Supplementary information for

Heterometallic BODIPY-based Molecular Squares Obtained by Self-Assembly: Synthesis and Biological Activities

Gajendra Gupta,[1,a,*] Yeji You,[2,a] Rizky Hadiputra,[3] Jaehoon Jung,[3] Dong-Ku Kang[2,*] and Chang Yeon Lee[1,*]

[1]Department of Energy and Chemical Engineering/Innovation Center for Chemical Engineering, Incheon National University, Yeonsu-gu, Incheon 22012, Republic of Korea.

[2]Department of Chemistry, Incheon National University, Yeonsu-gu, Incheon 22012, Republic of Korea.

[3]Department of Chemistry, University of Ulsan, Namgu, Ulsan 44776, Republic of Korea.

Corresponding author: gjngupt@gmail.com (G.G.); dkkang@inu.ac.kr (D.K.); cylee@inu.ac.kr (C. Y. L.)

Biological Experiments:

Cell culture

Human kidney epithelial cells (HEK-293), colorectal carcinoma cells (HCT116) and prostate adenocarcinoma cells (PC-3) were grown in Roswell Park Memorial Institute 1640 (RPMI 1640). Breast adenocarcinoma cells (MCF-7) and lung adenocarcinoma cells (A549) were grown as adherent monolayers in Dulbecco's modified eagle medium (DMEM). Both cell culture media were supplemented with 10% (v/v) fetal bovine serum (FBS) and 1.0% penicillin–streptomycin (10,000 IU and 10,000 μg/ml, respectively). All cell lines were cultivated at 37℃ and 5% CO_2 incubator under a humidified atmosphere.

Cytotoxic activity of synthesized supramolecules in cancer cells

Cytotoxic activities were tested on HEK-293, HCT116, PC-3, MCF-7 and A549 cells using conventional MTT assay. Cells were seeded in 96 well cell culture plate at a density of 1×10^4 /well and incubated for 24 h at 37℃ and 5% CO_2. Post incubation, the culture medium was removed and cells were treated with increasing doses of supramolecules and standard drug cisplatin (100 fM - 100 μM) mixed in complete medium. Supramolecules were first dissolved in DMSO and it was diluted with cell culture medium to make designated (final) concentration in the complete medium. Then precipitants were removed from the complete medium by filtration with 0.2 μm syringe filter prior to the treatment in the cells. After 48 h of incubation at 37℃ and 5% CO_2, 5 mg/mL thiazolyl blue tetrazolium bromide was added to each well and the plates were incubated for 4 h. Supernatant was removed and 200 μL of dimethyl sulfoxide (DMSO) was added to each well. After shaking for 30 minutes, absorbance was measured at 560 nm wavelength and then percentage of cell viability was calculated using below equation.

Cell Viability (%) = $(A_a/A_b) \times 100\%$

A_a = Mean of absorbance of treatment group, A_b = Mean of absorbance of control group

The half maximal inhibitory concentration (IC_{50}) was obtained by fitting viabilities to trend dose-response curves using GraphPad Prism 5.0 (GraphPad Software, Inc., CA, USA).

Analysis of cellular localization of synthesized supramolecules

The 18×18 mm cover slides were shortly sanitized with flame and placed into the wells in 6 well cell culture plate. Then HEK-293, HCT116, PC-3, MCF-7 and A549 cells were seeded in 6 well cell culture plate at a density of 7×10^4 and incubated for 24 h at 37℃ and 5% CO_2. The culture medium was removed post incubation and **3**-**6** were treated at a concentration of 3 μM in complete medium. After 1 h incubation, cells were stained with 5 mg/mL 4',6-diamidino-2-phenylindole (DAPI) for 10 minutes. The cells were washed thrice with PBS and fixed using 4% paraformaldehyde. Cell-seeded cover slides were mounted on slide glass (76×26 mm) with mounting solution followed by analyses under a confocal laser scanning microscopy (LSM 700, Carl ZEISS, Oberkochen, Germany). For the confocal imaging, all samples were excited by using 532 nm laser and emission intensity were measured using TRITC filter (590 ± 34 nm).

Apoptosis assay

Apoptosis assay was performed with Annexin V-FITC Apoptosis Staining/Detection kit (abcam) and flow cytometry (Gallios, Beckman Coulter, CA, USA). HCT116 and A549 cells were seeded at a density of 3×10^5 cells in 6 well cell culture plate and cultured for 24 h at 37℃ and 5% CO_2. Cell culture medium was removed and **3**-**6** or cisplatin were treated at a concentration of 10 μM or 10 -1000 μM in complete medium. Post 48 h incubation, cells were carefully harvested using trypsin treatment. The 1×10^5 cells were stained with Annexin-V and PI using Annexin V-FITC Apoptosis Staining/Detection kit and analysed by flow cytometry. The same number of cells were prepared without Annexin-V and PI staining after **3** and **4** treatments, and the values of fluorescence intensity were measured to remove initial fluorescence intensity because emission intensity of Annexin-V FITC overlaps with **3** and **4**.

Stability in biological media

For the determination of structural stability over time, pre-incubated supramolecules were tested on HCT 116 cells by MTT assay. The supramolecules were pre-incubated in DMSO or cell culture media (complete RPMI 1640 medium) at a concentration of 100 μM for 0, 12, 24, or 48 h at 37℃ and 5% CO_2. HCT116 cells were seeded in 96 well cell culture plate at a density of 1×10^4/well. After 24 h culture, the culture media was removed and cells were treated with 10 μM concentration of **3**-**6** at the in complete medium. After 48 h of incubation, 5 mg/mL Thiazolyl Blue Tetrazolium Bromide was added and incubated for 4 h. Then, supernatant was removed and 200 μL of DMSO was added into the wells. After shaking for 30 minutes, absorbance was measured at 560 nm wavelength. Percent cell viability was calculated in each case, using the following equation.

Cell Viability (%) = $(A_a/A_b) \times 100\%$

A_a = absorbance of treatment group, A_b = absorbance of control group

Then, percentage of inhibition rate was calculated using the following equation.

Inhibition rate (%) = $(V_b - V_a)/V_b \times 100\%$

V_a = cell viability of treatment group, V_b = viability of control group

Fluorescence emission spectra of synthesized supramolecules was measured in DMSO, 10 μM hydrogen peroxide (H_2O_2) in DMSO, 10 μM Dithiothreitol (DTT), 10 μM in DMSO, phosphate-buffered saline (PBS), RPMI 1640 and DMEM. Prior to the study, synthesized supramolecules were dissolved in distilled water at a concentration of 50 μM and then diluted with designated solutions so it reaches as a working concentration of 5 μM. Fluorescence emission spectra was analysed on a FluoroMate FS-2 fluorescence spectrometer (Sinco, Seoul, Republic of Korea).

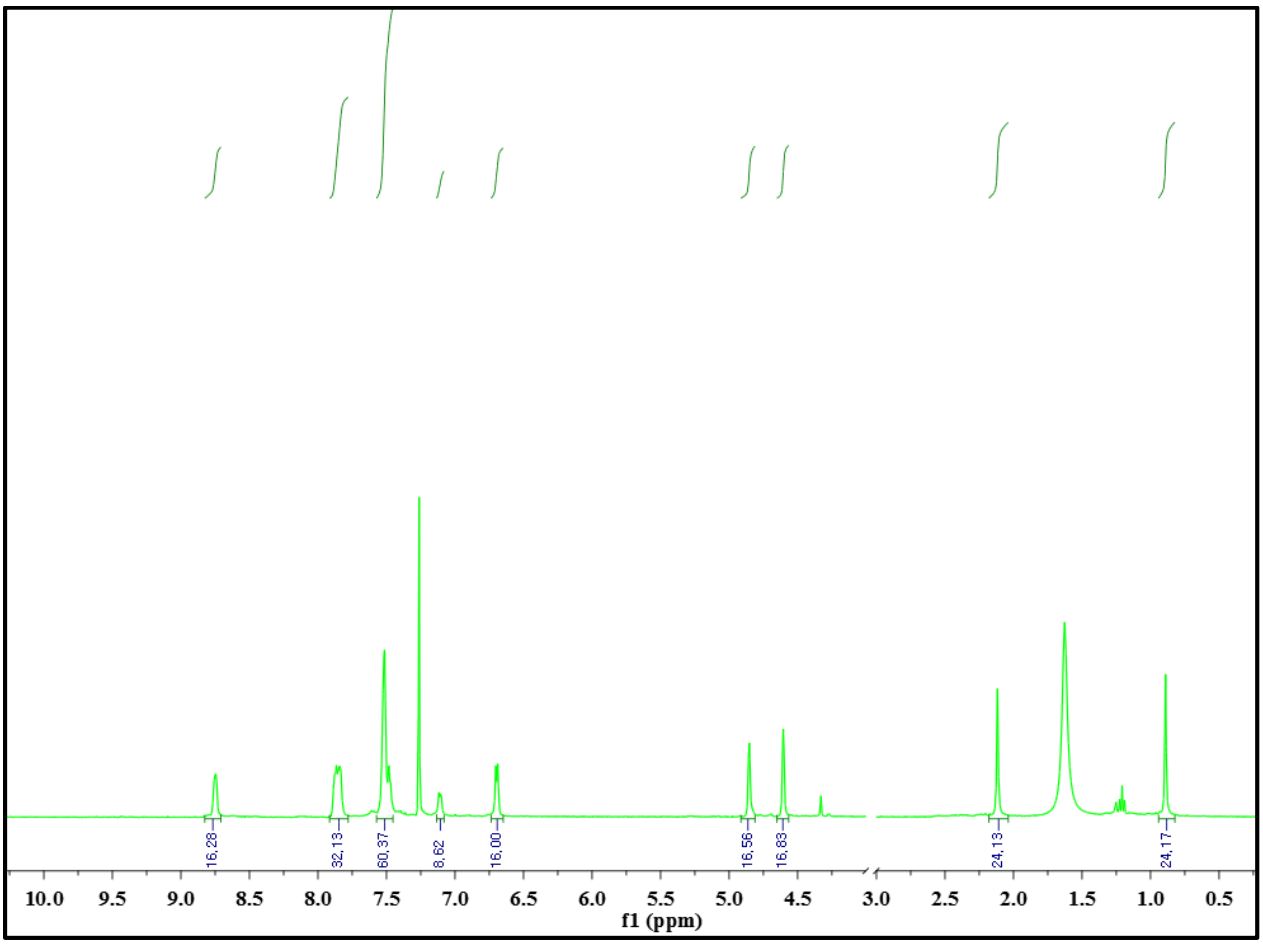

Figure S1a: ^{1}H NMR spectra of palladium BDP square **3** in $CDCl_3$.

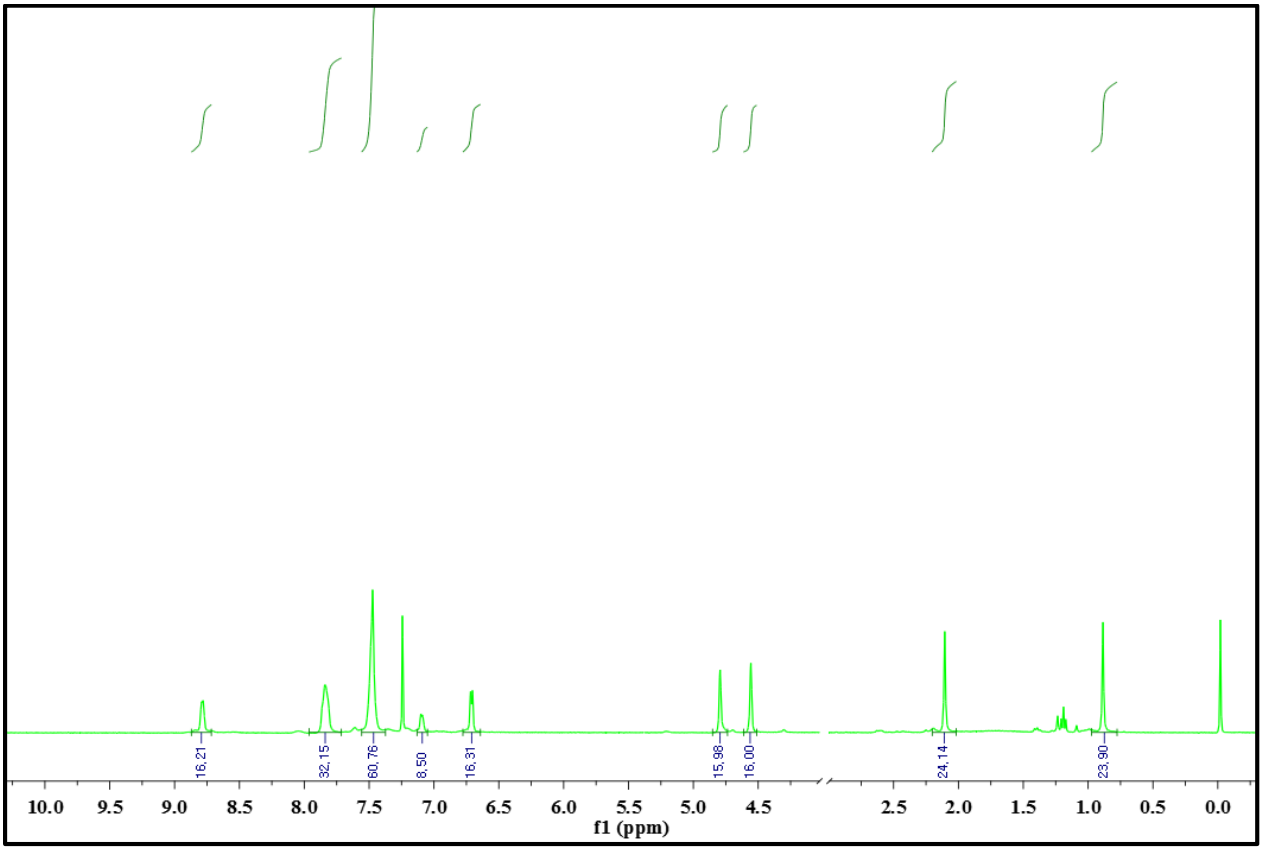

Figure S1b: ^{1}H NMR spectra of platinum BDP square **4** in $CDCl_3$.

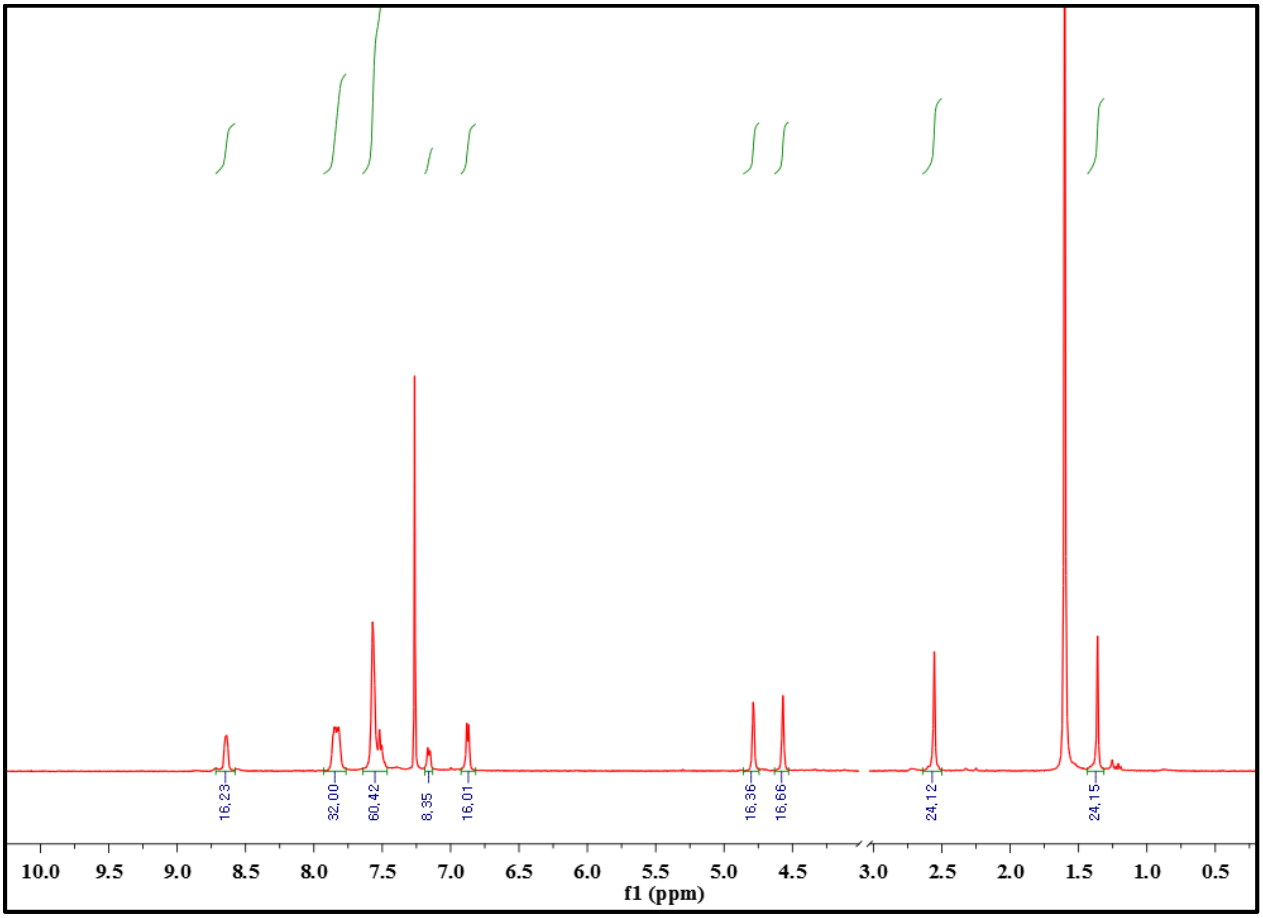

Figure S1c: ^{1}H NMR spectra of palladium BDPCC square **5** in $CDCl_3$.

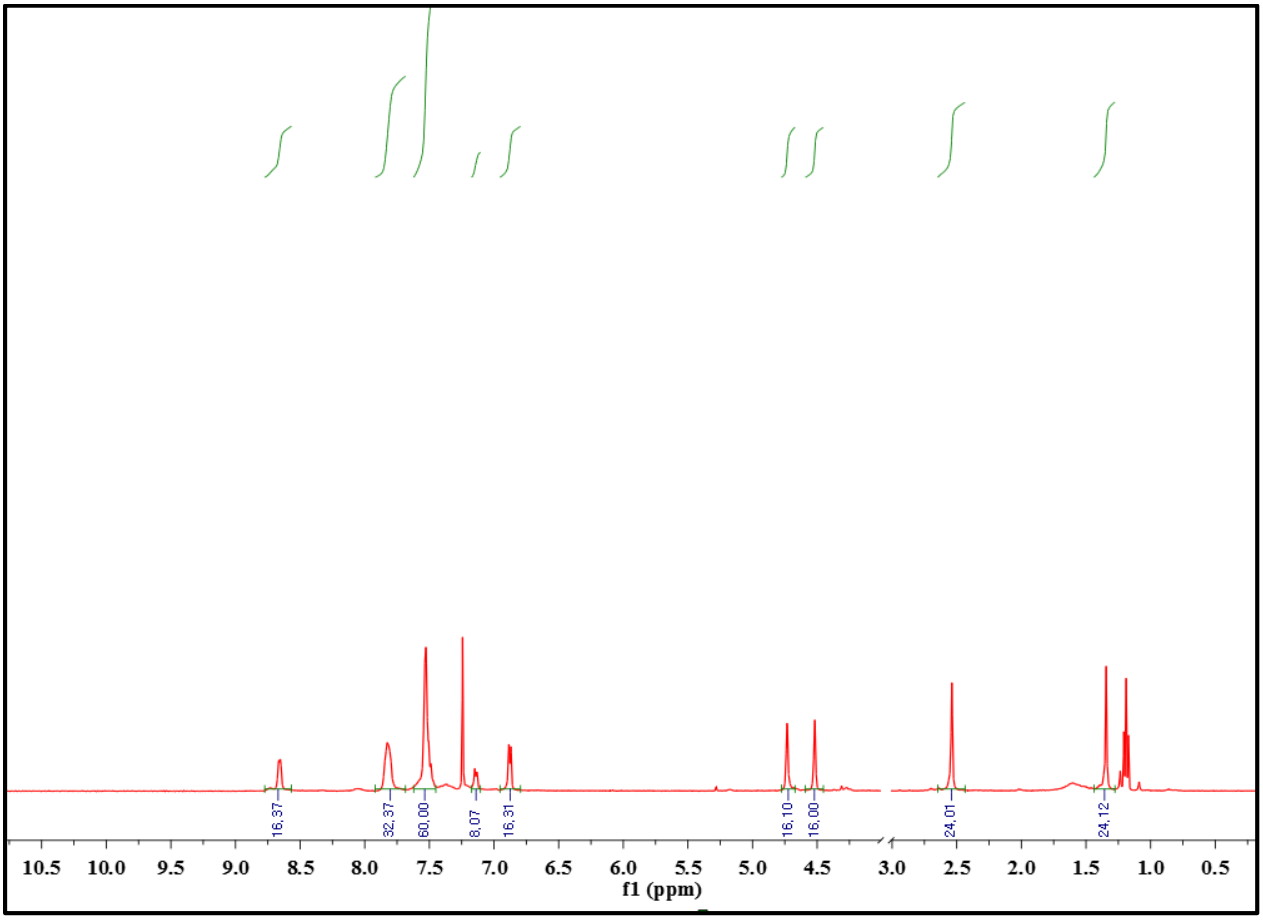

Figure S1d: ^{1}H NMR spectra of platinum BDPCC square **6** in $CDCl_3$.

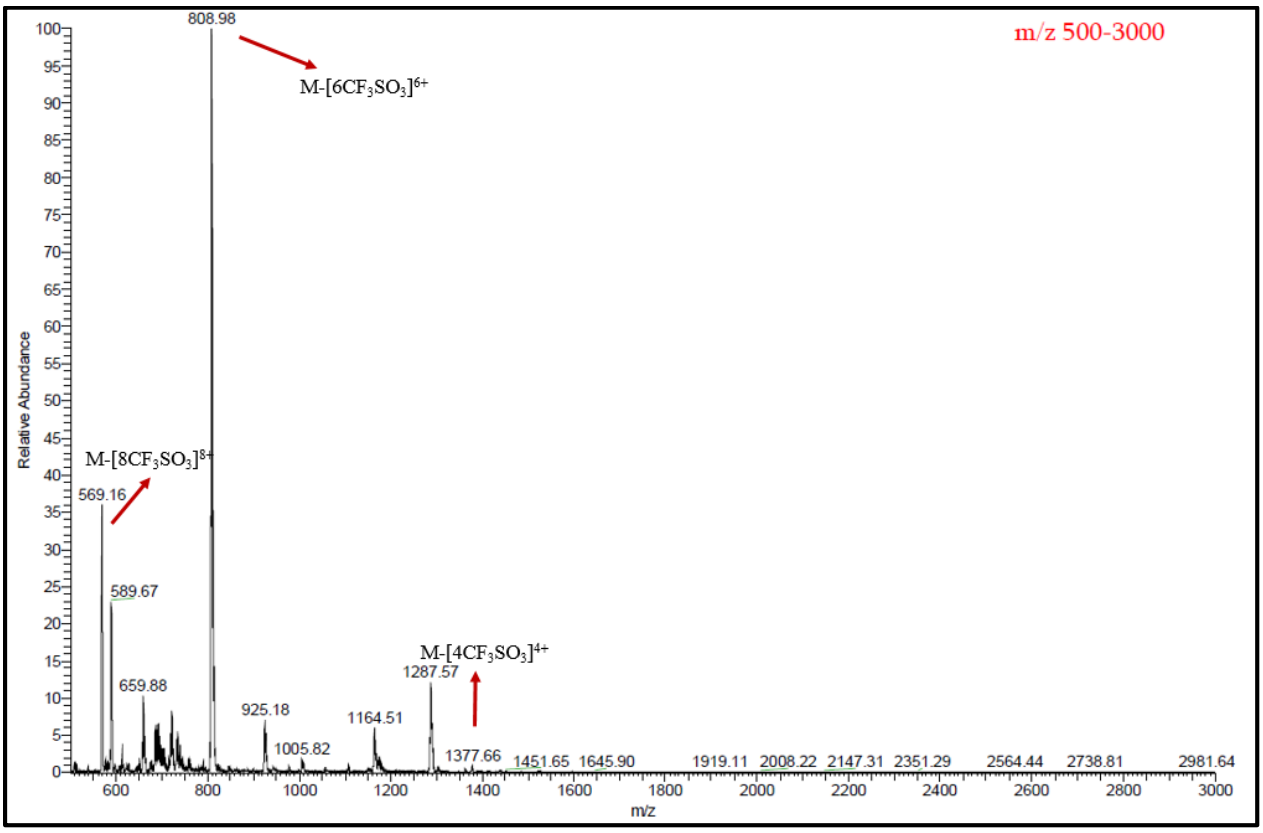

Figure S2a: ESI-MS spectra of palladium BDP square **3** in dichloromethane.

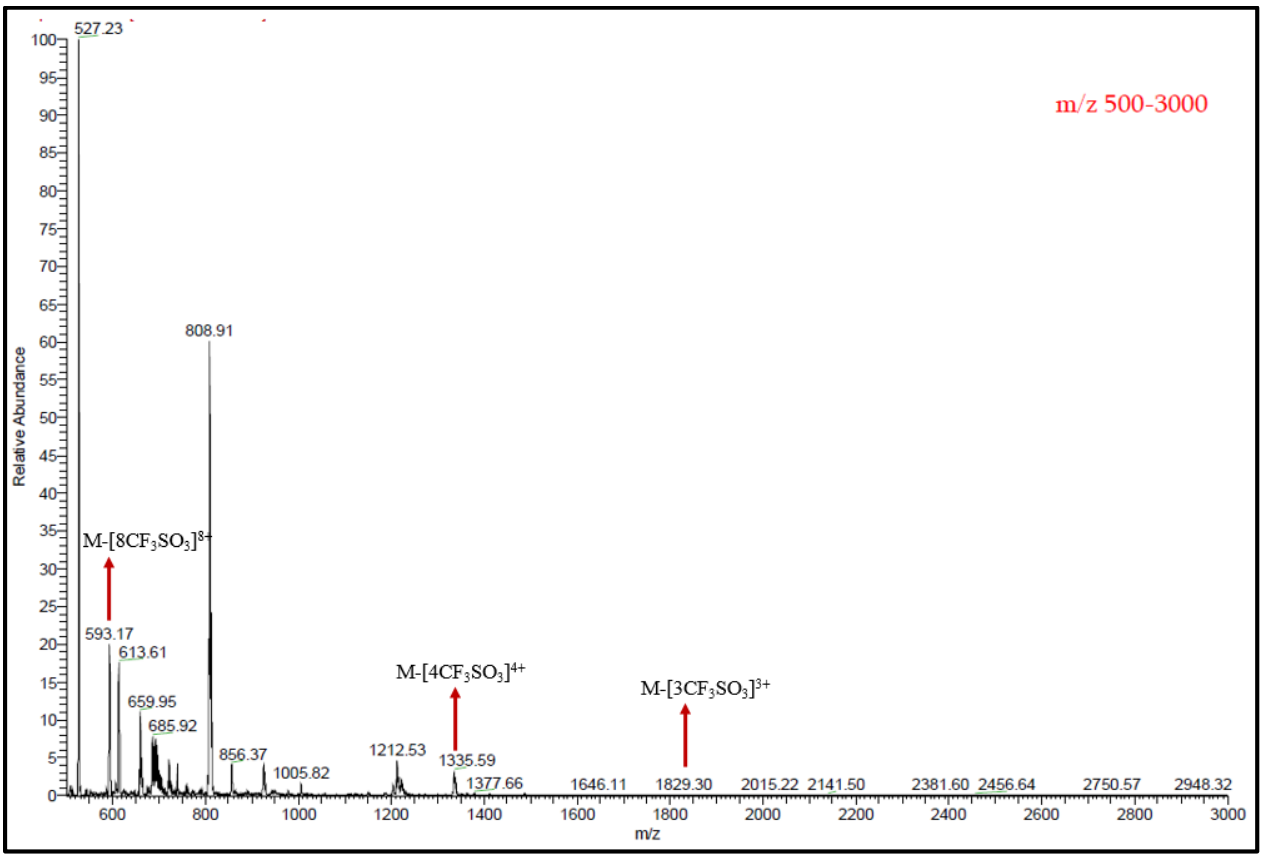

Figure S2b: ESI-MS spectra of palladium BDPCC square **4** in dichloromethane.

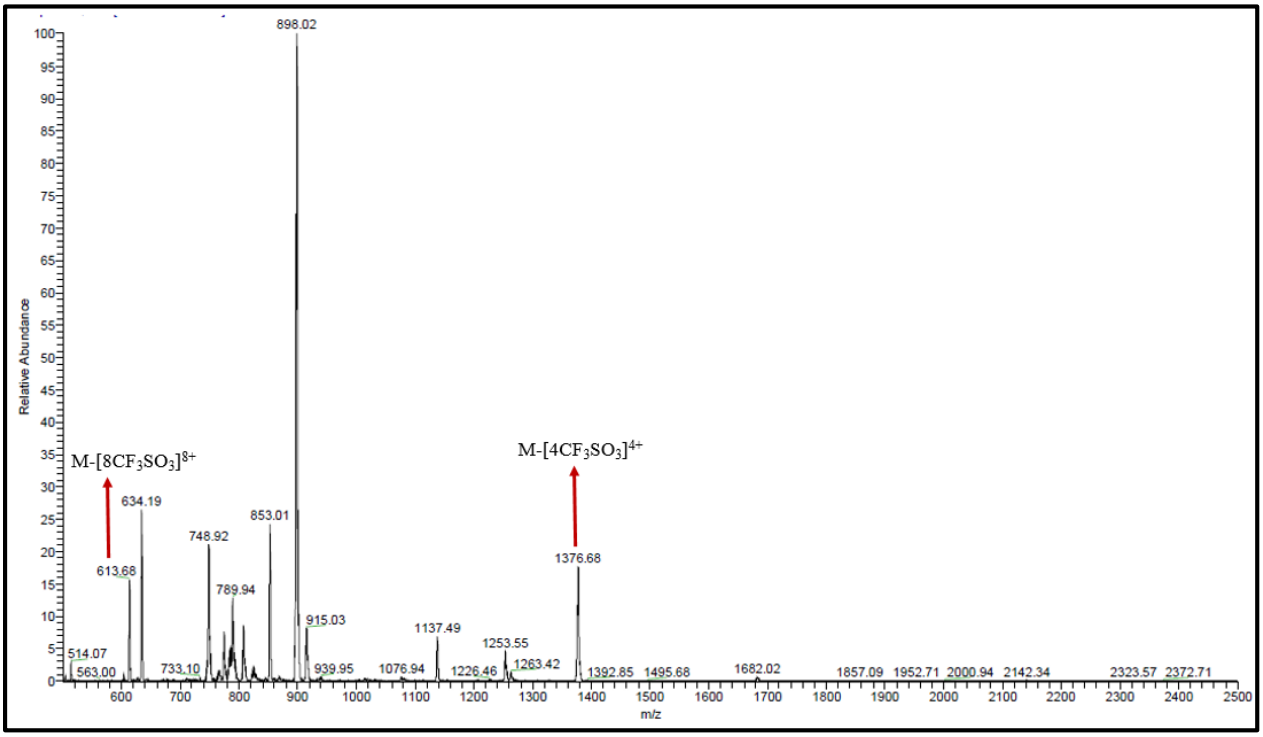

Figure S2c: ESI-MS spectra of platinum BDP square **5** in dichloromethane.

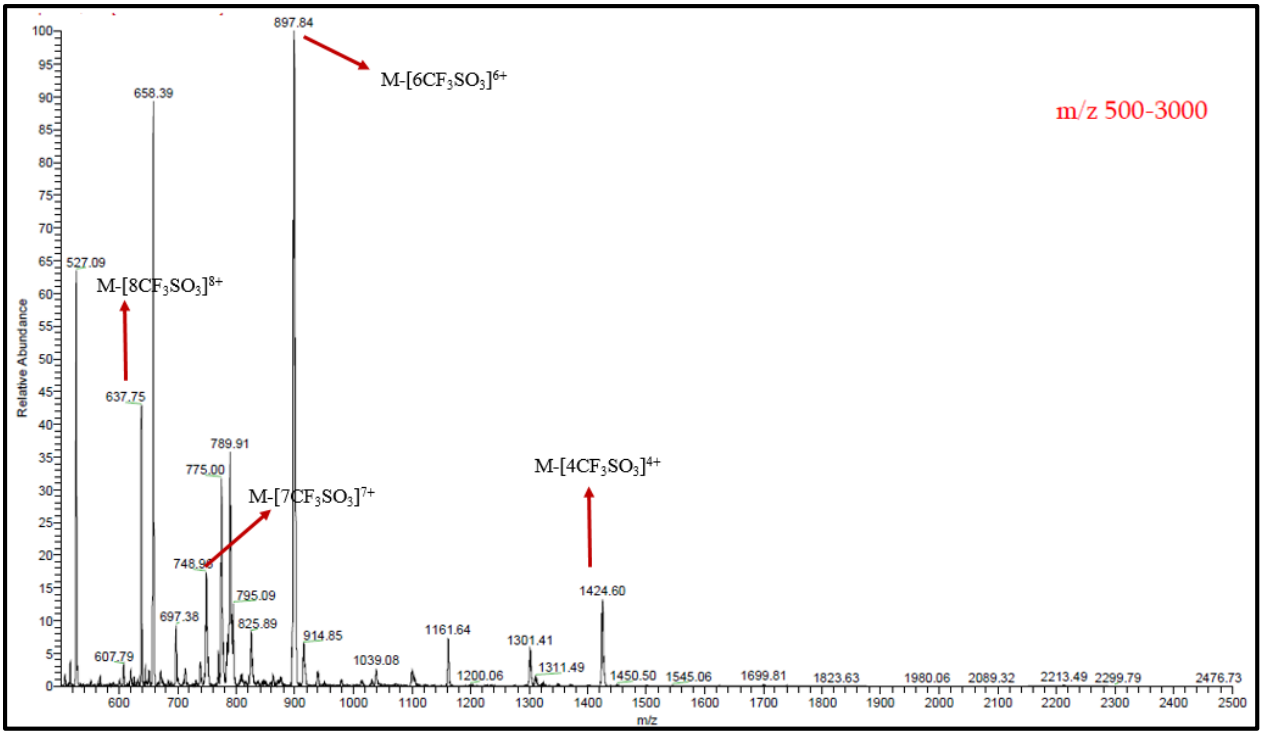

Figure S2d: ESI-MS spectra of platinum BDPCC square **6** in dichloromethane.

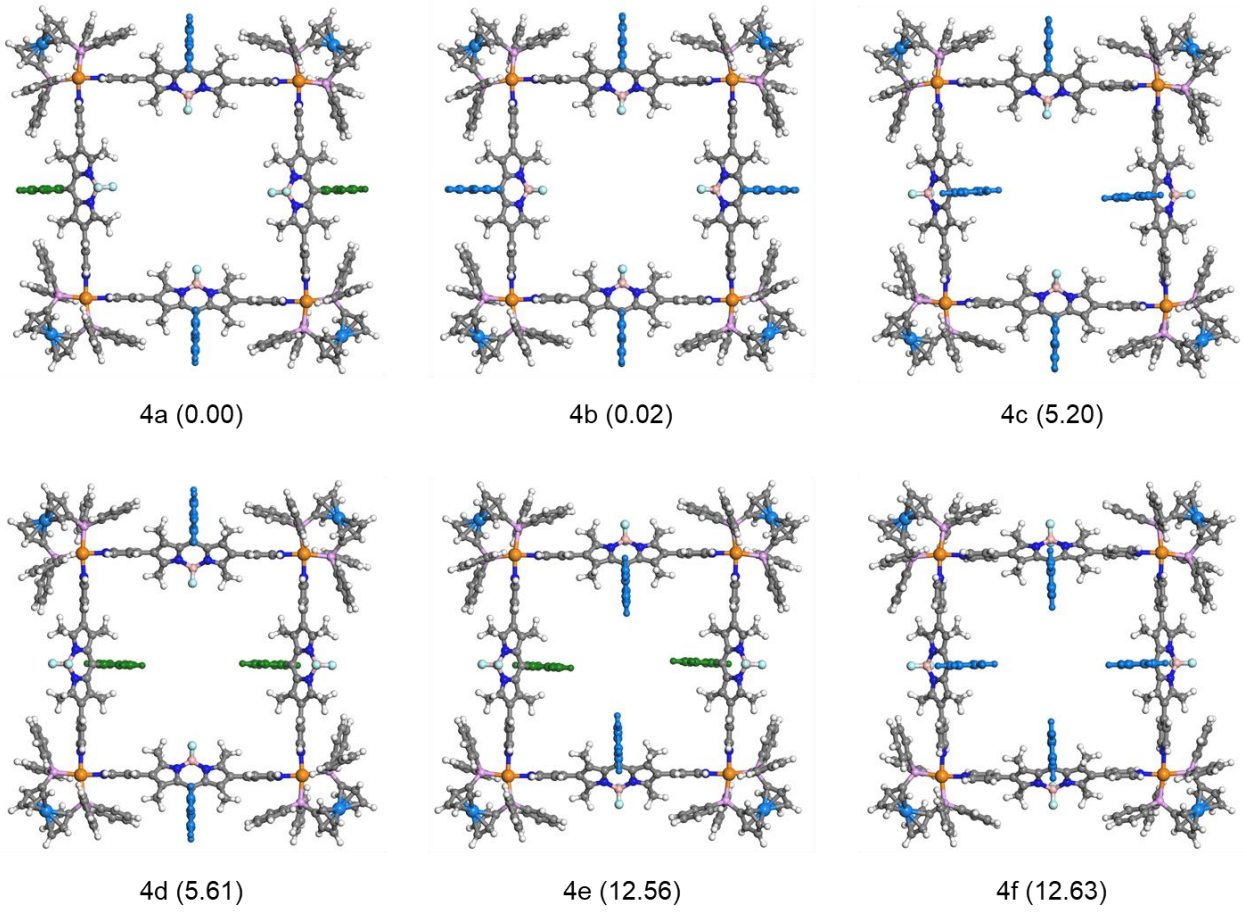

Figure S3a: PM7-optmized local minimum structures of complex **4** depending on the orientation of bodipy moiety (4a-4f). Phenyl-rings attached to bodipy moiety are colored to indicate their positions, i.e, up (blue) and down (green), with respect to square plane of four Pt ions. The relative energies (in kcal/mol) are presented in parentheses.

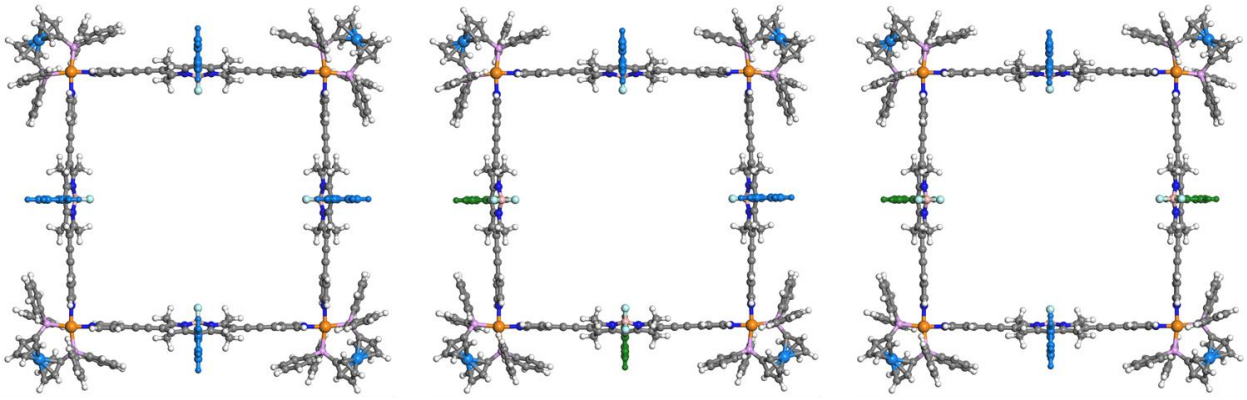

Figure S3b: PM7-optmized local minimum structures of complex **6** depending on the orientation of bodipy moiety (6a-6c). Phenyl-rings attached to bodipy moiety are colored to indicate their positions, i.e, up (blue) and down (green), with respect to the square plane of four Pt ions. The relative energies (in kcal/mol) are presented in parentheses.

Table S1: Selected geometric parameters for PM7-optmized structures of complexes **4** and **6**.

	interplane angle between square plane of 4 Pt ions and bodipy moiety	interior angle (∠ Pt-Pt-Pt)	torsional angle for 4 Pt ions	torsional angle for 4 Fe ions
complex 4				
4a	38.1°	87.3°, 92.7°	0.0°	0.0°
4b	34.1°	90.0°	0.0°	0.0°
4c	32.2°, 48.3°	87.2°, 92.8°	0.7°	1.3°
4d	38.8°, 43.8°	89.7°, 90.3°	1.0°	1.9°
4e	44.5°	87.3°, 92.7°	0.0°	0.1°
4f	52.0°	90.0°	0.0°	0.0°
complex 6				
6a	68.4°	90.0°	0.0°	0.0°
6b	71.5°	89.8°, 90.2°	0.0°	0.0°
6c	73.1°	90.0°	0.0°	0.0°