Supplementary Information Available

Highly luminescent metallacages featuring bispyridyl ligands functionalised with BODIPY for imaging in cells

Ben Woods,†[a] Daniel Döllerer,†[a,b] Brech Aikman,[a] Margot Wenzel,[a] Edward J. Sayers,[c] Fritz. E. Kühn,[b] Arwyn T. Jones,[c] and Angela Casini*[a]

Experimental section

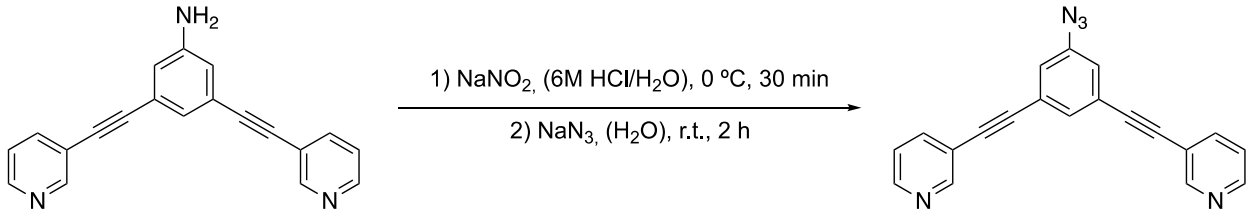
General

Chemicals. All reagents and solvents were obtained from commercial suppliers and used without further purification, unless otherwise stated. Triethylamine was distilled under nitrogen before use. ^{1}H NMR, ^{13}C{^{1}H} NMR, ^{11}B NMR and ^{19}F NMR spectra were recorded on a 500 MHz DMX (Bruker) or 400 MHz AV spectrometer (Bruker). Chemical shifts are given in parts per million (ppm). Abbreviations for NMR multiplicities are: singlet (s), doublet (d), triplet (t), quartet (q), doublet of doublets (dd), doublet of triplets (dt) and multiplet (m). Coupling constants *J* are given in Hz. The following solvents were used as internal standards: DMSO-d_6: 2.50 ppm (^{1}H NMR) and 39.52 ppm (^{13}C NMR); $CDCl_3$: 7.26 ppm (^{1}H NMR) and 77.16 ppm (^{13}C NMR); acetone-d_6: 2.05 ppm (^{1}H NMR) and 29.84 ppm (^{13}C NMR); DMF-d_7: 8.03 ppm (^{1}H NMR) and 163.15 ppm (^{13}C NMR).[1] High resolution ESI-MS spectra were recorded on a Walter Synapt G2SI QTOF.

Synthesis of ligands

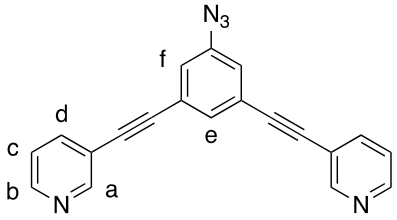
BODIPY **B1**,[1] **B2**[2] and ligand **L1**[3] have been synthesised adapting previously reported procedures and the analytical data is in accordance with the literature.

- *3,3'-((5-azido-1,3-phenylene)bis(ethyne-2,1-diyl))dipyridine* **(L2)**



L1 **L2**

Scheme S1. Synthesis of ligand **L2**.



Chemical Formula: $C_{20}H_{11}N_5$
Exact Mass: 321.10145
Molecular Weight: 321.34300

L1 (136 mg, 459 μmol, 1.00 eq.) was dissolved in 6M HCl (4.5 mL) and cooled down to 0 °C (water/ice). $NaNO_2$ (39.7 mg, 575 μmol, 1.20 eq.) was dissolved in water (3 mL) and added dropwise to the reaction. After 30 min, the reaction was allowed to warm to r.t. and NaN_3 (59.3 mg, 912 μmol, 2.00 eq.) dissolved in water (3 mL) was added dropwise and stirred for 2 h. The pH-value was adjusted to 8 with 2M NaOH, the

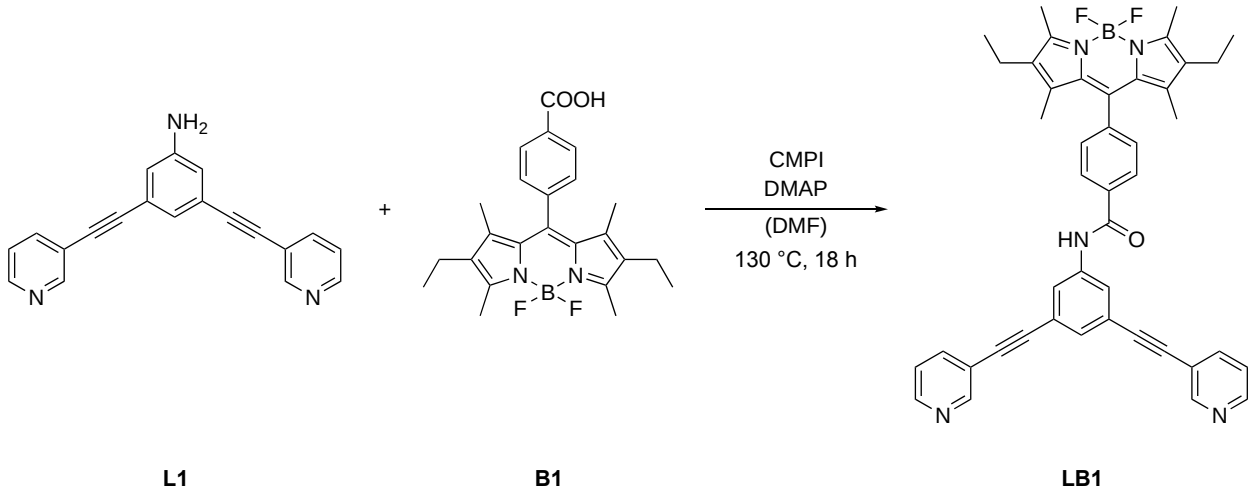
formed precipitate filtered over a glass-fritted funnel (porosity 3) and washed with a small amount of water, EtOH and Et_2O to give **L2** as a pale brown solid (96.3 mg, 300 μmol, 65%).

^{1}H NMR (400 MHz, $CDCl_3$): δ [ppm] = 8.78 (d, *J* = 1.3 Hz, 2H, H_a), 8.59 (dd, *J* = 1.7, 4.9 Hz, 2H, H_b), 7.82 (dt, *J* = 1.9, 7.9 Hz, 2H, H_d), 7.51 (t, *J* = 1.4 Hz, 1H, H_e), 7.31 (dd, *J* = 4.9, 7.9 Hz, 2H, H_c), 7.19 (d, *J* = 1.4 Hz, 2H, H_f).

^{13}C NMR (101 MHz, DMSO-d_6): δ [ppm] 151.8. 149.5, 141.0, 138.8, 130.7, 123.9, 123.7, 122.5, 118.8, 90.3, 87.8.

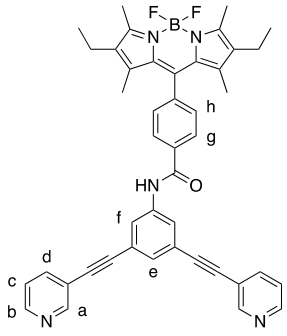
HRMS (ESI, MeCN): calcd. for $C_{20}H_{12}N_5$ $[M+H]^+$: *m/z* = 322.1094; found: 322.1112, δ = 5.9 ppm.

- *N-(3,5-bis(pyridin-3-ylethynyl)phenyl)-4-(2,8-diethyl-5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ⁴,5λ⁴-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)benzamide* **(LB1)**



L1 **B1** **LB1**

Scheme S2. Synthesis of **LB1**.



Chemical Formula: $C_{44}H_{38}BF_2N_5O$
Exact Mass: 701.31375
Molecular Weight: 701.62881

A mixture of **B1** (152 mg, 357 μmol, 1.00 eq.), **L1** (105 mg, 356 μmol, 1.00 eq.), CMPI (360 mg, 1.41 mmol, 4.00 eq.) and DMAP (433 mg, 3.54 mmol, 10.0 eq.) was dissolved in anhydrous DMF (18 mL) and stirred under a nitrogen atmosphere at 130 °C. After 18 h, the solvent was removed under reduced pressure, the residue dissolved in DCM (50 mL) and washed with water (3x 40 mL) and brine (1x 40 mL). The organic layer was dried over $MgSO_4$ and filtered over a glass-fritted funnel (porosity 3). The solvent was removed

under reduced pressure and the crude compound purified *via* silica column chromatography (EtOAc:*n*-hexane = 1:1 → 3:1, R_f = 0.21) to give product **LB1** as a red/pink solid (108 mg, 154 μmol, 43%).

^{1}H NMR (400 MHz, acetone-d_6): δ [ppm] = 9.97 (s, 1H, N*H*), 8.80 (d, *J* = 1.3 Hz, 2H, H_a), 8.61 (dd, *J* = 1.7, 4.9 Hz, 2H, H_b), 8.30 (d, *J* = 8.3 Hz, 2H, H_g), 8.21 (d, *J* = 1.5 Hz, 2H, H_f), 7.99 (dt, *J* = 1.9, 7.9 Hz, 2H, H_d), 7.61 (d, *J* = 8.2 Hz, 2H, H_h), 7.57 (t, *J* = 1.4 Hz, 1H, H_e), 7.47 (dd, *J* = 4.9, 7.9 Hz, 2H, H_c), 2.51 (s, 6H, NCCH_3), 2.36 (q, *J* = 7.5 Hz, 4H, CH_2CH$_3$), 1.36 (s, 6H, CCH_3), 0.99 (t, *J* = 7.5 Hz, 6H, CH$_2$CH_3).

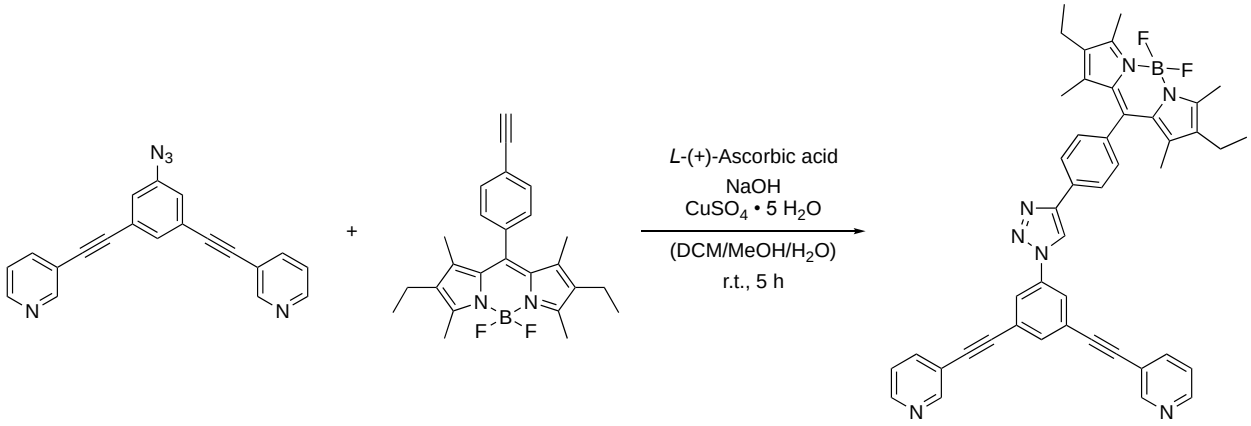
^{13}C NMR (101 MHz, acetone-d_6): δ [ppm] = 165.4. 156.0, 154.8, 152.9, 150.1, 148.3, 141.0, 140.2, 139.3, 139.0, 133.8, 130.5, 129.7, 129.4, 124.4, 124.3, 124.2, 120.6, 96.1, 91.8, 87.5, 17.5, 14.9, 12.7, 12.1.

^{11}B NMR (128 MHz, acetone-d_6): δ [ppm] = 0.76.

^{19}F NMR (376 MHz, acetone-d_6): δ [ppm] = -145.1.

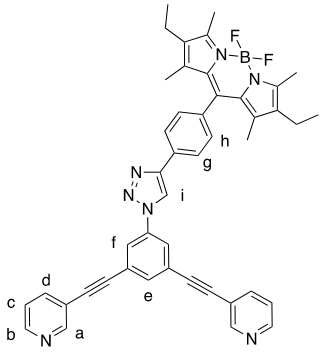
HRMS (ESI, MeCN): calcd. for $C_{44}H_{38}BFN_5O$ [M-F]$^+$: *m/z* = 682.3125; found: 682.3139, δ = -2.1 ppm.

- *10-(4-(1-(3,5-bis(pyridin-3-ylethynyl)phenyl)-1H-1,2,3-triazol-4-yl)phenyl)-2,8-diethyl-5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ⁴,5λ⁴-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinine* **(LB2)**



L2 **B2** **LB2**

Scheme S3. Synthesis of **LB2**.



Chemical Formula: $C_{45}H_{38}BF_2N_7$
Exact Mass: 725.32498
Molecular Weight: 725.65481

L2 (15.8 mg, 49.2 μmol, 1.00 eq.) was dissolved in DCM (0.5 mL) and diluted with MeOH:water (3:1, 6 mL). **B2** (54.5 mg, 135 μmol, 3.00 eq.), DCM (1 mL) and a solution of *L*-(+)-ascorbic acid (11.6 mg, 65.9 μmol, 1.35 eq.), NaOH (3.10 mg, 77.5 μmol, 1.60 eq.) and $CuSO_4 \cdot 5\ H_2O$ (1.10 mg, 4.41 μmol, 0.10 eq.) in water (2 mL) was added to the reaction mixture and stirred at room temperature. After 5 h, the reaction was quenched with a saturated NH_4OAc solution (20 mL) and stirred for 1 h. The reaction solution was washed with DCM (3x 20 mL) and the combined organic layers with brine (1x 30 mL). The organic layer was dried over Na_2SO_4, filtered over a glass-fritted funnel (porosity 3) and the solvent removed under reduced pressure. The crude compound was purified *via* silica column chromatography (DCM:MeOH = 100:5, R_f = 0.34) to give product **LB2** as a purple solid (19.7 mg, 27.1 μmol, 55%).

^{1}H NMR (400 MHz, acetone-d_6): δ [ppm] = 9.34 (s, 1H, H_i), 8.84 (d, J = 1.2 Hz, 2H, H_a), 8.64 (dd, J = 1.6, 4.9 Hz, 2H, H_b), 8.35 – 8.20 (m, 4H, H_f, H_g), 8.04 (dt, J = 1.9, 8.0 Hz, 2H, H_d), 7.92 (t, J = 1.4 Hz, 1H, H_e), 7.55 (d, J = 8.3 Hz, 2H, H_h), 7.50 (dd, J = 4.9, 7.9 Hz, 2H, H_c), 2.52 (s, 6H, NCCH_3), 2.37 (q, J = 7.5 Hz, 4H, CH_2CH$_3$), 1.44 (s, 6H, CCH_3), 1.00 (t, J = 7.5 Hz, 6H, CH$_2$CH_3).

^{13}C NMR (126 MHz, acetone-d_6): δ [ppm] = 154.5. 153.0, 150.5, 148.4, 139.4, 139.1, 138.7, 136.5, 134.9, 132.2, 130.0, 127.2, 126.5, 125.7, 125.7, 124.4, 123.8, 123.6, 120.5, 120.2, 90.7, 89.1, 17.5, 15.0, 12.6, 12.1.

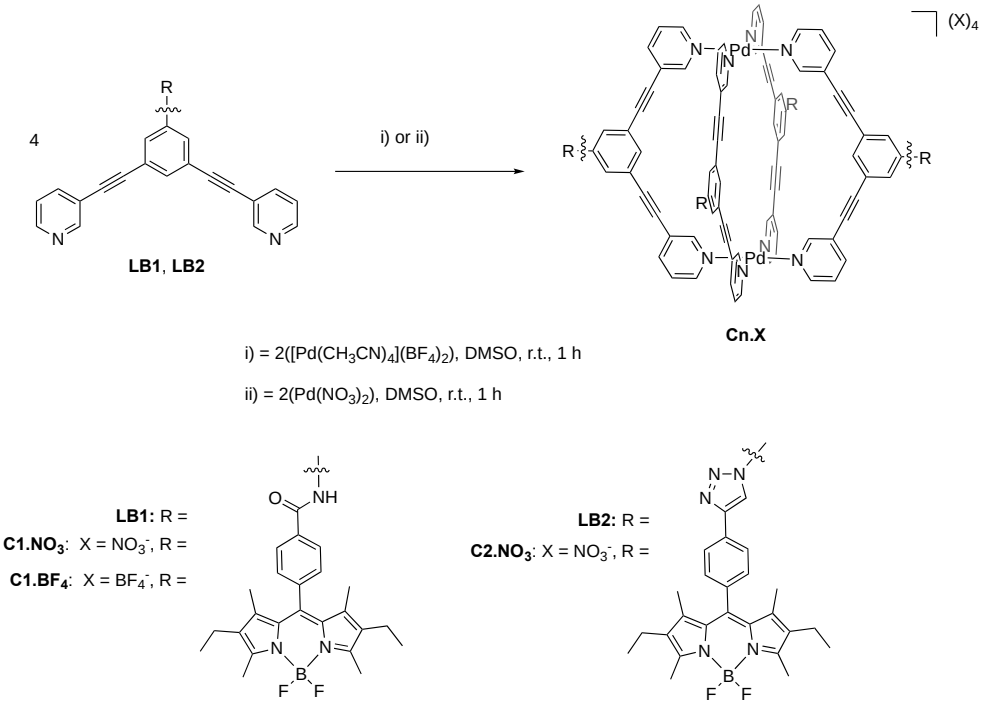
^{11}B NMR (160 MHz, acetone-d_6): δ [ppm] = 0.83.

^{19}F NMR (471 MHz, acetone-d_6): δ [ppm] = -145.1.

HRMS (ESI, MeCN): calcd. for $C_{45}H_{38}BFN_7$ $[M\text{-}F]^+$: m/z = 706.3281; found: 706.3214, δ = -7.7 ppm.

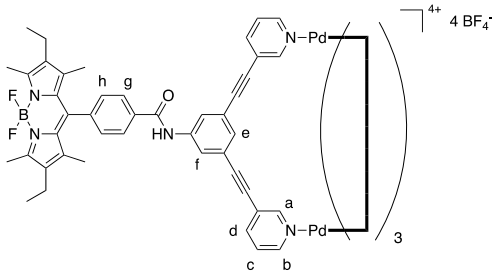
Synthesis of metallacages

A solution of Pd precursor (2 eq.) and ligand (4 eq.) in DMSO was stirred at r.t. for 1 h (Scheme S4). Afterwards, precipitation by addition of acetone and diethyl ether and consecutive filtration gave the respective cages **C1.BF$_4$**, **C1.NO$_3$** and **C2.NO$_3$**.



Scheme S4. General synthesis scheme for **C1.BF$_4$**, **C1.NO$_3$** and **C2.NO$_3$**.

- **C1.BF$_4$**



A solution of Pd(MeCN$_4$)$_4$(BF$_4$)$_2$ (6.00 mg, 14.3 µmol, 2.00 eq.) and **LB1** (20.0 mg, 28.5 µmol, 4.00 eq.) in DMSO (1.5 mL) was stirred for 1 h at r.t.. Following precipitation by addition of acetone (3 ml) and diethyl ether (excess), the solid was filtered over a glass-fritted funnel (porosity 4) to give cage **C1.BF$_4$** as a red solid (19.0 mg, 5.56 µmol, 78%).

^{1}H NMR (400 MHz, acetone-d_6): δ [ppm] = 9.95 (s, 1H, N*H*), 9.91 (s, 2H, H_a), 9.55 (d, J = 4.9 Hz, 2H, H_b), 8.39 – 8.13 (m, 6H, H_d, H_g, H_h,), 7.96 (s, 1H, H_e), 7.88 – 7.73 (m, 2H, H_c), 7.57 (s, 2H, H_f), 2.52 (s, 6H, NCCH_3), 2.48 (s, 4H, CH_2CH$_3$), 1.30 (s, 6H, CCH_3), 0.96 (t, J = 6.94 Hz, 6H, CH$_2$CH_3).

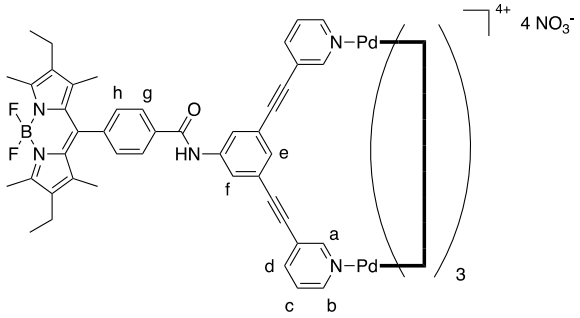
^{13}C NMR (101 MHz, acetone-d_6): δ [ppm] = 165.1, 161.8, 153.9, 153.5, 150.6, 143.0, 139.5, 138.0, 134.9, 132.9, 130.2, 129.3, 128.9, 128.5, 127.4, 124.5, 123.4, 122.7, 116.3, 93.9, 84.5, 16.5, 14.0, 11.7, 11.2.

^{11}B NMR (128 MHz, acetone-d_6): δ [ppm] = 0.66 (BF$_2$), -0.38 (BF$_4$).

^{19}F NMR (376 MHz, acetone-d_6): δ [ppm] = -145.1 (d, J = 33.2 Hz, BF_2), -145.3 (d, J = 33.2 Hz, BF_2), -149.9 (s, BF_4).

HRMS (ESI, MeCN): calcd. for $C_{161}H_{130}B_3F_6N_{18}O_4Pd_2$ $[M\text{-}4BF_4\text{-}(C_{17}H_{22}BF_2N_2)+2H]^{2+}$: *m/z* = 1367.9508; found: 1367.6577.

- **C1.NO$_3$**



A solution of Pd(NO$_3$)$_2$ · 2 H$_2$O (4.60 mg, 17.3 μmol, 2.00 eq.) and **LB1** (23.1 mg, 32.9 μmol, 4.00 eq.) in DMSO (1.5 mL) was stirred for 2 h at r.t. Following precipitation by addition of acetone (2 mL) and diethyl ether (excess), the solid was filtered over a glass-fritted funnel (porosity 4) to give cage **C1.NO$_3$** as a red solid (24.6 mg, 7.53 μmol, 91%).

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 10.69 (s, 1H, N*H*), 9.69 (s, 2H, H_a), 9.42 (s, 2H, H_b), 8.30 (d, J = 7.9 Hz, 2H, H_g), 8.24 (s, 2H, H_f), 8.16 (d, J = 7.4 Hz, 2H, H_d), 7.84 (s, 2H, H_h), 7.72 (s, 1H, H_e), 7.59 (d, J = 7.8 Hz, 2H, H_c), 2.44 (s, 6H, NCCH_3), 2.28 (s, 4H, CH_2CH$_3$), 1.25 (s, 6H, CCH_3), 0.93 (s, 6H, CH$_2$CH_3).

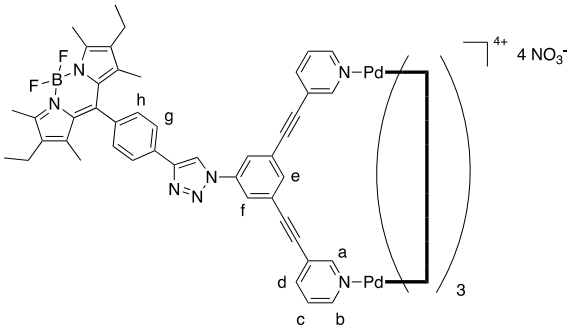
^{13}C NMR (126 MHz, DMSO-d_6): δ [ppm] = 183.5. 165.1, 157.9,153.6, 153.0, 150.6, 143.0, 140.2, 139.3, 138.5, 137.9, 132.8, 129.6, 128.6, 127.4, 122.2, 122.1, 93.7, 86.8, 85.9, 85.1, 16.4, 14.5, 12.3, 11.5.

^{11}B NMR (128 MHz, DMSO-d_6): δ [ppm] = 0.65.

^{19}F NMR (376 MHz, DMSO-d_6): δ [ppm] = -142.9.

HRMS (ESI, MeOH): calcd. for $C_{178}H_{157}B_4F_7N_{20}O_6Pd_2Na_2$ $[M\text{-}4NO_3\text{-}3H\text{-}F+2MeOH+2Na]^{2+}$: *m/z* = 1552.0486; found: 1552.0068.

- **C2.NO$_3$**



A solution of $Pd(NO_3)_2 \cdot 2\ H_2O$ (4.10 mg, 15.4 μmol, 2.00 eq.) and **LB2** (22.3 mg, 30.7 μmol, 4.00 eq.) in DMSO (1.5 mL) was stirred for 1 h at r.t.. Following precipitation by addition of acetone (3 mL) and diethyl ether (excess), the solid was filtered over a glass-fritted funnel (porosity 4) to give cage **C2.NO$_3$** as a dark purple solid (24.0 mg, 7.14 μmol, 93%).

^{1}H NMR (500 MHz, DMSO-d_6): δ [ppm] = 9.83 (s, 2H, H_a), 9.59 (s, 1H, C*H*), 9.45 (s, 2H, H_b), 8.38 (s, 2H, H_f), 8.31 (d, J = 8.3 Hz, 2H, H_d), 8.30 (d, J = 7.9 Hz, 2H, H_g), 8.05 (s, 1H, H_e), 7.89 (s, 2H, H_c), 7.51 (d, J = 8.1 Hz, 2H, H_h), 2.44 (s, 6H, NCCH_3), 2.22 – 2.32 (m, 4H, CH_2CH$_3$), 1.31 (s, 6H, CCH_3), 0.93 (t, J = 7.4 Hz, 6H, CH$_2$CH_3).

^{11}B NMR (128 MHz, DMSO-d_6): δ [ppm] = 0.77.

^{19}F NMR (376 MHz, DMSO-d_6): δ [ppm] = -142.9.

HRMS (ESI, MeOH): calcd. For $C_{140}H_{103}B_2F_4N_{24}Pd_2$ [M-4NO$_3$-($C_{17}H_{22}BF_2N_2$)-($C_{23}H_{26}BF_2N_2$)]$^{2+}$: m/z = 1214.8926; found: 1214.3605.

Quantum Yield Determination

Quantum yield of fluorescence was calculated by comparison to a reference standard (Rhodamine 6G in degassed ethanol, ϕ = 94% at room temperature). UV-Visible absorption spectra were recorded on a Cary 60 UV-Vis spectrometer from *Agilent Technologies*. Emission spectra were recorded on a Cary Eclipse Fluorescence Spectrophotometer from *Agilent Technologies*. The selected fluorophore was dissolved in degassed DMSO to a concentration corresponding to UV-Visible absorbance 0.8 A.U. ($\lambda_{(max)}$ = 523 - 535 nm; 25 °C). The solution was transferred to a fluorescence spectrophotometer and an emission spectrum was recorded (excitation wavelength 595 nm).

Stability studies by UV-Visible Spectroscopy

UV-visible absorption spectra to investigate the stability of the metallacages in solution were recorded on a Cary 60 UV-Vis spectrometer from *Agilent Technologies*. For each compound, stock solutions at a concentration of $3 \cdot 10^{-3}$ M were prepared. An aliquot was diluted either with 1x PBS (pH 7.4) or deionised water and the UV-Vis spectra measured at different times immediately after dilution at room temperature over 24 h. The cuvette was then shaken, and another spectrum recorded, to determine if the compound was altered during the 24 h or if the reduction in absorption was only due to precipitation.

Metallacage stability in the presence of L-glutathione

C1.BF$_4$ was dissolved in a 9:1 ratio of DMSO-d_6:D_2O (0.5 mL) and a ^{1}H NMR spectrum was recorded. L-glutathione was added to the solution to achieve a final concentration of 2 mM L-glutathione (0.3 mg) and the first ^{1}H NMR spectrum immediately recorded. Afterwards, spectra were recorded every 5 min for the first hour, followed by every hour for the following 17 hours. Finally, another 0.3 mg of L-glutathione were added after 18 hours to ensure that the reaction was complete. The ratio of the metallacage: ligand in solution was calculated by comparing the integral value of peak H_b of the metallacage to the peak of H_b of the ligand.

Cisplatin encapsulation studies

^{1}H NMR spectroscopy

Each metallacage (4.4 µM, 1.00 eq.) was dissolved in 1 mL DMF-d_7 and a ^{1}H NMR spectrum was recorded. Afterwards, Cisplatin (8.8 µM, 2.00 eq.) was added to the NMR tube and the deuterated solution was stirred for 10 min before ^{1}H NMR spectrum was recorded. Finally, NMR spectra were compared to evaluate any chemical shifts due to the encapsulation of cisplatin. Both spectra were calibrated to the residual solvent signal of the carbonyl proton of DMF (8.03 ppm).

^{195}Pt NMR spectroscopy

Cisplatin (2 mg, 6.7 µM, 1 eq.) was dissolved in DMF (0.5 mL) and transferred to an NMR tube with a capillary insert filled with DMF-d_7. Thus, the ^{195}Pt NMR spectra was recorded. To this sample, the selected

metallacage (**C1.BF$_4$**) (6.7 µM, 1 eq.) was added and the solution stirred for 10 min before another ^{195}Pt NMR spectrum of 1 equiv. of metallacage and 1 equiv. of cisplatin was recorded. Afterward, a second equivalent of cisplatin (2 mg, 6.7 µM, 1 eq.) was added to the solution to achieve a cisplatin:cage ratio of 2:1, and a final ^{195}Pt NMR spectrum was recorded. The resulting spectra were compared to observe any chemical shifts between the free cisplatin and metallacage complex.

Cell culture maintenance

Human malignant melanoma cell line A375 was obtained from ATCC and maintained in culture according provider instructions. Cells were cultured in a humidified atmosphere at 37 °C and 5% CO_2 using DMEM Dulbecco's Modified Eagle Medium (DMEM, 4.5 g/L glucose, Corning, Thermo Fischer Scientific) supplemented with 10% fetal bovine serum (FBS, Eu-approved South American Origin, Thermo Fisher Scientific) and 1% penicillin/streptomycin (Gibco) and passaged when reaching confluence.

Antiproliferative assay

To evaluate the inhibition of cell growth, 96-well tissue culture-treated plates (Corning) were seeded in a concentration of 15000 cells/well with 200 µL full medium. Working solutions of ligand and cage samples were prepared in the required concentration by diluting fresh stock solutions (5 x 10^{-3} M in DMSO) of the corresponding compound in aqueous complete DMEM medium accordingly. Dilutions (1 mM stock) of reference compound cisplatin (Sigma-Aldrich) were freshly prepared in aqueous solution and mixed with the metallacages prior each experiment. Cage formation and cisplatin encapsulation were confirmed by ^{1}H NMR spectroscopy as previously reported.[4] Following the initial 24 h incubation required for cell adhesion, cells were incubated for an additional 24 h with 200 µL of the compounds' dilution per well. Afterwards, 20 µL/well of CellTiter-Blue® reagent was added to the assay plate, shaken 10 sec and incubated for 4 hours at 37 °C and 5% CO_2. Fluorescent intensity ($531_{Ex}/595_{Em}$ nm) from each well was quantified in quadruplicates for each experiment using a multi-well plate reader (VICTOR X5, Perking Elmer). The percentage of surviving cells was calculated, using GraphPad Prism software, from the ratio of fluorescence intensity of treated to untreated cells, corrected for the interfering fluorescence of the BODIPY. The EC_{50} value for each compound was calculated as the concentration showing 50% decrease in cell growth, when compared to controls, using a nonlinear fitting of [concentration] vs response. Data is presented as mean ± SEM of at least three independent experiments.

Fluorescence microscopy assays

Round glass coverslips (Ø 13mm, VWR) sterilised by UV-light were inserted in 24-well tissue culture-treated plates (Corning). Cells were seeded at a concentration of 50.000 cells/well and incubated at 37 °C under humidified atmosphere with 5% CO_2 for 48 h. The medium was discarded and fresh medium containing 5 µM of either cage or ligand was added. Following 2 h of incubation at either 37 °C under tissue culture

conditions or 4 °C in the fridge, the glass coverslips were removed from the wells, washed 4x with 1x Phosphate Buffered Saline (PBS, Corning) and fixed with 4% formaldehyde (Alfa-Aesar) for 20 min at room temperature (r.t.). The coverslips were washed 3x with 1x PBS and incubated for 1 min with 40 µL 1:1000 from a 1 mg/mL stock solution of 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI, Sigma-Aldrich/ MERCK) at r.t.. After washing the coverslips thrice with 1x PBS they were mounted on glass microscope slides (VWR) using Mowiol® 4-88 (Sigma-Aldrich). Fluorescence images were obtained using either a Zeiss Axio Vert.A1 epifluorescent microscope or a Leica SP5 confocal laser-scanning microscope. For confocal imaging, two laser lines were used: 405 Blue Diode (excitation wavelength 405 nm, laser intensity 30%) for DAPI and Argon (excitation wavelength 514 nm, laser intensity 30%) for the complexes, captured sequentially to avoid bleedthrough. A HCX PL APO 63x 1.4 NA oil immersion objective was used for all images with Leica Type F immersion oil. Captured "XYZ" images from the Leica SP5 and "XY" images from the Zeiss were analysed using ImageJ. All images were captured under the same settings within each experiment and treated equally following acquisition.

Figures

NMR spectra

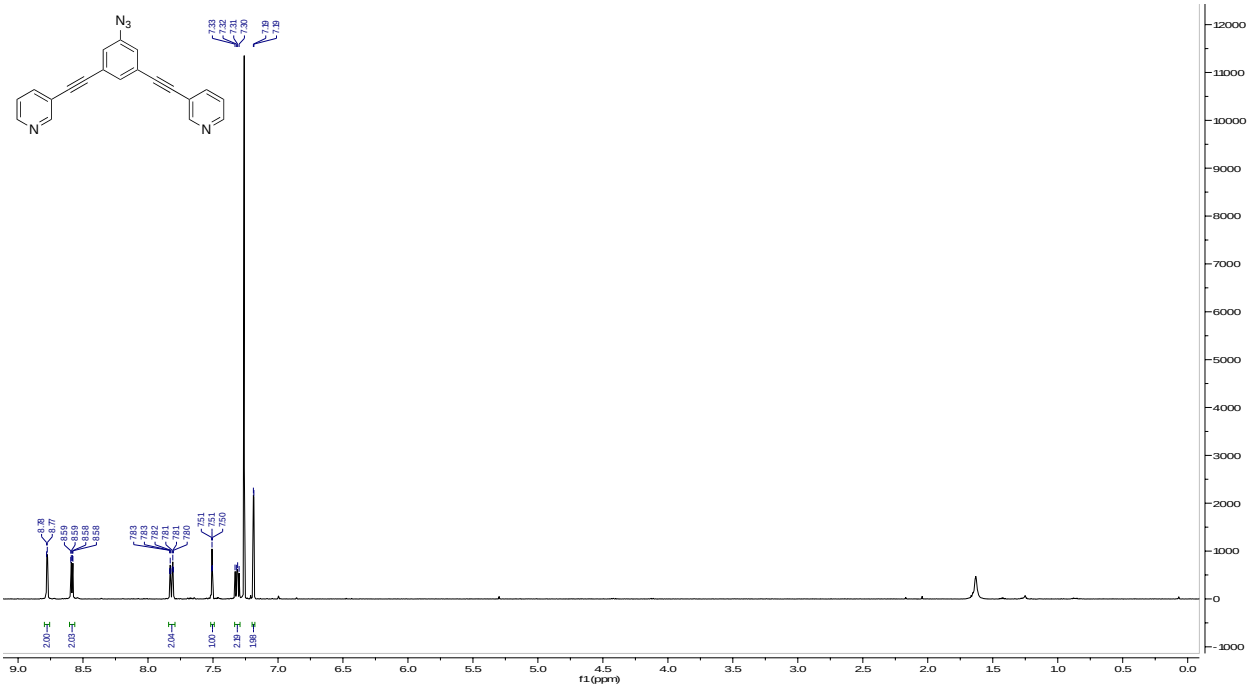


Figure S1. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **L2**.

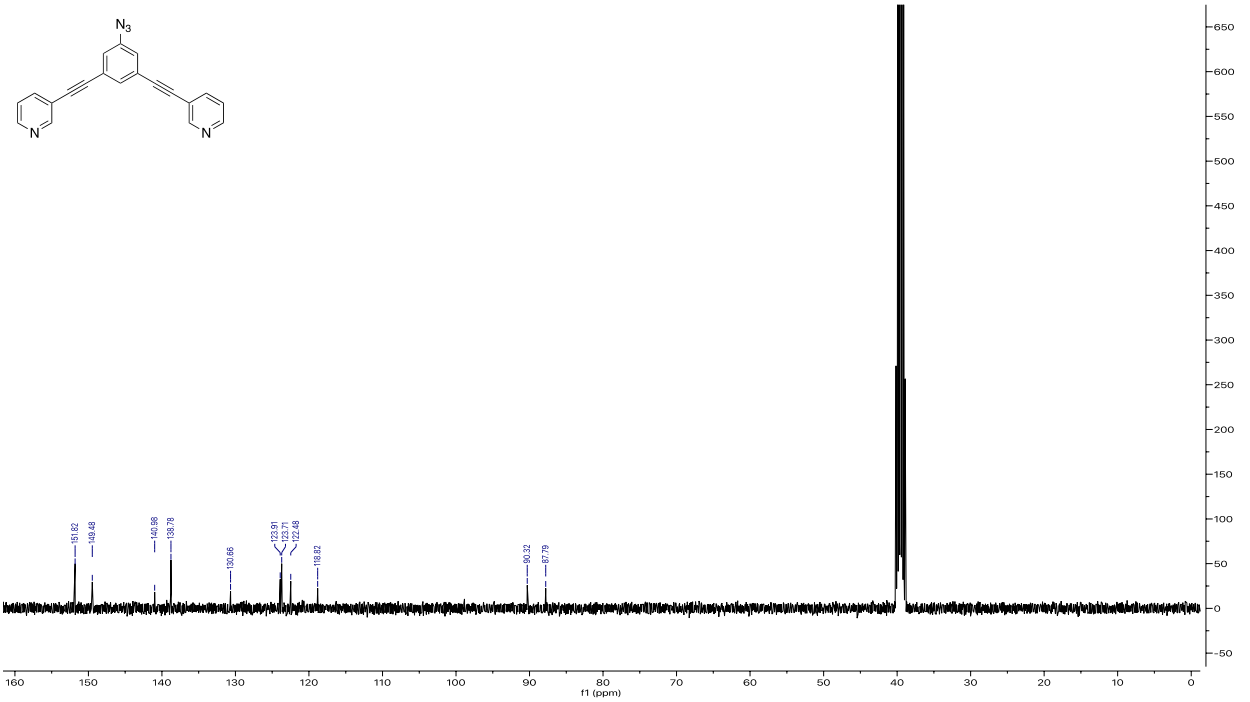


Figure S2. ^{13}C NMR (101 MHz, DMSO-d_6) spectrum of **L2**.

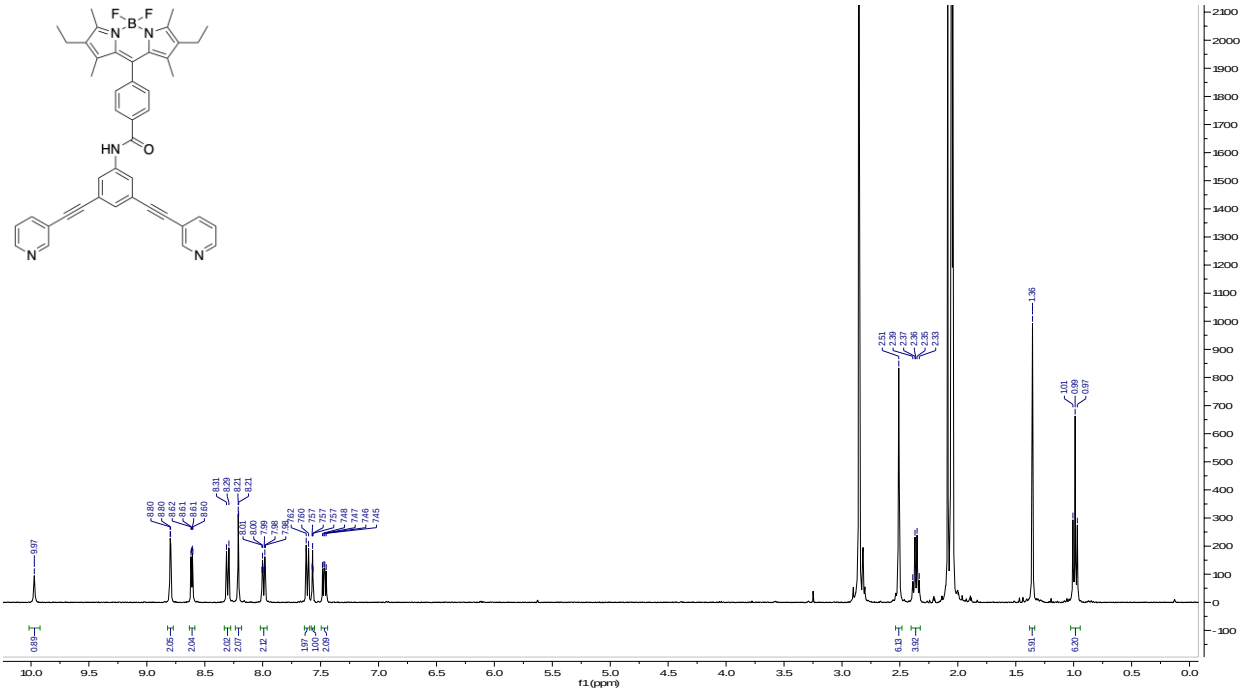


Figure S3. ^{1}H NMR (400 MHz, acetone-d_6) spectrum of **LB1**.

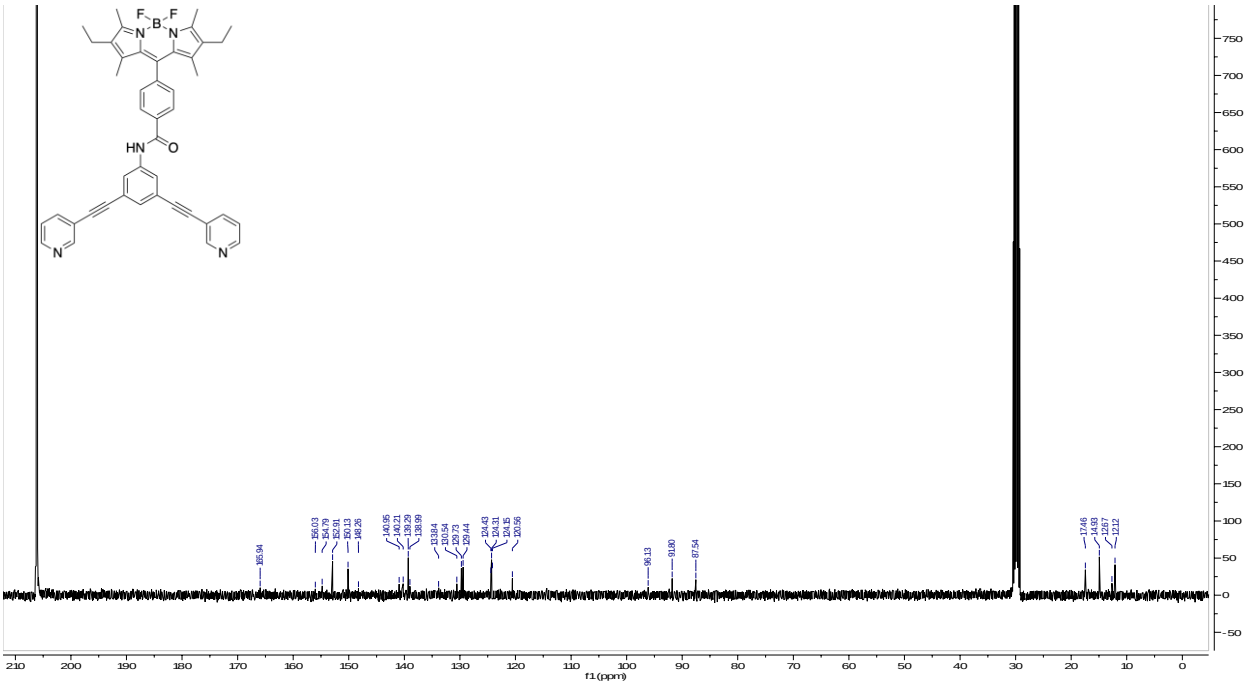


Figure S4. ^{13}C NMR (101 MHz, acetone-d_6) spectrum of **LB1**.

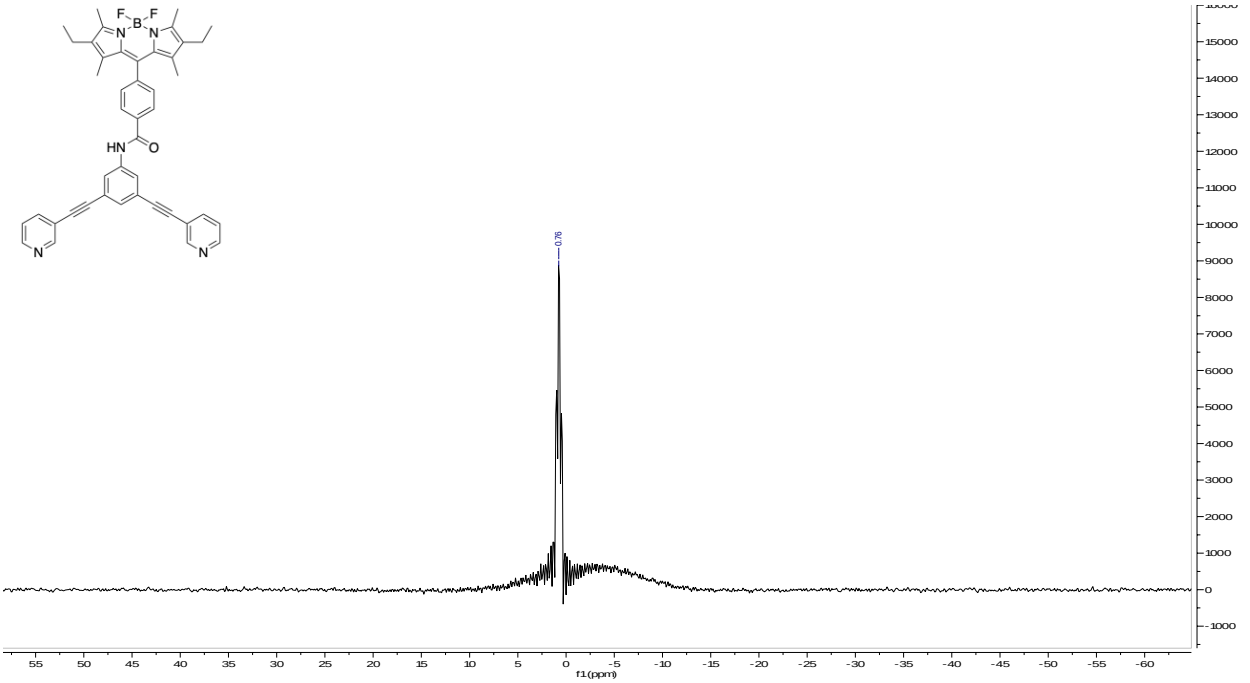


Figure S5. ^{11}B NMR (128 MHz, acetone-d_6) spectrum of **LB1**.

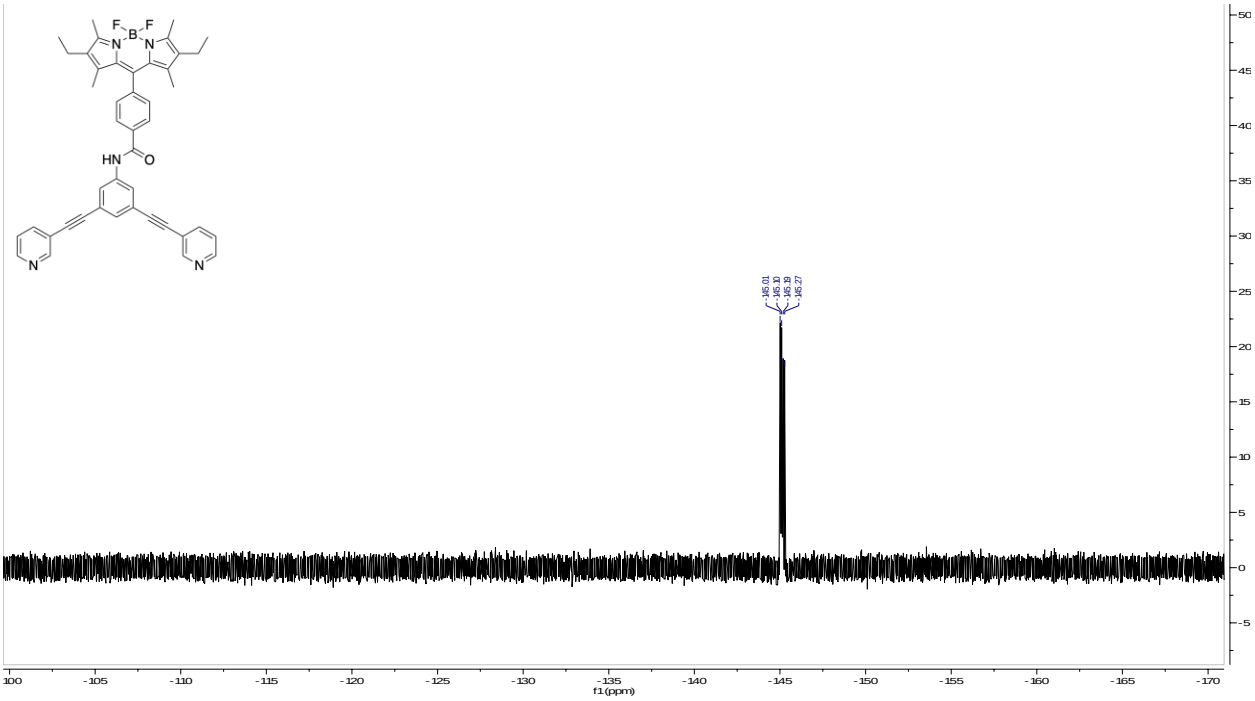


Figure S6. ^{19}F NMR (376 MHz, acetone-d_6) spectrum of **LB1**.

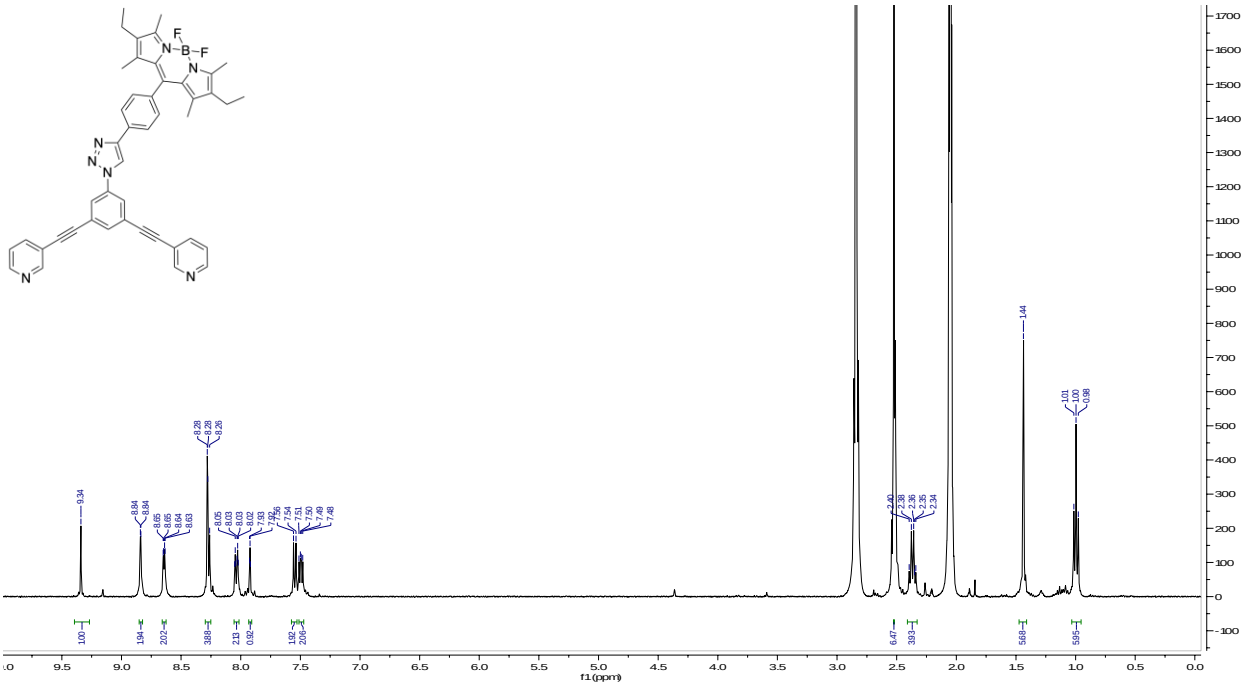


Figure S7. ^{1}H NMR (400 MHz, acetone-d_6) spectrum of **LB2**.

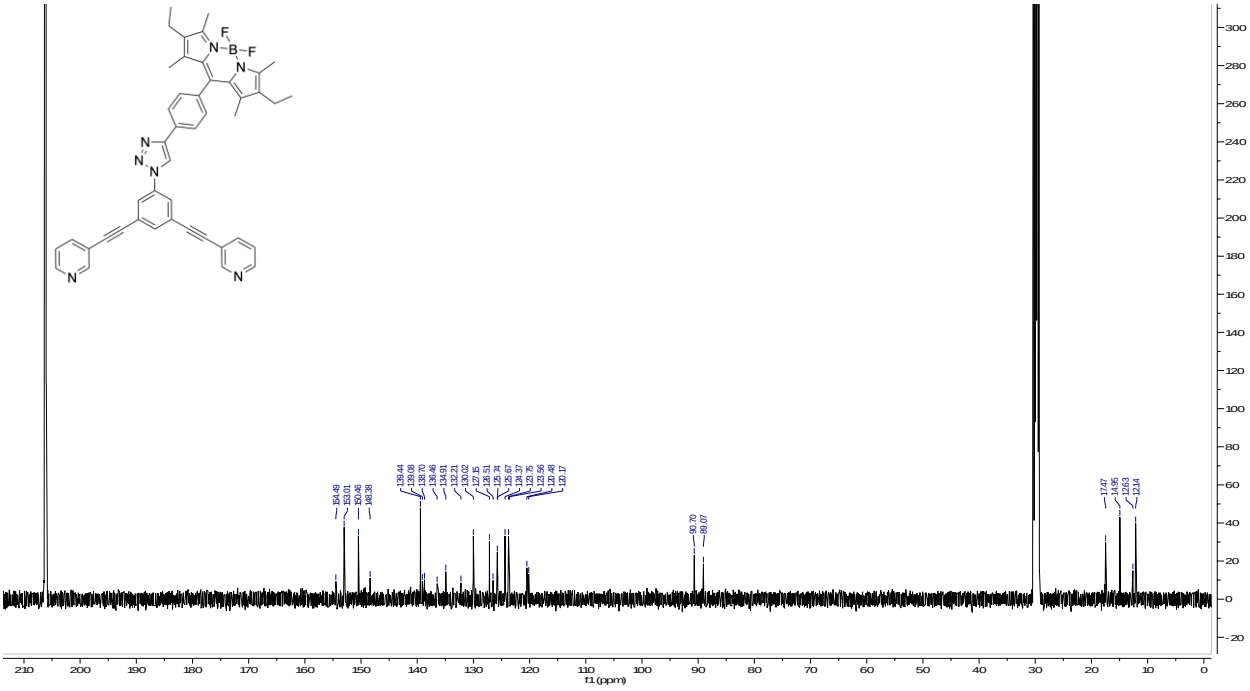


Figure S8. ^{13}C NMR (126 MHz, acetone-d_6) spectrum of **LB2**.

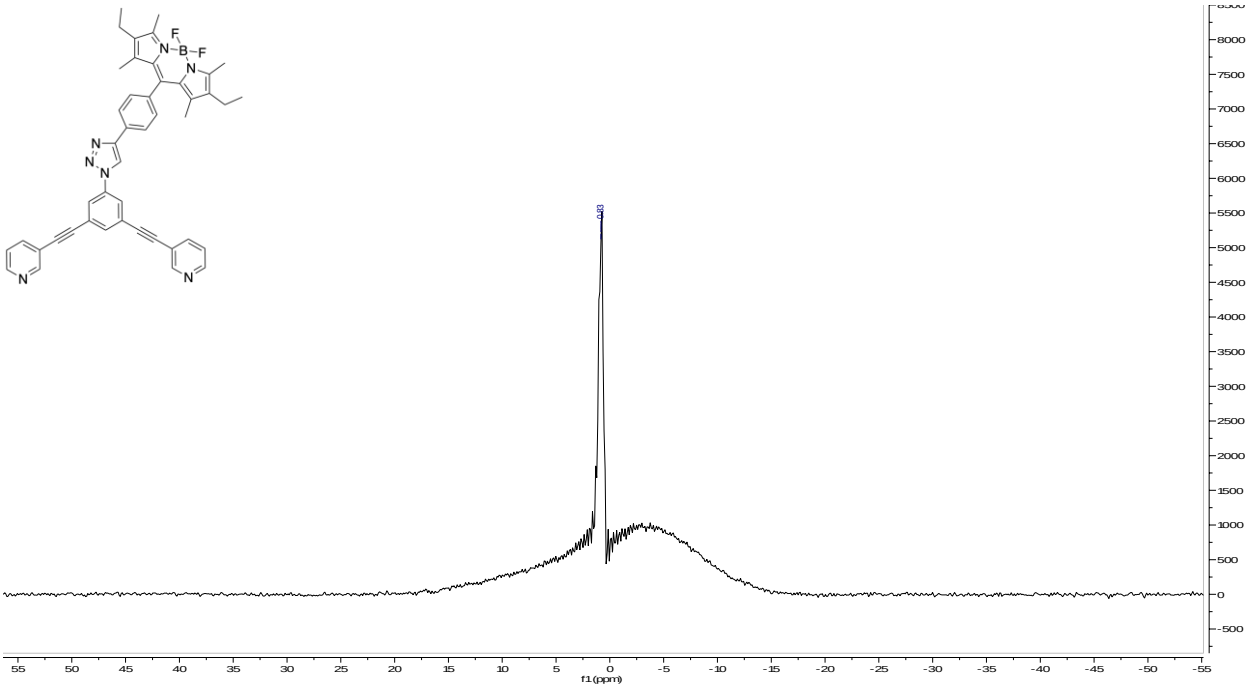


Figure S9. ^{11}B NMR (160 MHz, acetone-d_6) spectrum of **LB2**.

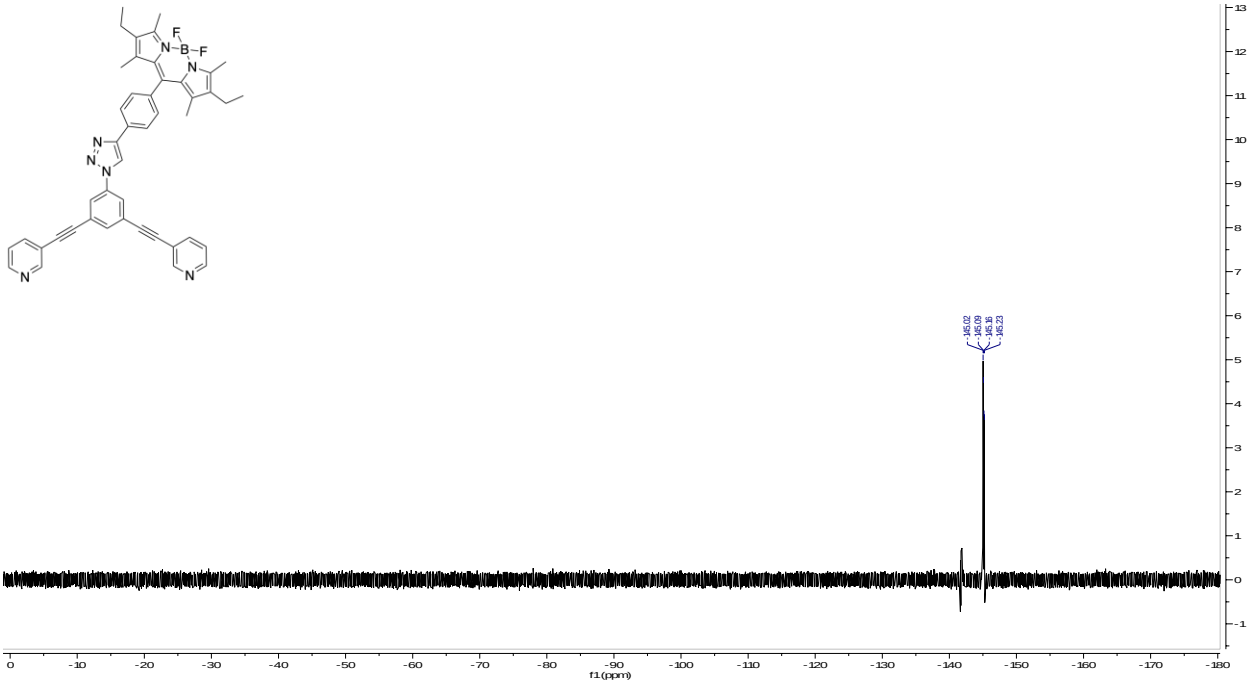


Figure S10. ^{19}F NMR (471 MHz, acetone-d_6) spectrum of **LB2**.

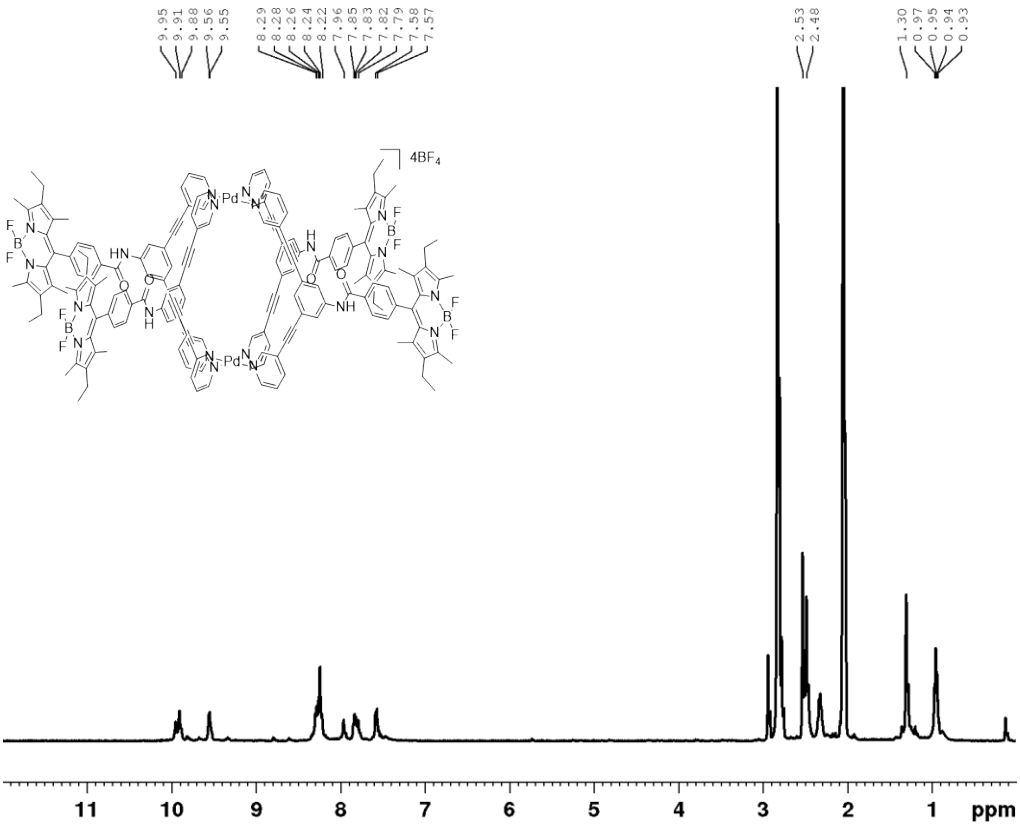


Figure S11. ^{1}H NMR (400 MHz, acetone-d_6) spectrum of **C1.BF$_4$**.

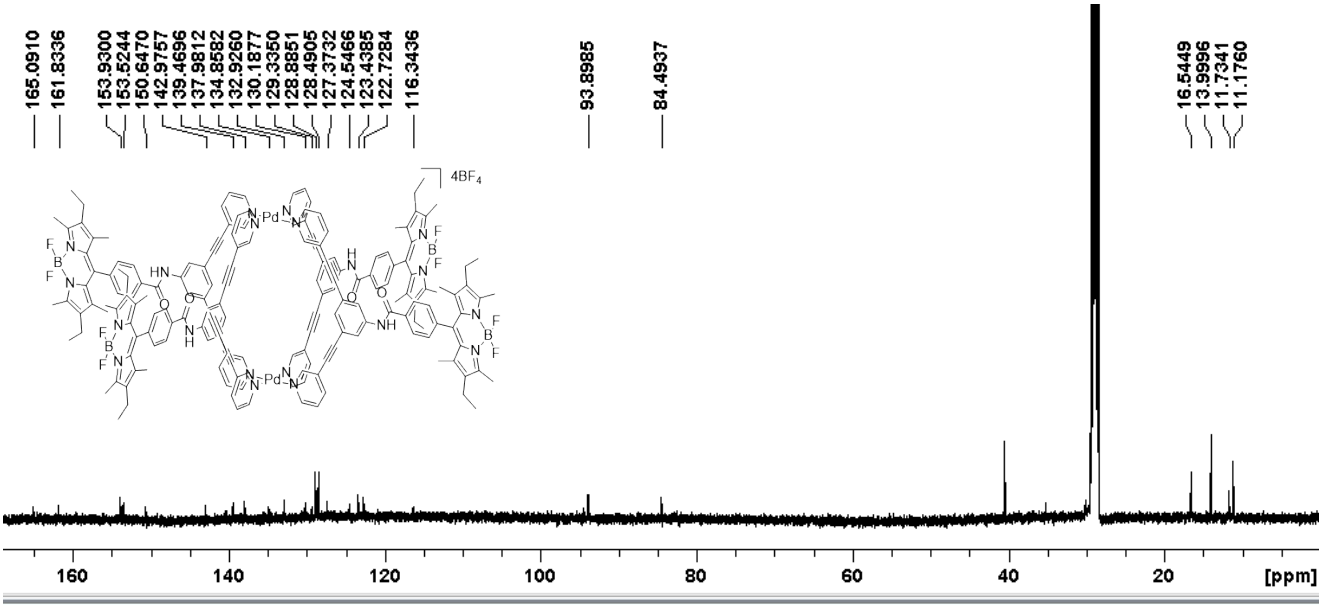


Figure S12. ^{13}C NMR (126 MHz, acetone-d_6) spectrum of **C1.BF$_4$**.

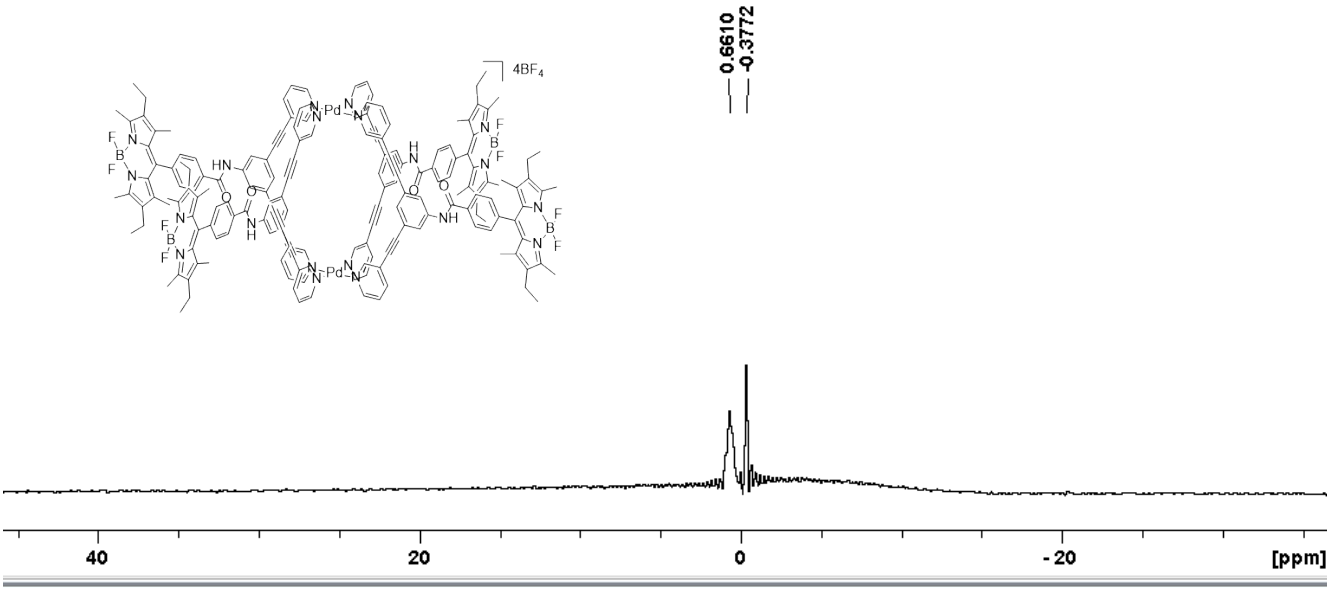


Figure S13. ^{11}B NMR (160 MHz, acetone-d_6) spectrum of **C1.BF$_4$**.

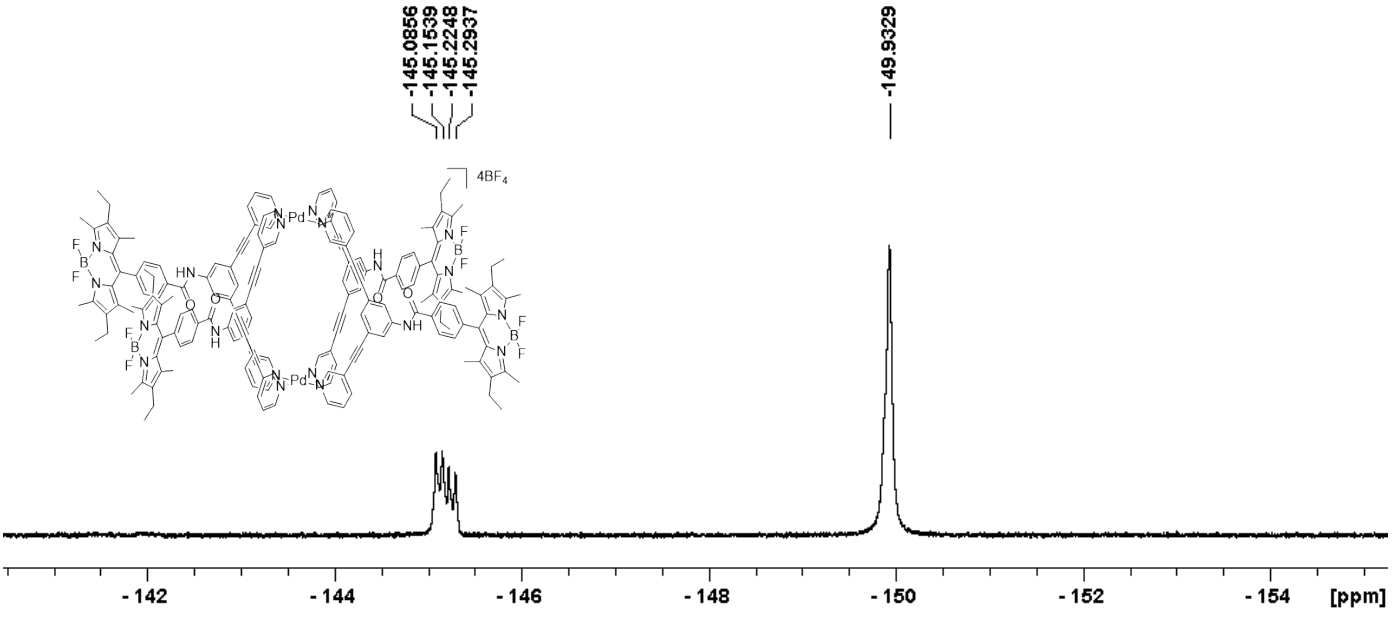


Figure S14. ^{19}F NMR (471 MHz, acetone-d_6) spectrum of **C1.BF$_4$**.

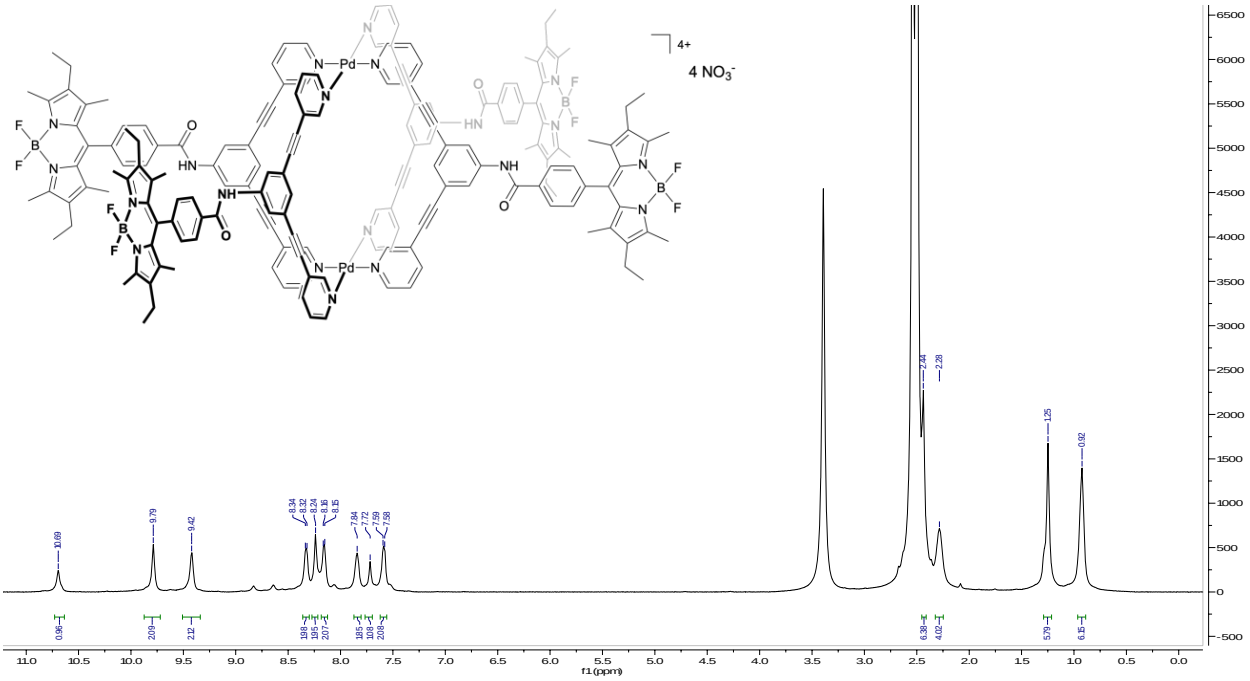


Figure S15. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of **C1.NO$_3$**.

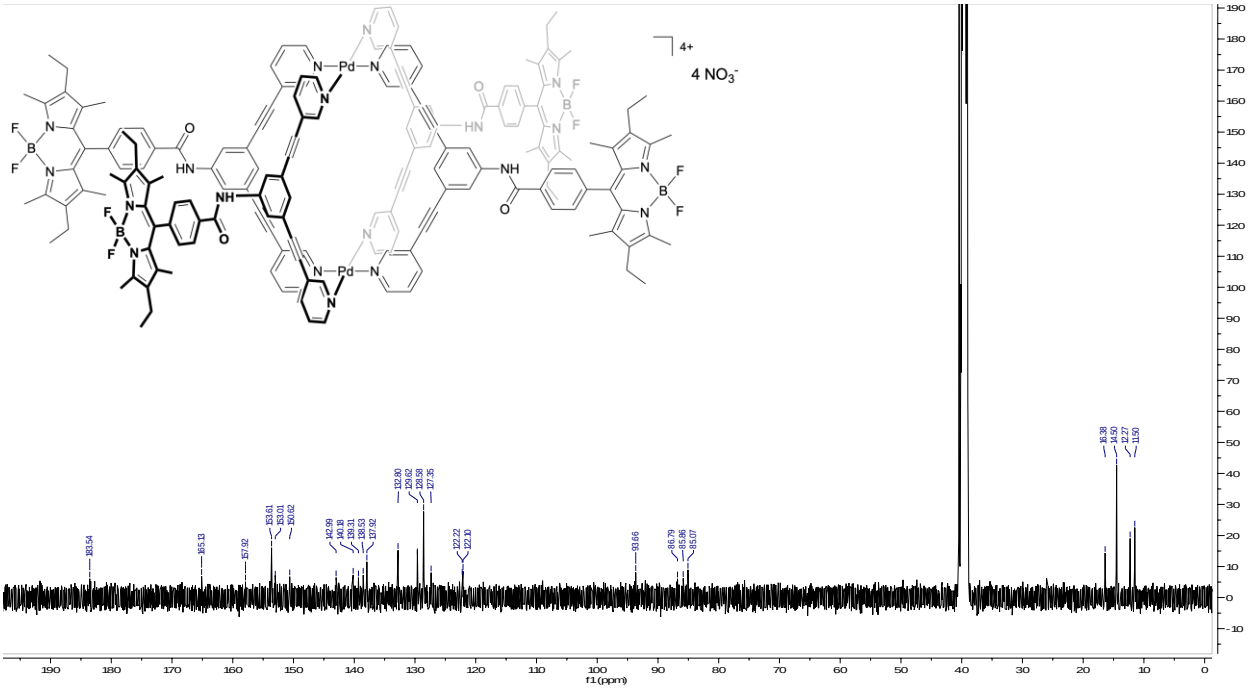


Figure S16. ^{13}C NMR (126 MHz, DMSO-d_6) spectrum of **C1.NO$_3$**.

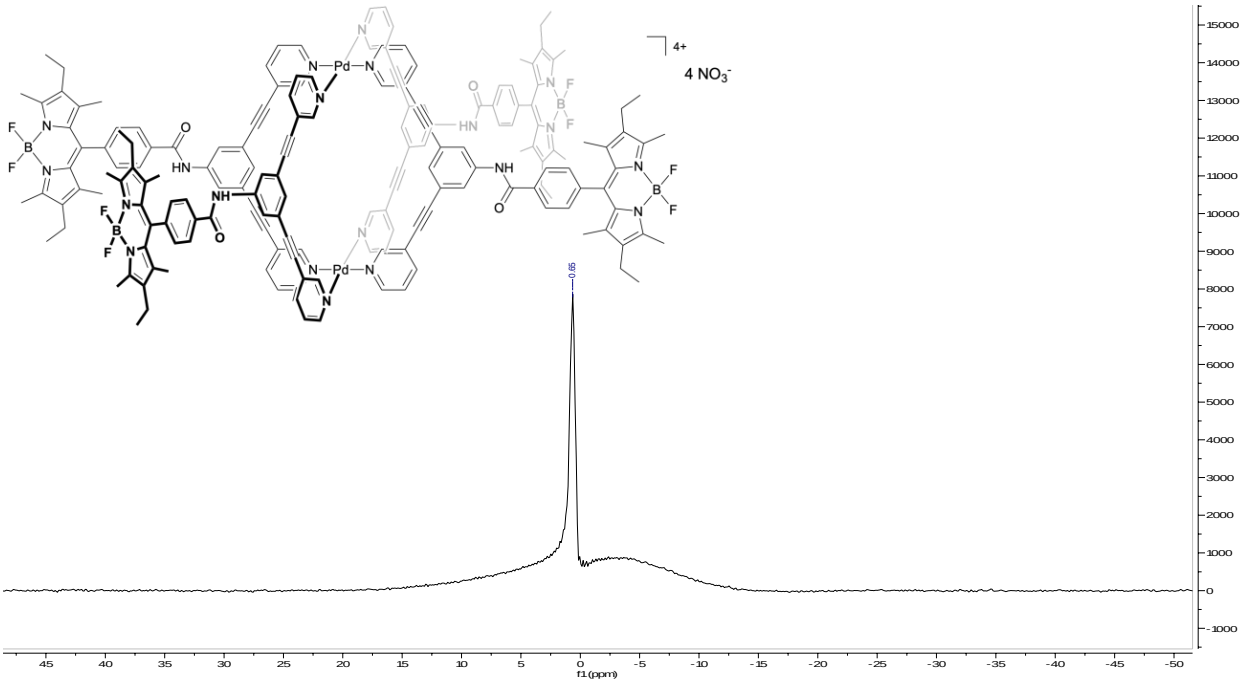


Figure S17. ^{11}B NMR (160 MHz, DMSO-d_6) spectrum of **C1.NO$_3$**.

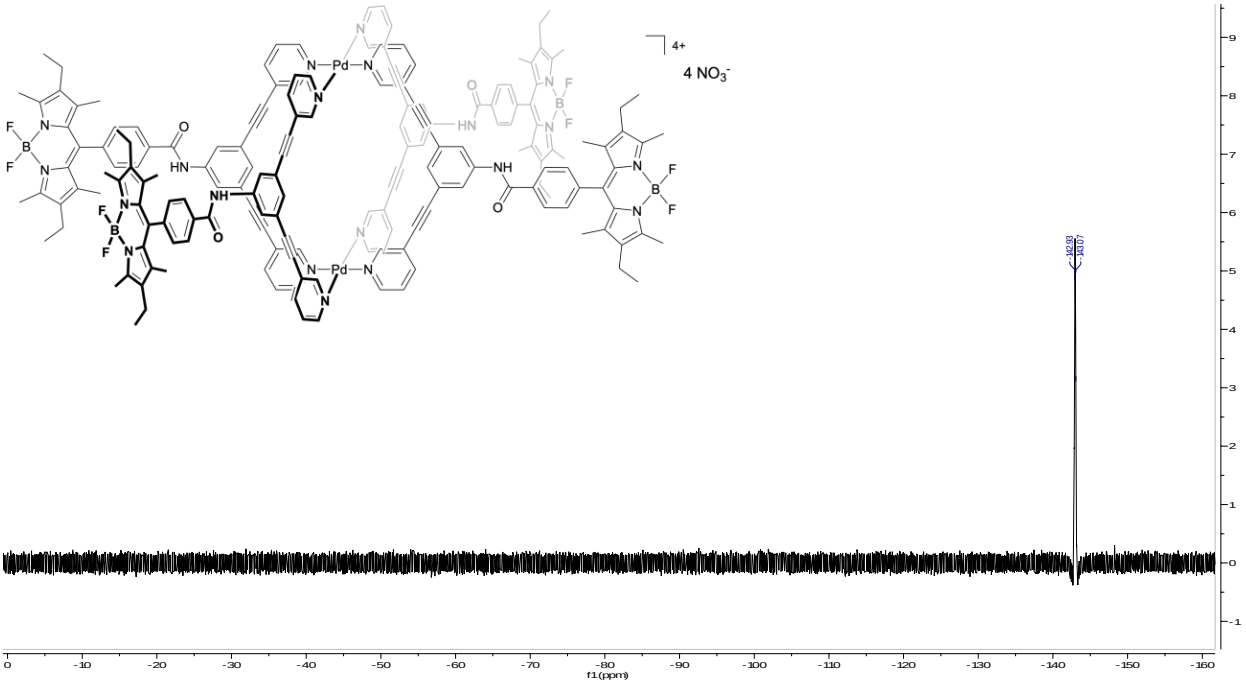


Figure S18. ^{19}F NMR (471 MHz, DMSO-d_6) spectrum of **C1.NO$_3$**.

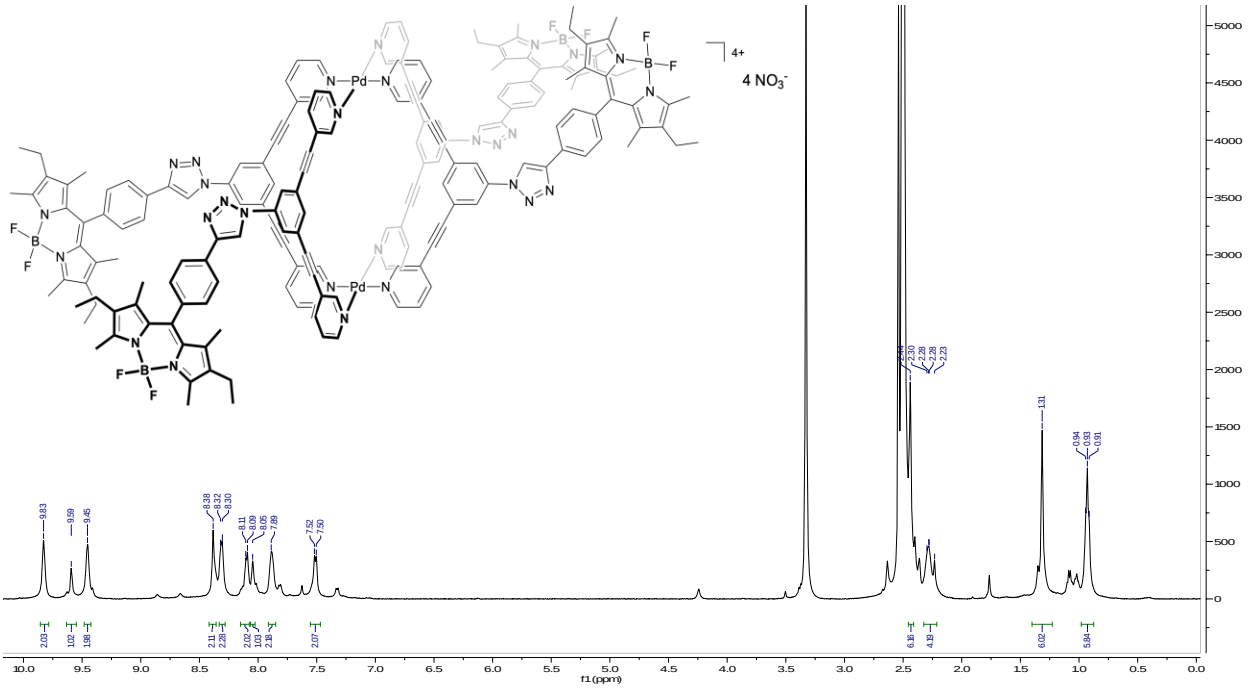


Figure S19. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of **C2.NO$_3$**.

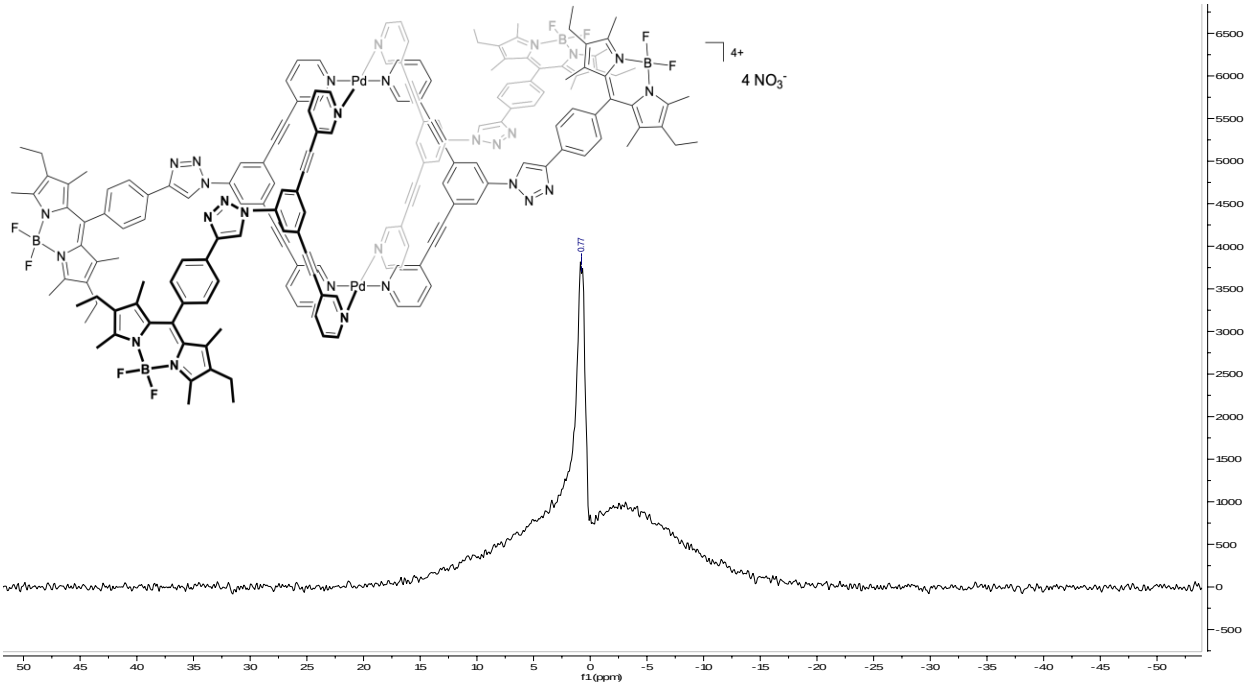


Figure S20. ^{11}B NMR (128 MHz, DMSO-d_6) spectrum of **C2.NO$_3$**.

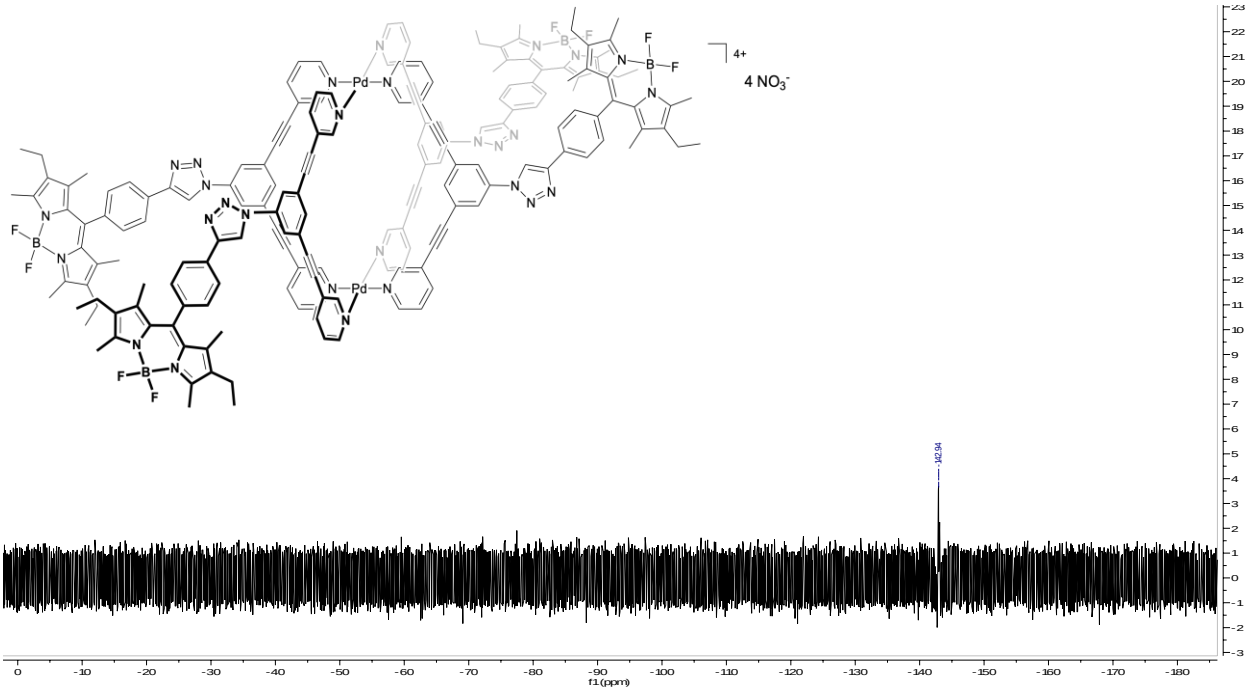


Figure S21.^{19}F NMR (376 MHz, DMSO-d_6) spectrum of **C2.NO$_3$**.

Stability studies by UV-Visible Spectroscopy

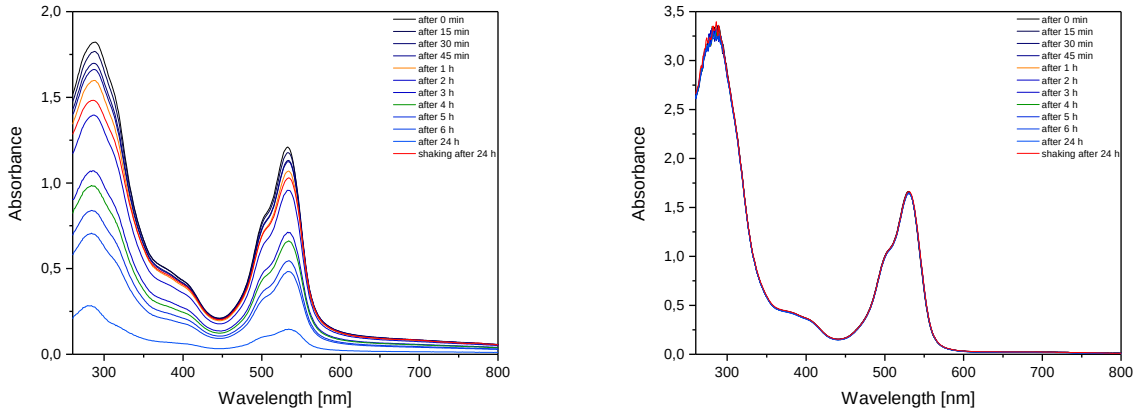


Figure S22. UV-Visible spectra of **C1.BF$_4$** [25 x 10^{-6} M] in 1x PBS (left); and in water (right) recorded over 24 h and shaken cuvette afterwards.

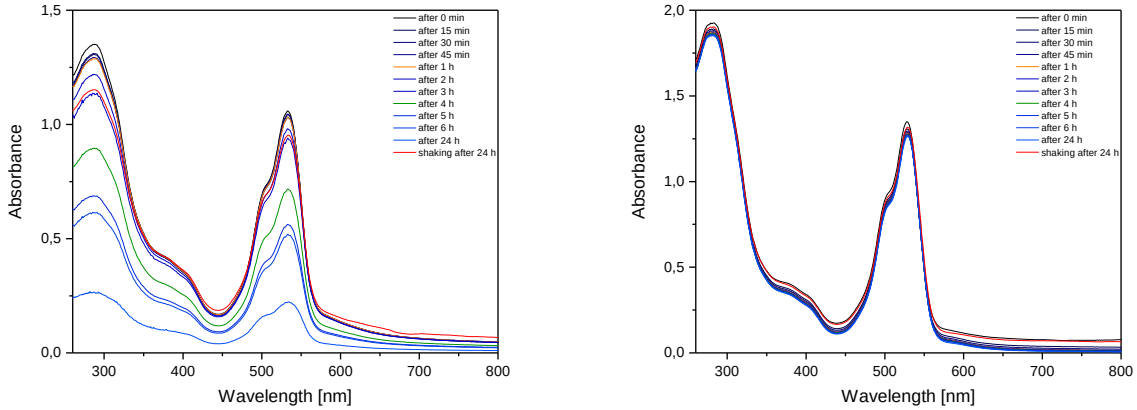


Figure S23. UV-Visible spectra of **C1.NO$_3$** [55 x 10^{-6} M] in 1x PBS (left); and in water (right) recorded over 24 h and shaken cuvette afterwards.

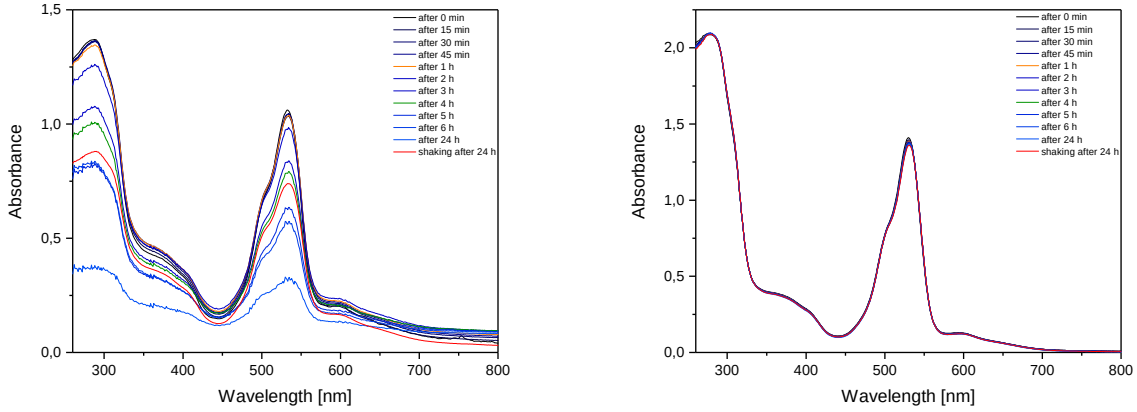


Figure S24. UV-Visible spectra of **C2.NO$_3$** [86 x 10^{-6} M] in 1x PBS (left); and in water (right) recorded over 24 h and shaken cuvette afterwards.

Cisplatin encapsulation studies

^{1}H NMR Spectra

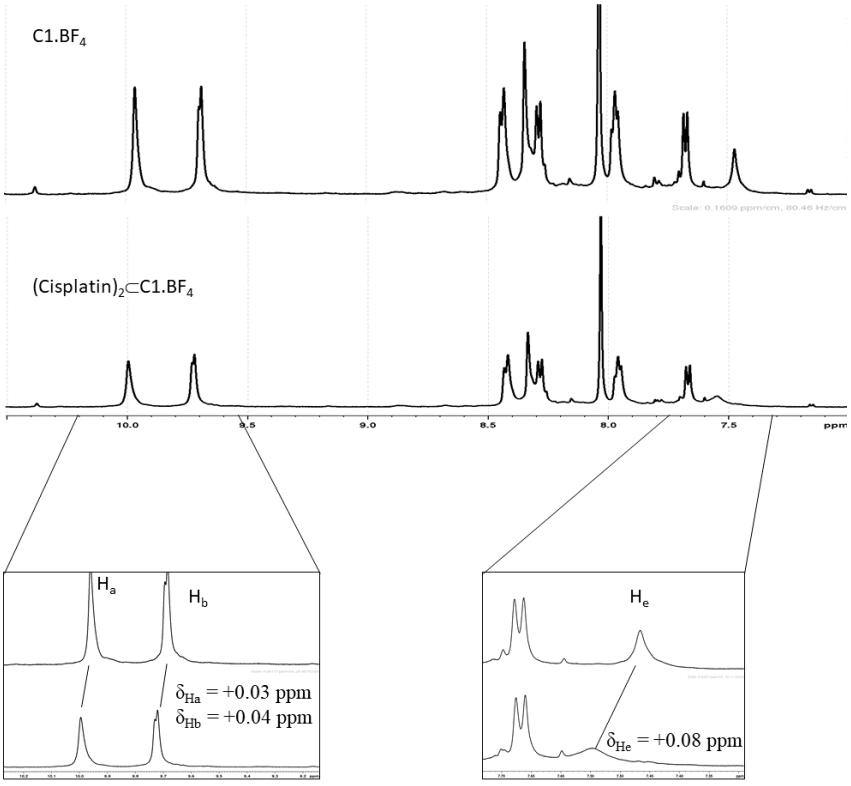


Figure S25. Stack ^{1}H NMR spectra in DMF-d_7 of the aromatic region (7-11 ppm) of the cage **C1.BF$_4$** before (top) and after (bottom) addition of 2 eq. of cisplatin. Both the cavity facing proton peaks H_a and H_e undergo downfield shifts, as well as H_b. Broadening of H_e peak upon encapsulation of cisplatin may also be indicative of intermolecular forces between the host and guest complex.

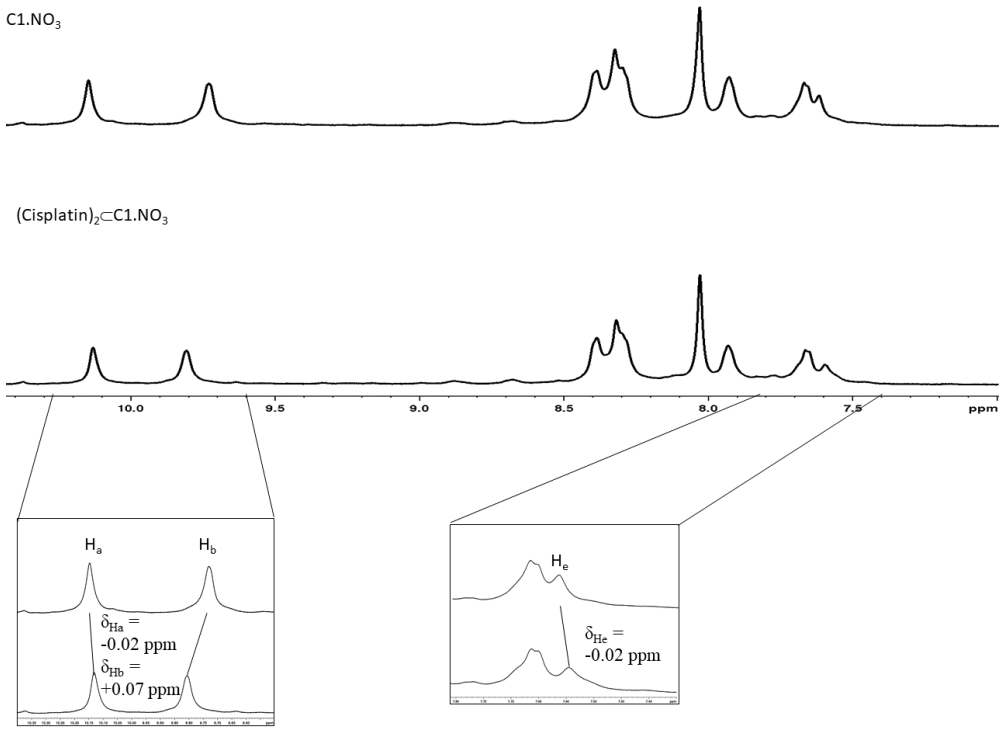


Figure S26. Stack ^{1}H NMR spectra in DMF-d_7 of the aromatic region (7-11 ppm) of the cage **C1.NO$_3$**, before (top) and after (bottom) addition of 2 eq. of cisplatin. The cavity facing proton peaks H_a and H_e both undergo upfield shifts, consistent with replacement of the encapsulated negatively charged NO_3^- counterion by cisplatin. H_b signal undergoes a slight downfield shift upon cisplatin addition.

Cell Uptake Studies

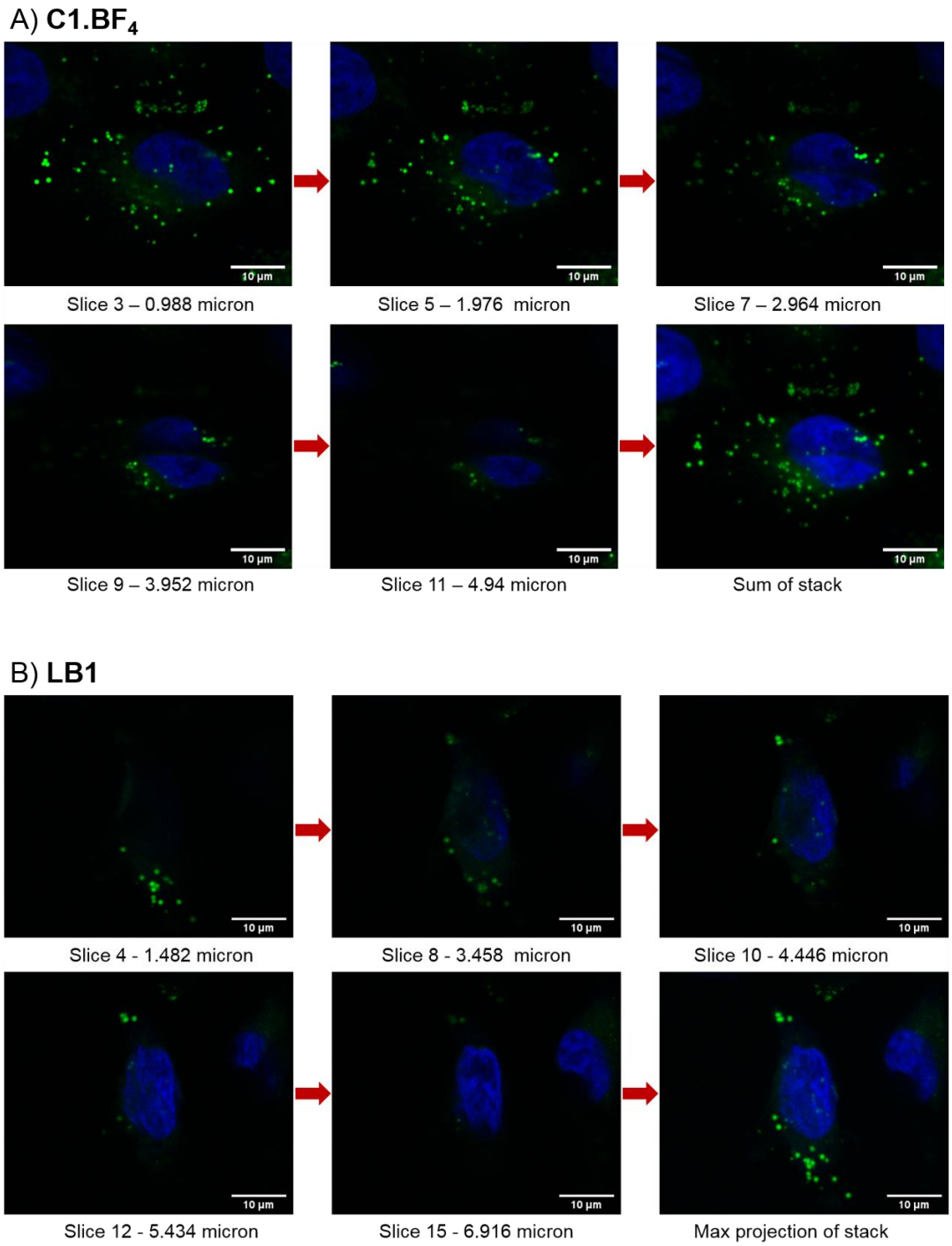


Figure S27. CLSM images of a representative fixed A375 melanoma cell incubated for 2 h with A) 5 µM **C1.BF$_4$** or B) 5 µM **LB1**. Counterstaining with DAPI. Different z-slices from top to bottom are shown as well as the sum of stack/maximum projection of stack. Scale bar represents 10 µm.

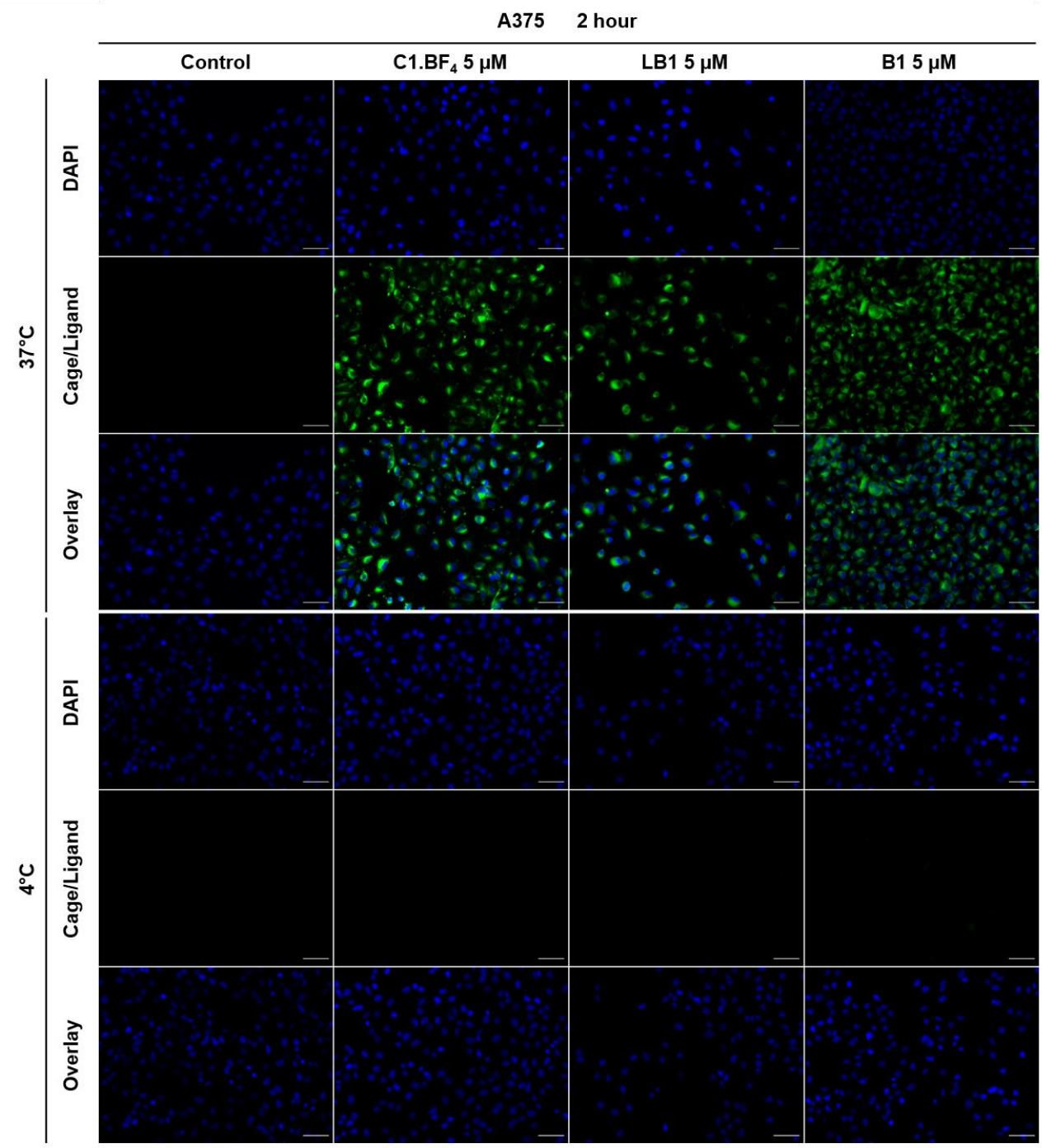


Figure S28. Fluorescence widefield microscopy (Zeiss) images of fixed cells comparing control to samples incubated with 5 µM of **C1.BF$_4$**, **LB1** or **B1** for 2 h at 37 °C (top three rows) or 4 °C (bottom three rows). Scale bar represents 50 µm.

References

[1] B. Brizet, C. Bernhard, Y. Volkova, Y. Rousselin, P. D. Harvey, C. Goze, F. Denat, Org. & Biomol. Chem. 2013, 11, 7729-7737.

[2] N. W. Smith, A. Alonso, C. M. Brown, S. V. Dzyuba, Biochem Biophys Res Comm. 2010, 391, 1455-1458.

[3] A. Schmidt, M. Hollering, J. Han, A. Casini, F. E. Kühn, Dalton Trans. 2016, 45, 12297-12300.

[4] J. Han, A. F. B. Räder, F. Reichart, B. Aikman, M. N. Wenzel, B. Woods, M. Weinmüller, B. S. Ludwig, S. Stürup, G. M. M. Groothuis, H. P. Permentier, R. Bischoff, H. Kessler, P. Horvatovich, A. Casini, Bioconjug. Chem. 2018, 29, 3856-3865.