S*,6*S*)-3,4,5-trihydroxy-6-(((2*S*,3*S*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)tetrahydro-2*H*-pyran-2-yl)methyl)propanamide 14

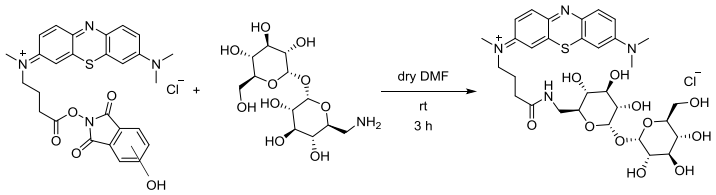


Compound **14** was synthesized and isolated according to the general procedure **GP-1**.

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.

^{1}H 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* = 3.4, 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); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 170.3, 152.9, 138.7, 133.1, 130.6, 128.7, 127.8, 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; **^{11}B NMR** (160 MHz, DMSO-d_6) δ 0.50 (t, *J* = 31.6 Hz, 1B); **HRMS** (ESI) $[M]^-$ calcd. for $C_{36}H_{44}BF_2N_3O_{11}S_2$: 806.2478, observed: 806.2407.

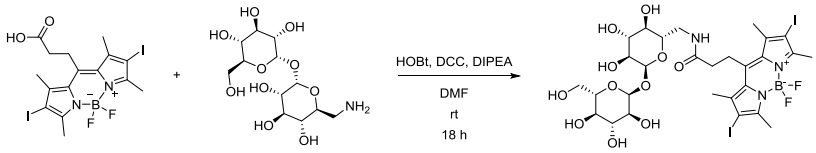
***N*-((*Z*)-7-(dimethylamino)-3*H*-phenothiazin-3-ylidene)-*N*-methyl-4-oxo-4-((((2*S*,3*R*,4*R*,5*S*,6*S*)-3,4,5-trihydroxy-6-(((2*S*,3*S*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)tetrahydro-2*H*-pyran-2-yl)methyl)amino)butan-1-aminiumchloride 15**



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.

^{1}H 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); **^{13}C NMR** (101 MHz, DMSO-d_6) 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-diiodo-1,3,7,9-tetramethyl-5*H*-4λ^4,5λ^4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)-*N*-(((2*S*,3*R*,4*R*,5*S*,6*S*)-3,4,5-trihydroxy-6-(((2*S*,3*S*,4*R*,5*R*,6*S*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)tetrahydro-2*H*-pyran-2-yl)methyl)propanamide <u>16</u>



Compound **16** was synthesized and isolated according to the general procedure **<u>GP-1</u>**.

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). R_t = 52 min.

^{1}H NMR (400 MHz, Methanol-d_4) 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) **^{13}C NMR** (101 MHz, Methanol-d_4) 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; **^{11}B NMR** (128 MHz, Methanol-d_4) δ 0.32 (t, *J* = 31.6 Hz, 1B); **HRMS** (ESI) $[M+Na]^+$ calcd. for $C_{28}H_{38}BF_2I_2N_3O_{11}Na$: 918.0555, observed: 918.0549.

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Supporting Figures

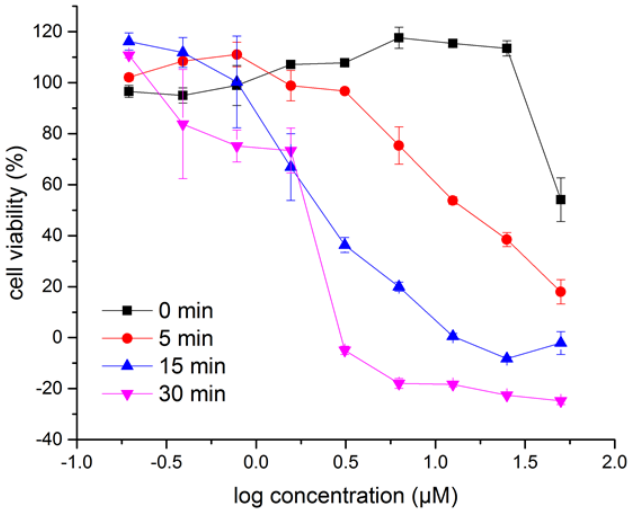


Figure S1: Cell killing efficiency of compound **16** is depended on the applied irradiation time. *M. smegmatis* mc^2155 cells were grown in the presence of compound **16** and irradiated after 24 hours with a high pressure sodium lamp for 0, 5, 15 or 30 min (10 mW/cm^2). Subsequently cell viability was determined using the resazurin reduction assay. Data represents means of triplicate measurements, error bars indicate +/- SEM.

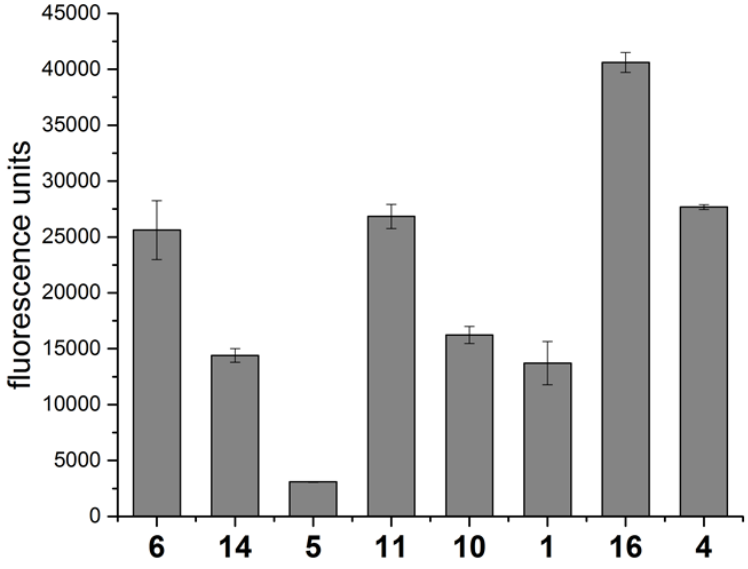


Figure S2: Fluorescence based assay, in which the probe *Singlet Oxygen Sensor Green* (SOSG) is incubated with the respective photosensitizers during irradiation (high pressure sodium lamp, 30 min, 10 mW/cm^2). SOSG selectively reacts with singlet oxygen producing a fluorescent product, which can be quantified following excitation/emission at 504/525 nm. Data represents means of triplicate measurements, error bars indicate +/- SEM.

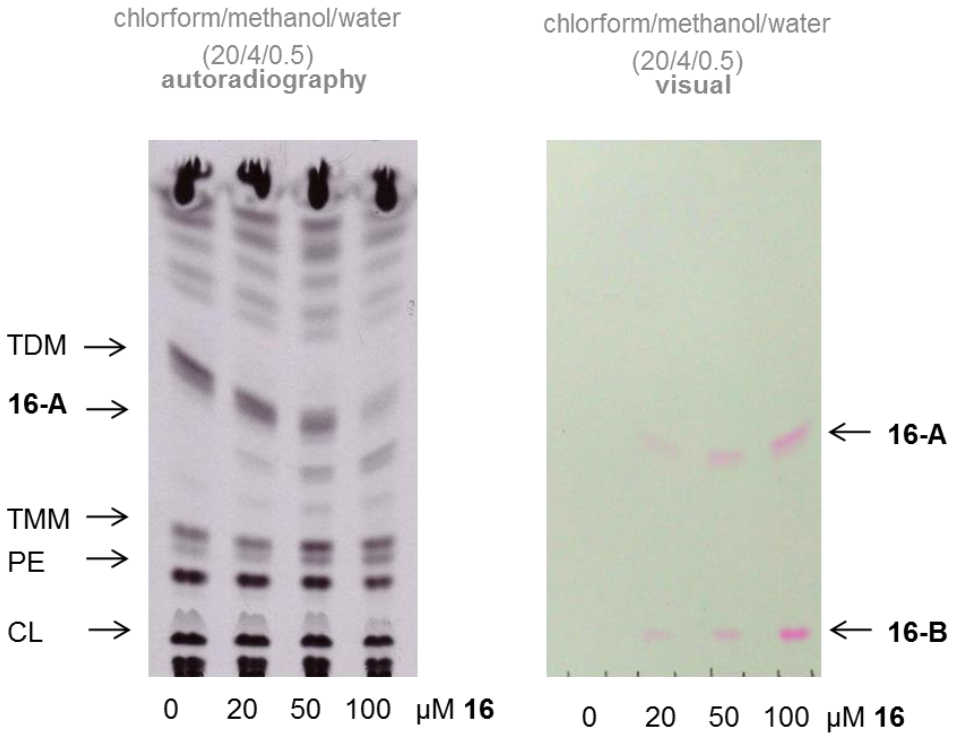


Figure S3: TLC analysis of lipids isolated from *M. smegmatis* mc^{2}155 treated with 6AT-I-BODIPY (**16**) at different concentrations. ^{14}C acetate was added as metabolic label 16 h after addition of **16** and the cells were cultivated for further 3 h. PE: phosphatidylethanolamine, CL: cardiolipin. Two independent experiments were performed, yielding similar results.

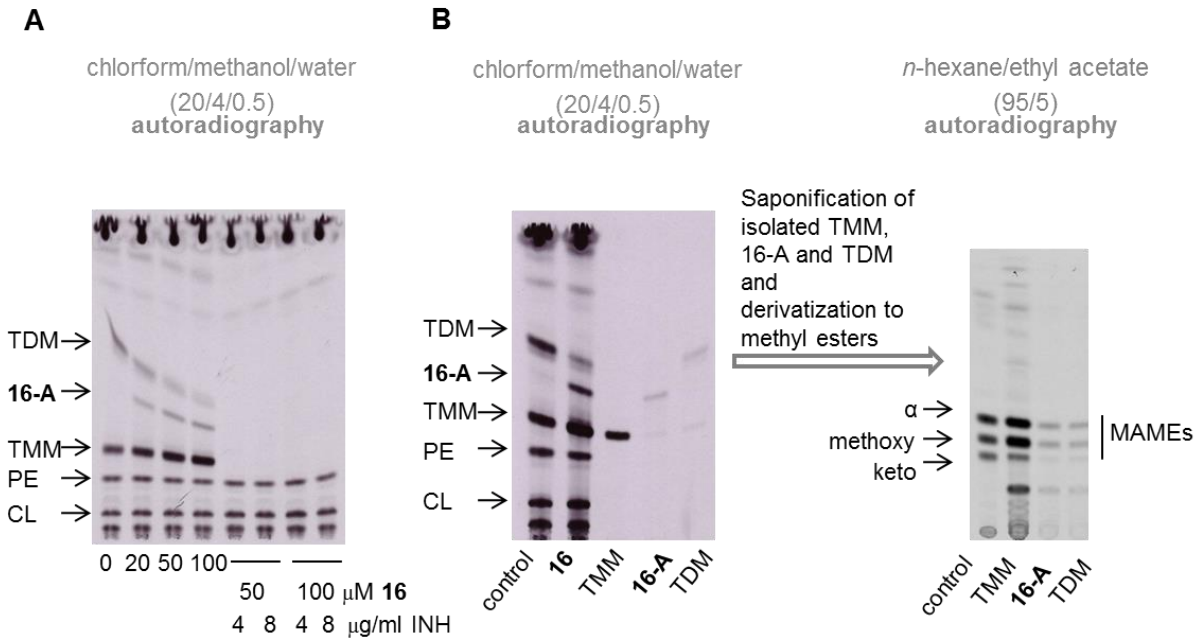


Figure S4: Isolation of lipids after incubation of *M. tuberculosis* H37Ra with 6AT-I-BODIPY (**16)**. A) Analysis of lipids extracted from *M. tuberculosis* H37Ra treated with **16** and **INH**. B*)* Isolated TMM, **16-A** and TDM by preparative TLC and subsequent MAME analysis after saponification. TLC in *n*-hexane/ethyl acetate 3 times run. PE: phosphatidylethanolamine, CL: Cardiolipin. Two independent experiments were performed, yielding similar results.

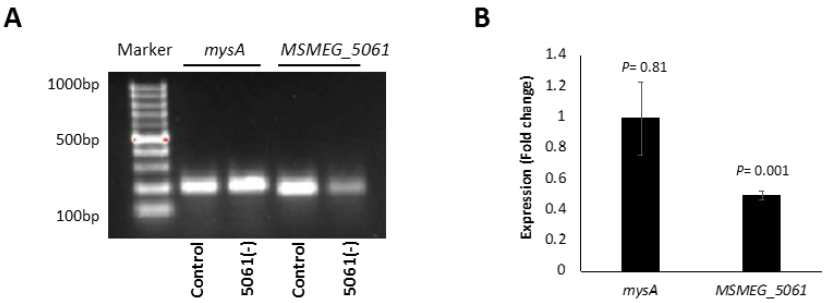


Figure S5: Silencing of *lpqY* in *M. smegmatis*. To achieve the repression of *MSMEG_5061* (*lpqY*), a pair of complementary oligonucleotides specific to the target ORF near 5'-end (Pr360: 5'-GTCGGCCTCGGCGAGACCAG-3' and Pr361: 5'-CGCTGGTCTCGCCGAGGCCGACCATG-3') were synthesized, annealed and cloned in 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^2155 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 $\pm$ S.D. values from three experiments are shown. Statistical significance is determined by paired Student's t-test.

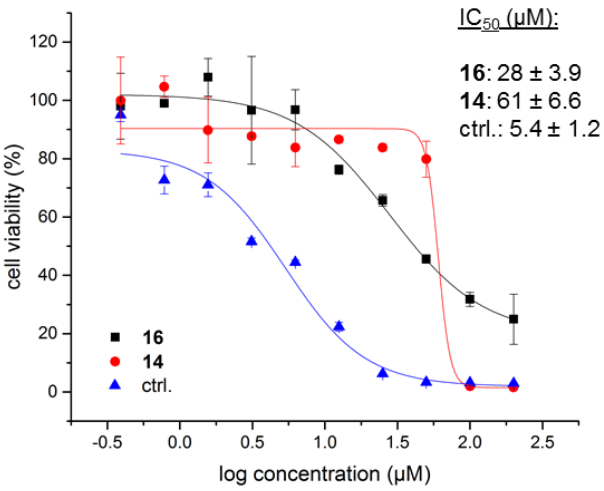


Figure S6: Viability of Hela cells treated with compound **16**, **14** and control compound (Blasticidin S) for 24 hours. Cell viability was assayed by MTT reduction assay (see SI text). Analysis was done in triplicates. Error bars represent +/- SEM.

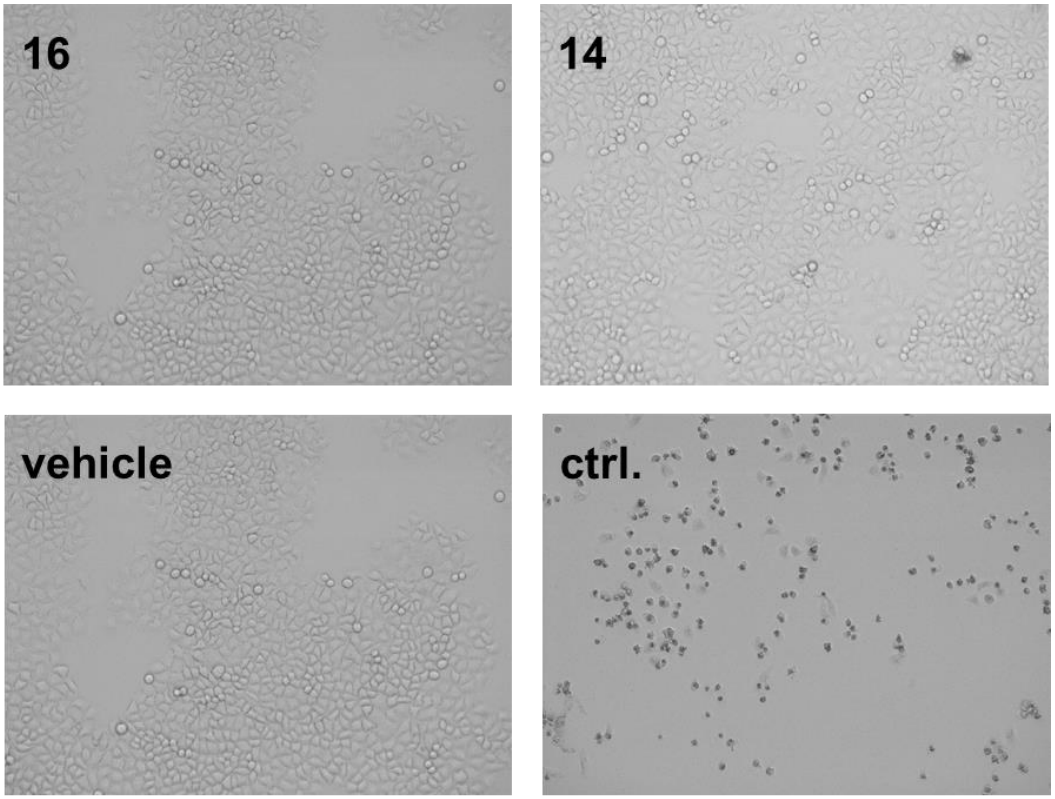


Figure S7: Overall appearance of Hela cells treated for 24 hours at 20 µM concentration with compound **16**, **14,** vehicle or control compound (Blasticidin S). Cells are visually not affected after treatment with compound **16** and **14**. Pictures show representative 20 x micrographs.

Attachment: NMR Files and LC chromatograms

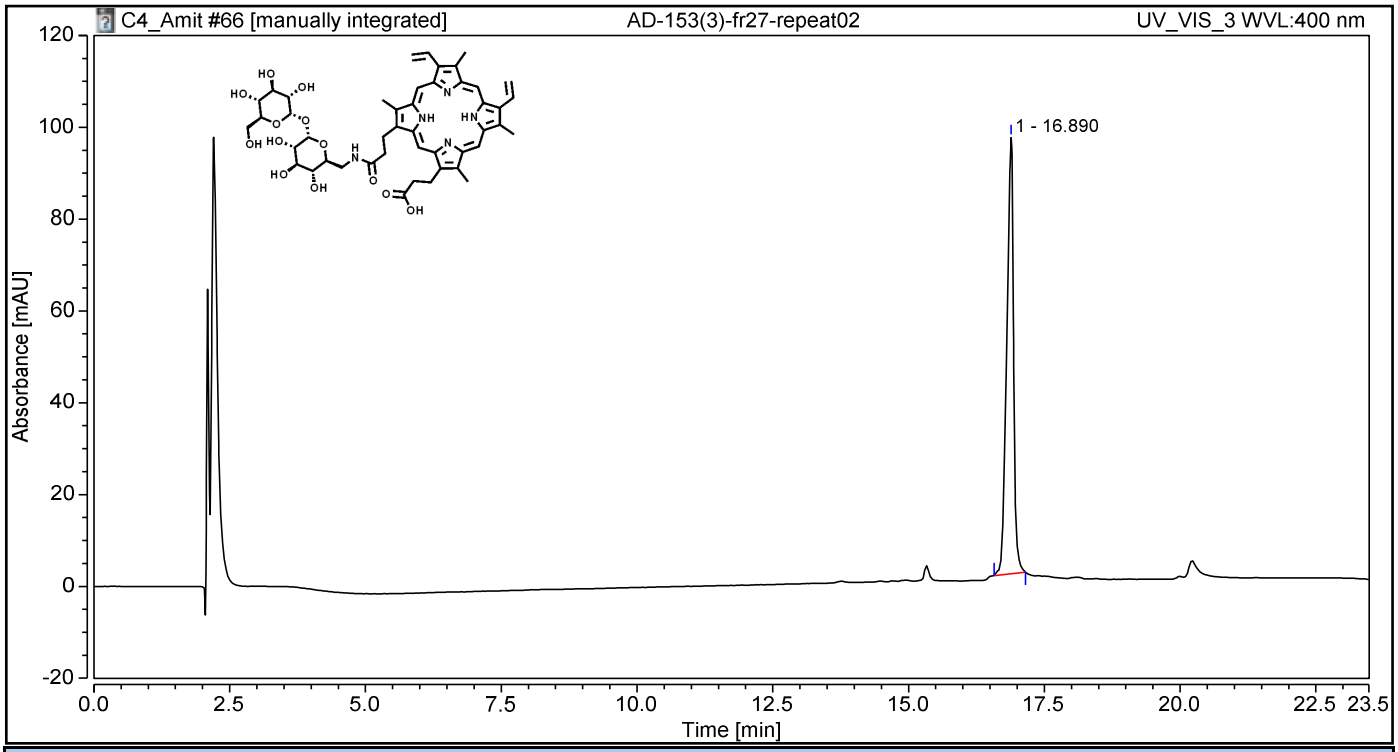
HPLC traces of Compound 9 (mixture of regioisomers; 400 nm)

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)_PPIX002**	*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**	

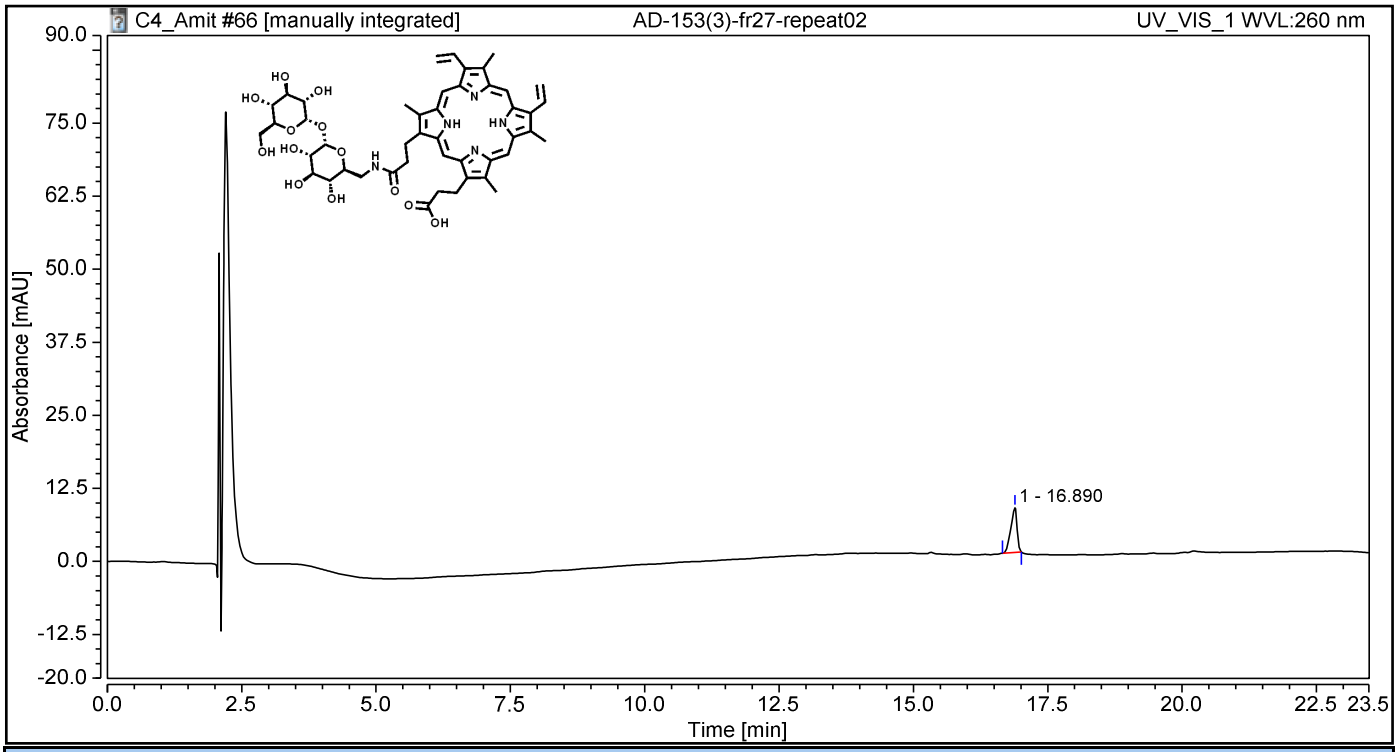
HPLC traces of Compound 9 (mixture of regioisomers; 260 nm)

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_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**	

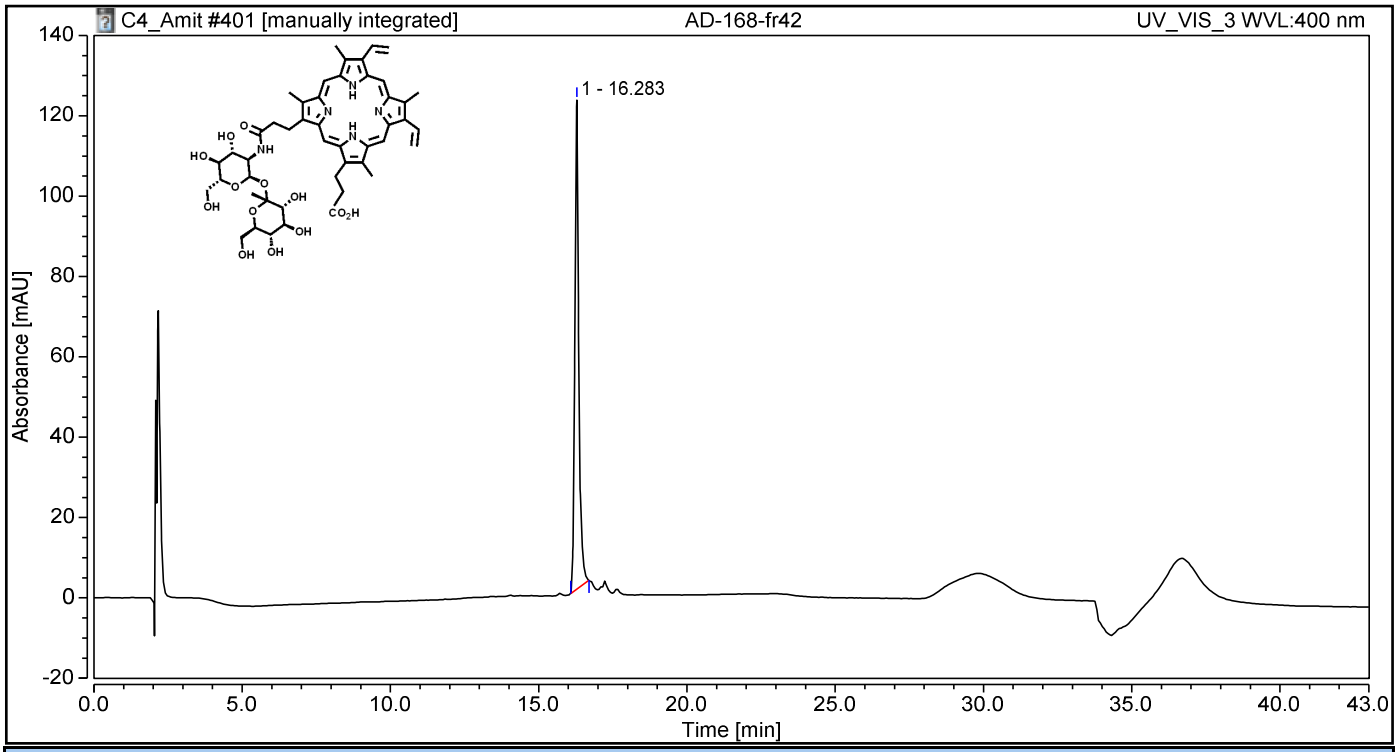
HPLC traces of Compound 10 (mixture of regioisomers; 400 nm)

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_3**
Calibration Level:		*Wavelength:*	**400**
Instrument Method:	**AD_10000-0595_30_ESI (without buffer)_PPIX002**	*Bandwidth:*	**4**
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	18.650	121.718	100.00	100.00	n.a.
Total:			**18.650**	**121.718**	**100.00**	**100.00**	

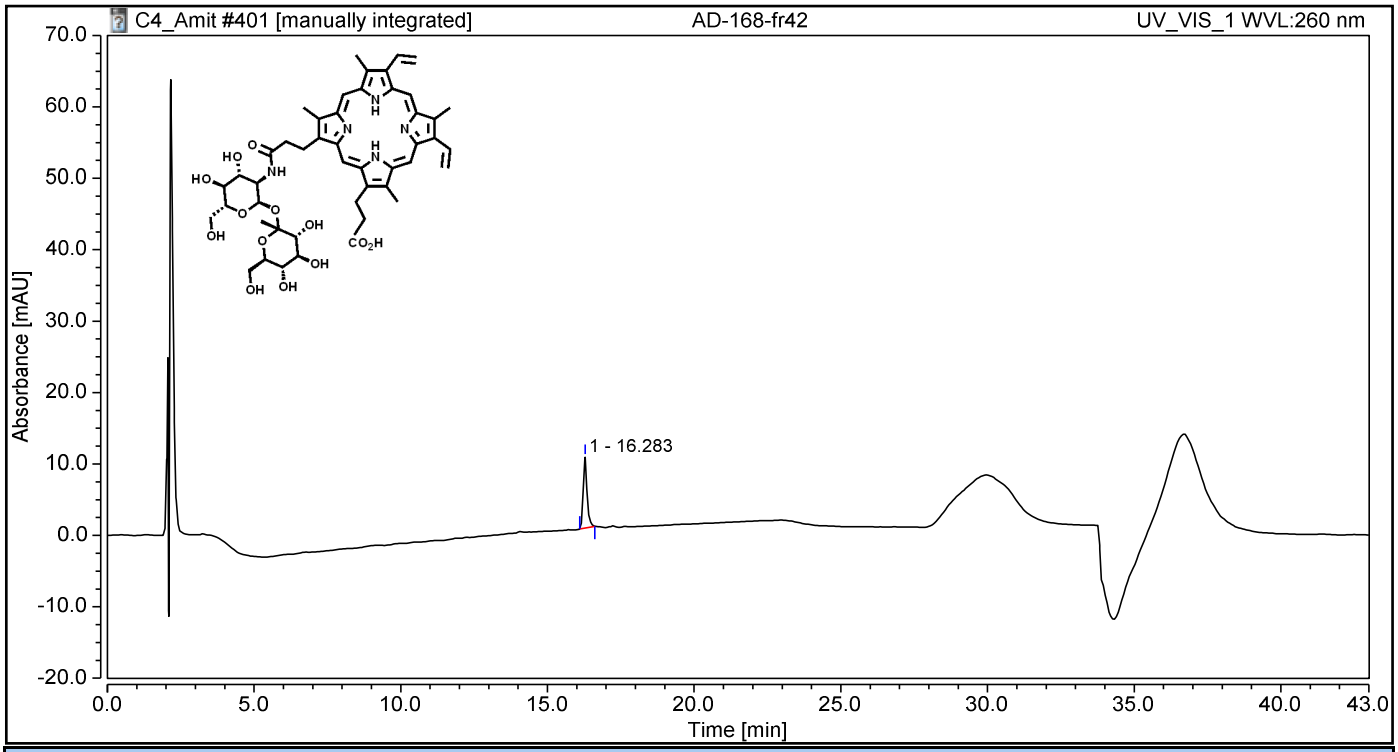
HPLC traces of Compound 10 (mixture of regioisomers; 260 nm)

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**	

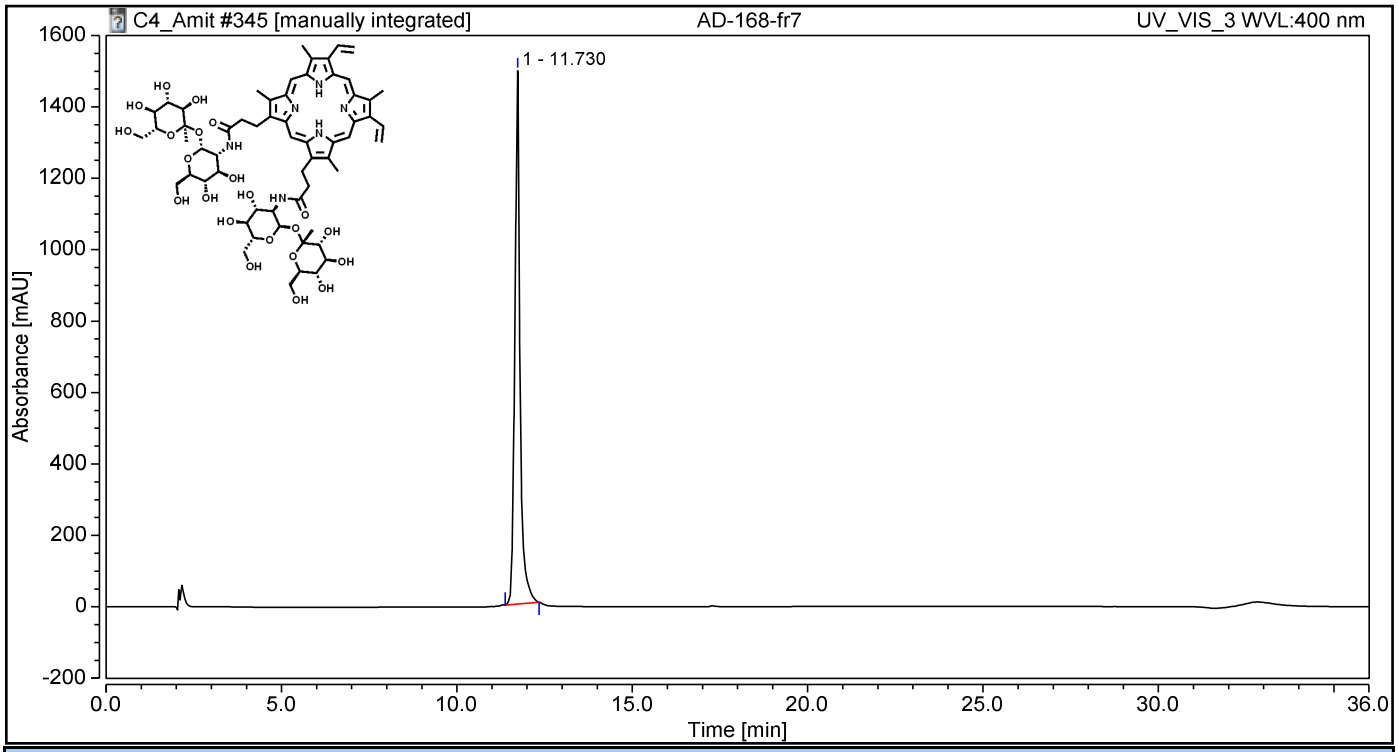
HPLC traces of Compound 11 (400 nm)

Chromatogram and Results

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_3**
Calibration Level:		*Wavelength:*	**400**
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**

Chromatogram



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:			**254.926**	**1494.144**	**100.00**	**100.00**	

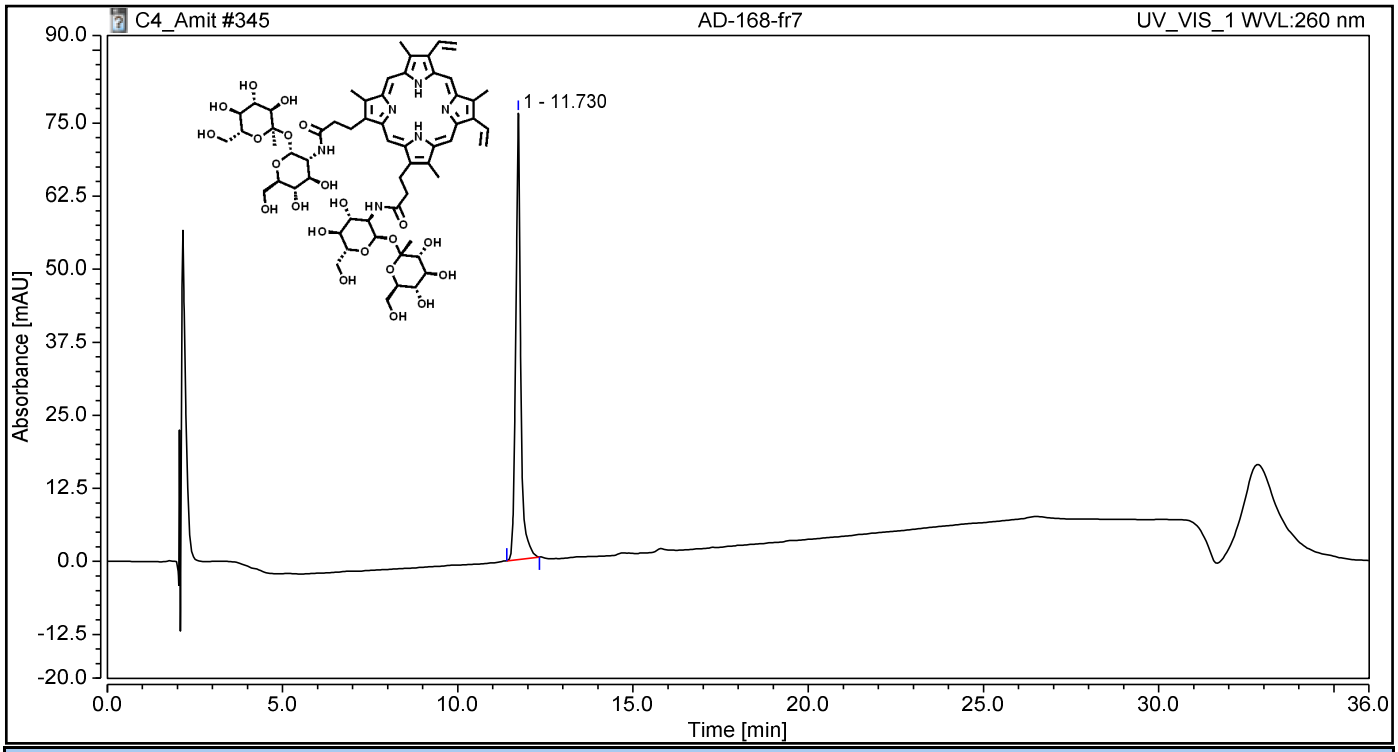
HPLC traces of Compound 11 (260 nm)

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:		*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**	

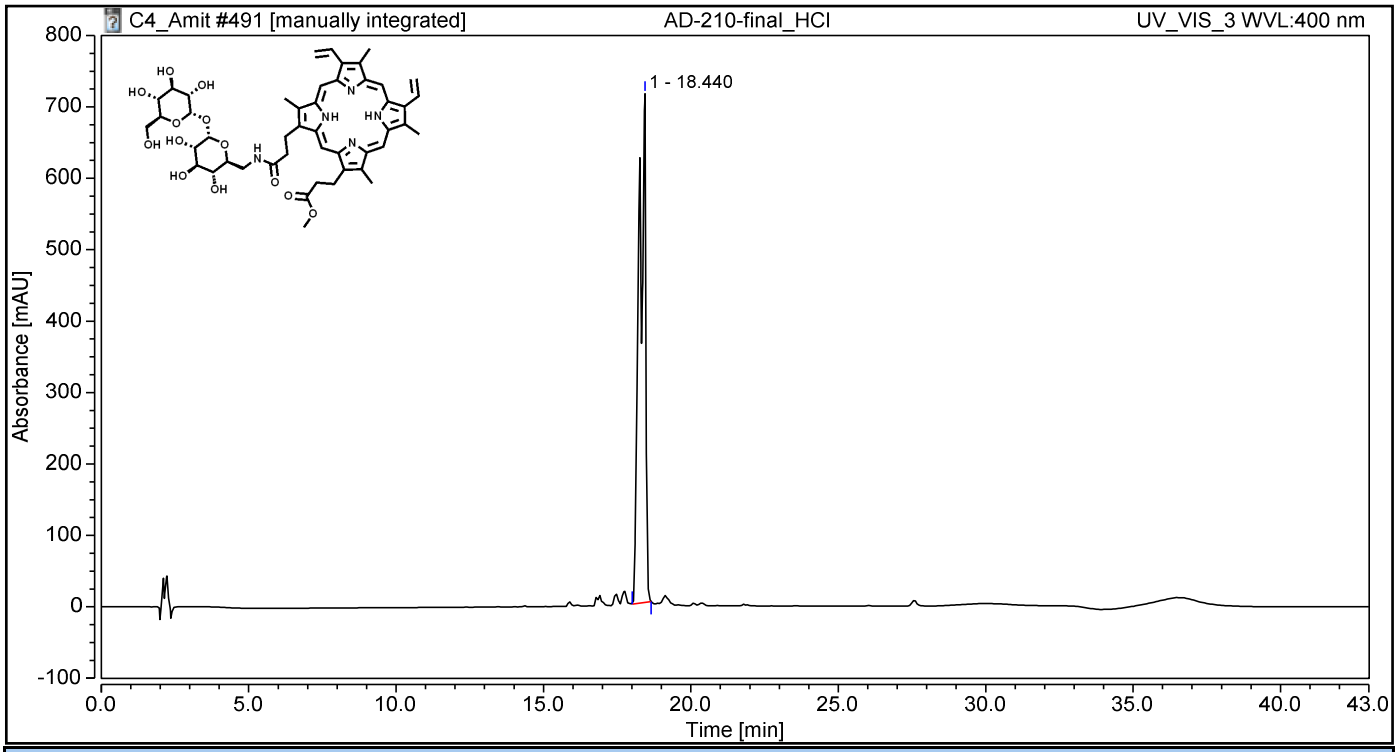
HPLC traces of Compound 12 (mixture of regioisomers; 400 nm)

Chromatogram and Results

Injection Details

Injection Name:	**AD-210-final_HCl**	*Run Time (min):*	**43.00**
Vial Number:	**BE1**	*Injection Volume:*	**4.00**
Injection Type:	**Unknown**	*Channel:*	**UV_VIS_3**
Calibration Level:		*Wavelength:*	**400**
Instrument Method:	**AD_10000-0595_30_ESI (without buffer)_PPIX002**	*Bandwidth:*	**4**
Processing Method:	**MS standard**	*Dilution Factor:*	**1.0000**
Injection Date/Time:	**13/Dec/18 16:04**	*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		18.440	178.118	711.958	100.00	100.00	n.a.
Total:			**178.118**	**711.958**	**100.00**	**100.00**	

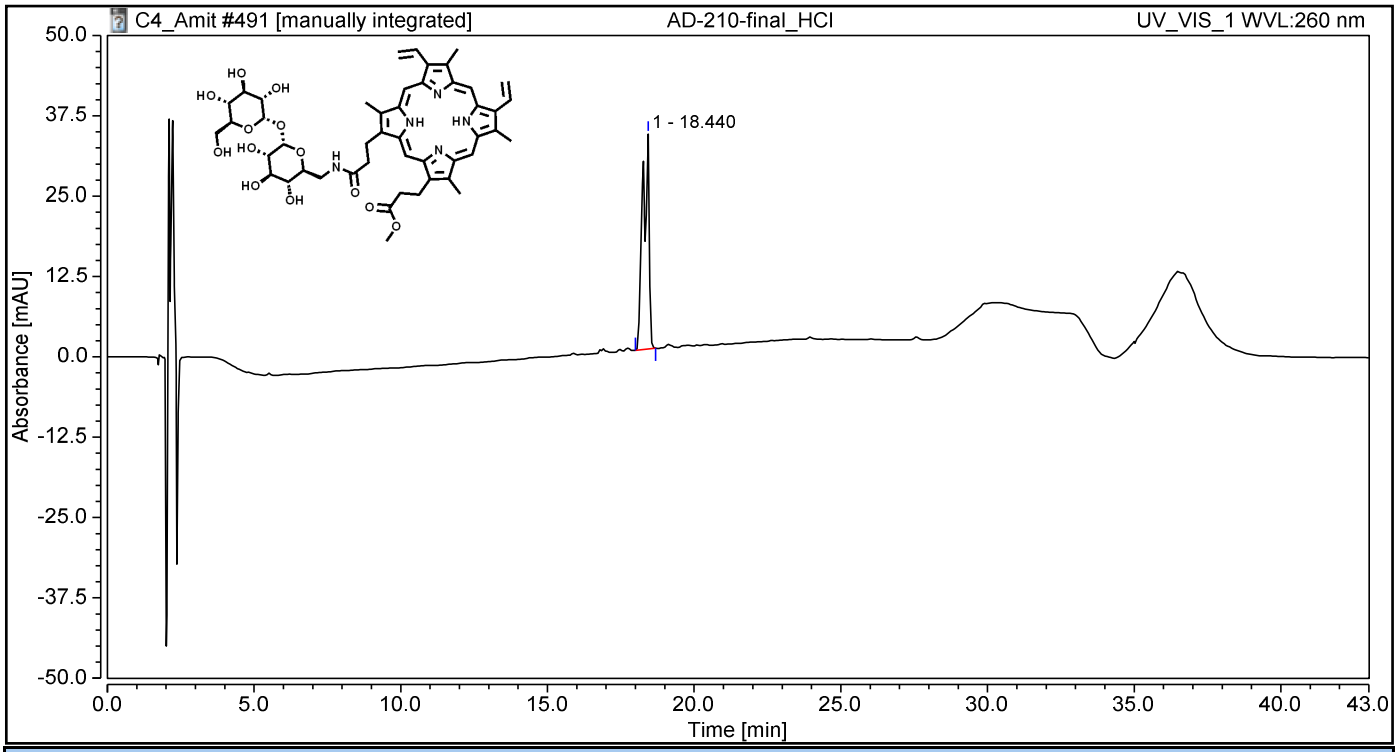
HPLC traces of Compound 12 (mixture of regioisomers; 260 nm)

Chromatogram and Results

Injection Details

Injection Name:	**AD-210-final_HCl**	*Run Time (min):*	**43.00**
Vial Number:	**BE1**	*Injection Volume:*	**4.00**
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:	**13/Dec/18 16:04**	*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		18.440	8.335	33.457	100.00	100.00	n.a.
Total:			**8.335**	**33.457**	**100.00**	**100.00**	

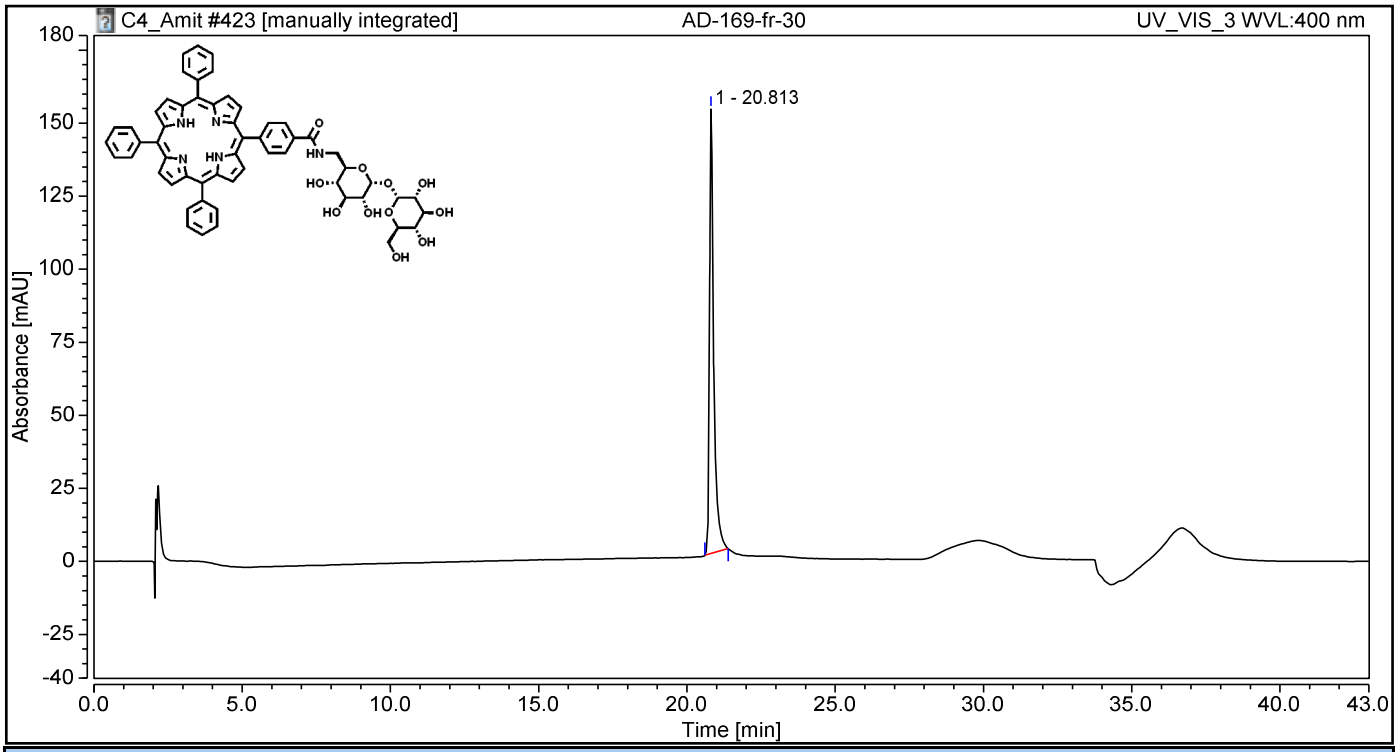
HPLC traces of Compound 13 (400 nm)

Chromatogram and Results

Injection Details

Injection Name:	**AD-169-fr-30**	*Run Time (min):*	**43.00**
Vial Number:	**BC1**	*Injection Volume:*	**5.00**
Injection Type:	**Unknown**	*Channel:*	**UV_VIS_3**
Calibration Level:		*Wavelength:*	**400**
Instrument Method:	**AD_10000-0595_30_ESI (without buffer)_PPIX002**	*Bandwidth:*	**4**
Processing Method:	**MS standard**	*Dilution Factor:*	**1.0000**
Injection Date/Time:	**29/May/18 20:49**	*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		20.813	24.331	152.037	100.00	100.00	n.a.
Total:			**24.331**	**152.037**	**100.00**	**100.00**	

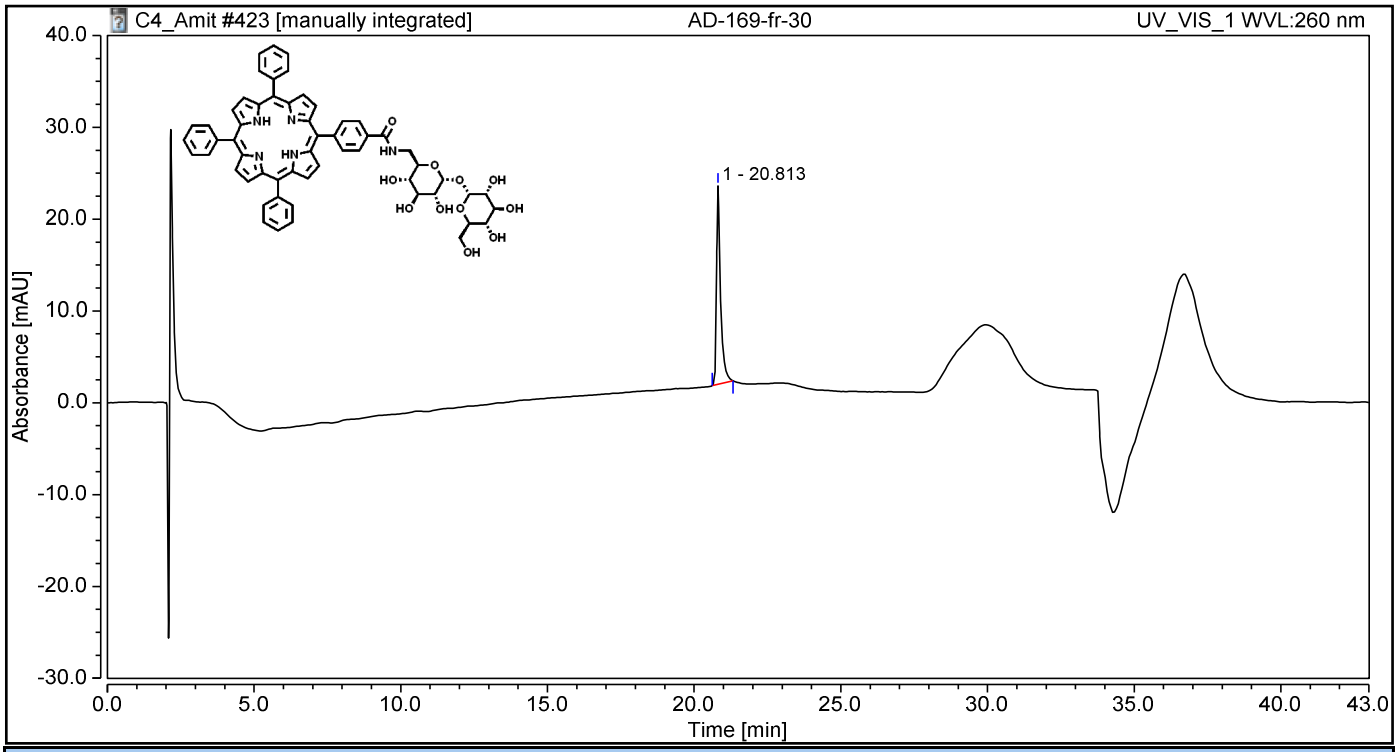
HPLC traces of Compound 13 (260 nm)

Chromatogram and Results

Injection Details

Injection Name:	**AD-169-fr-30**	*Run Time (min):*	**43.00**
Vial Number:	**BC1**	*Injection Volume:*	**5.00**
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:	**29/May/18 20:49**	*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		20.813	3.393	21.610	100.00	100.00	n.a.
Total:			**3.393**	**21.610**	**100.00**	**100.00**	

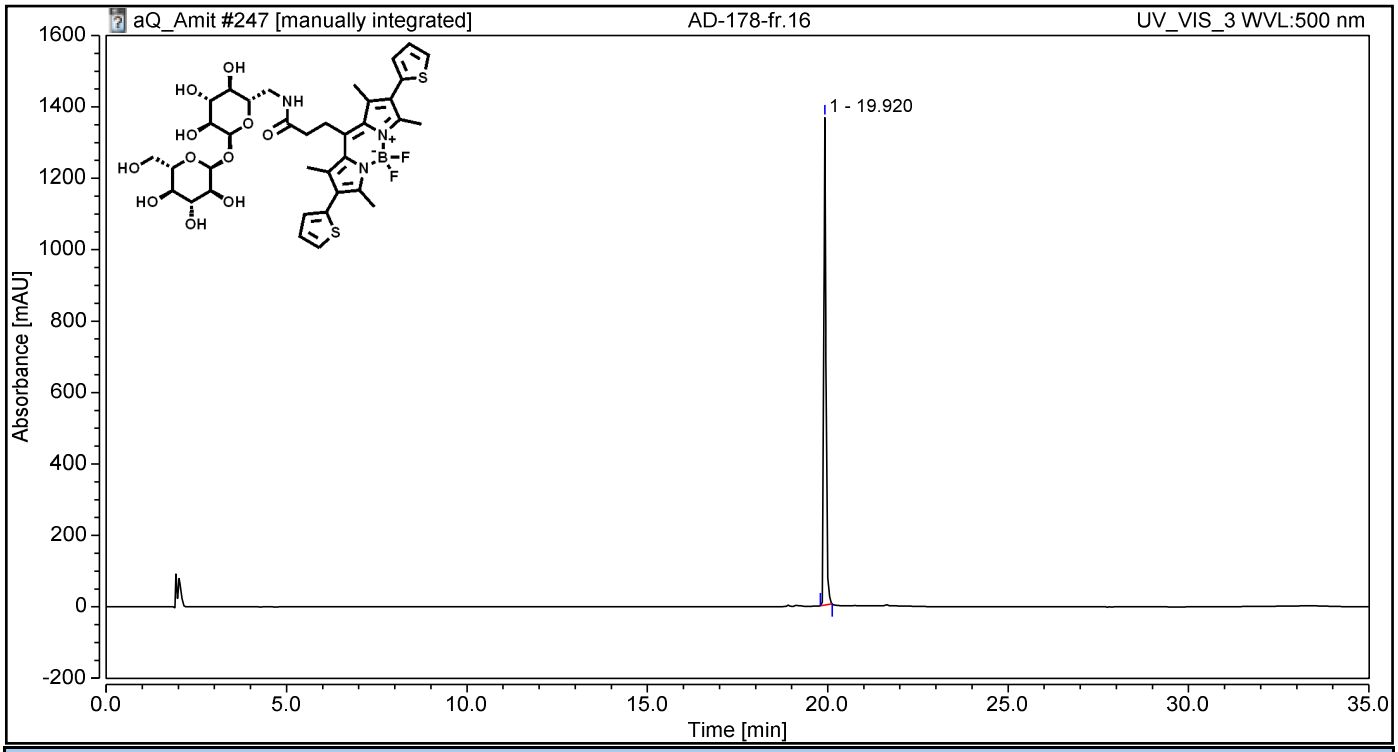
HPLC traces of Compound 14 (500 nm)

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_3**
Calibration Level:		*Wavelength:*	**500**
Instrument Method:	**AD_10000-0595_30_ESI (without buffer)_BODIPY**	*Bandwidth:*	**4**
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		19.920	88.103	1364.543	100.00	100.00	n.a.
Total:			**88.103**	**1364.543**	**100.00**	**100.00**	

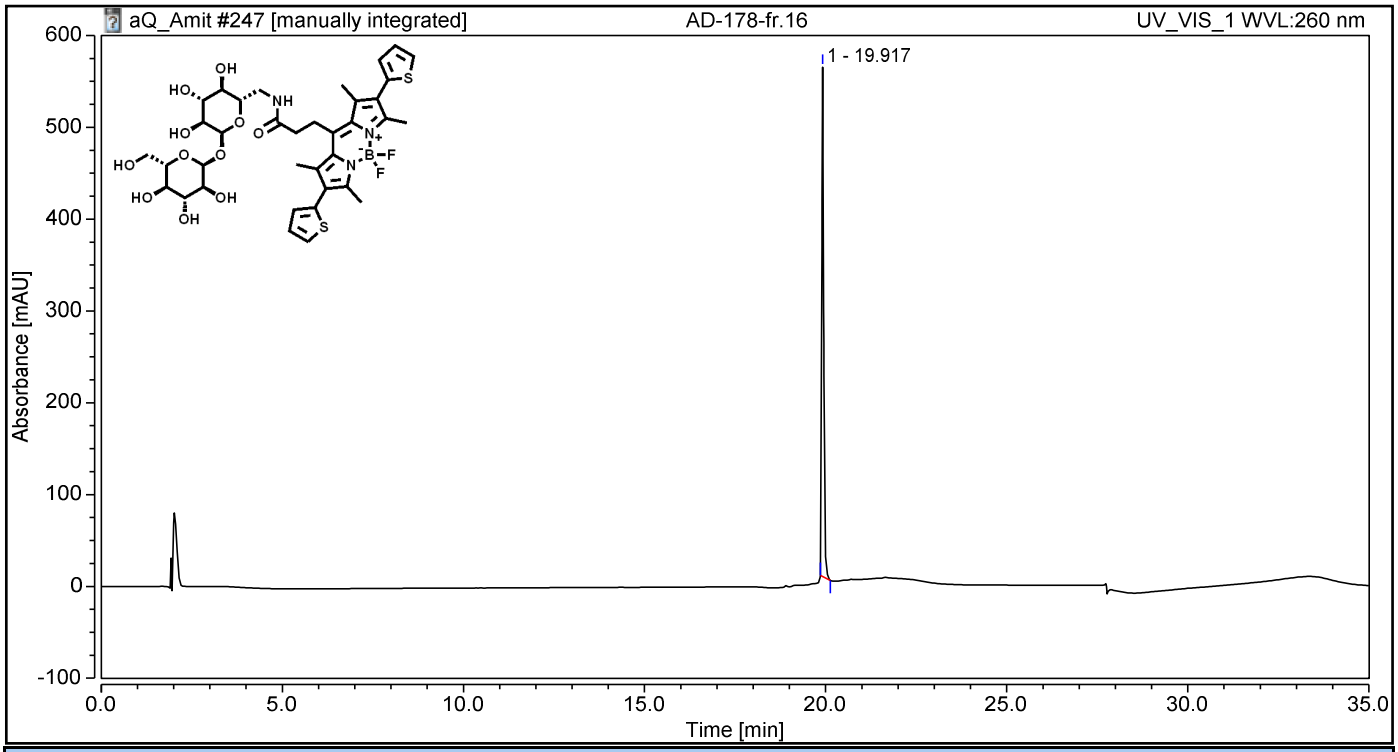
HPLC traces of Compound 14 (260 nm)

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**	

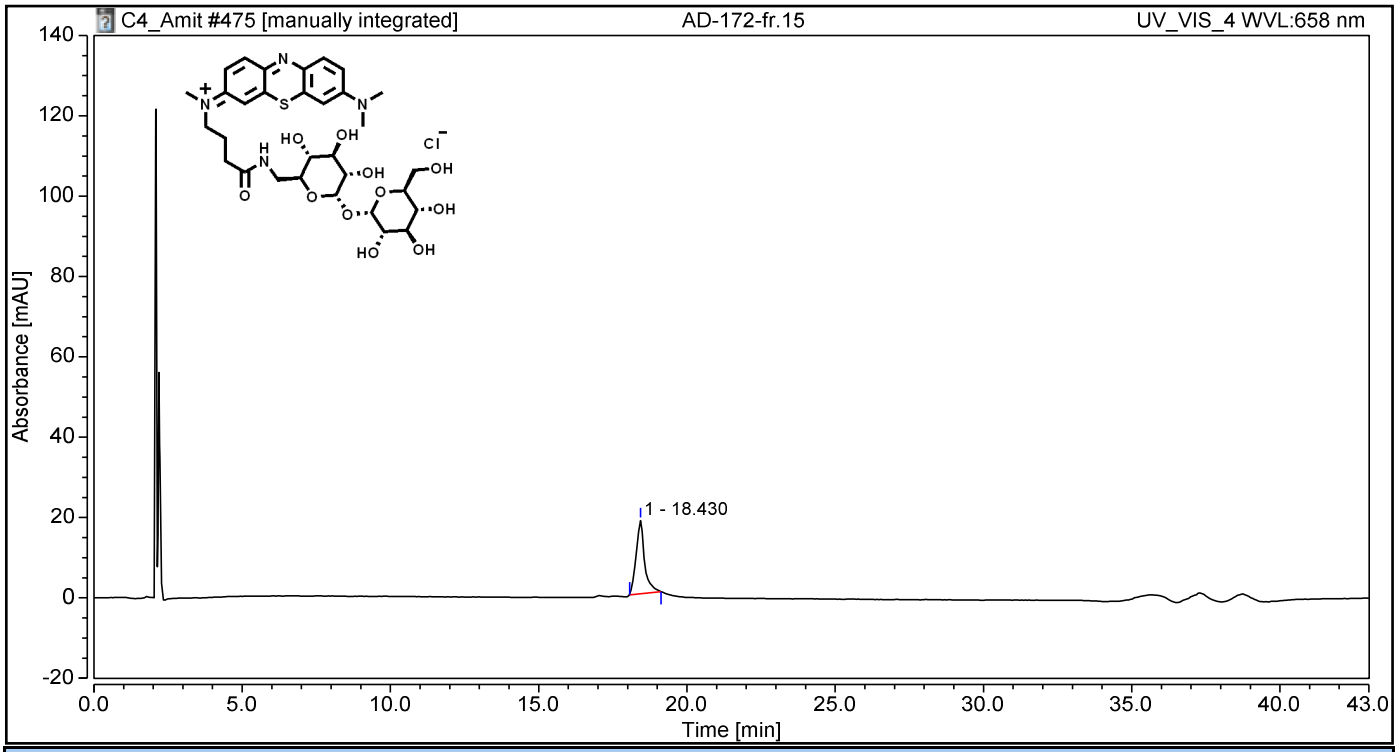
HPLC traces of Compound 15 (658 nm)

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**

Chromatogram



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**	

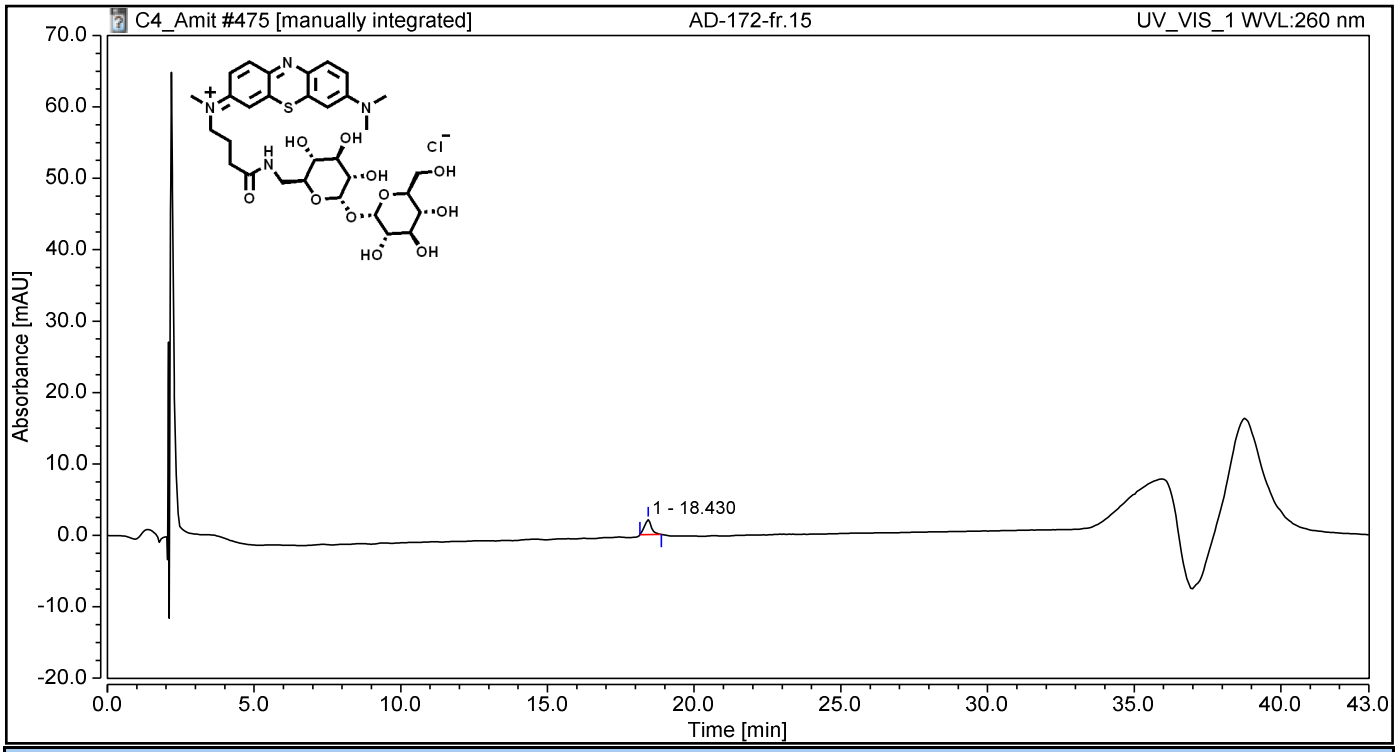
HPLC traces of Compound 15 (260 nm)

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_1**
Calibration Level:		*Wavelength:*	**260**
Instrument Method:	**AD_10000-0595_30_ESI (without buffer)_MB003**	*Bandwidth:*	**2**
Processing Method:	**MS standard**	*Dilution Factor:*	**1.0000**
Injection Date/Time:	**05/Jun/18 09:43**	*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		18.430	0.588	2.091	100.00	100.00	n.a.
Total:			**0.588**	**2.091**	**100.00**	**100.00**	

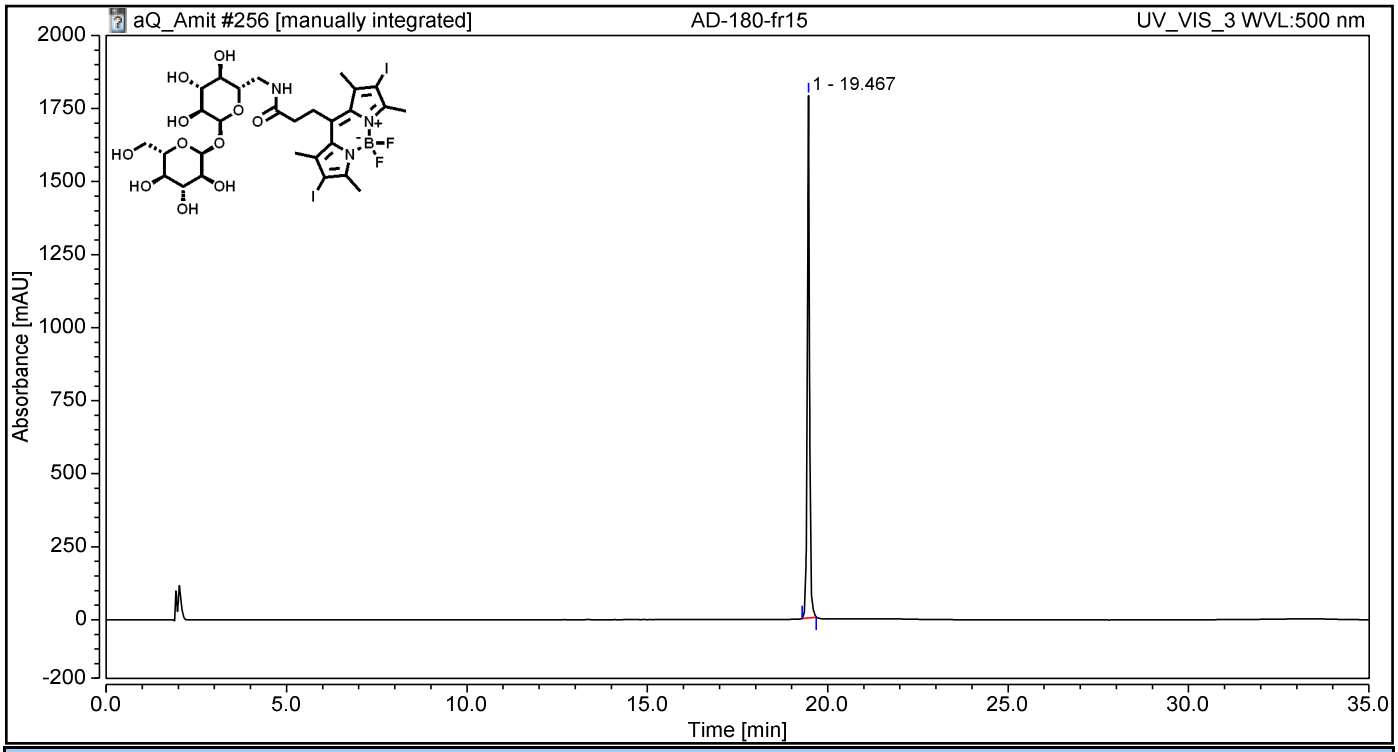
HPLC traces of Compound 16 (500 nm)

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_3**
Calibration Level:		*Wavelength:*	**500**
Instrument Method:	**AD_10000-0595_30_ESI (without buffer)_BODIPY**	*Bandwidth:*	**4**
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		19.467	126.001	1787.914	100.00	100.00	n.a.
Total:			**126.001**	**1787.914**	**100.00**	**100.00**	

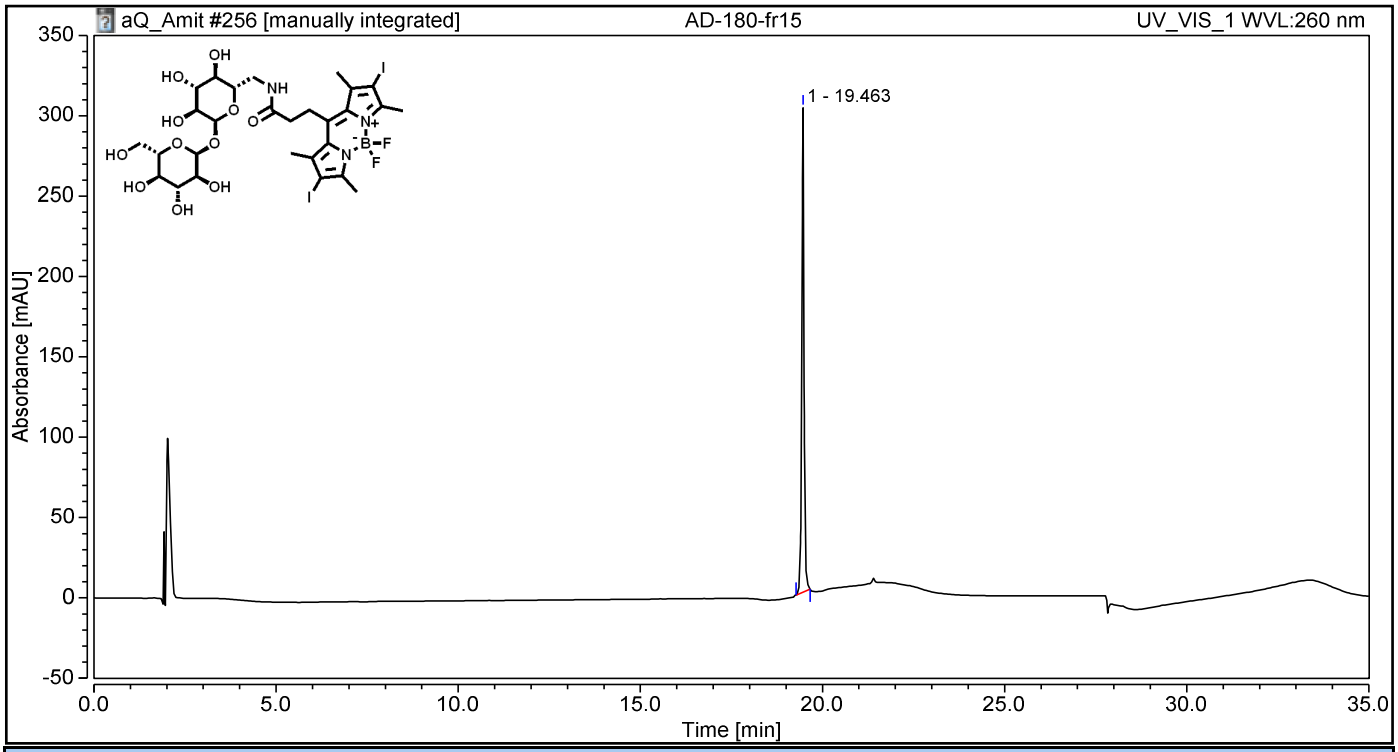
HPLC traces of Compound 16 (260 nm)

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**	

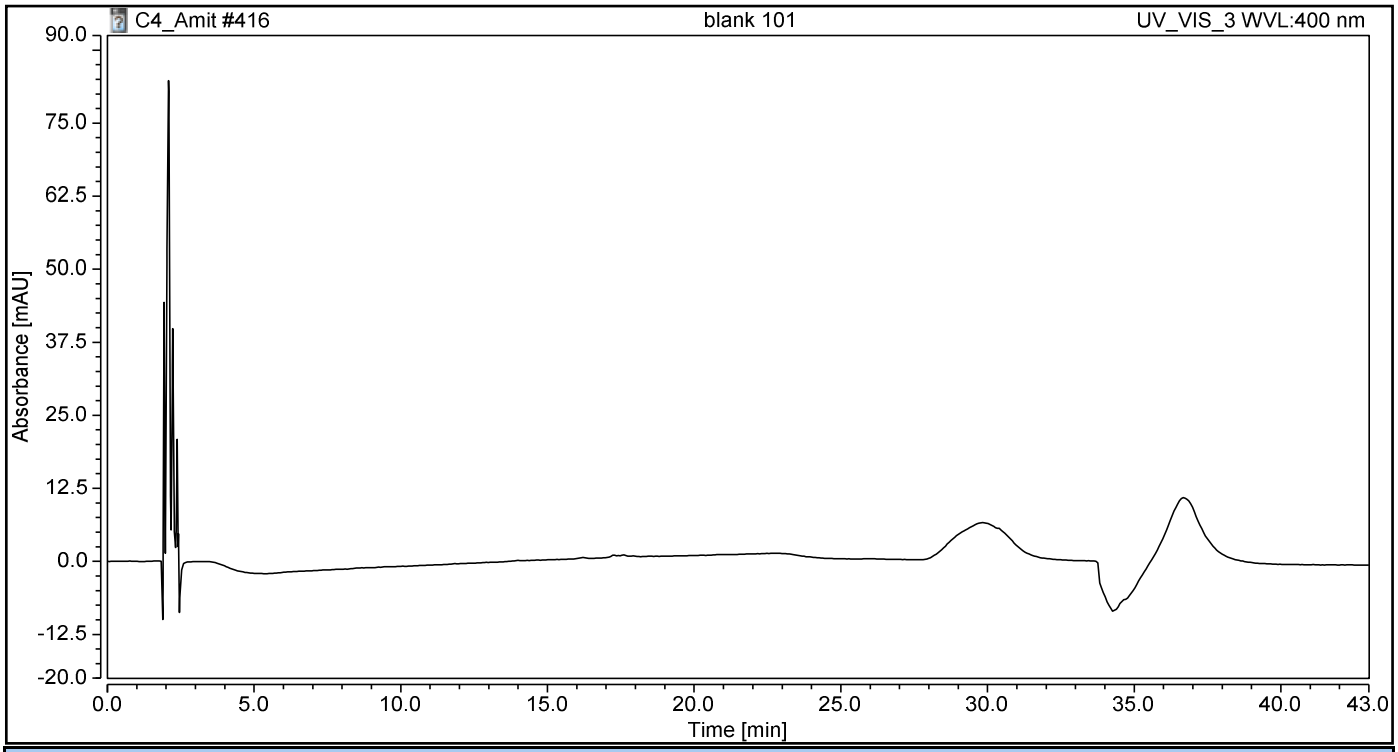
HPLC traces of blank sample (400 nm)

Chromatogram and Results

Injection Details

Injection Name:	**blank 101**	*Run Time (min):*	**43.00**
Vial Number:	**BA1**	*Injection Volume:*	**20.00**
Injection Type:	**Unknown**	*Channel:*	**UV_VIS_3**
Calibration Level:		*Wavelength:*	**400**
Instrument Method:	**AD_10000-0595_30_ESI (without buffer)_PPIX002**	*Bandwidth:*	**4**
Processing Method:	**MS standard**	*Dilution Factor:*	**1.0000**
Injection Date/Time:	**29/May/18 06:35**	*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.
Total:			**0.000**	**0.000**	**0.00**	**0.00**	

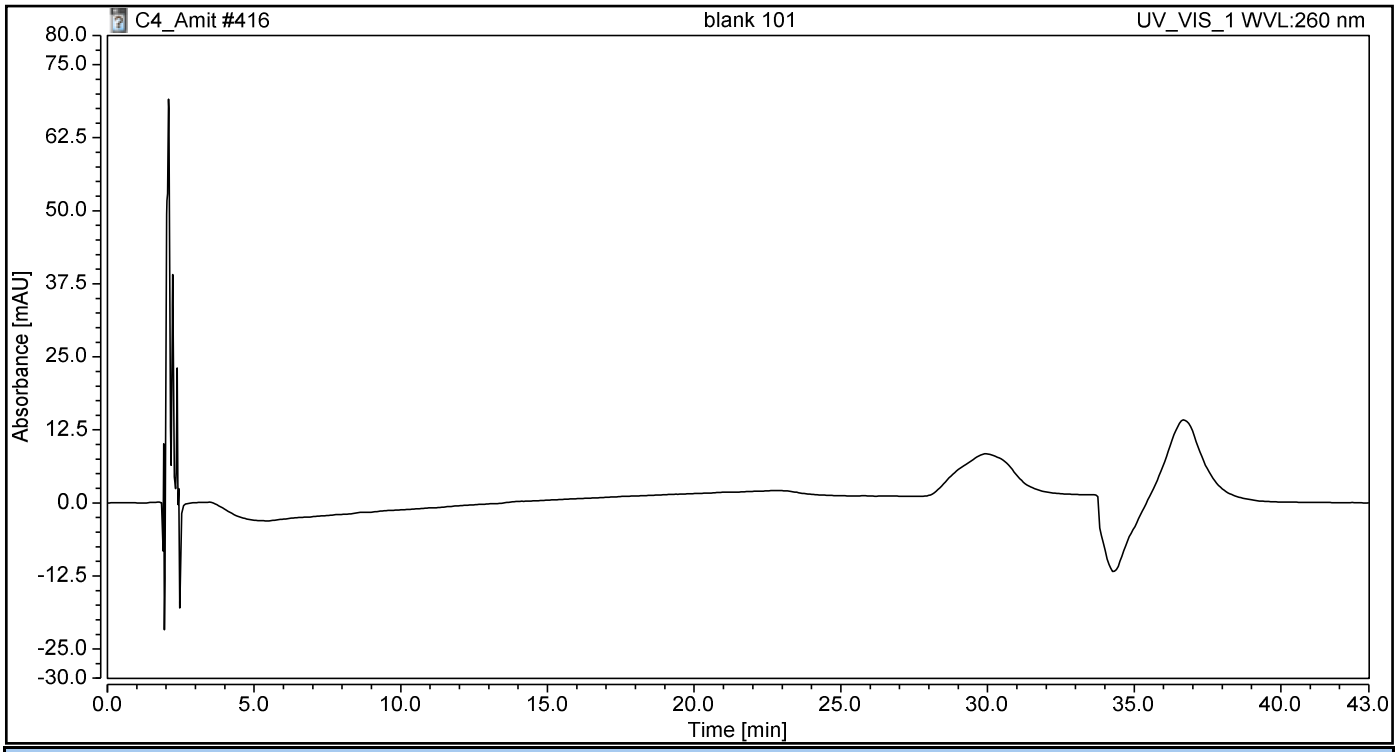
HPLC traces of blank sample (260 nm)

Chromatogram and Results

Injection Details

Injection Name:	**blank 101**	*Run Time (min):*	**43.00**
Vial Number:	**BA1**	*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:	**29/May/18 06:35**	*Sample Weight:*	**1.0000**

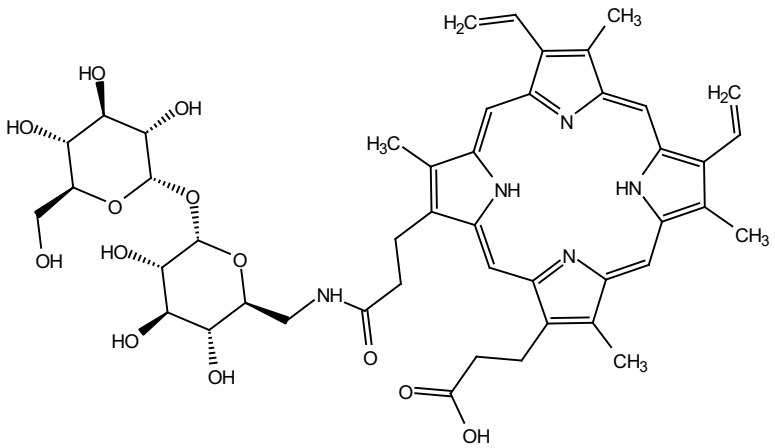
Chromatogram

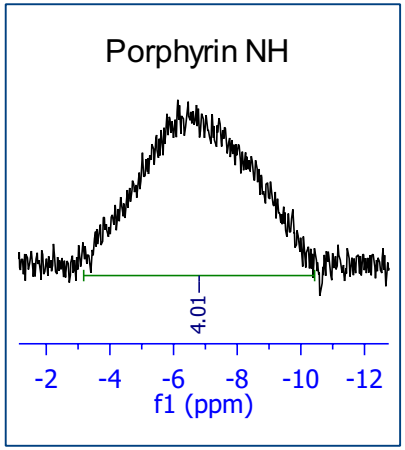


Integration Results

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

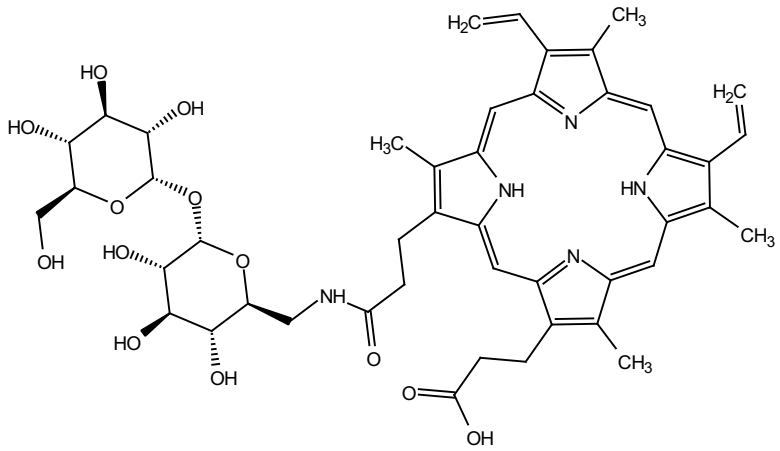
^{1}H of Compound **9** (mixture of regioisomers; DMSO-d_6)





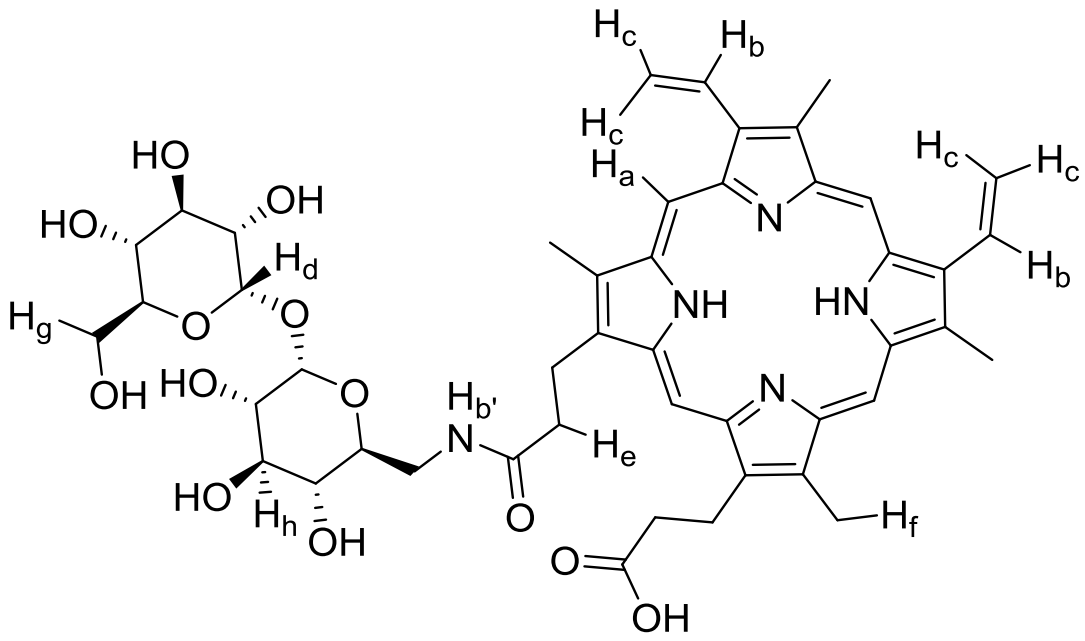
NMR 1

^{13}C of Compound **9** (mixture of regioisomers; DMSO-d_6)



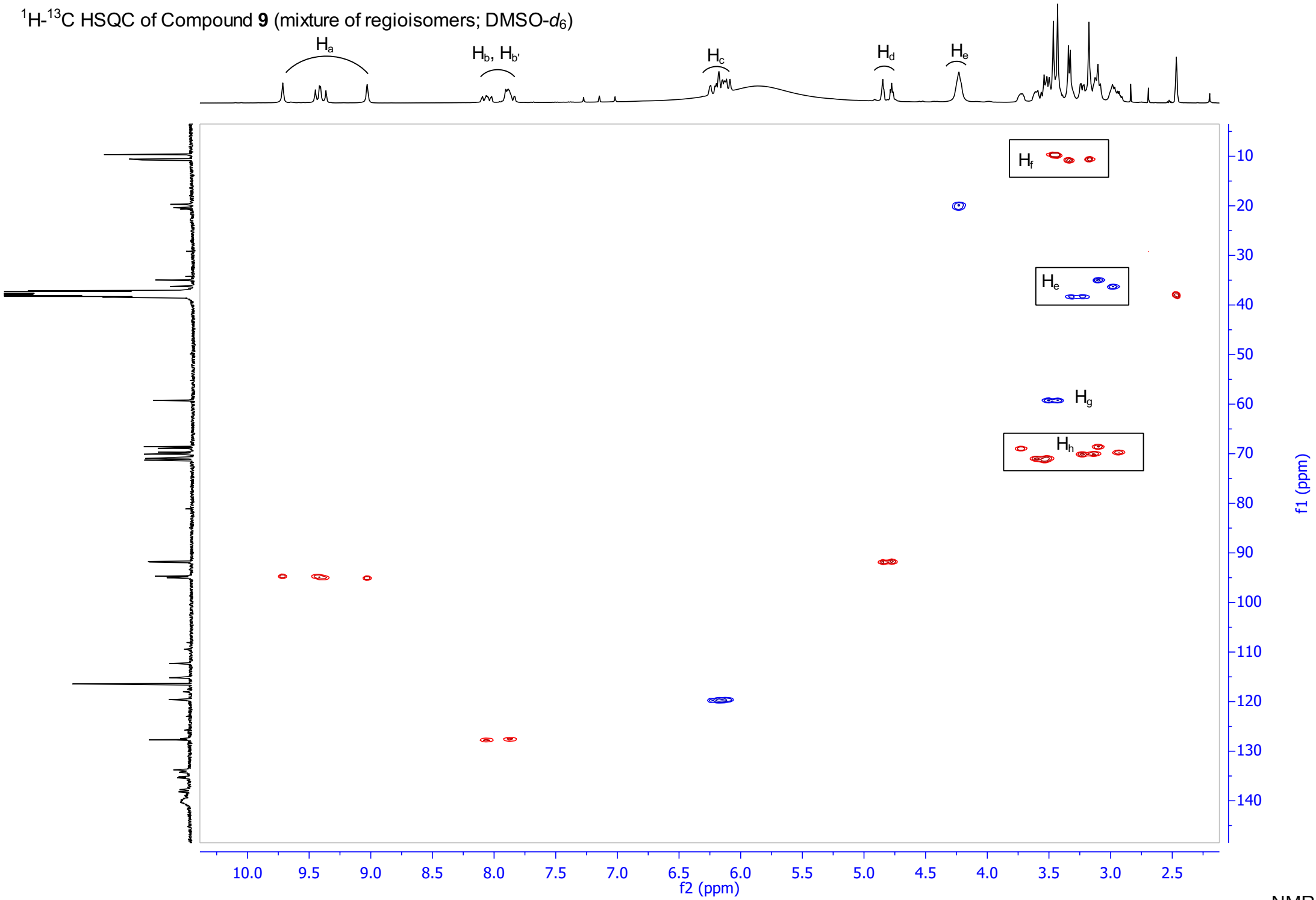
NMR 2

2D NMR analysis of Compound **9**

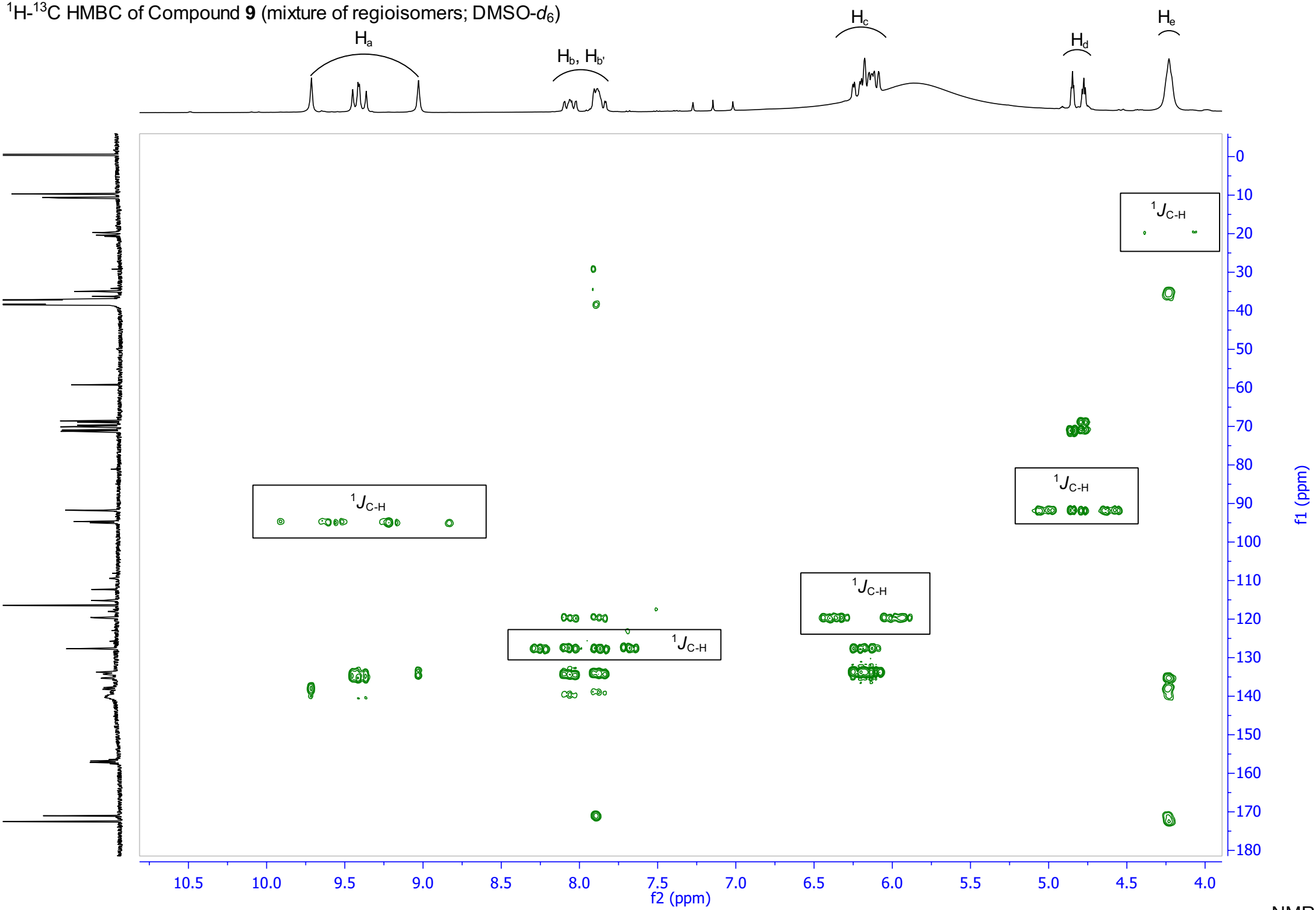


Trehalose unit	**Porphyrin unit**
H_d = Trehalose anomeric C-H	H_a = Porphyrin ring C-H
H_g = Trehalose CH_2	H_e = Porphyrin CH_2
H_h = Trehalose C-H (except H_d)	H_f = Porphyrin CH_3

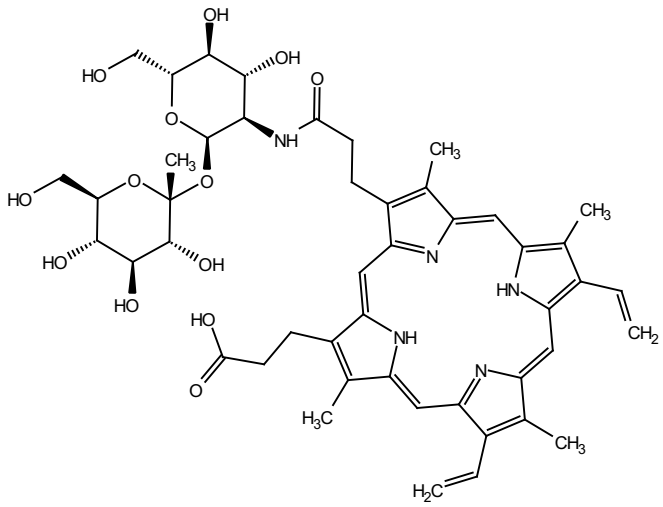
^{1}H-^{13}C HSQC of Compound **9** (mixture of regioisomers; DMSO-d_6)

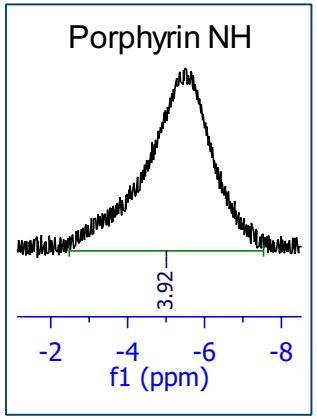


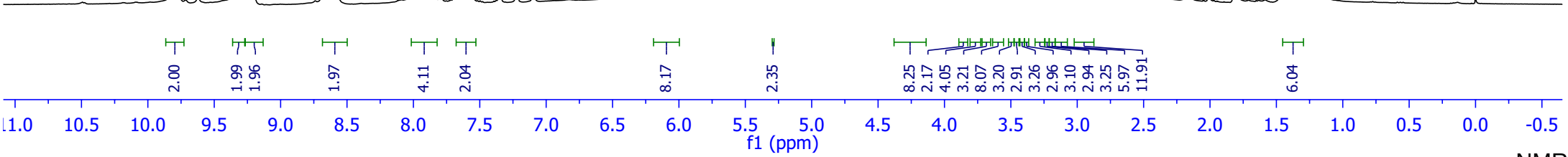
^{1}H-^{13}C HMBC of Compound **9** (mixture of regioisomers; DMSO-d_6)



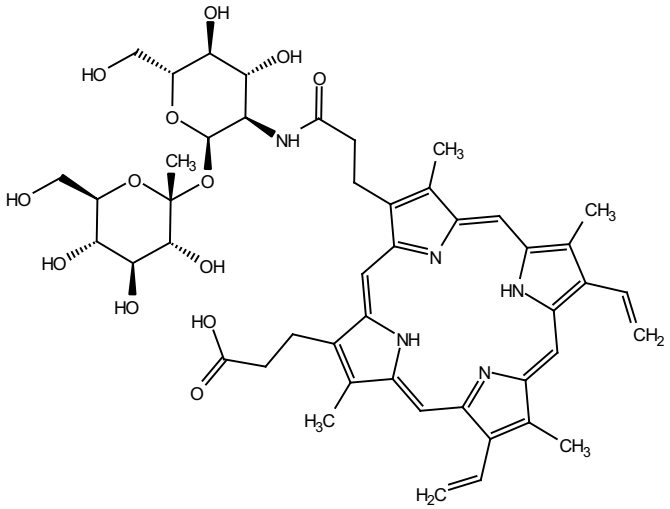
^{1}H of Compound **10** (mixture of regioisomers; DMSO-d_6)



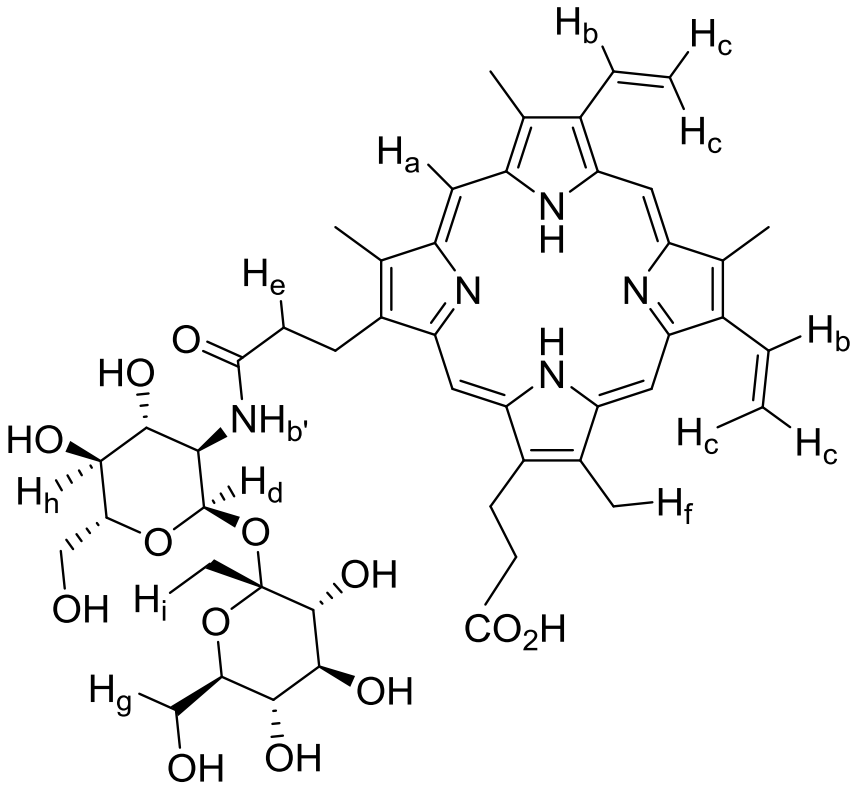




^{13}C of Compound **10** (mixture of regioisomers; DMSO-d_6)

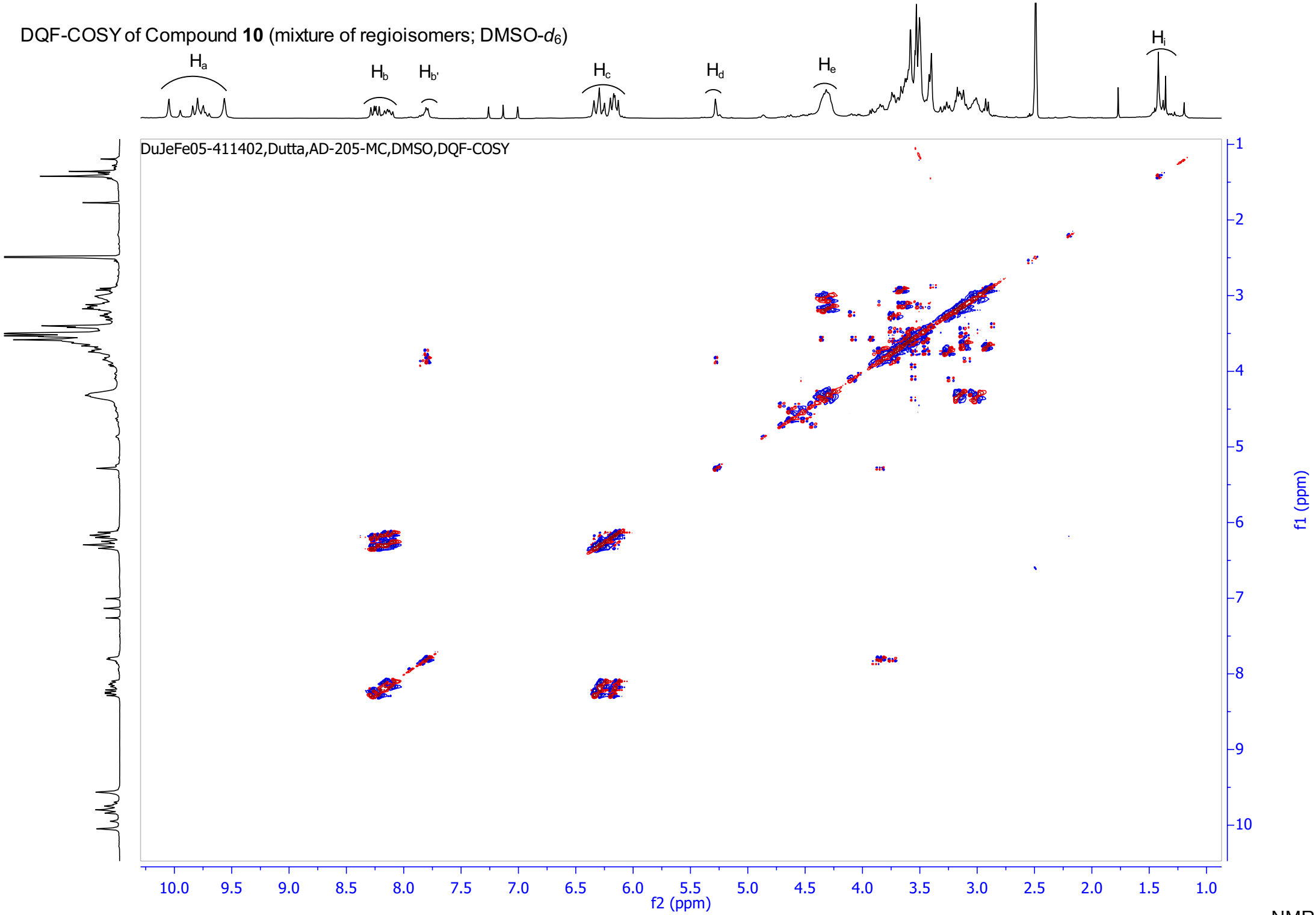


2D NMR analysis of Compound **10**

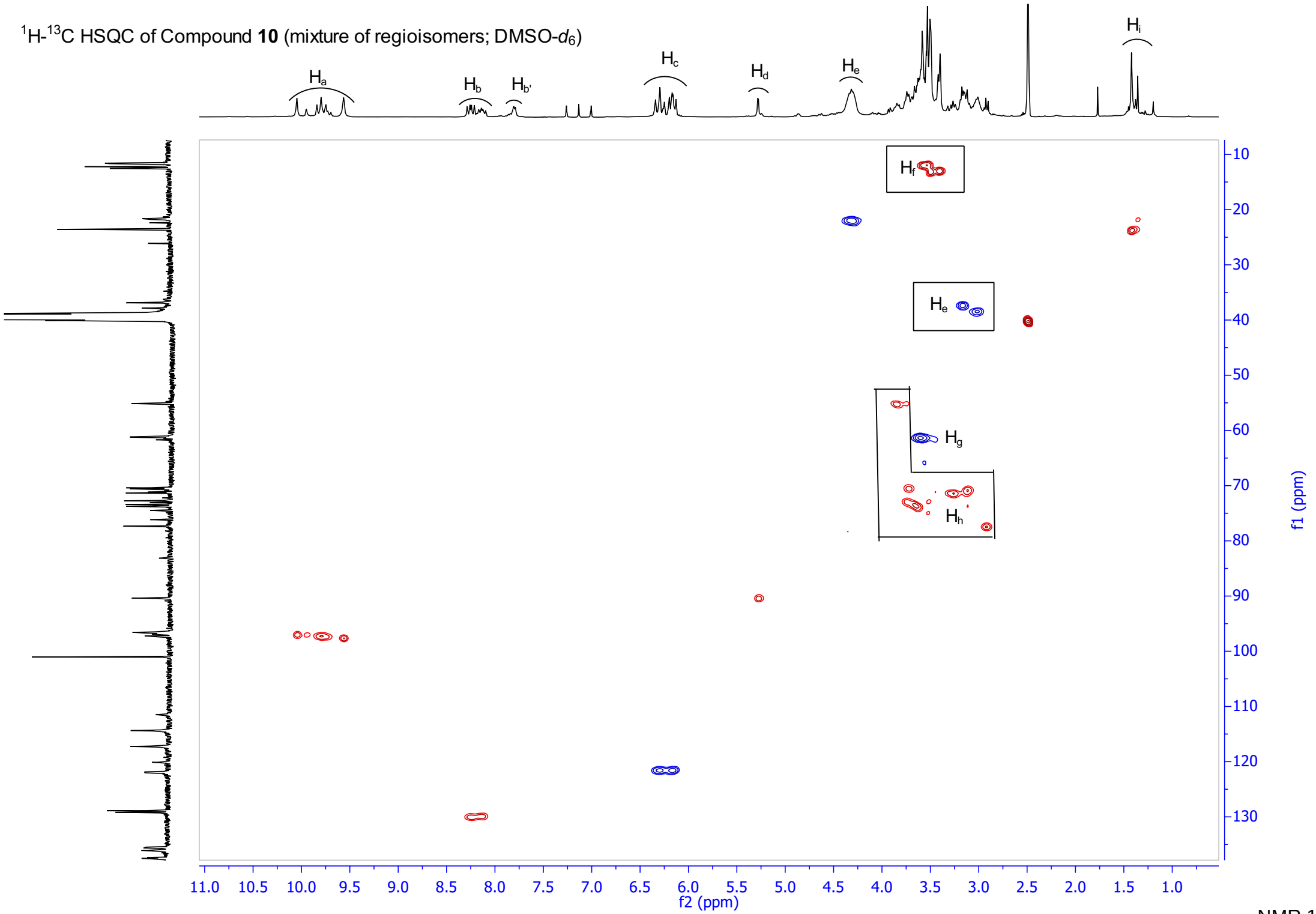


Trehalose unit	**Porphyrin unit**
H_d = Trehalose anomeric C-H	H_a = Porphyrin ring C-H
H_g = Trehalose CH_2	H_e = Porphyrin CH_2
H_h = Trehalose C-H (except H_d)	H_f = Porphyrin CH_3
H_i = Trehalose CH_3	

DQF-COSY of Compound **10** (mixture of regioisomers; DMSO-d_6)

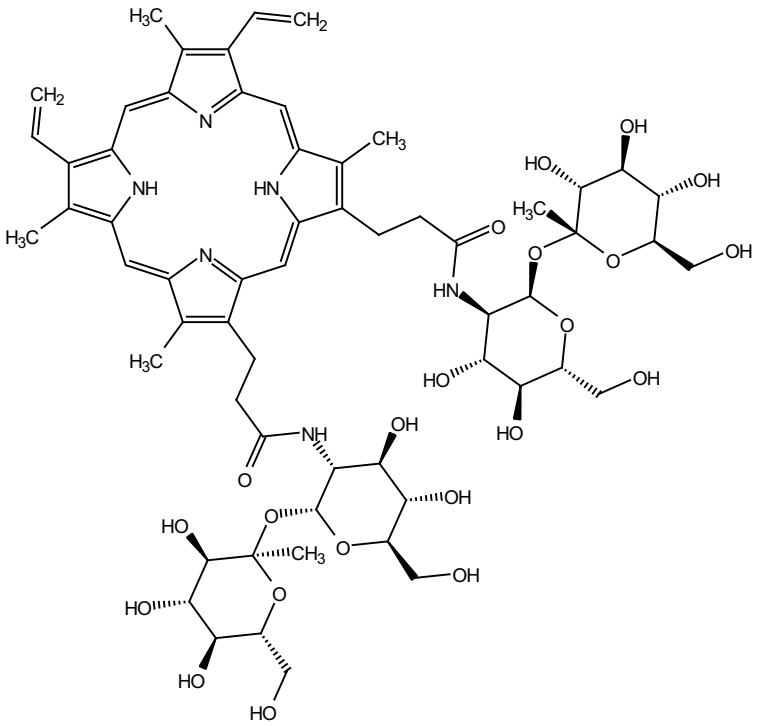


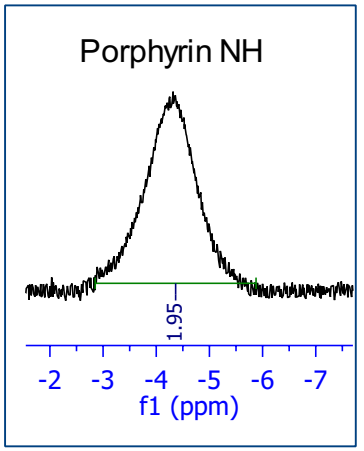
^{1}H-^{13}C HSQC of Compound **10** (mixture of regioisomers; DMSO-d_6)



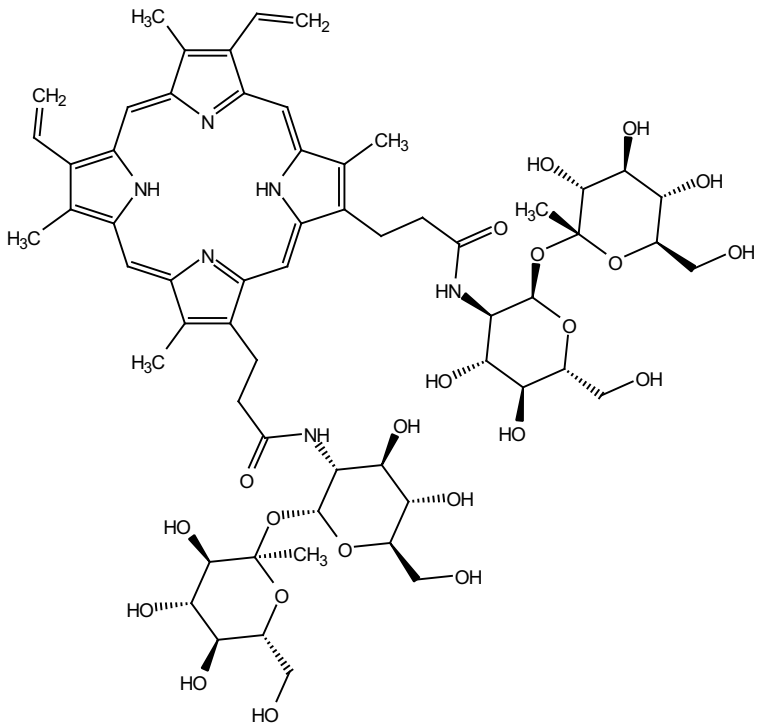
NMR 10

^{1}H of Compound **11** (DMSO-d_6)

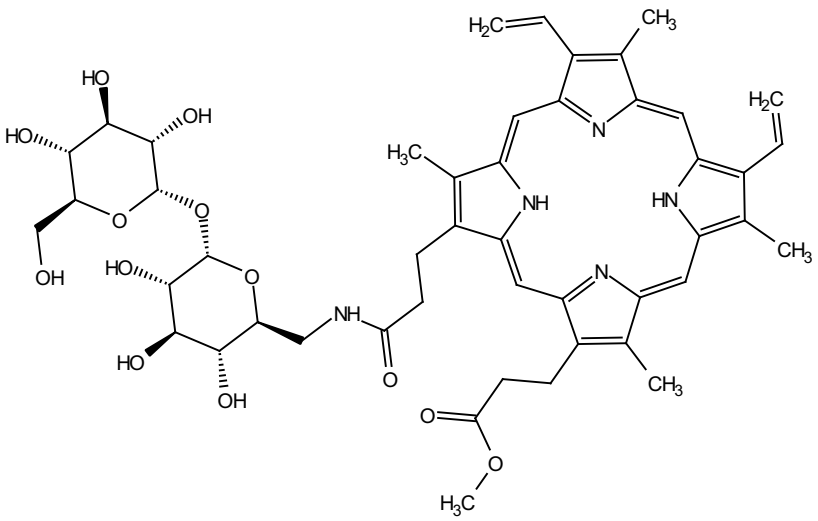




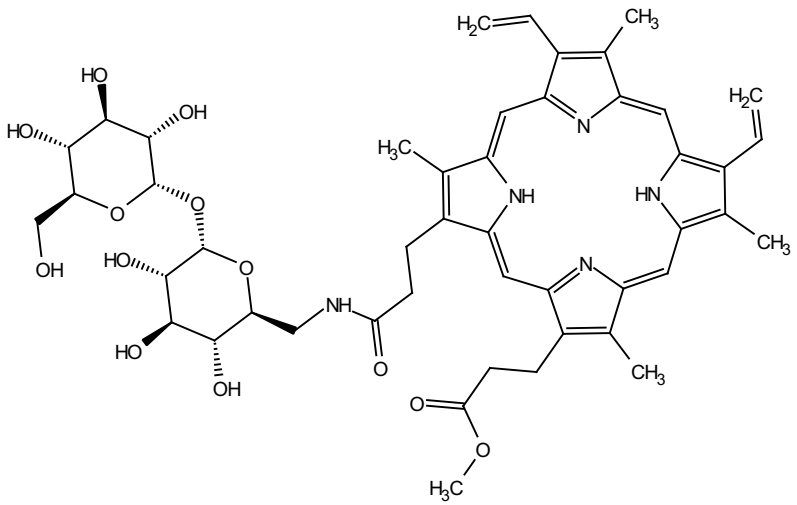
^{13}C of Compound **11** (DMSO-d_6)



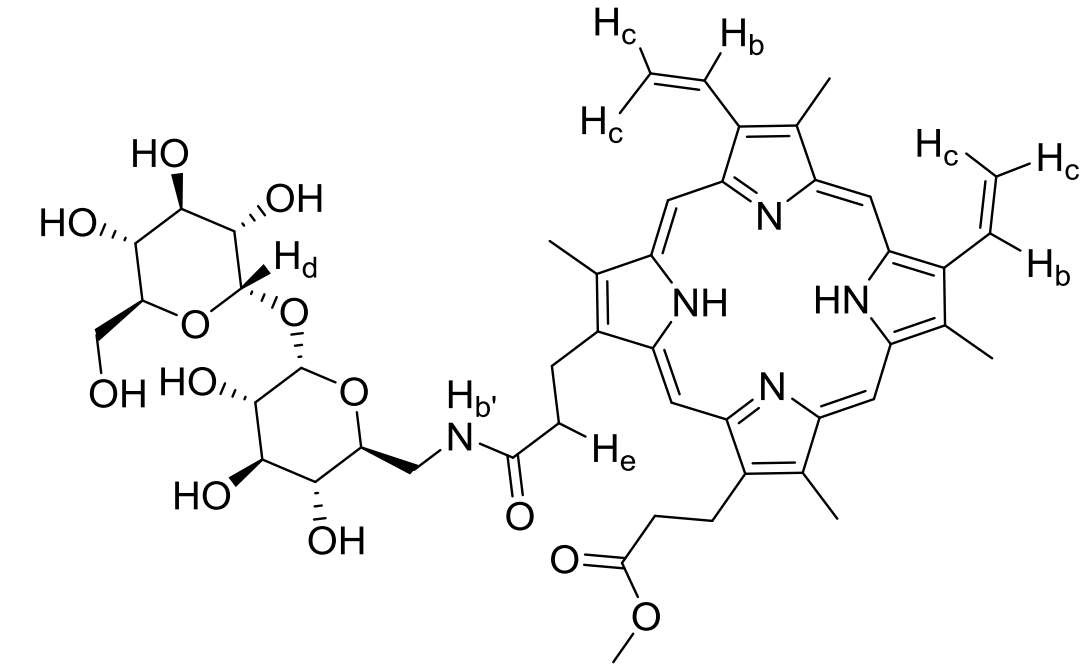
^{1}H of Compound **12** (mixture of regioisomers; DMSO-d_6)



^{13}C of Compound **12** (mixture of regioisomers; DMSO-d_6)



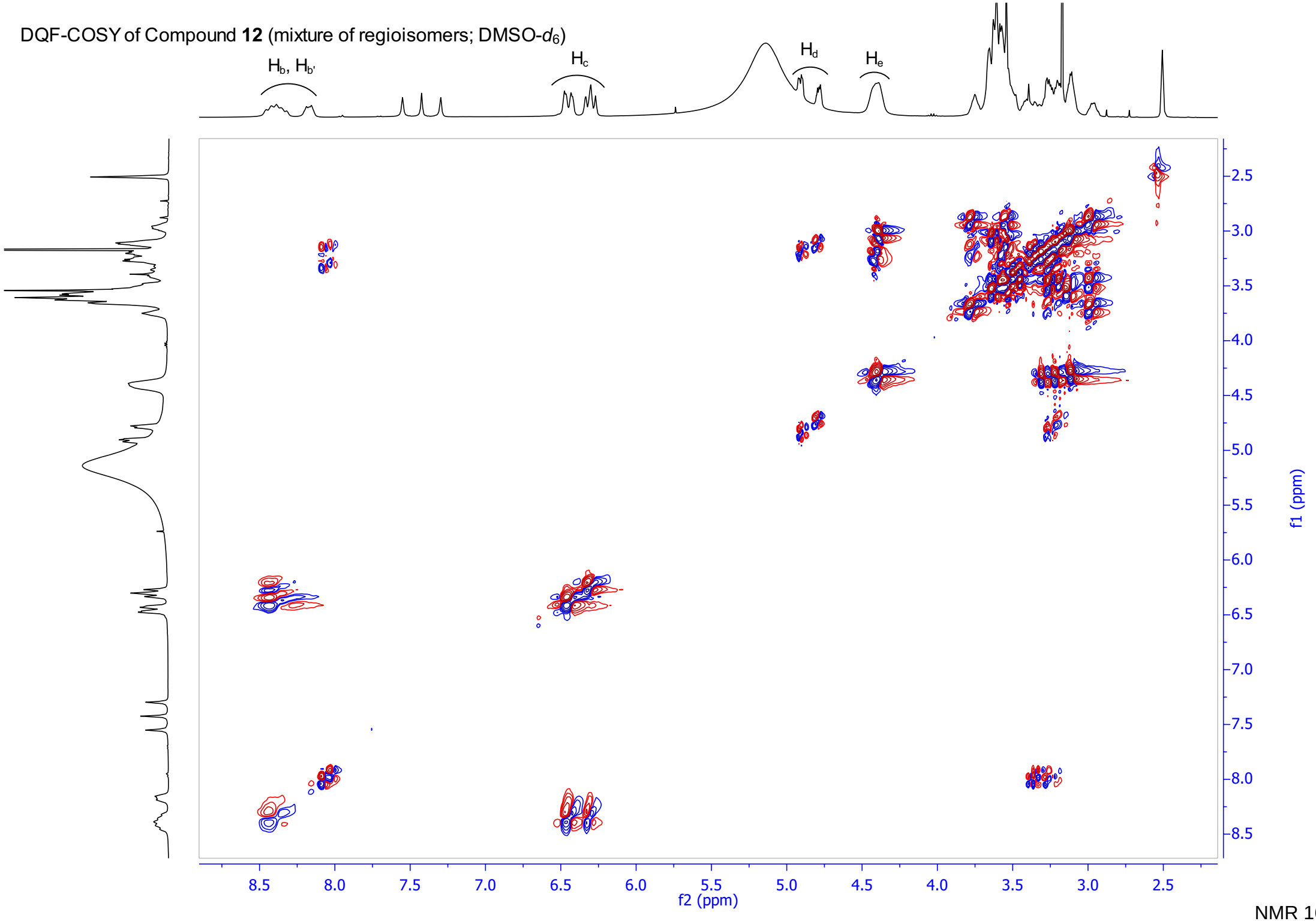
2D NMR analysis of Compound **12**



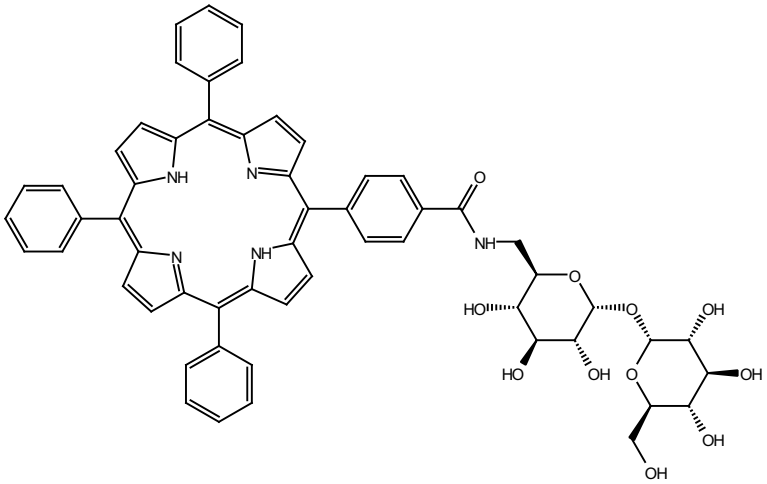
Trehalose unit | **Porphyrin unit**

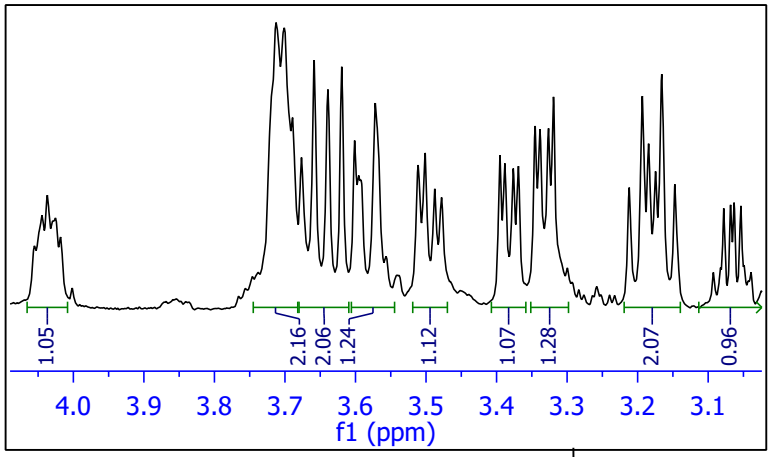
H_d = Trehalose anomeric C-H | H_e = Porphyrin CH_2

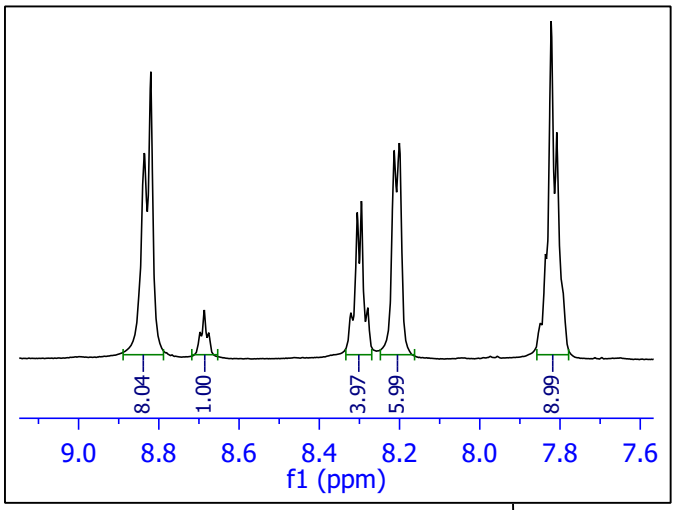
DQF-COSY of Compound **12** (mixture of regioisomers; DMSO-d_6)

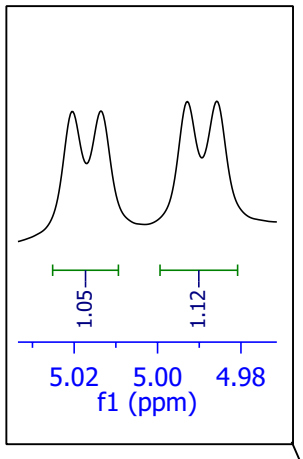


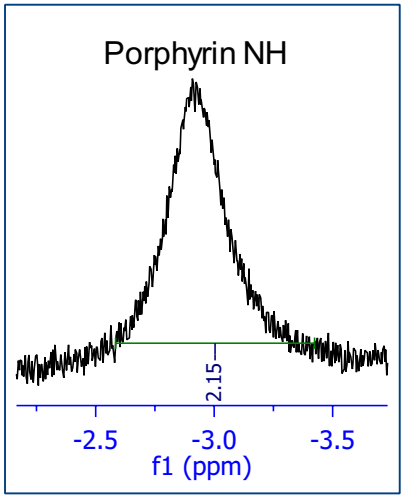
^{1}H of Compound **13** (DMSO-d_6)



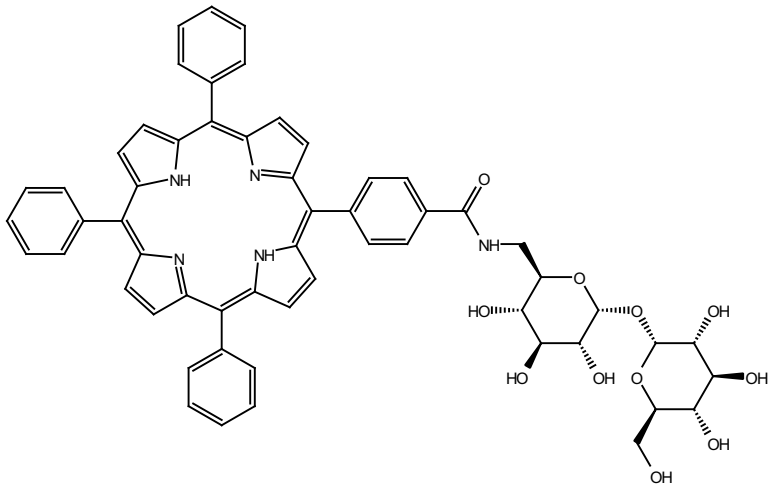




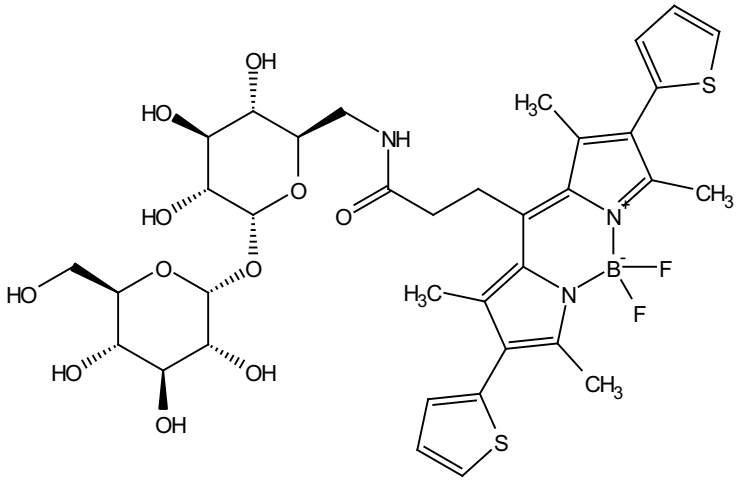


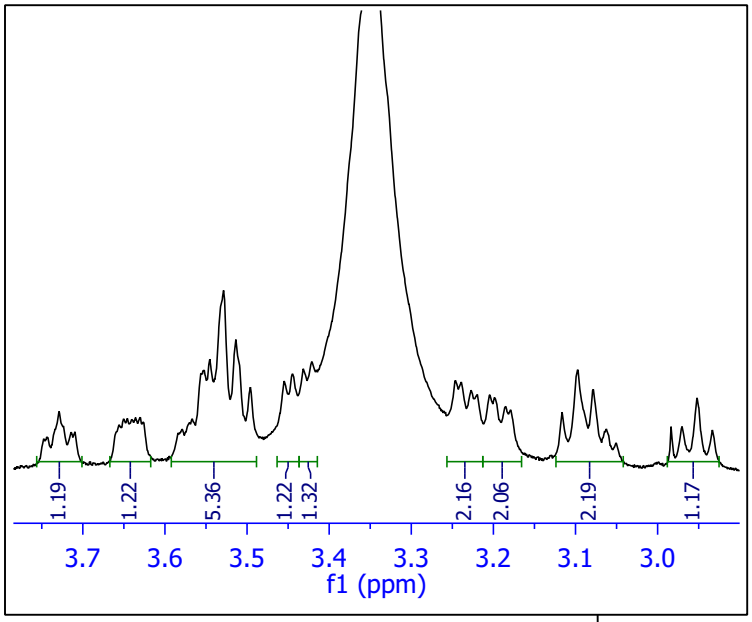


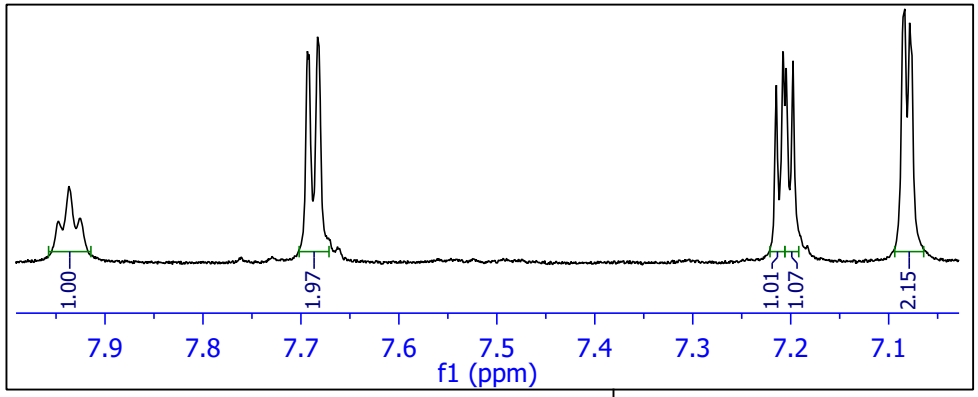
^{13}C of Compound **13** (DMSO-d_6)

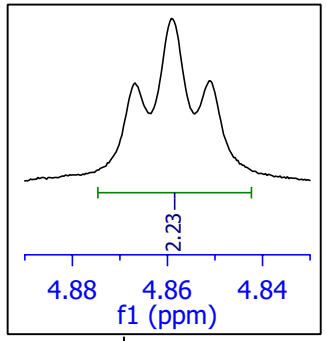


^{1}H of Compound **14** (DMSO-d_6)

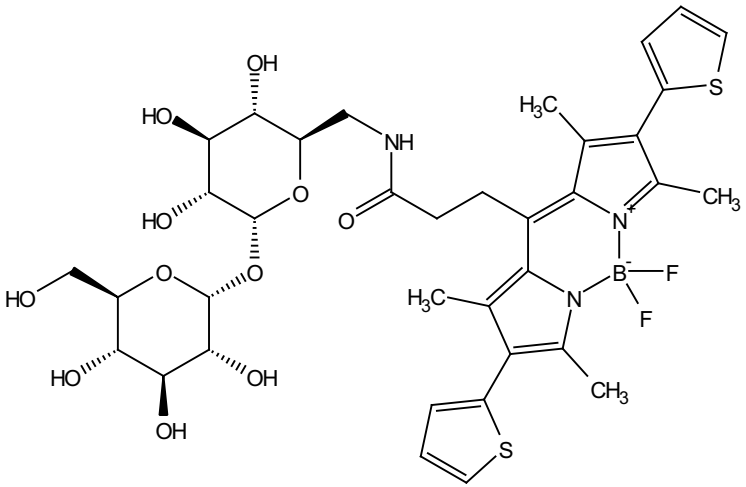




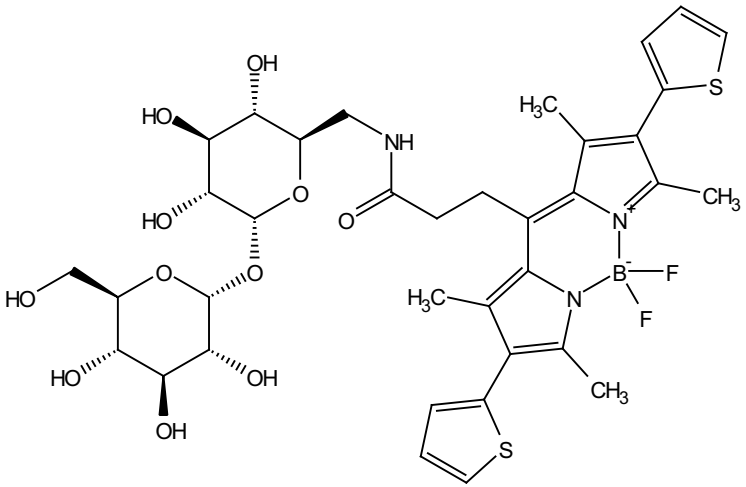


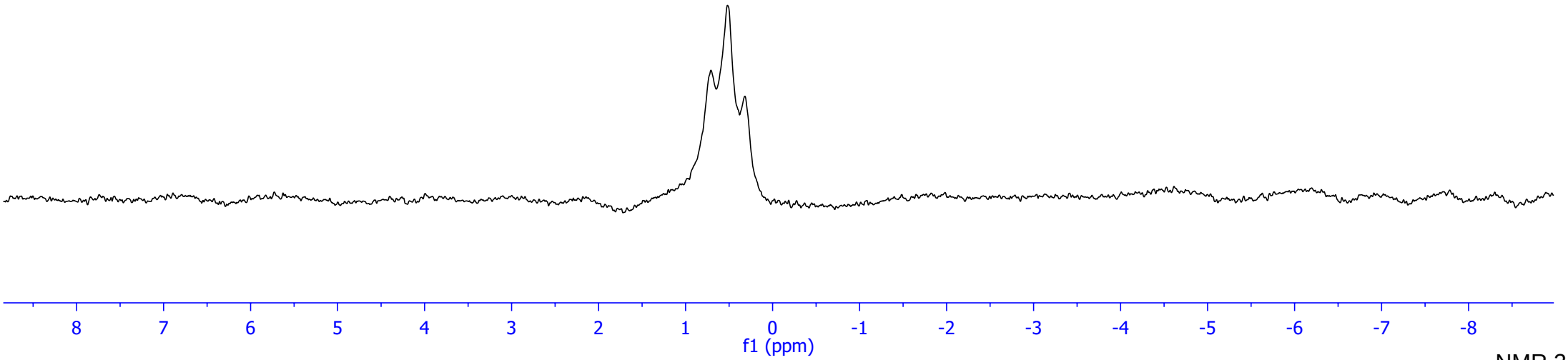


^{13}C of Compound **14** (DMSO-d_6)

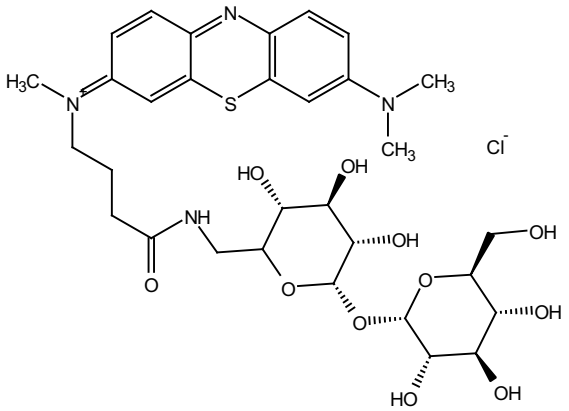


^{11}B of Compound **14** (DMSO-d_6)

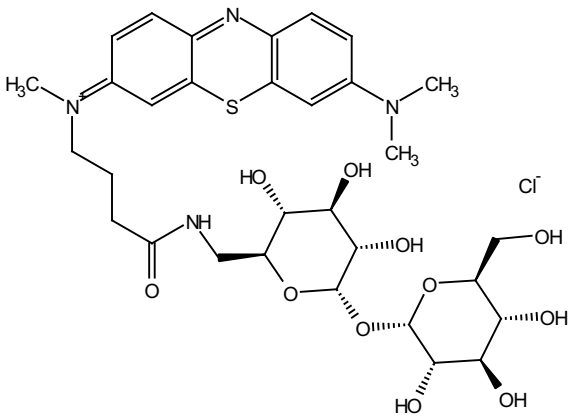




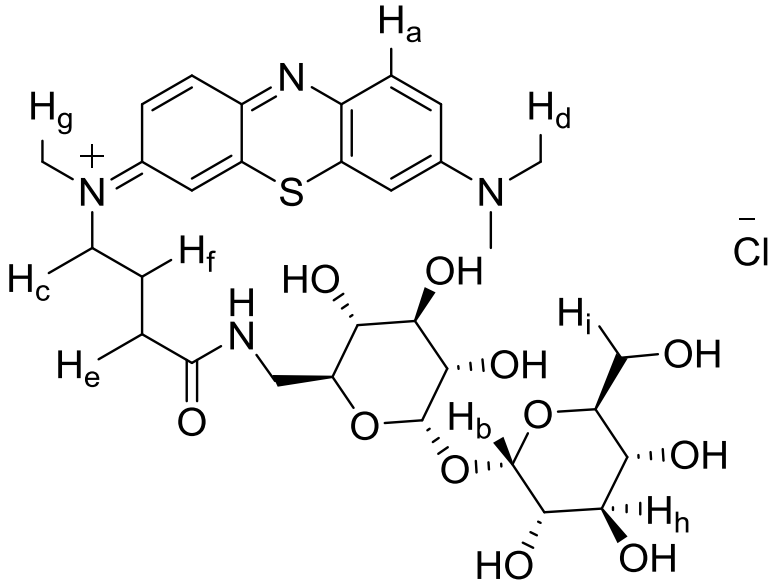
^{1}H of Compound **15** (DMSO-d_6)



^{13}C of Compound **15** (DMSO-d_6)

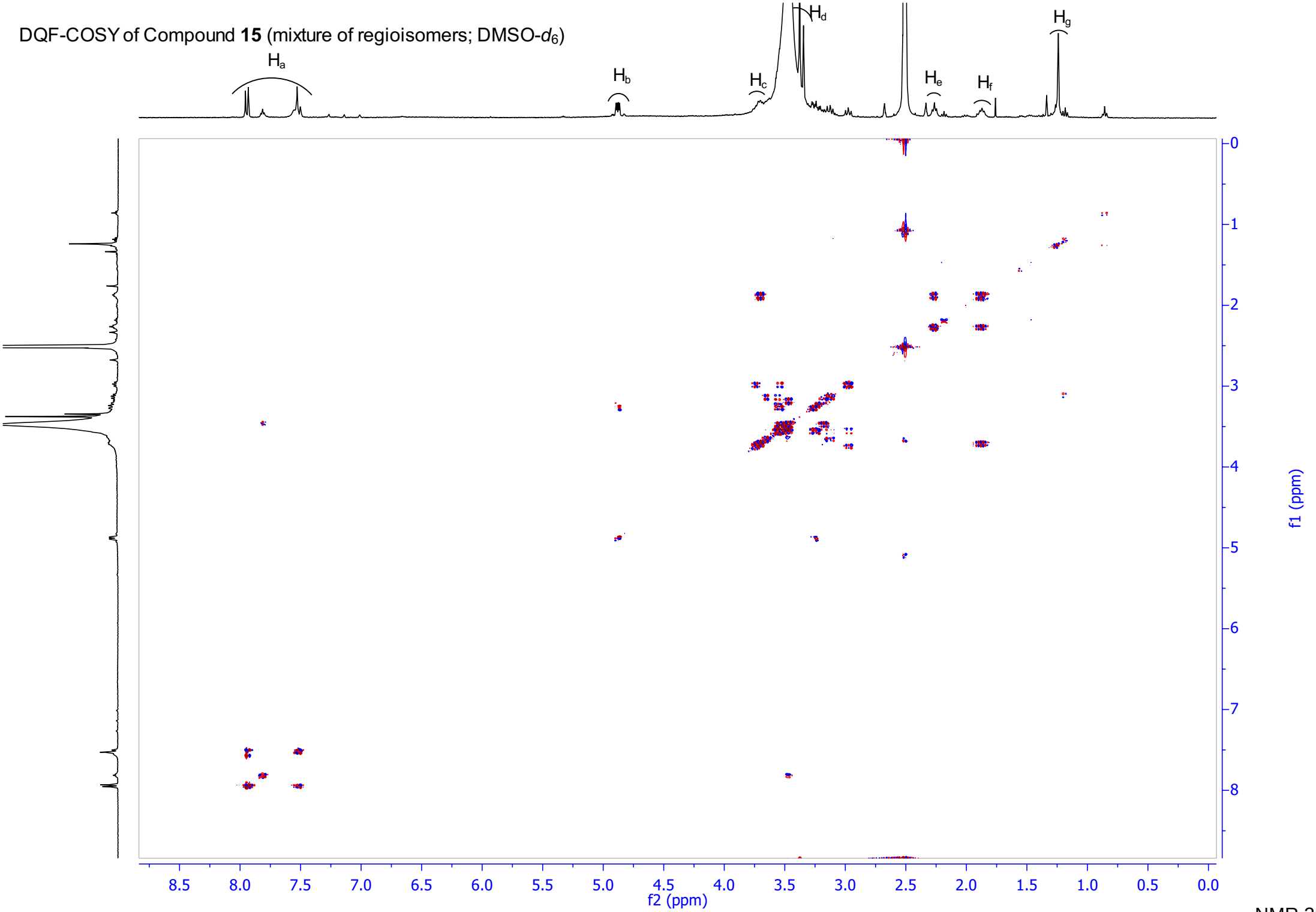


2D NMR analysis of Compound **15**

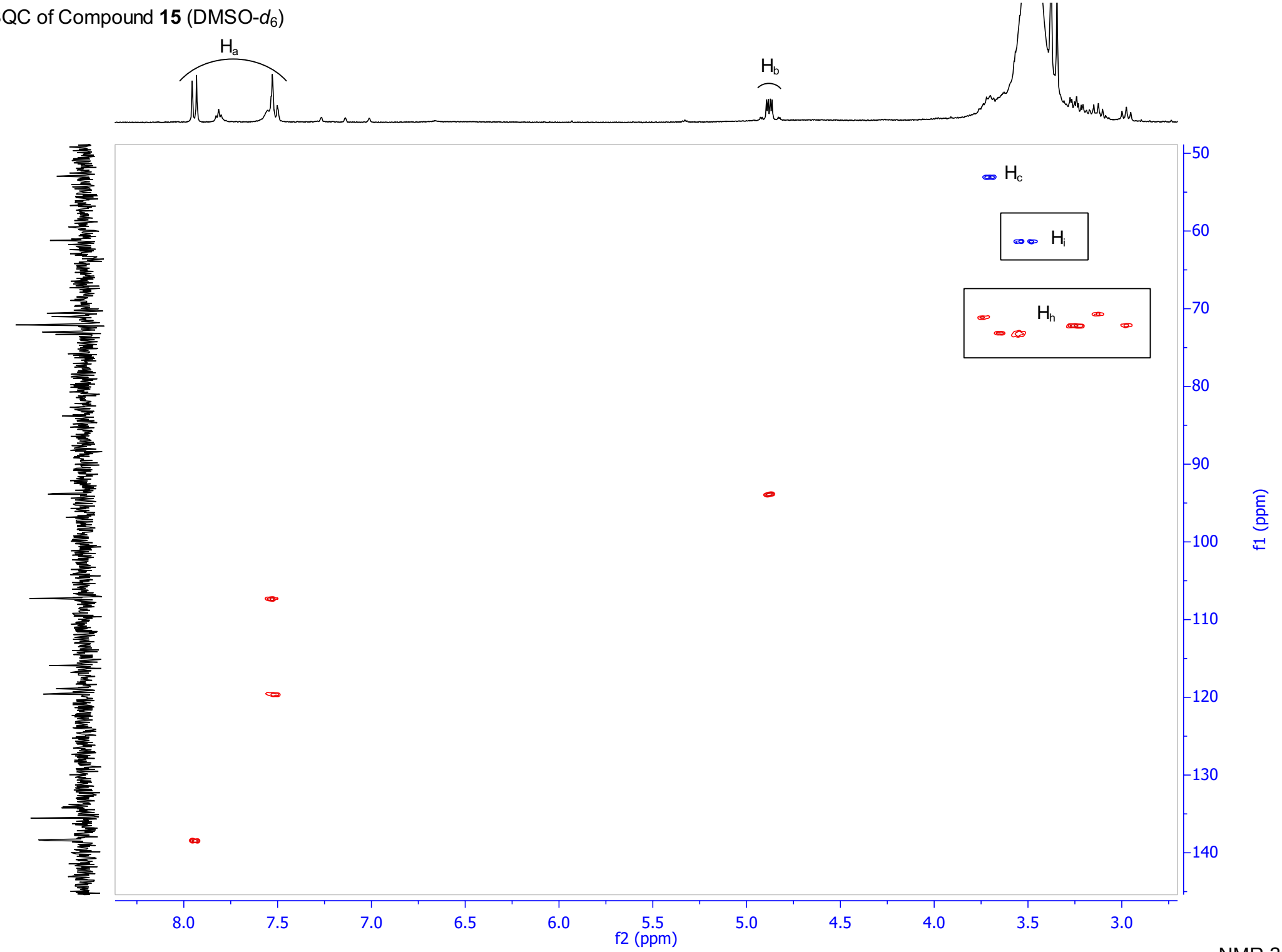


H_a = Aromatic C-H
H_b = Trehalose anomeric C-H
H_h = Trehalose C-H (except H_b)
H_i = Trehalose CH_2

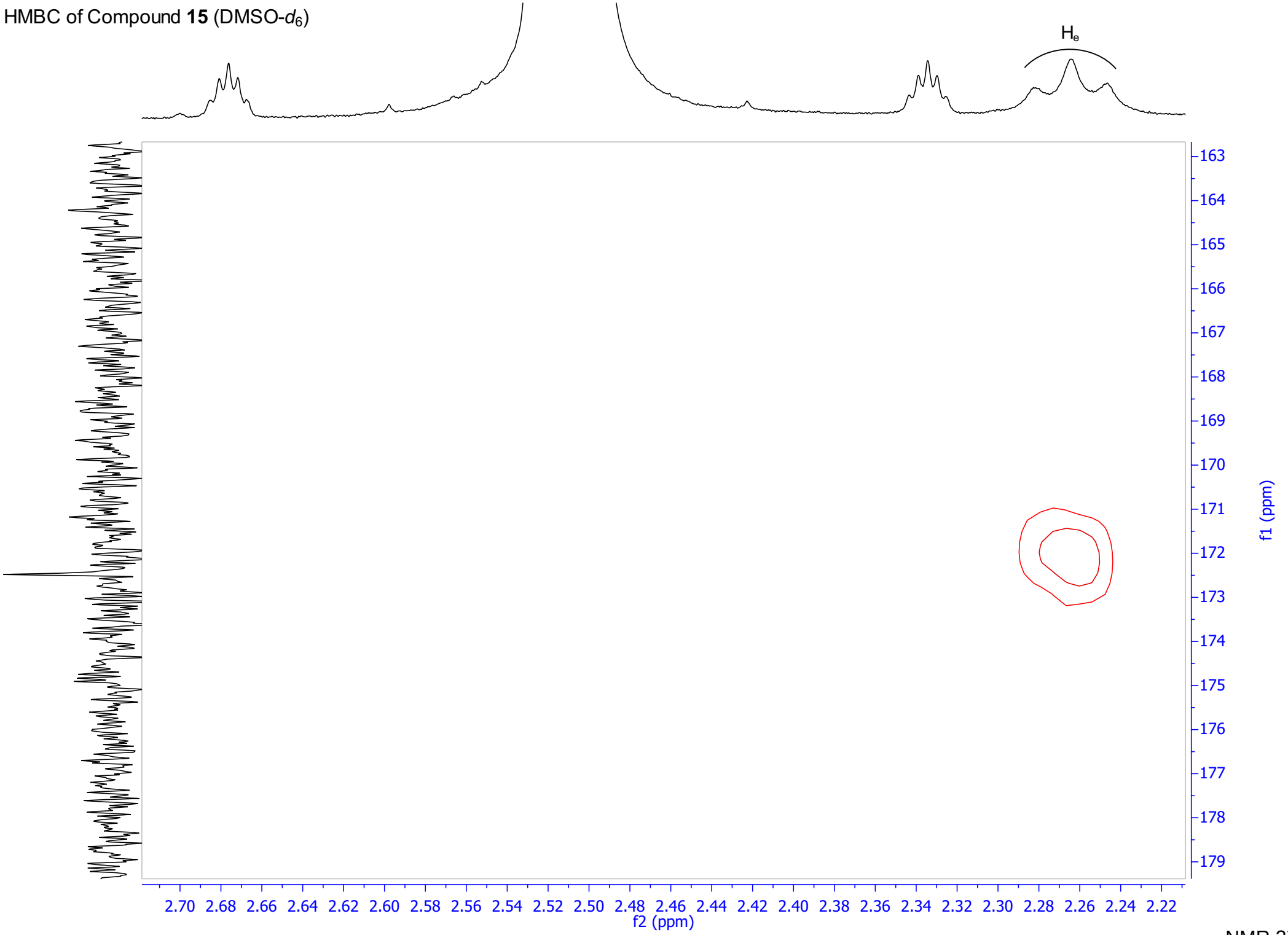
DQF-COSY of Compound **15** (mixture of regioisomers; DMSO-d_6)



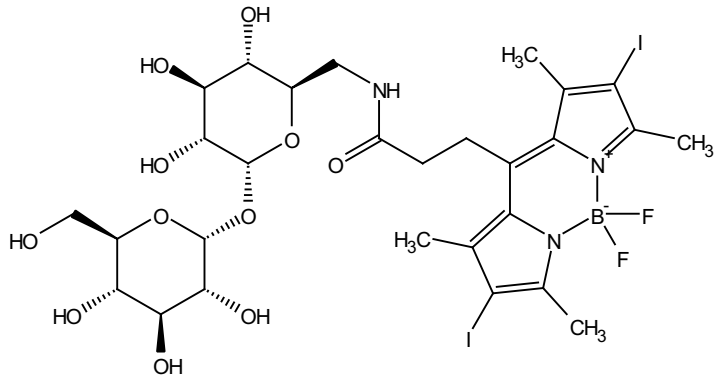
^{1}H-^{13}C HSQC of Compound **15** (DMSO-d_6)

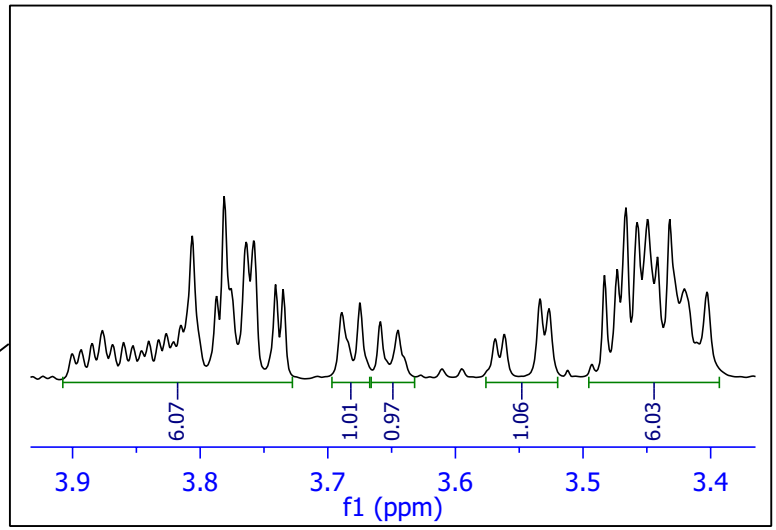


^{1}H-^{13}C HMBC of Compound **15** (DMSO-d_6)



^{1}H of Compound **16** (MeOH-d_4)



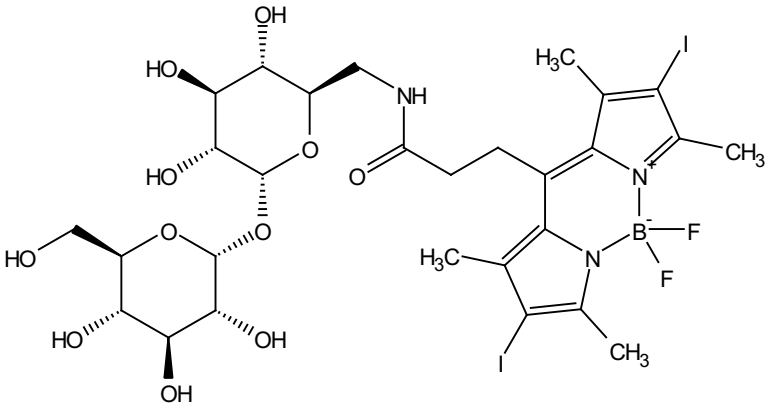


1.00 1.05 6.07 1.01 0.97 1.06 6.03 1.11 6.03 6.56

6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6 0.4 0.2 0.0

f1 (ppm)

^{13}C of Compound **16** (MeOH-d_4)



^{11}B of Compound **16** (MeOH-d_4)