Electronic Supplementary Information (ESI)

Investigation of photophysical, photochemical, and multifunctional biological properties of fluorinated zinc (II) phthalocyanine functionalized with carboxylic acid

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Experimental

1. Chemical and Materials

4-(2',3',4',5',6'-pentafluorobenzyloxy)phthalonitrile was prepared according to the reported procedure.[1] Silica gel Kieselgel 60, 200–400 mesh) was used in the separation and purification of compounds by column chromatography. All reagents and solvents were of reagent grade quality obtained from commercial suppliers. All solvents were dried and purified, as described by Perrin and Armarego.[2] The homogeneity of the products was tested in each step by TLC (silica gel). The chemical substances and solvents used in experimental studies, 1,3-diphenylisobenzofuran (DPBF) and zinc phthaocyanine (ZnPc) were supplied commercially from Sigma-Aldrich and used without further purification.

2. Instruments

^{1}H-NMR results of the synthesized structures were obtained from Agilent VNMRS 500 MHz. ^{13}C-NMR and ^{19}F-NMR results of the synthesized structures were obtained from Agilent VNMRS 126 MHz and 470 MHz, respectively. UV–Vis spectra were recorded on a Scinco SD 1000 diode array, single-beam ultraviolet–visible (UV–Vis) spectrophotometer. Costech ECS 4010 was used for elemental analysis. Bruker microflex LT MALDI-TOF MS was used to obtain mass analyzes of phthalocyanines. FT-IR spectra were recorded on an Agilent Technologies Cary 630 FTIR instrument over the range 4000–400 cm^{-1}. Absorption spectra in the UV-Visible region were obtained with a Shimadzu 2001 UV spectrophotometer. Fluorescence spectra were measured using a Varian Eclipse spectrofluorometer using 1 cm path length cuvettes at room temperature. Photo-irradiations were measured using a General Electric quartz line lamp (300W). A 600 nm glass cut off filter (Schott) and a water filter were used to filter off ultraviolet and infrared radiations respectively. Interference filters (Intor, 670 nm

with a bandwidth of 40 nm for 2 were additionally placed in the light path before the sample). Light intensities were measured with a POWER MAX5100 (Mol electron detector incorporated) power meter.

3. Photophysical and Photochemical Studies

Initially, the aggregation behavior of compound **2** was investigated in DMSO. DMSO was chosen as the solvent due to its excellent compatibility and low cytotoxicity, as well as its capacity to enhance membrane permeability while minimizing cellular damage, making it particularly suitable for photophysical and photochemical studies.[3] Macrocyclic compounds such as phthalocyanines tend to aggregate through strong intermolecular interactions, which can result in either a hypsochromic (blue) or bathochromic (red) shift in the absorption bands of their supramolecular assemblies. To evaluate this tendency, a series of solutions with increasing concentrations of compound **2** was prepared, and their absorption spectra were recorded. In addition, to determine their emission characteristic, compound **2** was dissolved in DMSO at fixed concentrations: 1.31×10^{-5} M.

The singlet oxygen generation capability of compound **2** was evaluated using a photochemical approach. For this experiment, a solution containing equal amounts of sample (5.25×10^{-5} M for **2**) and 1,3-diphenylisobenzofuran (DPBF, 8.5×10^{-5} M) was prepared under low-light conditions to prevent undesired photodegradation. The solutions were then irradiated with light at an intensity of 7.05×10^{15} photons s^{-1} cm^{-2} for photochemical analysis.

For photodegradation analysis, compound **2** was subsequently exposed to high-intensity light in 20-minute intervals. Details of each method are summarized in our previous studies[4-7] and below.

3.1. The Parameters for Fluorescence Quantum Yield (Φ_F)

Fluorescence quantum yield (Φ_F) was determined by applying the comparative method (Eq. 1).[8]

$$\Phi_F = \Phi_{F(Std)} \frac{F \cdot A_{Std} \cdot n^2}{F_{Std} \cdot A \cdot n^2_{Std}} \quad (1)$$

Where F and F_{Std} are under the fluorescence emission curves of the sample and the standard, respectively. A and A_{Std} are the respective absorbances of the samples and standard (Unsubstituted ZnPc) at the excitation wavelengths, respectively. n^2 and n^2_{Std} are there fractive indices of solvents used for the sample and standard, respectively. Unsubstituted ZnPc (Φ_F = 0.20 in DMSO)[8] was used as the standard. Both the samples and standard were excited at the same wavelength.

3.2. The Parameters for Singlet Oxygen Quantum Yield (Φ_Δ)

Singlet oxygen efficiency was determined in air (no oxygen bubbled) using the elative method (Eq. 2) with unsubstituted ZnPc (zinc phthalocyanine) as standard and 1,3-Diphenylisobenzofuran (DPBF) as chemical quencher for singlet oxygen,

$$\Phi_\Delta = \Phi_\Delta^{std} \frac{R.I_{abs}^{std}}{R^{std}.I_{abs}} \quad (2)$$

where is the singlet oxygen quantum yield for the unsubstituted Standard ZnPc (Φ_Δ = 0.67 in DMSO).[9] R and R_{Std} are the DPBF photodegradation rates in the presence of the respective samples and standard, respectively. I_{abs} and I_{abs}^{Std} are the rates of light absorption by the sample and standard, respectively. The samples containing DPBF were prepared in the dark and irradiated at the Q band region. The absorption band of the DPBF reduced by light irradiation (The light intensity of 7.05×10^{15} photons s^{-1} cm^{-2}). The degradation of DPBF was monitored using UV-Vis spectroscopy after each 5 s light irradiation at 417 nm for photochemical study.

3.3 The Parameters for Photodegradation Quantum Yields

Photodegradation quantum yields were determined using Eq. 3,

$$\Phi_d = \frac{(C_0 - C_t).V.N_A}{I_{abs}.S.t} \quad (3)$$

where "C_{0}" and "C_t" are the sample concentrations before and after irradiation respectively, "V" is the reaction volume, "N_A" is the Avogadro's constant, "S" is the irradiated cell area, "t" is the irradiation time, "I_{abs}" is the overlap integral of the radiations of light intensity and the absorption of the sample. A light intensity of 2.42 $\times 10^{16}$ s^{-1} cm^{-2} was employed to determine the photodegradation.[9] The degradation of max. Q band was monitored after each 5 minute irradiation.

4. Biological properties

4.1 Antioxidant activity

The following stages were used to test this method. A fresh DPPH solution was prepared with methanol. Accordingly, 250 µL of 0.004% DPPH solution in methanol was taken and transferred to an aluminum foil-covered glass tube. After adding 1000 µL of test solutions with concentrations of 6.25, 12.5, 25, 50, and 100 mg/L, the DPPH solution was vortexed. Pure methanol was introduced to the control tube in place of 1000 µL of the test solution, and DPPH was used as the control. A spectrophotometer was used to evaluate the antioxidant activity against methanol at a wavelength of 517 nm following 30 minutes in the dark and at room temperature. The following formula was used to determine the percentage inhibition of the DPPH radical scavenging activity response (4):

$$Capacity\ (\%) = \left(\frac{Abs(control) - Abs(sample)}{Abs(control)} \right) \times 100 \quad (4)$$

4.2 Antidiabetic activity

The sample was put in test tubes at concentrations of 50, 100, and 200 mg/L. After adding phosphate buffer and α-amylase, the mixture was kept at 37°C for 15 minutes. 200 µL of a 1% potato starch solution was added to start the hydrolysis process. 400 µL of 3,5-dinitrosalicylic acid (DNS) was added to each test tube to stop the hydrolysis process after 20 minutes of incubation at 37°C. After that, the tubes were left in boiling water for five minutes. The same procedure was used without test samples as a control. After cooling, the mixture was diluted with 3 mL of distilled water, and spectrophotometric readings were taken at 540 nm. The following formula was used to calculate the antidiabetic activity (5).

$$Antidiabetic\ Activity\ (\%) = \left(\frac{Sample_{abs} - Control_{abs}}{Control_{abs}} \right) \times 100 \quad (5)$$

4.3 DNA cleavage ability

Different concentrations of test compounds were added to PCR tubes, which had a volume of 20 µL (5 µL of pBR322 plasmid DNA + 15 µL of test compounds) and then incubated for two hours at 37°C in a dark atmosphere. The electrophoresis tank was placed with the produced 1% agarose gel. After fixing the gel, the TAE buffer was poured into the tank to cover the gel. After adding 3 µL of loading dye, the test samples were loaded into the wells. 60 minutes of execution was carried out with the electrical power device set to 100 volts (V). After this period, the agarose gel was carefully taken out of the tank and brought to the UV imaging cabinet for imaging. The results have been saved.

4.4 Antimicrobial activity and antimicrobial-photodynamic therapy (APDT)

The microdilution technique was used to assess the antibacterial properties of newly synthesized MPcs. In U-bottom microplate wells, the compounds to be tested were serially diluted. The test compounds' antimicrobial properties were evaluated against microfungi and both Gram-positive and Gram-negative bacteria. After serial dilution procedures, microbial cultivation was carried out. The plates were cultivated and then incubated at 37°C for 24 hours. The minimal inhibitory concentration was the lowest concentration at which no growth occurred after 24 hours. In addition, the above-applied method in antimicrobial photodynamic therapy activity was applied after the samples were exposed to light irradiation for 30 min. A diode that emits red-orange light was used; its wavelength and energy were λ632 ± 2 nm and 12 J/cm^2.

4.5 Microbial cell viability

The inhibition of microbial cell viability by newly synthesized MPcs was also examined. *E. coli* (ATCC 10536) served as the model microbial strain used in this investigation. In our earlier study, the cultivation of *E. coli* was described in detail.[10] The cultivated and prepared *E. coli* was used in both bacterial viability inhibition and APDT studies. Pcs were added to the prepared *E. coli* at various concentrations. It was then incubated for an hour. Following the conclusion of the period, the cultivation process and the dilution stage were completed, and the mixture was incubated for 24 hours at 37°C. The colonies were then tallied. The percentages of microbial inhibition were computed using equation (3). Finally, the colonies were counted, and Eq. (6) was used to calculate the APDT.

$$Cell\ viability\ inhibition\ (\%) = \left(\frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100 \quad (6)$$

4.6 Antibiofilm activity

Using the method described below, the biofilm inhibition ability of MPcs was applied at different concentrations. MPcs were added at different concentrations to the sterile 24-well polystyrene plates containing Nutrient Broth (NB) in order to assess the antibiofilm activity. After that, the test microorganisms *S. aureus* and *P. aeruginosa* were cultured for 3 days at 37°C in a medium containing NB. Wells devoid of MPcs served as the controllers. Following incubation, the plate's medium was drained, and it was twice washed with sterile phosphate-buffered water (PBS) before being allowed to dry at 75°C for 20 minutes.

After that, 1% crystal violet dye solution was applied, and let to sit for 20 minutes, and then the dye on the plate was drained and given two PBS cleanings. The samples were then read at a wavelength of 595 nm after the biofilm was removed from the plate by adding 70% (v/v) ethyl alcohol to each well. Biofilm inhibition was computed using the following formula (7).

$$Biofilm\ Inhibition\ (\%) = \left(\frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \right) \times 100 \quad (7)$$

4.7 Cell culture

L929 cells (ATCC, CCL-1) were cultured in Dulbecco's Modified Eagle Medium (Thermo Fisher Scientific) supplemented with fetal bovine serum (10%, v/v) and penicillin-streptomycin (1%, v/v). Mycoplasma test was performed monthly using the MycoAlert™ PLUS Mycoplasma Detection Kit (Lonza) and measured with a Lucetta™ 2 Luminometer (Lonza).

4.8 Dark and photocytotoxicity assay

Cells were seeded in 96-well plates (5 × 10^3 cells/well) and incubated in a humidified incubator (37°C, 5% CO_2) for 24 h. After incubation, varying concentrations of Pc (0, 0.75, 1, 7.5, 10, 75, and 100 µM) were administered, followed by a 1 h incubation to facilitate cellular uptake. Photodynamic irradiation was conducted using a ceLED system (CETONI, Germany) with a wavelength range of 660-700 nm. The fluence rate was set at 75 mW/cm², and the light dose was 20 J/cm² (exposure time of 270 s). Following a 24 h treatment period, cell viability was quantified using the WST-1 assay (Roche Applied Science) according to the manufacturer's instructions [11]

SUPPLEMENTARY FIGURES

NMR spectra of Pcs

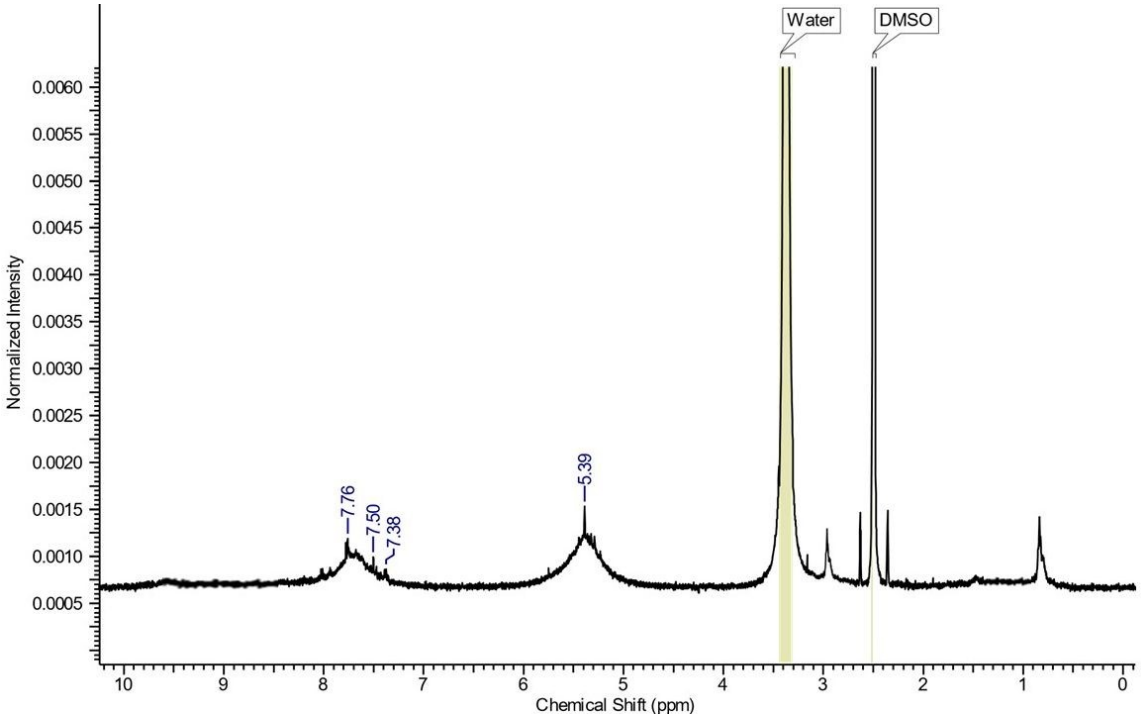

Figure S1. ^{1}H NMR spectrum of **1** in d_6-DMSO.

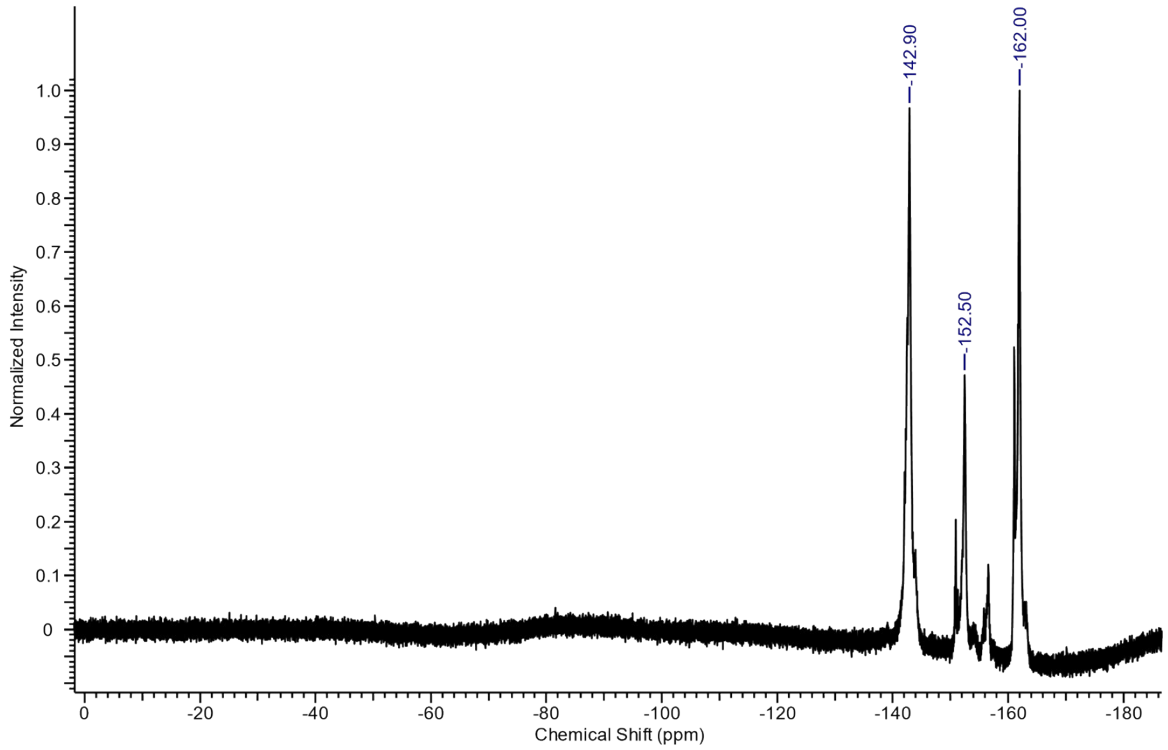

Figure S2: ^{19}F NMR spectrum of **1** in d_6-DMSO.

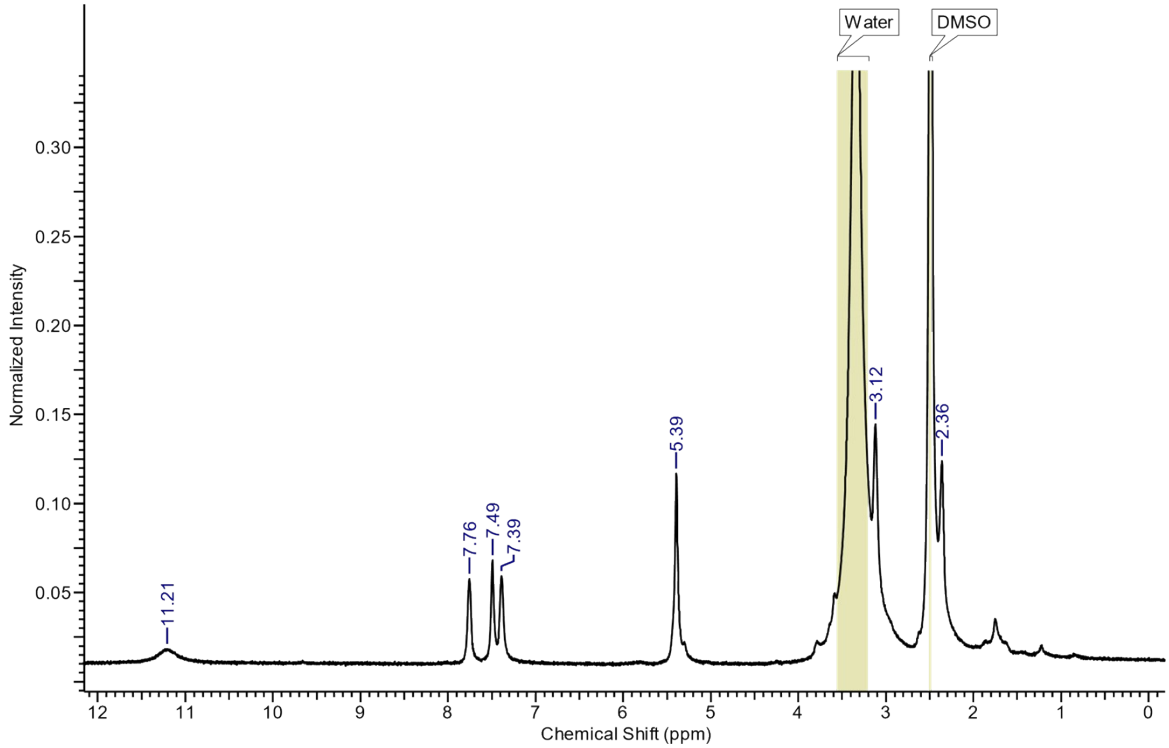

Figure S3: ^{1}H NMR spectrum of **2** in d_6-DMSO.

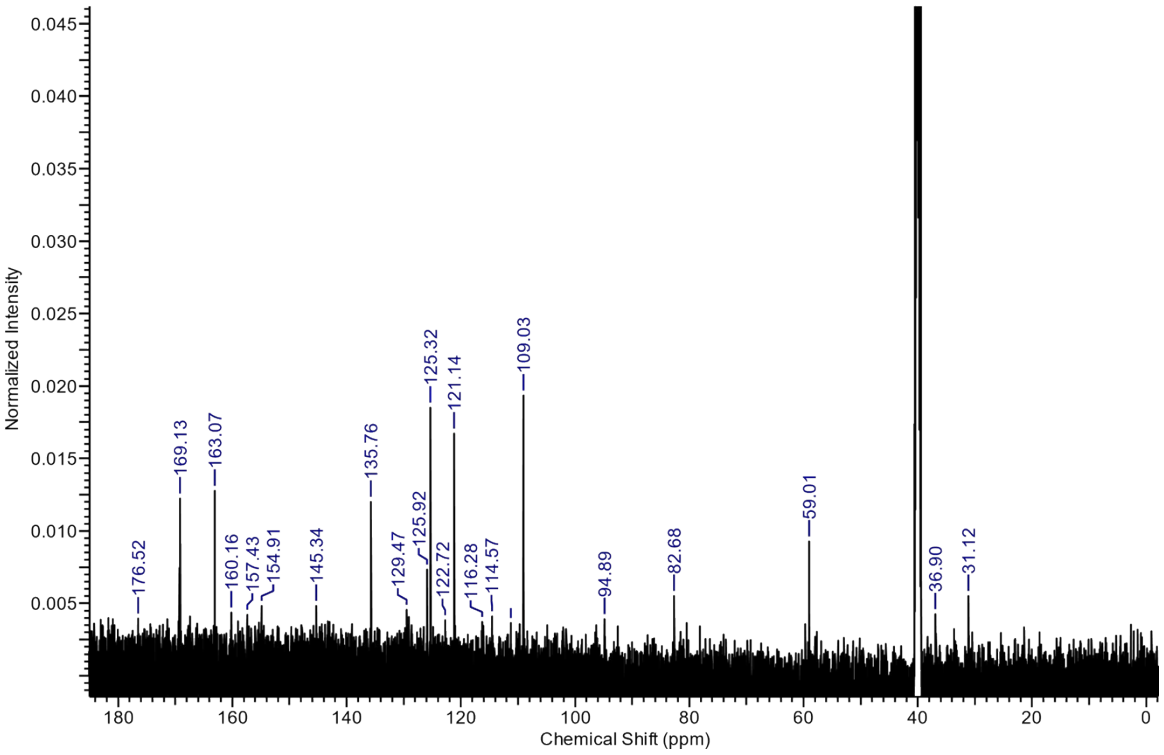

Figure S4: ^{13}C NMR spectrum of **2** in d_6-DMSO.

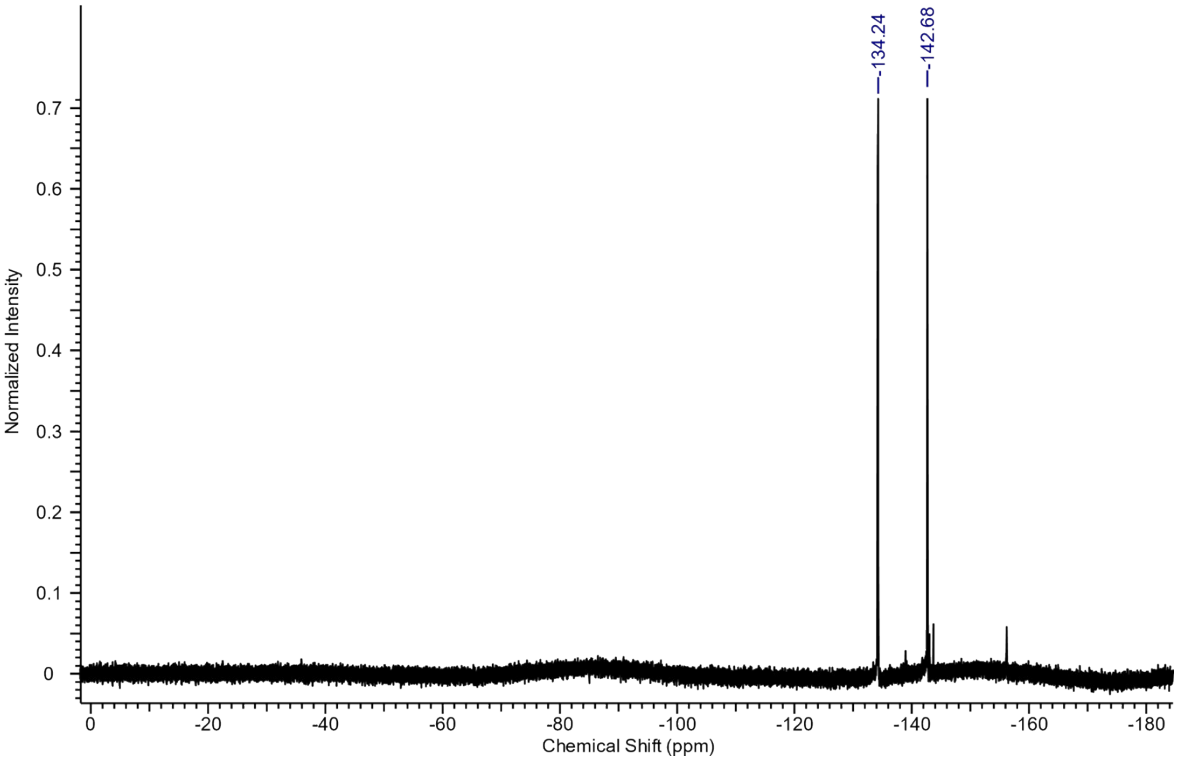

Figure S5: ^{19}F NMR spectrum of **2** in d_6-DMSO.

MALDI-TOF MS spectra of Pcs

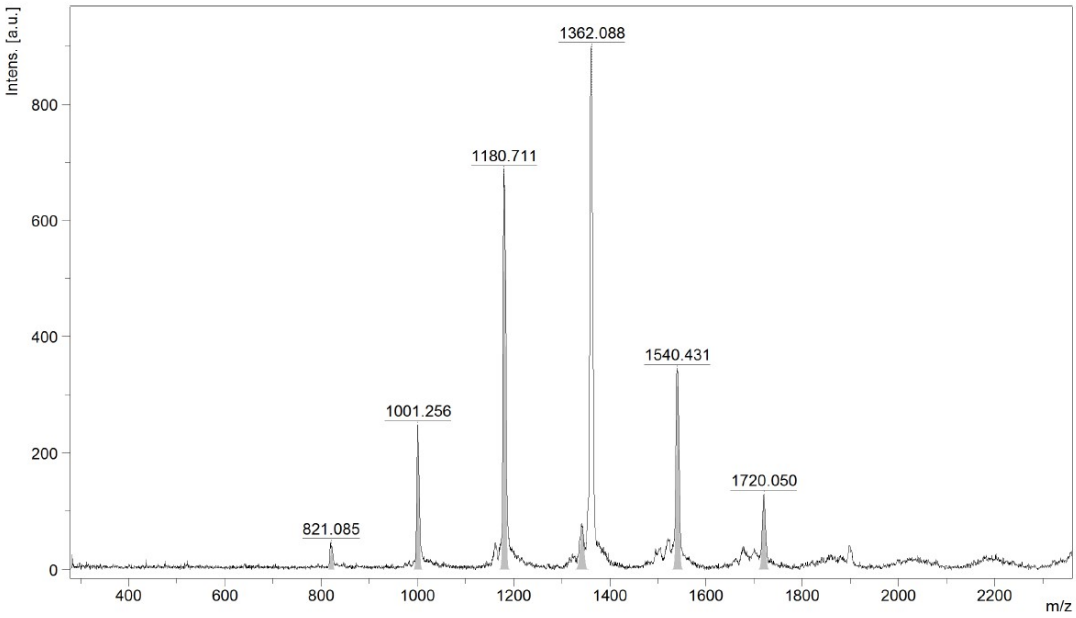

Figure S6. MALDI-TOF MS spectrum of **1**.

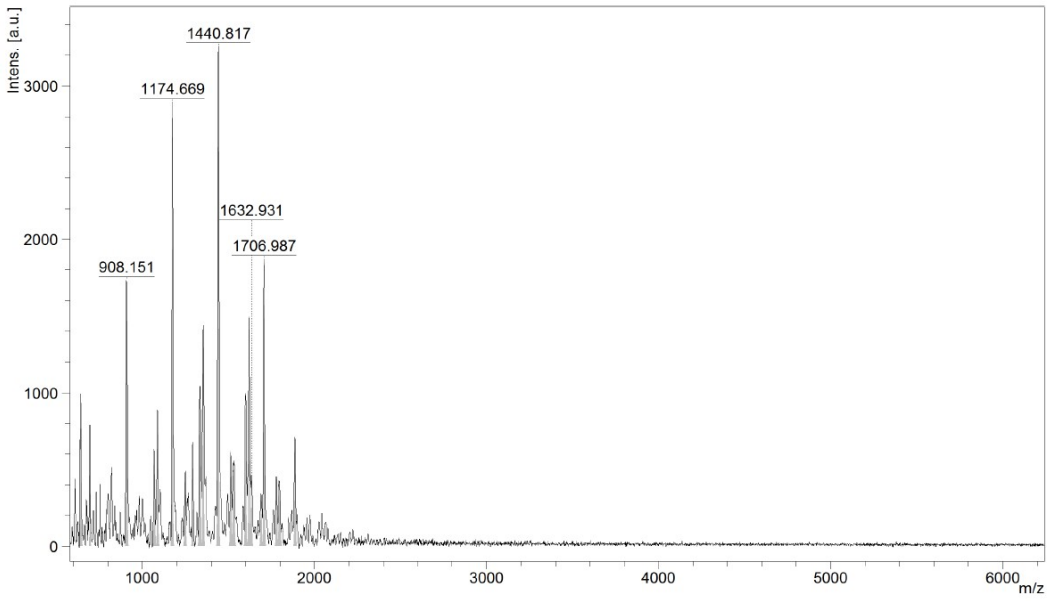

Figure S7: MALDI-TOF MS spectrum of **2**.

FT-IR spectra of Pcs

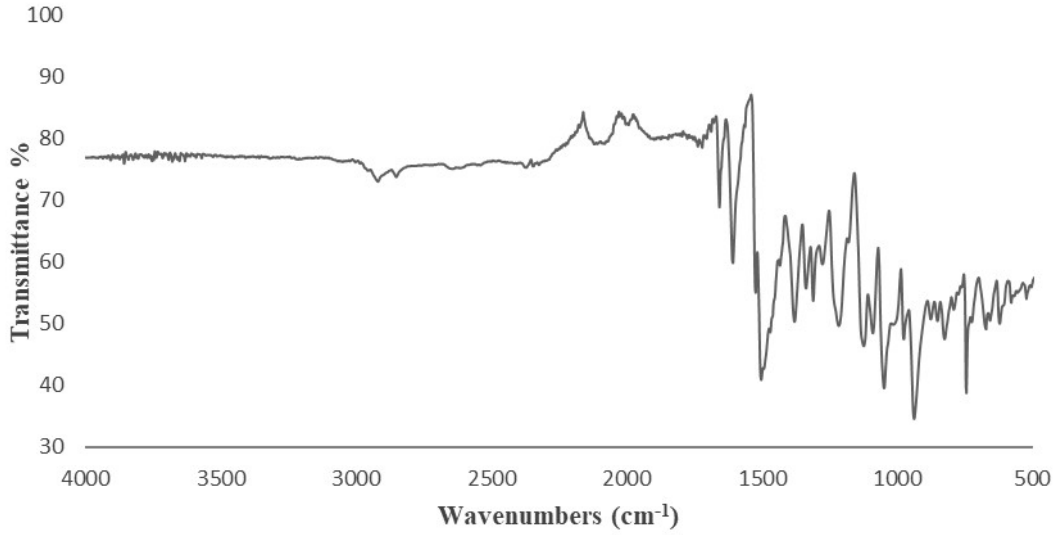

Figure S8: FT-IR spectrum of **1**.

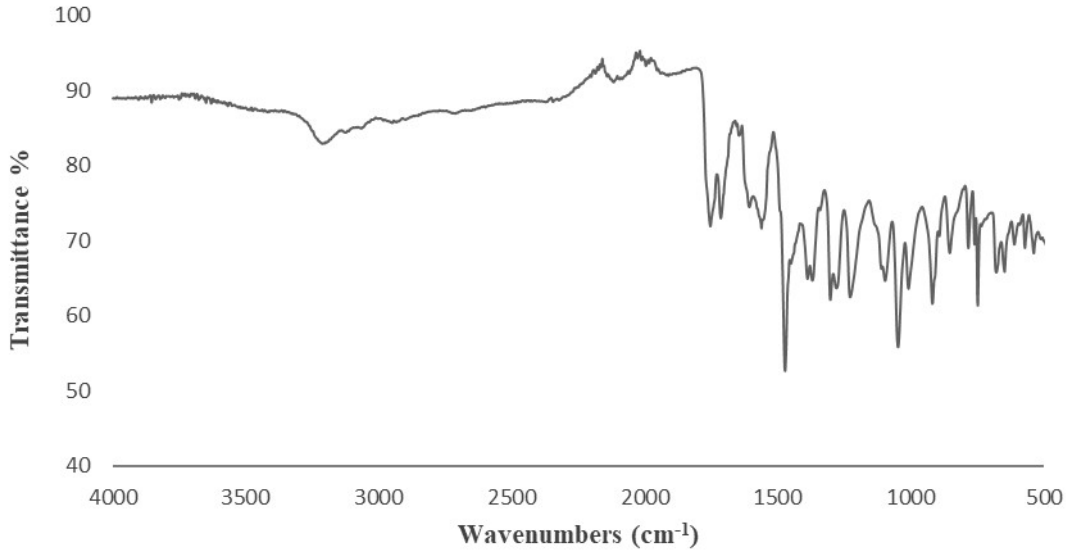

Figure S9: FT-IR spectrum of **2**.

References

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