Electronic Supplementary Material (ESI) for Dalton Transactions.

New AB_3-type Porphyrins with Piperidine and Morpholine Motifs; Synthesis, Photo-physicochemical and Biological Properties

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Content:

Materials and methods:

IR spectra were recorded between 4000 and 500 cm-1 using a PerkinElmer Spectrum 100 FT-IR spectrometer with an attenuated total reflection (ATR) accessory featuring a zinc selenide (ZnSe) crystal. MALDI- TOF mass spectrometry analyses were carried out on Bruker microflex LT MALDI-TOF MS spectrometer using dihydroxybenzoic acid as a matrix. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions on a Varian 500 MHz spectrometer. TMS standart was used as internal standart for ^{1}H-NMR measurements. Elemental analysis was carried out using Thermo Finnigan Flash 1112 Instrument. Steady-state fluorescence excitation and emission spectra were recorded using a Varian Cary Eclipse spectrofluorometer. Singlet oxygen quantum yield (FD) measurements were done by Horiba Jobin–Yvon Fluorometer with Hamamatsu NIR PMT 5509 by using direct method. All solvents and chemicals were of reagent-grade quality, purchased from Sigma Aldrich Chemical Co. and Merck

Ground state electronic absorption

Absorption spectra were recorded at room temperature on a Shimadzu UV-2600 UV-vis spectrometer using a 1 cm path length quartz cuvette between maximum range 300 and 1400 nm in THF. Solutions were freshly prepared in spectrophotometric grade solvents. Molar extinction coefficients (ε) were determined by measurement of the absorption solutions of differing concentration for each compound, followed by determination of the slope.

Fluorescence quantum yield (Φ_F) determination

Fluorescence quantum yields of the molecules were calculated employing the comparative William's method according to followed Equation (1) where Std designates standard, and Grad is the gradient of the plot and n is the refractive index of the solvent, respectively [1,2]. The fluorescence quantum yield (Φ_F) values of the compounds 3-5 were determined in THF by comparing it with the fluorescence of H_2TPP and ZnTPP (Φ_F = 0.13 and 0.06 and in THF, respectively) [3,4] as a standard molecule. Φ_F 's of compound was calculated by the comparative method (Eq. 1). Integrated fluorescence intensities versus absorbance for H_2TPP (Φ_F = 0.13 in THF) were plotted. Slopes of the plots were proportional to the quantity of the quantum yield. Equation 1 was used to calculate quantum yield ((Φ_F) values. In the equation, Grad is the gradient of the plot and n is the refractive index of the solvent used for samples and standards.

$$\Phi_F = \Phi_F^{Std}\left(\frac{Grad}{Grad_{Std}}\right)\left(\frac{n^2}{n_{Std}^2}\right) \quad (1)$$

Fluorescence lifetime (τ_F) measurements

Fluorescence lifetimes were recorded using Horiba FL3-2IHR with a Time Correlated Single Photon Counting (TCSPC) system using FLUOROLOG-3 spectrofluorometer. The instrument was equipped with a nanoLED-670-LH and a standard air cooled R928 PMT detector. A NanoLED light source (HORIBA Jobin Yvon) of λ_{max} 390 nm was used for 395 nm excitation. A non-fluorescence suspension of colloidal silica (LUDOX 30%, Sigma Aldrich) in water was used for fluorescence lifetimes measurement.

Singlet oxygen quantum yield (Φ_Δ) determination

The direct method in THF was applied for the determination of Φ_Δ and measurements were done by Horiba Jobin-Yvon Fluorometer with Hamamatsu NIR PMT 5509. For direct method, the calculation of the $^1O_2$ quantum yields was based on the detection of NIR $^1O_2$ luminescence by an optical method based on the comparison of single molecular oxygen phosphorescence produced by the porphyrin sample with that generated by the reference H_2TPP and ZnTPP (Φ_Δ = 0.58 and 0.61 in THF) [3,4] in the near infrared region at 1276 nm. Φ_Δ values were calculated according to Equation 2.

$$\phi_{\Delta s} = \phi_{\Delta r}\frac{\eta_s^2 A_r I_s}{\eta_r^2 A_s I_r} \quad (2)$$

In Eq. 2, $\Phi_{\Delta s}$ and $\Phi_{\Delta r}$ are the quantum yields of the sample and reference and η_s and η_r are refractive indexes of the solvents used for the measurements of the sample and reference. A_s and A_r are the absorbance of the sample and the reference, and I_s and I_r are the integrated areas under the emission spectra of the sample and the reference, respectively.

Microbial Cell Viability Inhibition

The microbial cell viability inhibition were performed using the process previously described in reference [1] with some modifications and *E. coli* (ATCC 25922) was used as model microbial cell. The microorganism was inoculated and then incubated for 24 h at 37 °C in a shaker at 120 rpm. *E. coli* was obtained by centrifugation for 7 min at 6000 rpm. The pellet was cleaned with sterile NaCl solution (0.9%) to remove the fermentation medium residue. The

cleaned bacterial strain was suspended with using 0.9% NaCl. This microbial suspension was utilized for *E. coli* viability inhibition assay (2.9×10^8 CFU/mL). *E. coli* was exposed with various concentrations of porphyrins for 90 min at 37 ℃. Then, the mixtures were diluted at various ratio, inoculated on Nutrient Broth(NB) agar, and incubated at 37 ℃ for 24 h. The colonies were counted and bacterial cell viability inhibiton was calculated by applying the Eq. (3) [5]. DMSO also used as negative control.

$$\text{Cell viability (\%)} = (A_{control} - A_{sample} / A_{control}) \times 100 \quad (3)$$

Biofilm inhibition ability and Biofilm inhibition activity with photodynamic therapy

The biofilm inhibition ability of the porphyrins was assayed by studying the following assay. *S. aureus* and *P. aeruginosa* were used as model organisms. A 24 well plate including different concentrations of porphyrins and the related organism (*S. aureus* or *P. aeruginosa*) were kept for 3 days at 37 ℃ in NB medium. The plate's wells were emptied and washed after 3 days. Adherent cells remaining in the wells were gently cleaned twice with distilled water and then plates were dried at 75 ℃ for 45 min. After the addition of crystal violet (CV) to stain the biofilms, they were incubated for 1 h. Then, the wells were cleaned twice with distilled water. Ethanol was added for the dissolution of the CV. The wells were left for 15 min for the recovery of the absorbed CV. The results were measured using a spectrophotometer at 595 nm. *S. aureus* and *P. aeruginosa* were also used as positive controls which wells not contain test compounds. Additionally, the same process was used for the determination of the biofilm inhibition activity with photodynamic therapy (PDT) but the porhyrins were exposed to LED light for 30 min. A red-orange light emitting diode was used at 632 ± 2 nm with a dosage of 12 J/cm^2. The results were calculated using Eq. (4) [5]. DMSO also used as negative control.

$$\text{Biofilm Inhibition (\%)} = (Abs_{(control)} - Abs_{(sample)} / Abs_{(control)}) \times 100 \quad (4)$$

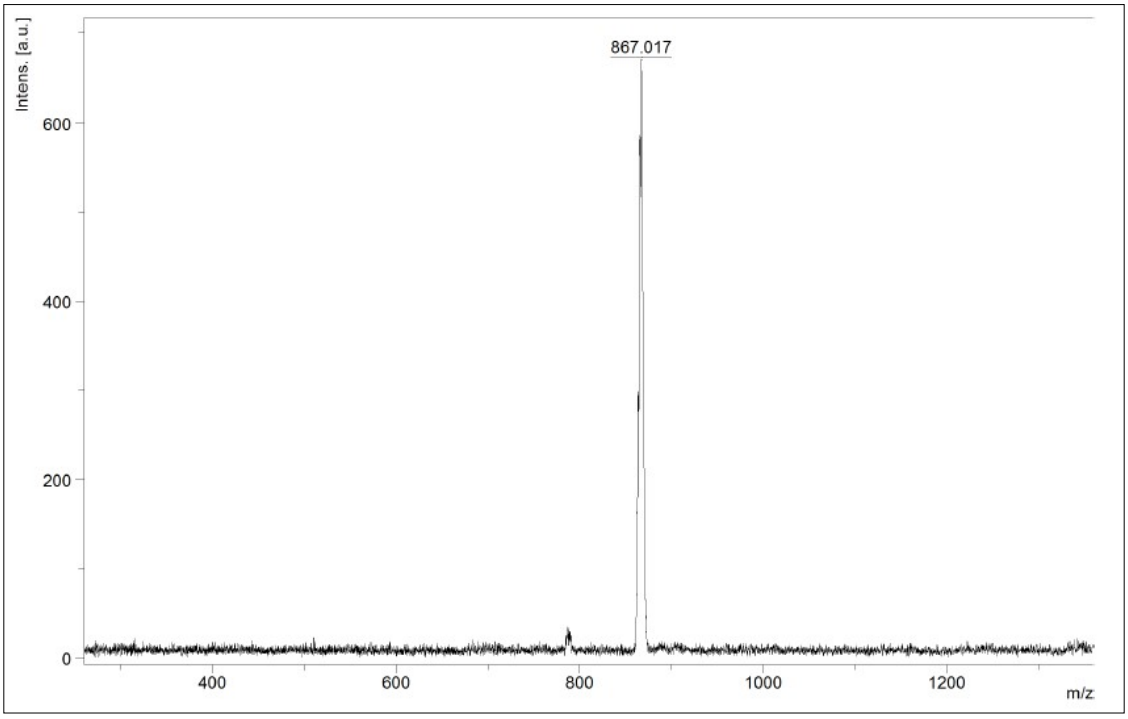

Fig. S1. MALDI-TOF mass spectrum of compound **1**.

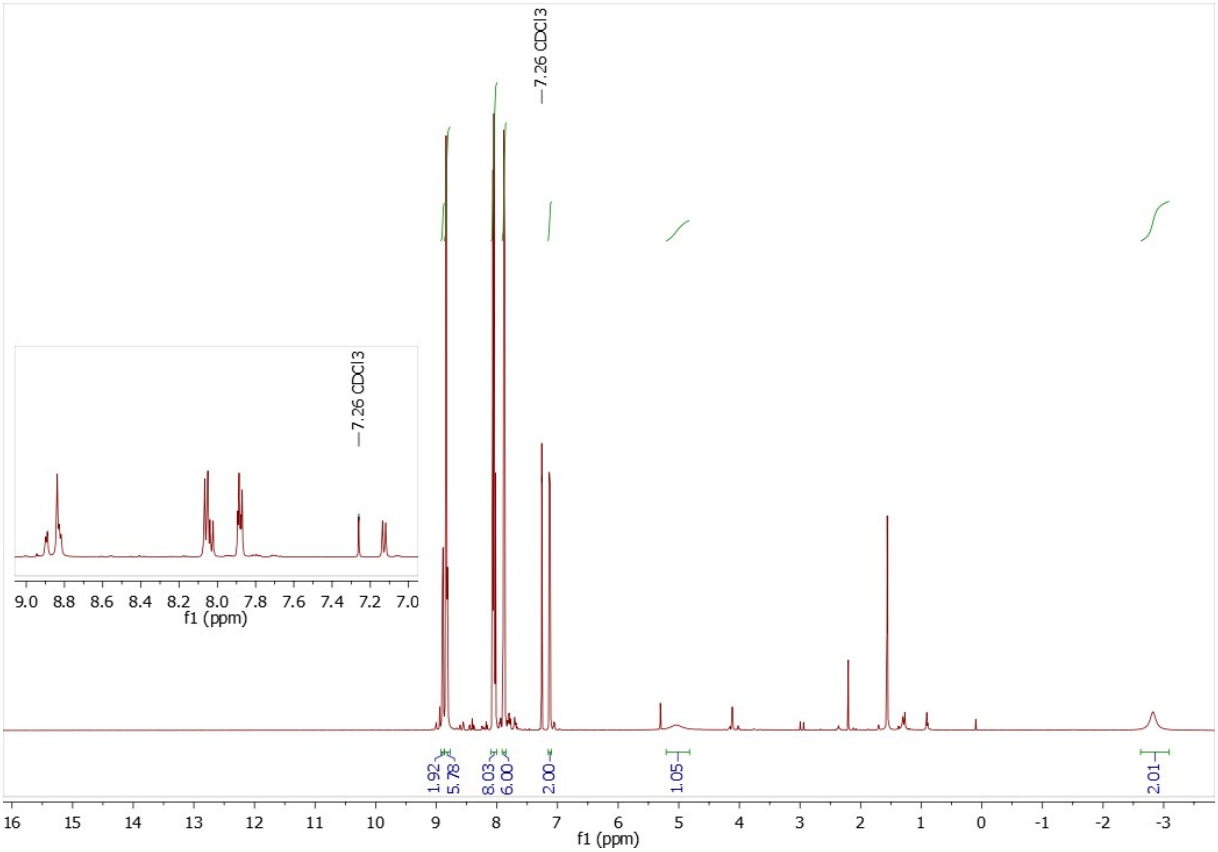

Fig. S2. ^{1}H NMR spectrum of compound **1** (500 MHz, in $CDCl_3$).

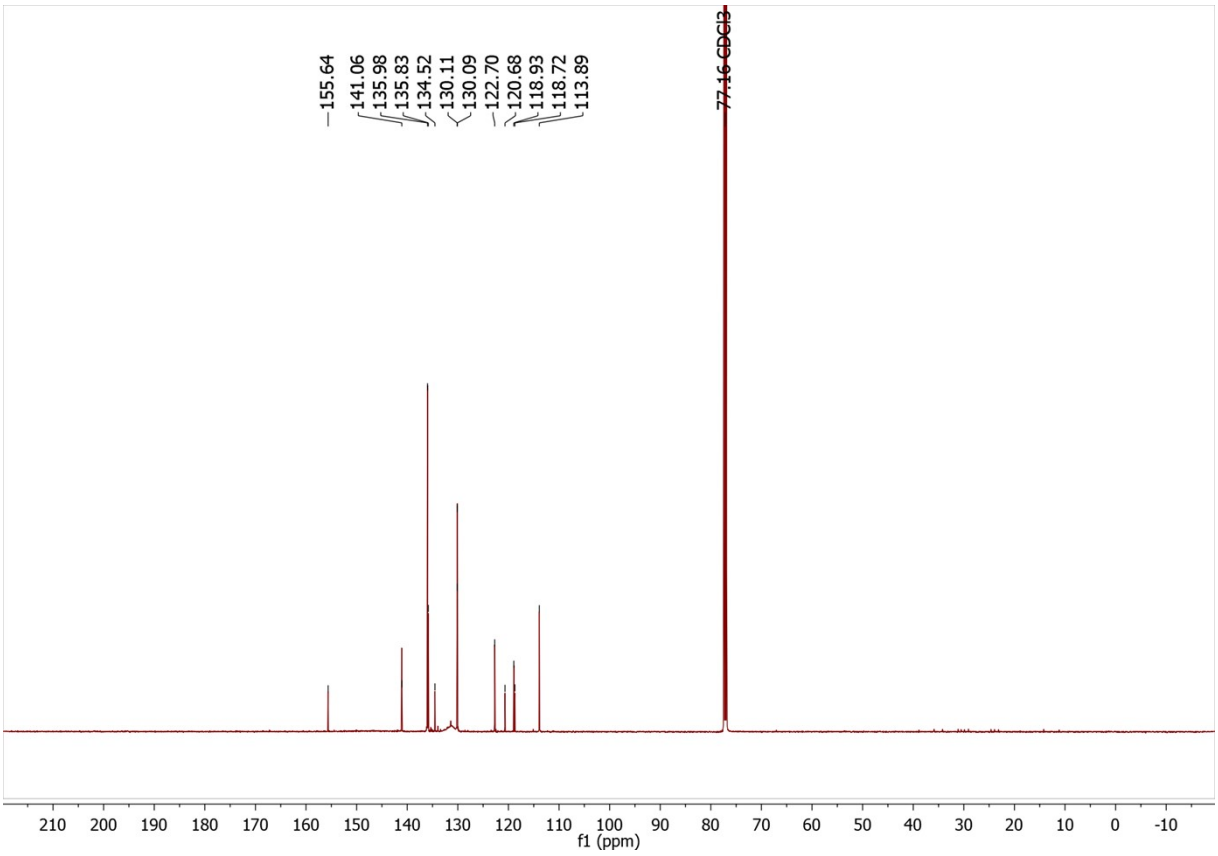

Fig. S3. ^{13}C NMR spectrum of compound **1** (125 MHz, in $CDCl_3$).

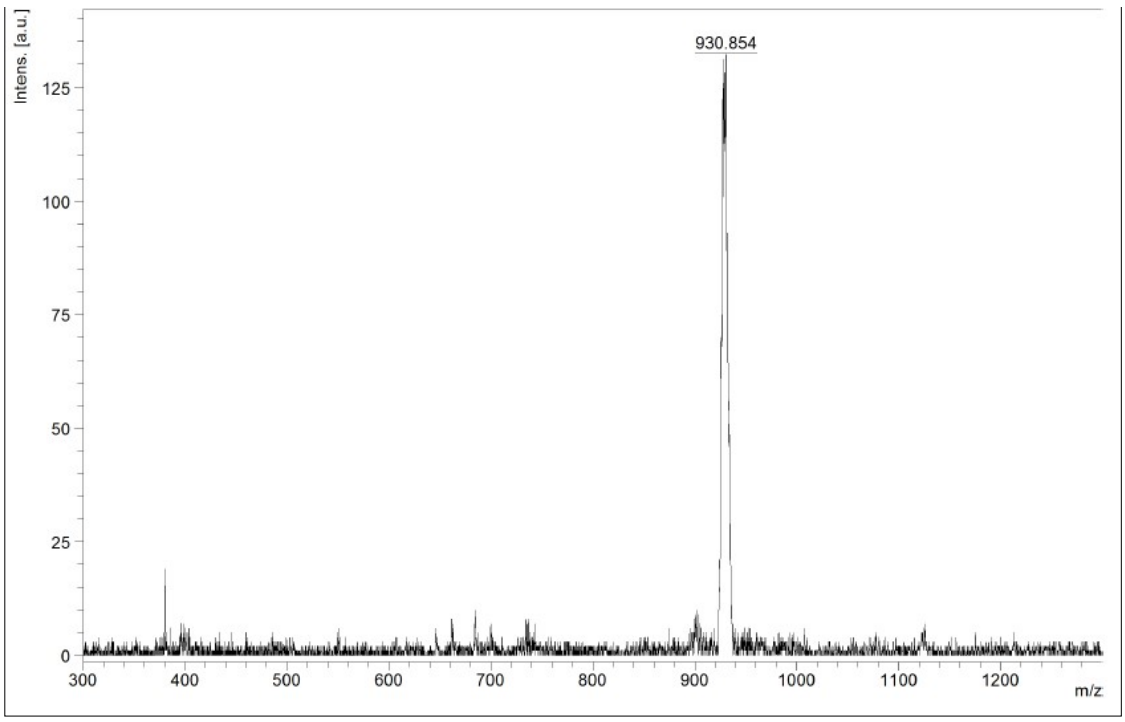

Fig. S4. MALDI-TOF mass spectrum of compound **2**.

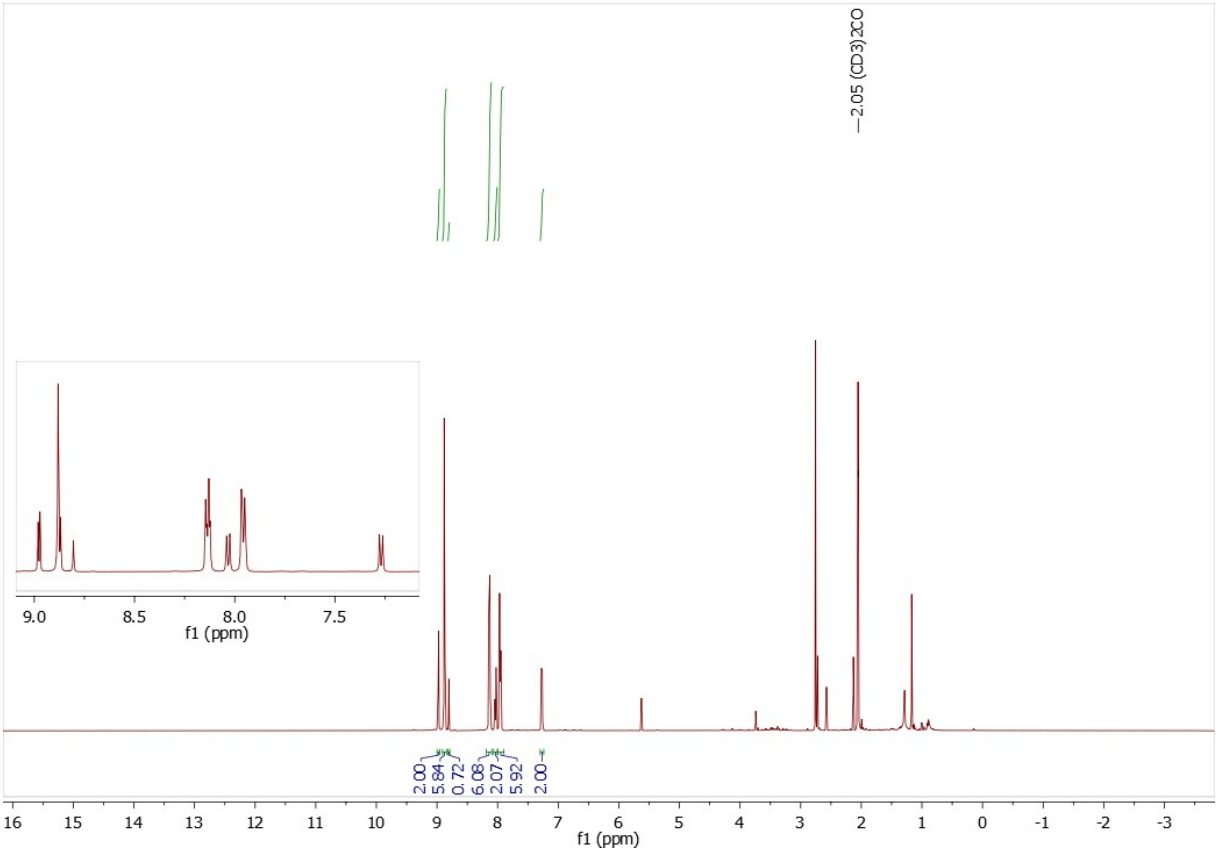

Fig. S5. ^{1}H NMR spectrum of compound **2** (500 MHz, in Acetone-d_6).

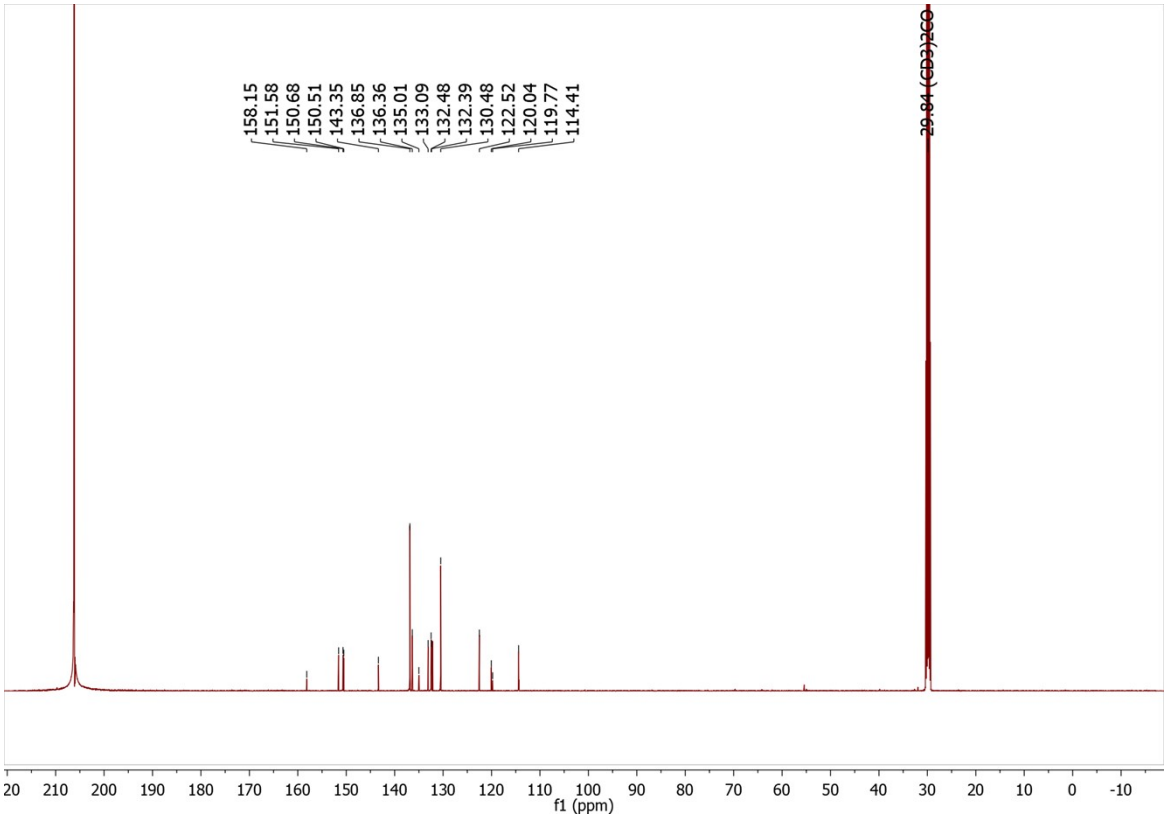

Fig. S6. ^{13}C NMR spectrum of compound **2** (125 MHz, in $CDCl_3$).

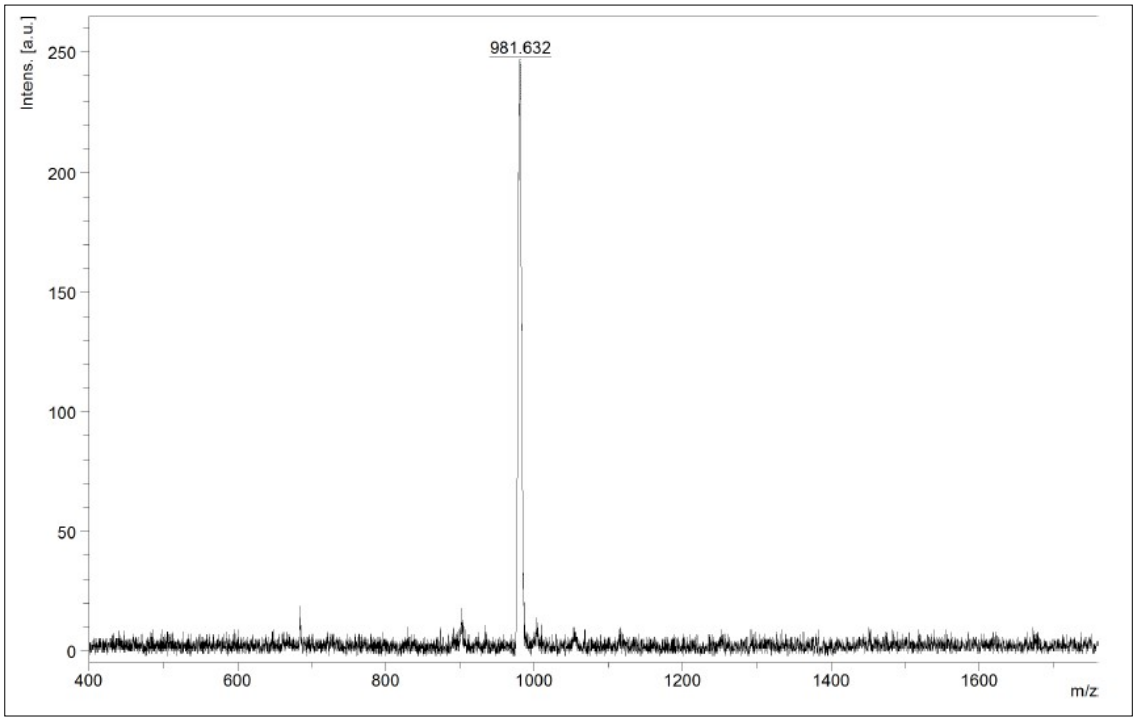

Fig. S7. MALDI-TOF mass spectrum of compound **3**.

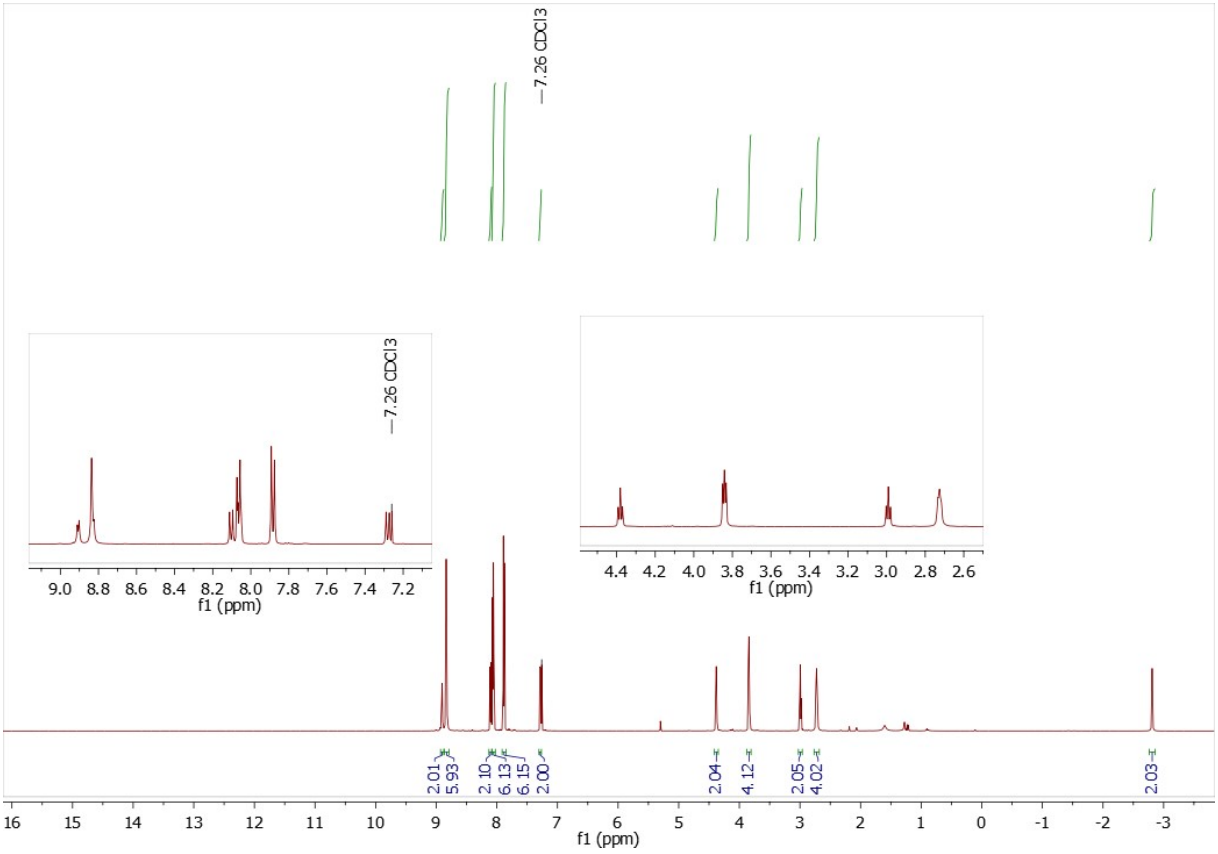

Fig. S8. ^{1}H NMR spectrum of compound **3** (500 MHz, in $CDCl_3$).

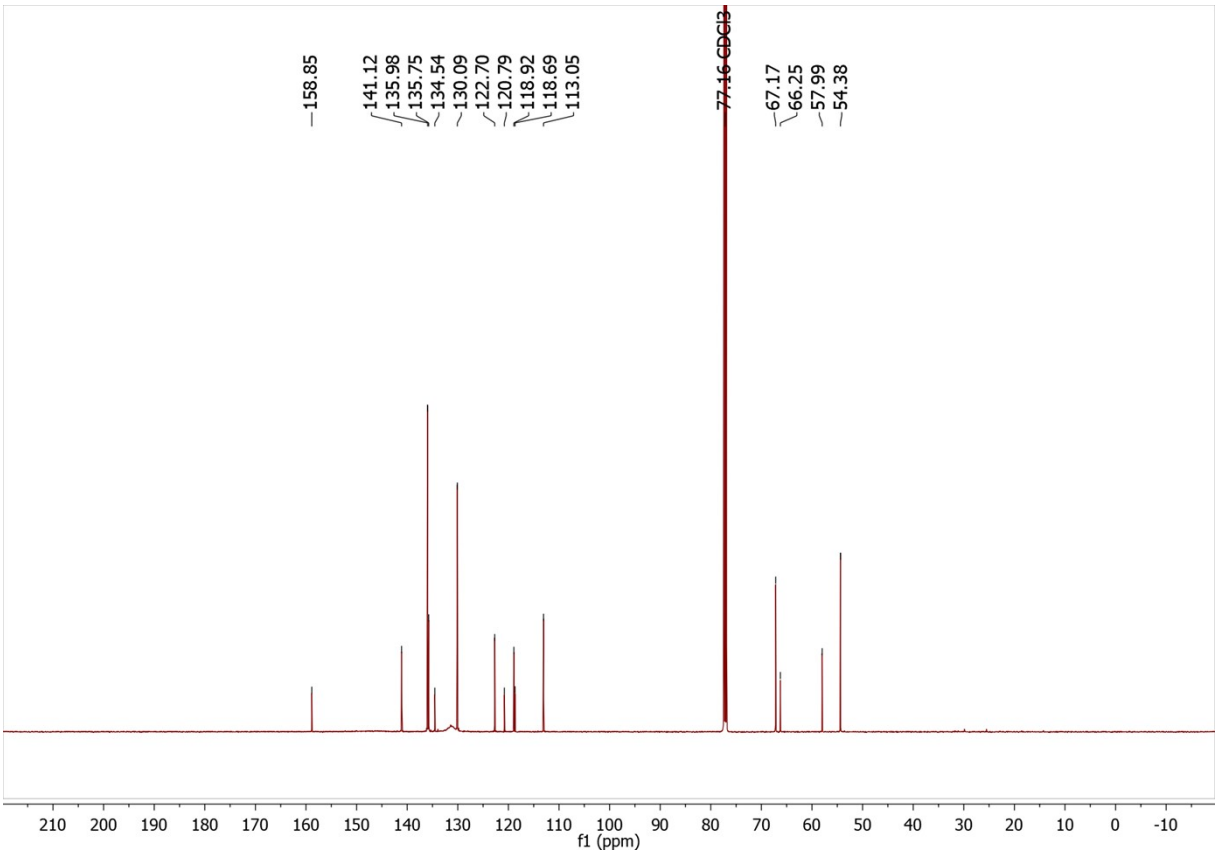

Fig. S9. ^{13}C NMR spectrum of compound **3** (125 MHz, in $CDCl_3$).

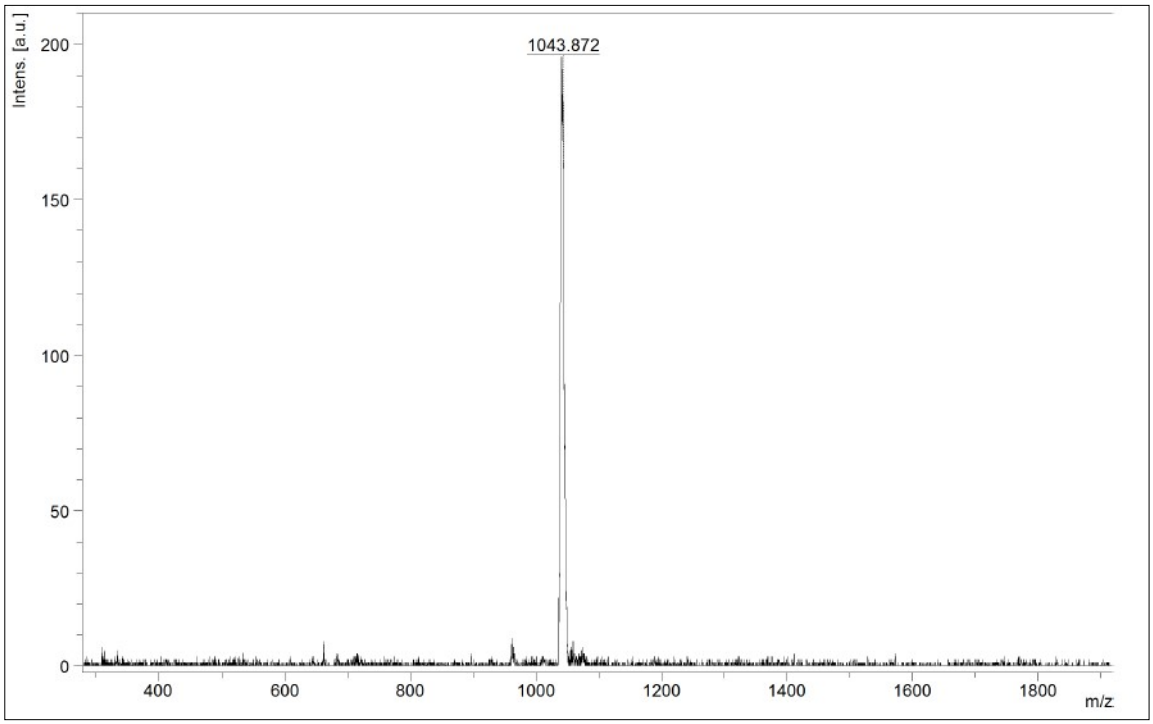

Fig. S10. MALDI-TOF mass spectrum of compound **4** (DIT).

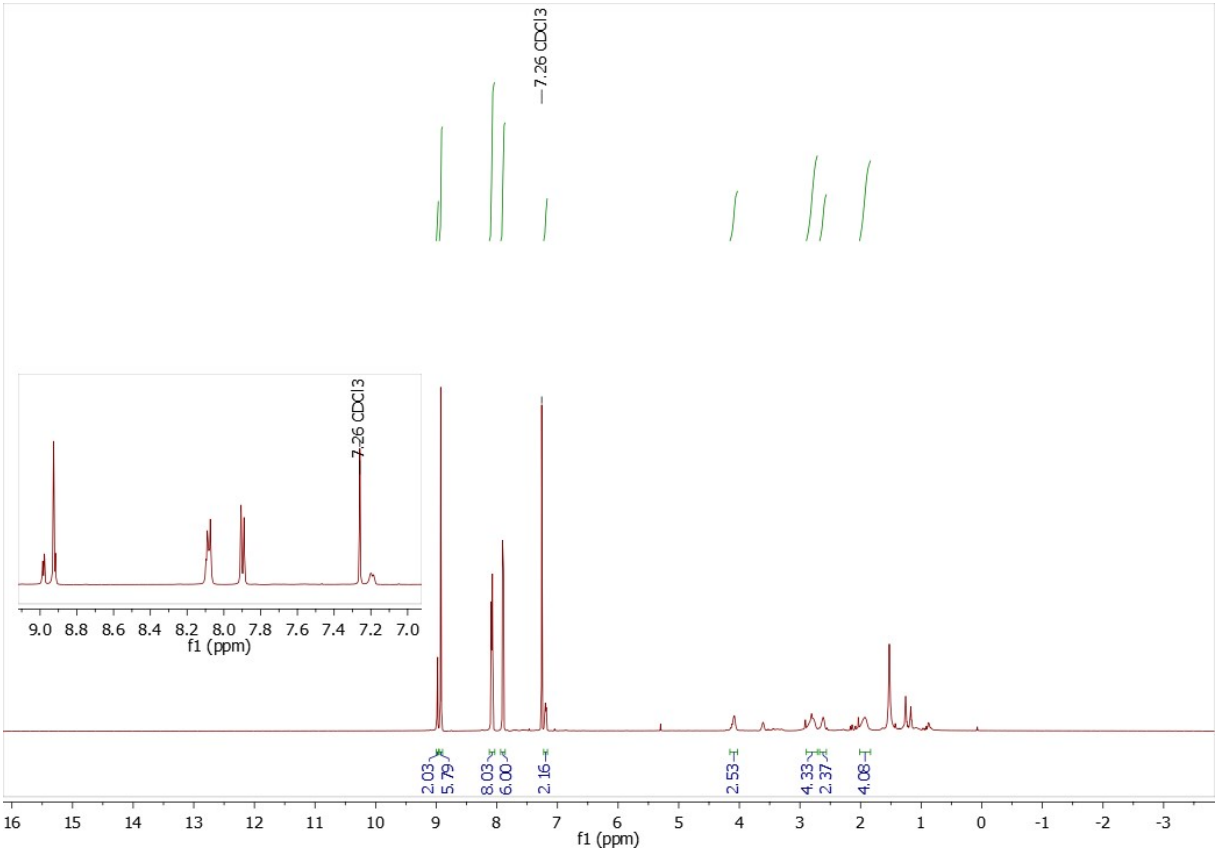

Fig. S11. ^{1}H NMR spectrum of compound **4** (500 MHz, in $CDCl_3$).

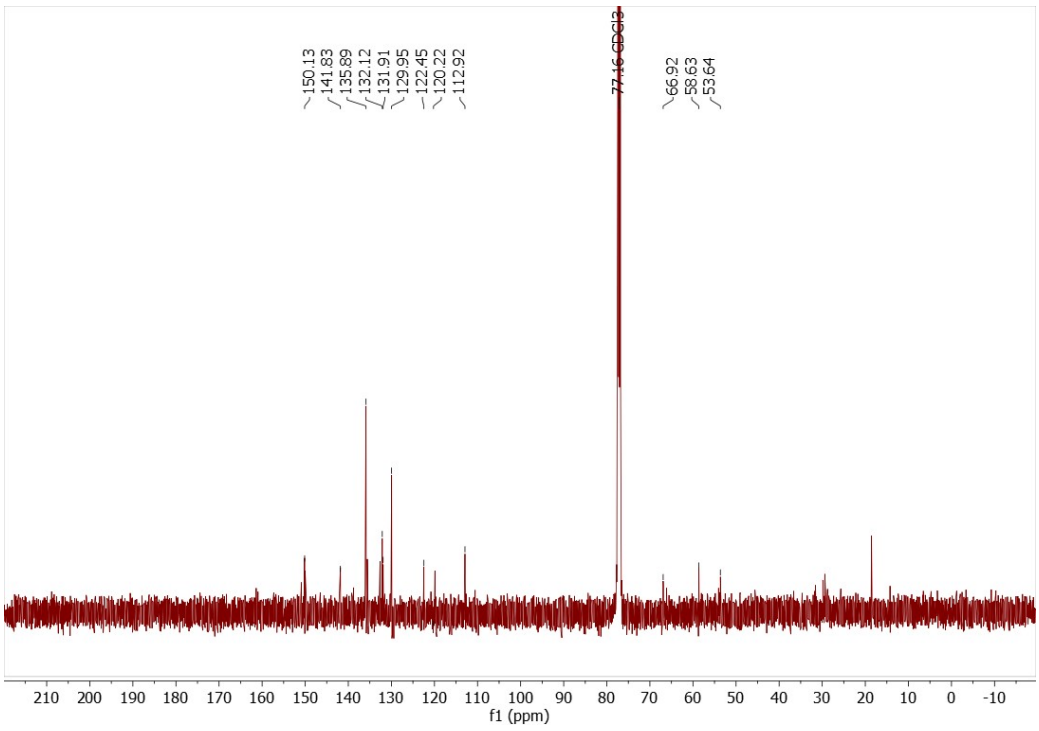

Fig. S12. ^{13}C NMR spectrum of compound **4** (125 MHz, in $CDCl_3$).

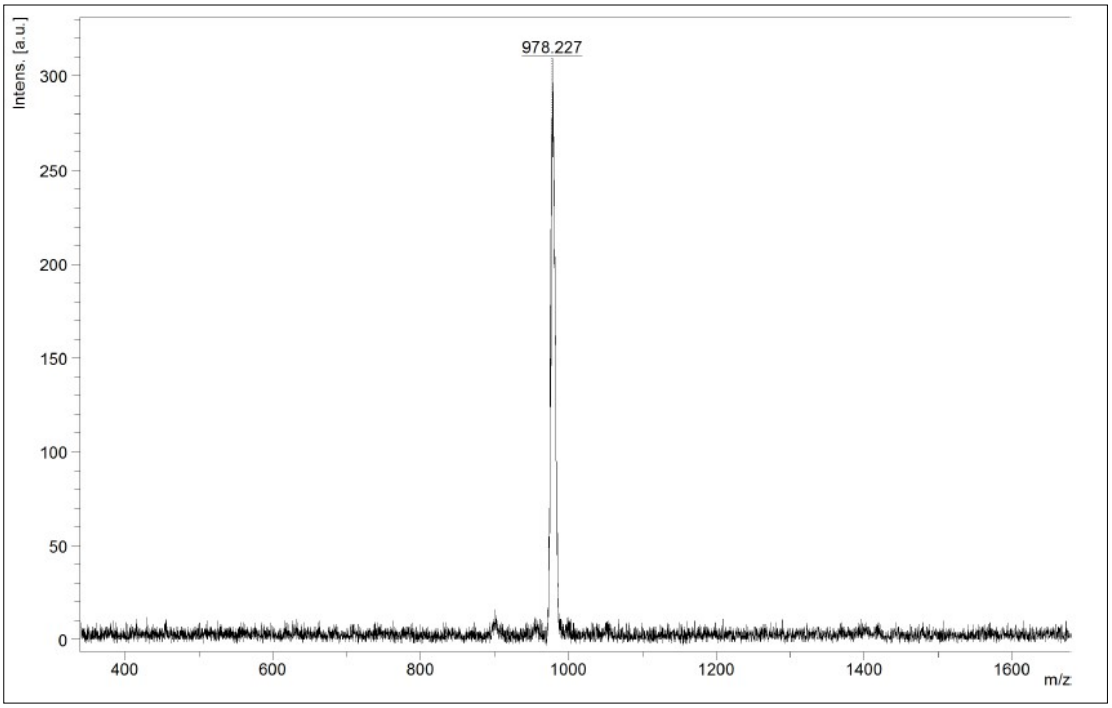

Fig. S13. MALDI-TOF mass spectrum of compound **5** (DIT).

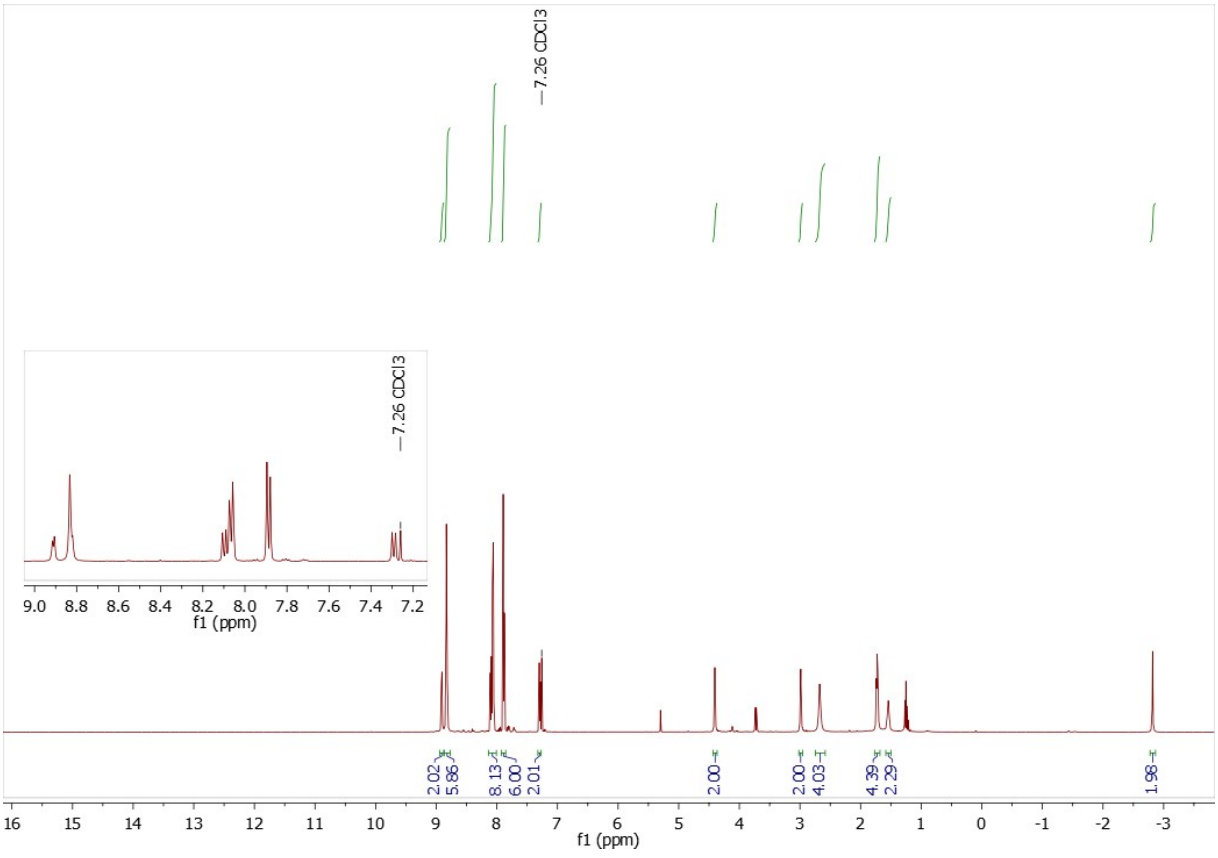

Fig. S14. ^{1}H NMR spectrum of compound **5** (500 MHz, in $CDCl_3$).

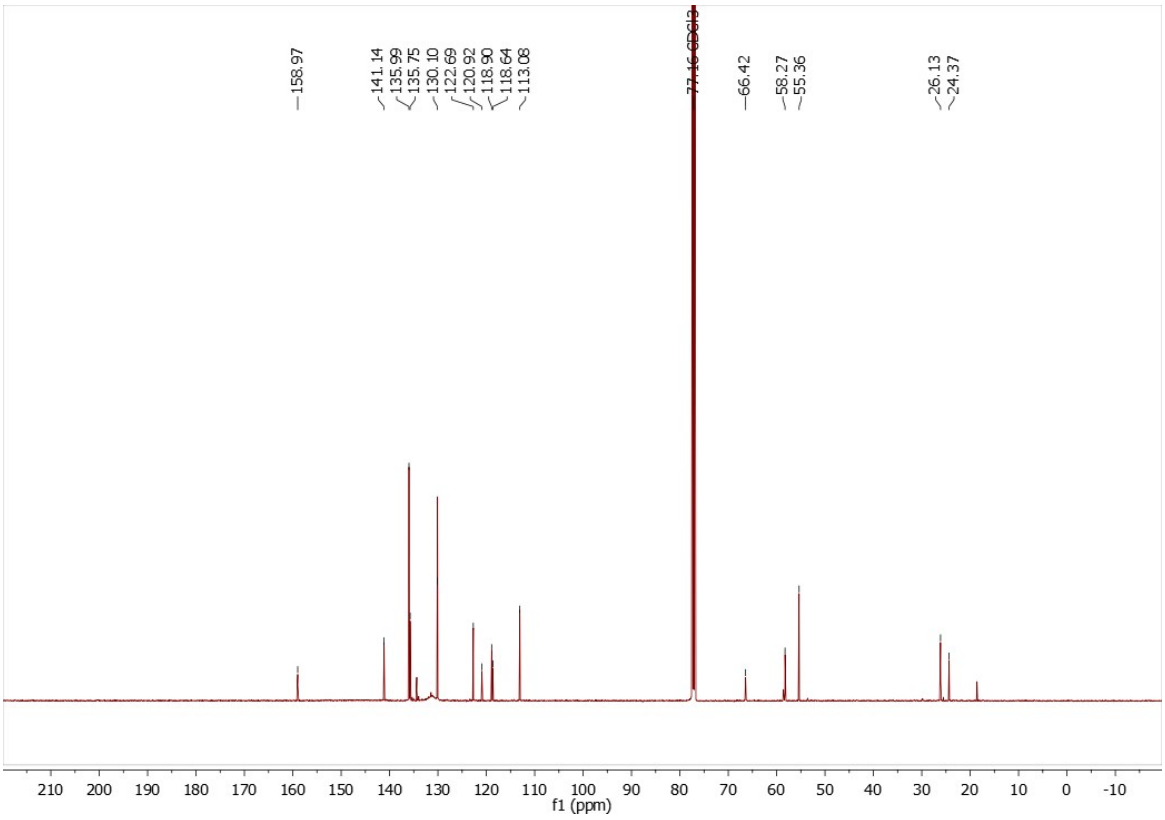

Fig. S15. ^{13}C NMR spectrum of compound **5** (125 MHz, in $CDCl_3$).

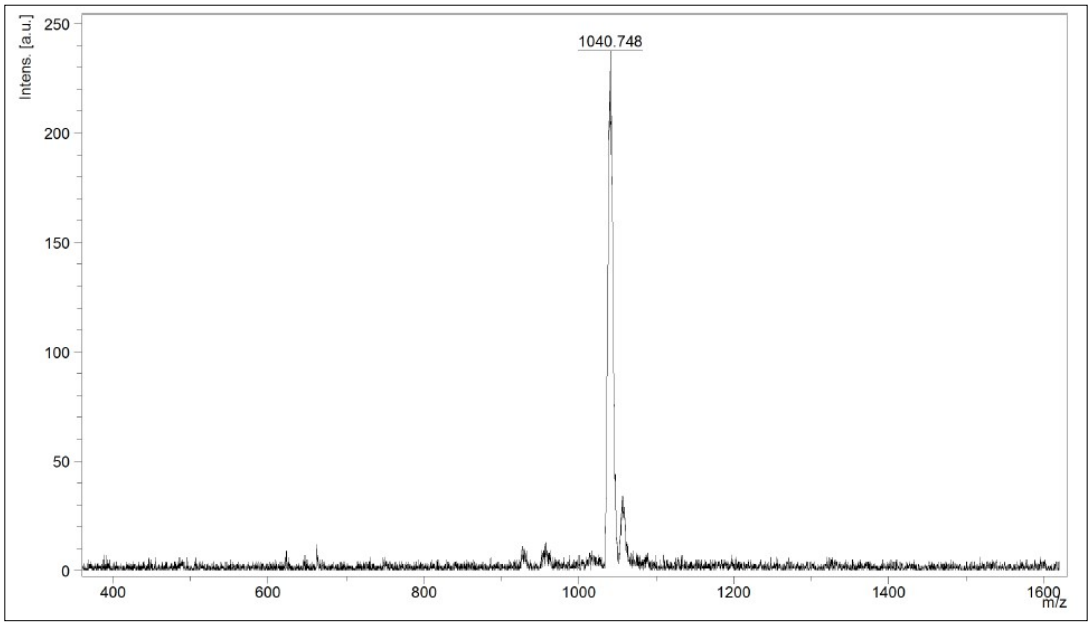

Fig. S16. MALDI-TOF mass spectrum of compound **6** (DIT).

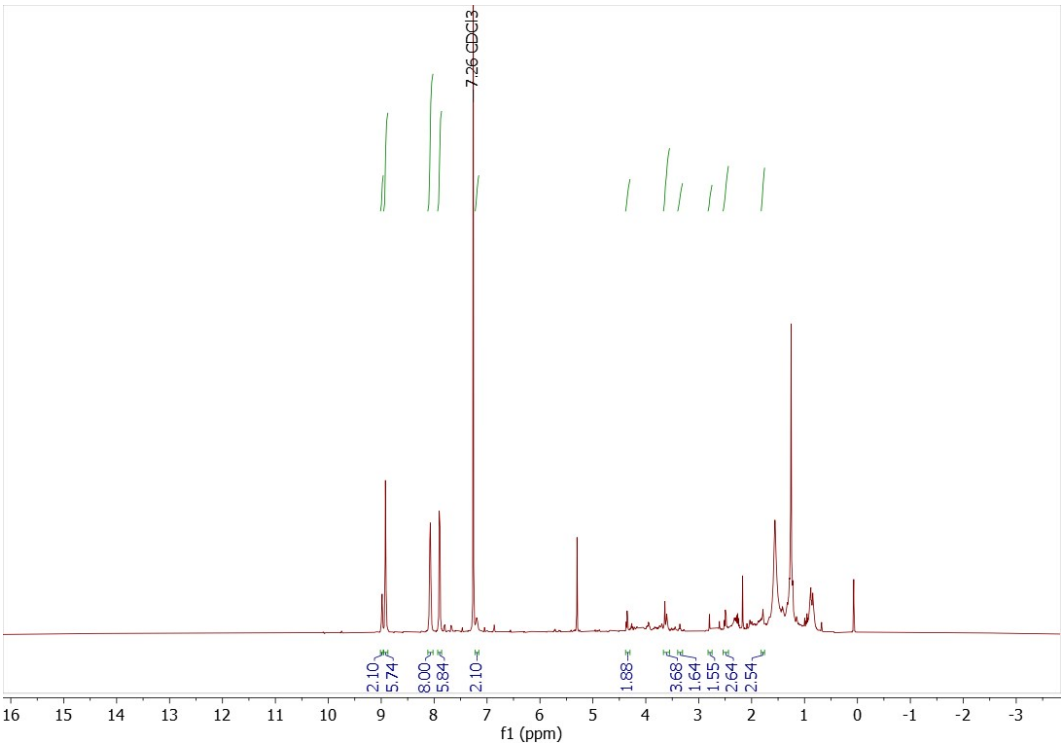

Fig. S17. ^{1}H NMR spectrum of compound **6** (500 MHz, in $CDCl_3$).

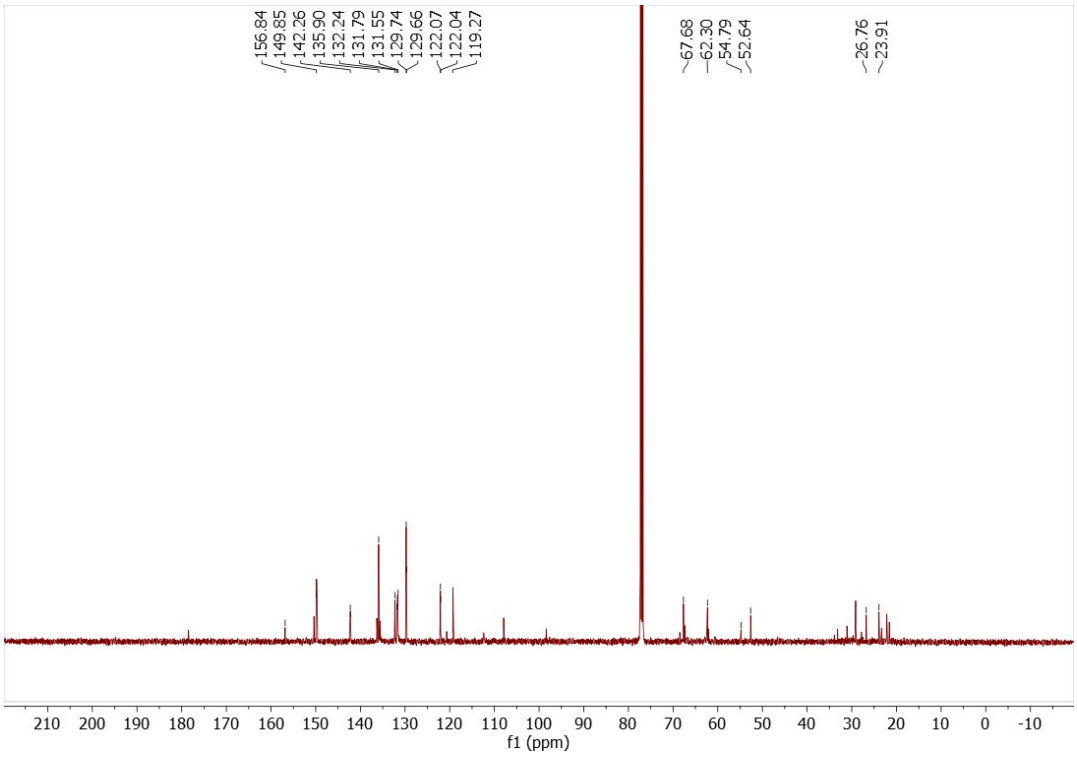

Fig. S18. ^{13}C NMR spectrum of compound **6** (125 MHz, in $CDCl_3$).

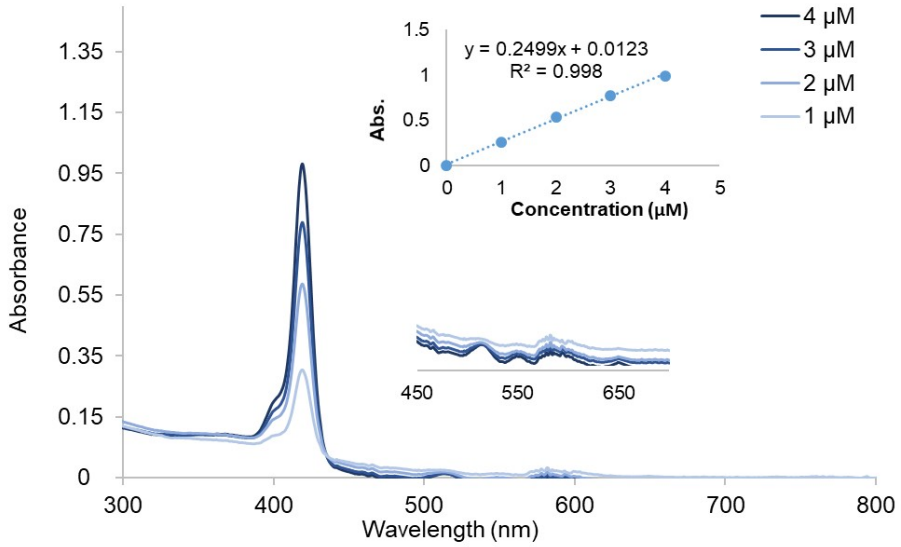

Fig. S19: UV-Vis absorption spectra of **1** in THF solutions of 1-4 µM. Insets: absorbance vs. concentration.

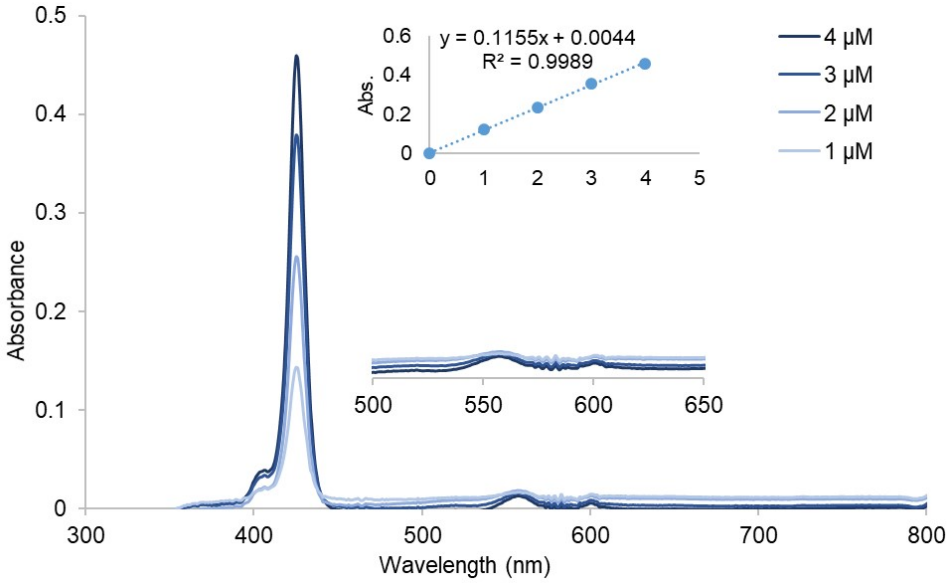

Fig. S20: UV-Vis absorption spectra of **2** in THF solutions of 1-4 µM. Insets: absorbance vs. concentration.

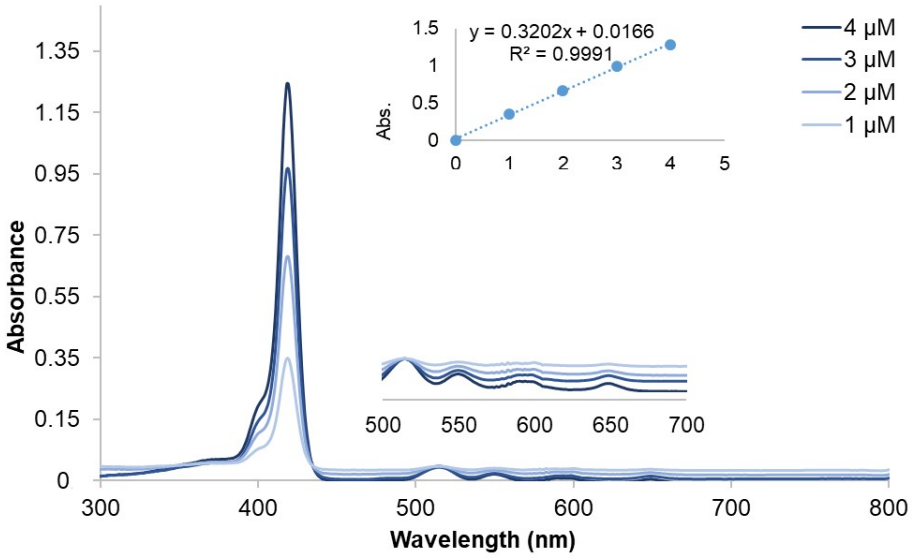

Fig. S21: UV-Vis absorption spectra of **3** in THF solutions of 1-4 µM. Insets: absorbance vs. concentration.

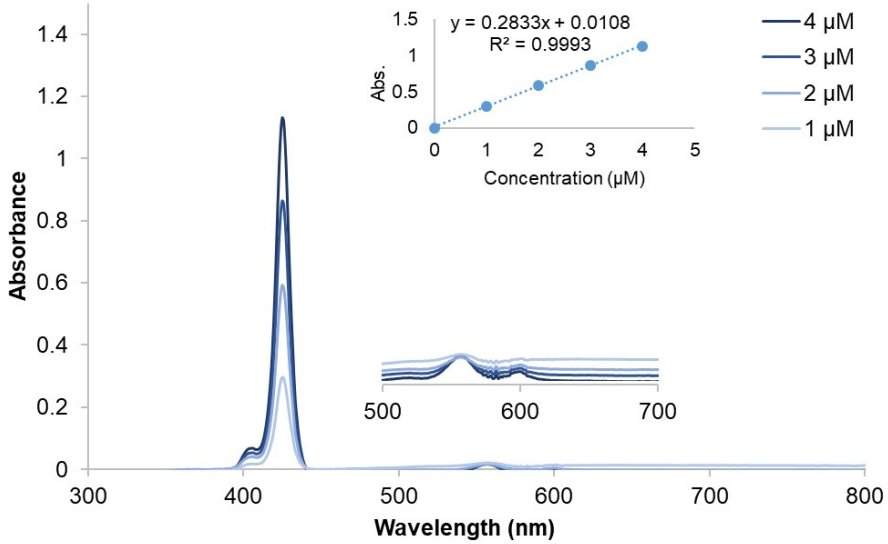

Fig. S22: UV-Vis absorption spectra of **4** in THF solutions of 1-4 µM. Insets: absorbance vs. concentration.

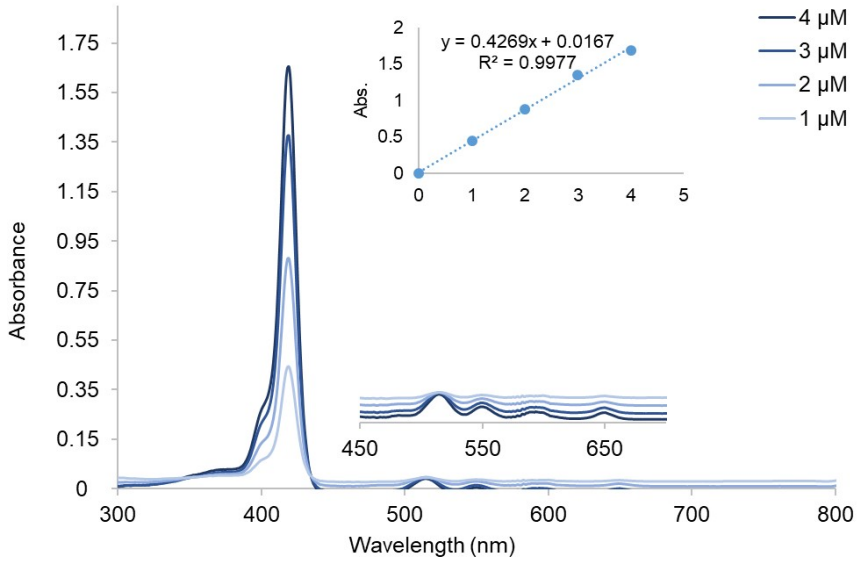

Fig. S23: UV-Vis absorption spectra of **5** in THF solutions of 1-4 µM. Insets: absorbance vs. concentration.

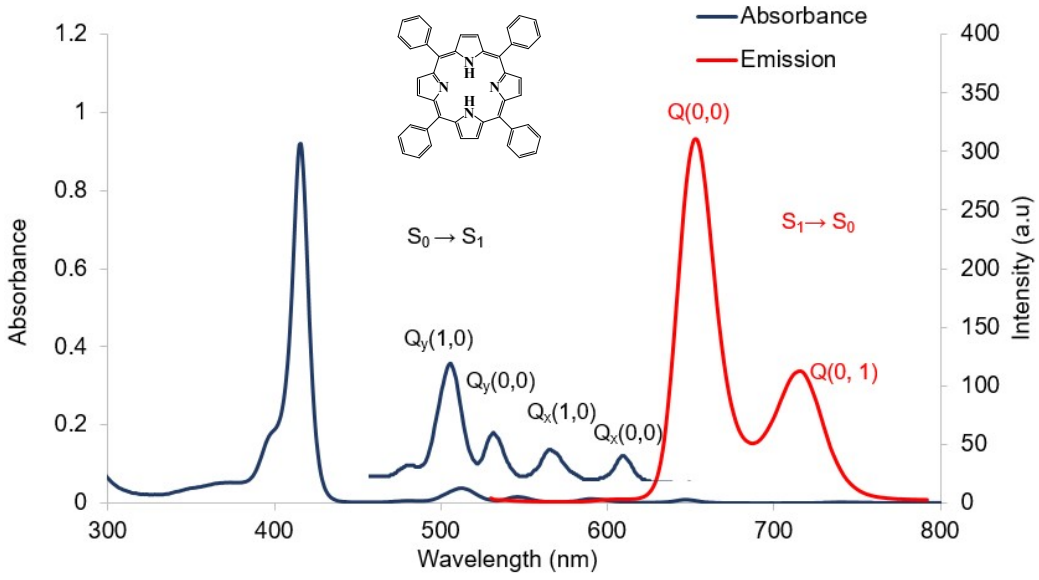

Fig. S24: Superimposed absorption and emission spectra of **H_2TPP** in THF (5µM)

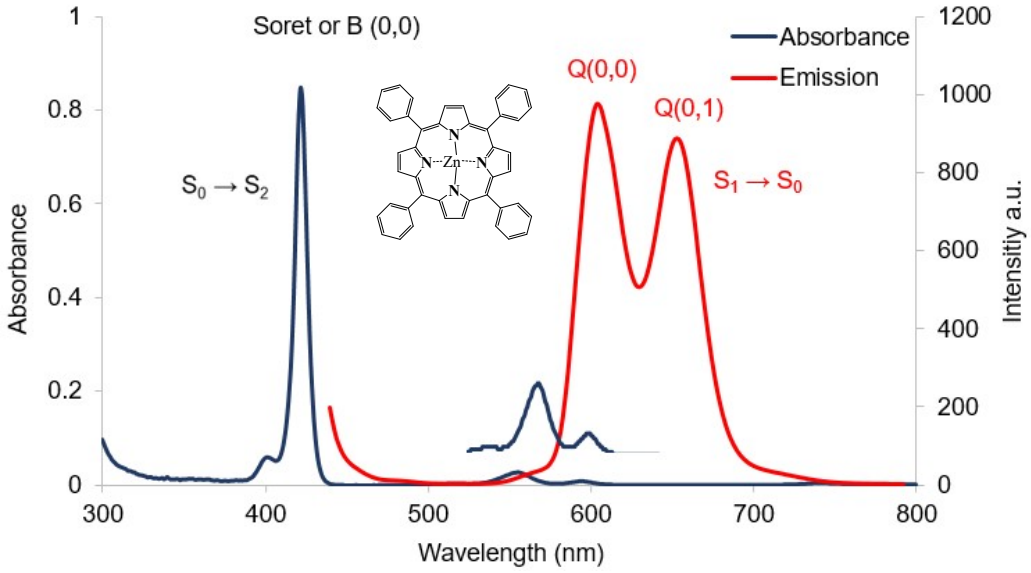

Fig. S25: Superimposed absorption and emission spectra of **ZnTPP** in THF (5 µM)

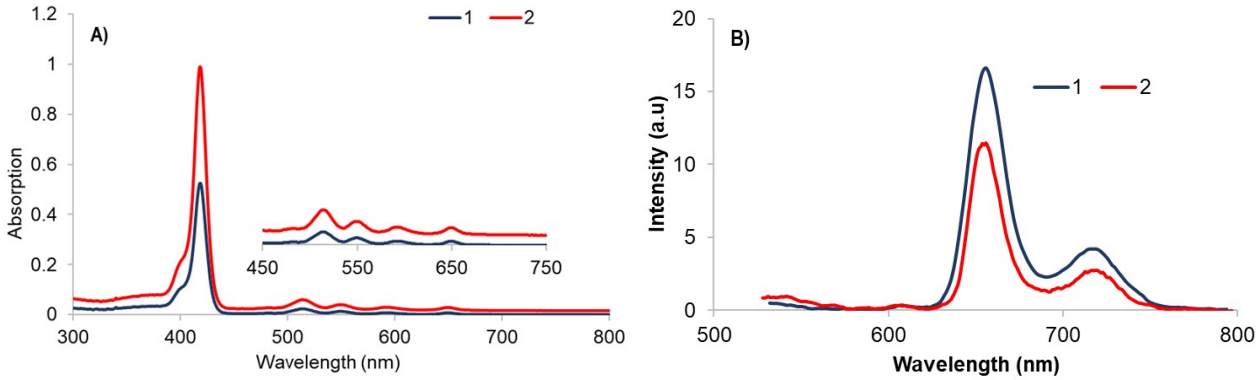

Fig. S26: (A)Absorption (1= 3.8µM, 2= 1.8µM) and (B) Emission spectra (λ_{ex} = 514 nm) of the porphyrin **1** in THF.

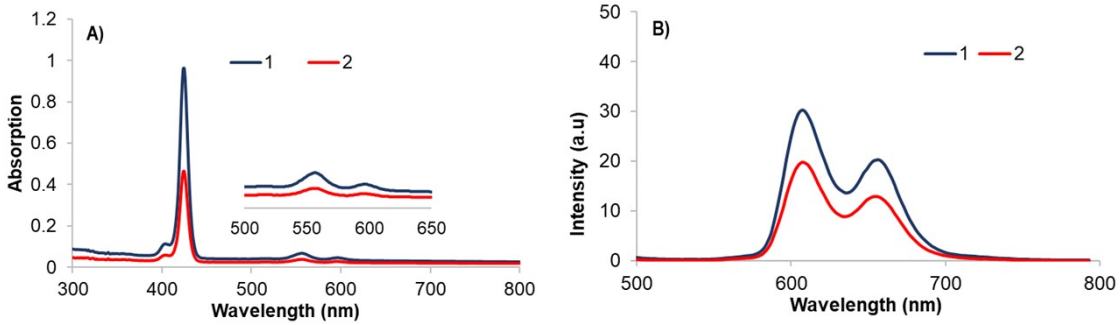

Fig. S27: (A)Absorption (1= 8µM, 2= 4µM) and Emission spectra (B, λ_{ex} = 425 nm) of the porphyrin **2** in THF.

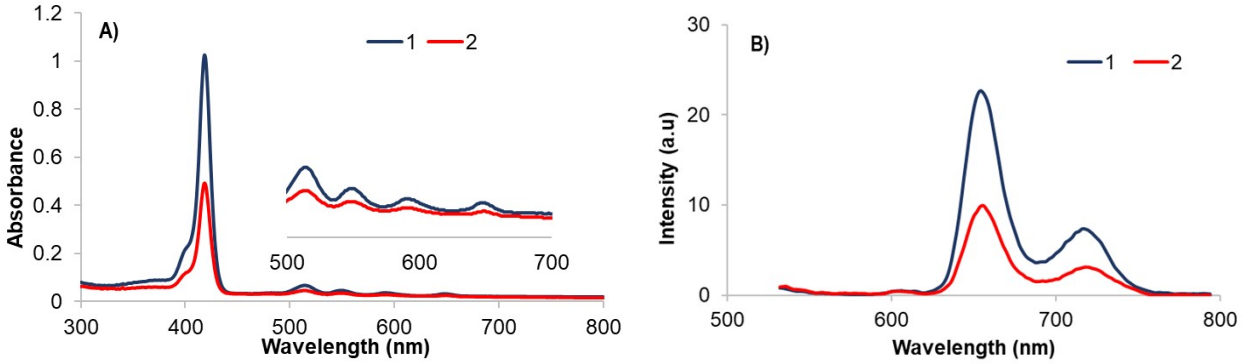

Fig. S28: (A)Absorption (1= 3µM, 2= 1.5µM) and Emission spectra (B, λ_{ex} = 514 nm) of the porphyrin **3** in THF.

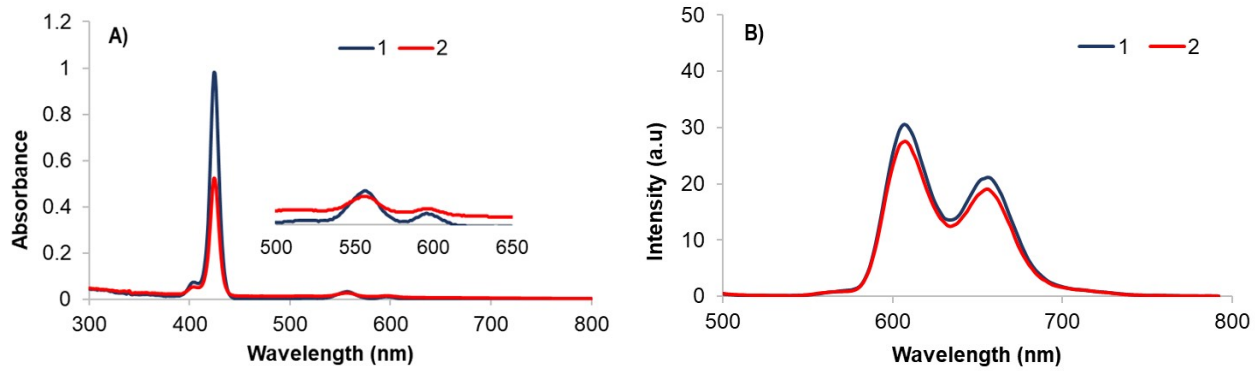

Fig. S29: (A)Absorption (1= 4µM, 2= 2µM) and Emission spectra (B, λ_{ex} = 424 nm) of the porphyrin **4** in THF.

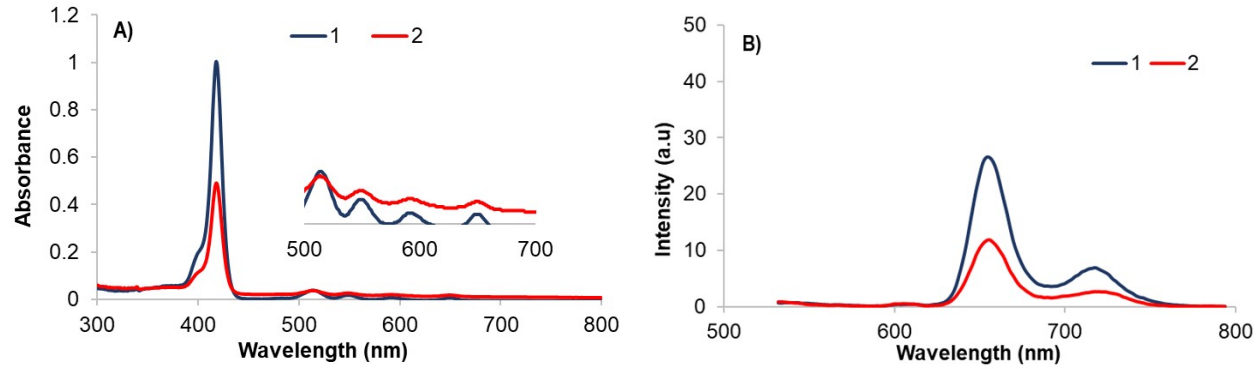

Figure 30: (A)Absorption (1= 2.5µM, 2= 1.25µM) and (B) Emission spectra (λ_{ex} = 514 nm) of the porphyrin **5** in THF.

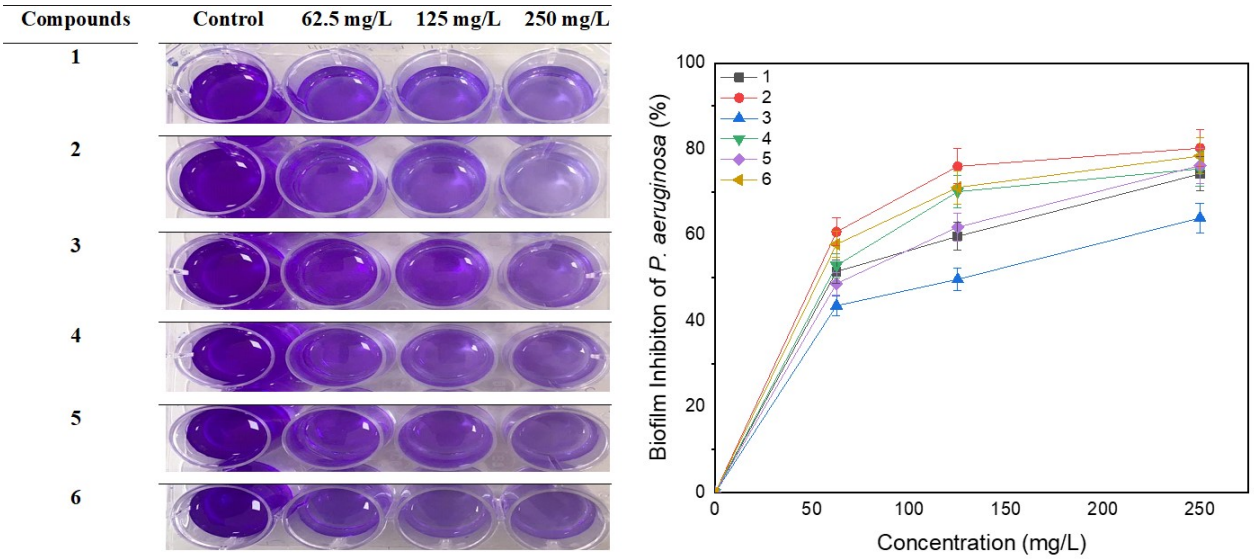

Fig. S31: Biofilm inhibition of compounds **1-6** by using *P. Aeruginosa*

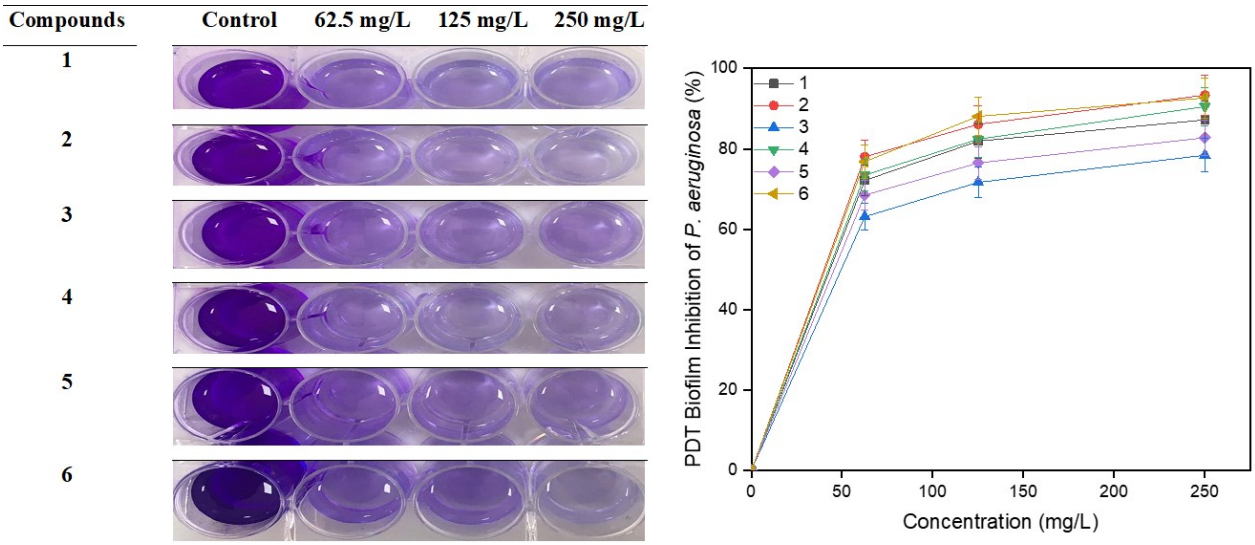

Fig. S32: Biofilm inhibition of with photodynamic therapy by using *P. aeruginosa*

Table S1: Absorption properties of complexes in THF.

Compound	Soret	$Q_y(1,0)$	$Q_y(0,0$	$Q_x(1,0)$	$Q_x(0,0)$
1	418(5.39)	514(3.99),	550(3.71)	583(3.23)	650 (3.46)
2	425(5.06)	557 (3.17)	596 (2.60)	-	-
3	418(5.50)	514(4.18)	549(3.88)	592(3.64)	648(3.56)
4	425(5.45)	556(4.02)	596(3.60)	-	-
5	419(5.63)	514(4.15)	549(3.95)	592(3.83)	649(3.81)
6	425(5.19)	557(3.46)	596(3.34)	-	-

Table S2: Photophysical parameters of complexes for different concentrations (in 1 and 0.5 abs. solutions in THF) at room temperature solutions.

Compound	λ_{ex} (nm)	λ^{em}_{max} Q(0,0)	Q(0,1)	λ^{ex}_{max} (nm)	Φ_F
1 (3.8 µM)	514 $Q_y(1,0)$	655	721	652	0.052
1 (1.8 µM)	514 $Q_y(1,0)$	654	722	653	0.050
2 (8 µM)	425 (Soret, B)	607	659	606	0.041
2 (4 µM)	425 (Soret, B)	607	659	606	0.039
3 (3 µM)	514 $Q_y(1,0)$	654	719	652	0.049
3 (1.5 µM)	514 $Q_y(1,0)$	655	718	652	0.045
4 (4 µM)	425 (Soret, B)	607	658	606	0.048
4 (2 µM)	425 (Soret, B)	607	657	605	0.046
5 (2.5 µM)	514 $Q_y(1,0)$	655	719	652	0.067
5 (1.25 µM)	514 $Q_y(1,0)$	655	718	651	0.063
6 (6 µM)	425 (Soret, B)	607	658	606	0.050
6 (3 µM)	425 (Soret, B)	608	657	605	0.049

References

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