Electronic Supplementary Information

Water-compatible Fluorescent [2]Rotaxanes for Au^{3+} Detection and Bioimaging

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General information

Unless otherwise stated, all reagents and anhydrous solvents were purchased from commercial sources and used without further purification. Flash column chromatography was performed using Aluminum oxide, neutral (Aluminum oxide, neutral, Brockmann I, for chromatography, 50-200 μm, 60A, ACROS Organics™). Analytical TLC was performed on pre-coated silica gel plates (0.25 mm thick, 60F254, Merck, Germany) and pre-coated ALUGRAM® (0.20 mm thick, F254, MN, Germany). Spots are observed under UV light. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Advance-III spectrometer (at 400 and 101 MHz, respectively). Chemical shifts are reported in parts per million from low to high field and referenced to residual solvent ($CDCl_3$: ^{1}H, 7.26 ppm; ^{13}C, 77.16 ppm. CD_3CN: ^{1}H, 1.94 ppm; ^{13}C, 1.32 ppm.). NMR data was processed using MestReNova software (Mestrelab). Coupling constants are reported in Hertz. Standard abbreviations indicating multiplicity were used as follows: m = multiplet, quint = quintet, q = quartet, t = triplet, d = doublet, s = singlet, app. = apparent, br = broad. High-resolution mass spectra were recorded on a Bruker Autoflex mass spectrometer (MALDI-TOF) and a Thermo Fisher Scientific UPLC-Q exactive focus hybrid quadrupole-orbitrap mass spectrometer in positive ion mode (ESI-MS). Fluorescent emission spectra of **RA**-H·PF_6 and **RRB** were collected on PTI-QM4. Fluorescent emission spectra of **RRA** were collected on Perkin Elmer LS55B. UV-vis spectra were collected on Agilent Cary 8454 UV-Vis Spectrometer.

$LiNO_3$, NaCl, KNO_3, $Mg(NO_3)_2$, $CaCl_2$, NH_4Cl, $AgNO_3$, $AlCl_3$, $Ni(NO_3)_2·6H_2O$, $ZnCl_2$, $CdCl_2$, $Co(NO_3)_2·9H_2O$, $HgCl_2$, $FeCl_2·4H_2O$, $FeCl_3$, $Cr(NO_3)_3·9H_2O$, CsCl, $Pb(NO_3)_2$, $Cu(NO_3)·3H_2O$, K_2PtCl_4, $AuCl_3$, $MnCl_2$, VCl_3, K_2PdCl_4, $IrCl_3·xH_2O$, $RhCl_3$ and $RuCl_3·3H_2O$ were prepared as 10 mM aqueous solution and used in the fluorescence analysis. For NMR titration, metal salts were prepared as 500 mM aqueous solution.

For NMR titration of metal ions with rotaxanes, probe solution (10 mM) in MeCN was prepared. Then, metal ions (500 mM) were added. The NMR spectra were obtained immediately after the addition. For fluorescence titration of Au^{3+}, multiple sets of probe solutions were prepared. Then, various amount of Au^{3+} were added into the probe solutions to give the required concentration of Au^{3+} in probe solution. The fluorescence intensities were measured after the equilibrium of the detection.

For adjusting the pH of solution, HCl and NaOH were prepared in pH = 1 and 13

aqueous solution respectively, and further diluted to corresponding pH. The aqueous solution was mixed with MeCN and 1 mM probe solution to give the results as shown in red line. 10 mM Metal ion in solution was then added another solution to give the red line results in Figure 4.

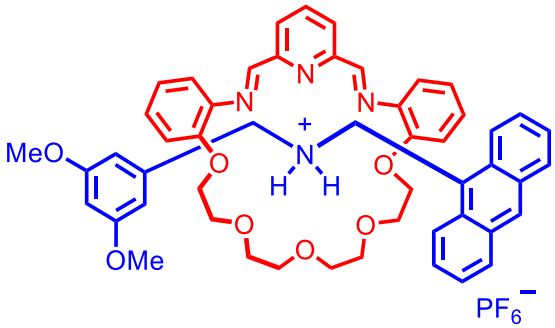
Cell culture and cytotoxicity assays

HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) and 100 units per mL of penicillin and 100 mg per mL of streptomycin at 37 °C with 5% CO_2 in a humidified incubator. After reaching the confluence, the cells were seeding in 96-well plate at the density of 1×10^4 cells/well. Then, the cells were treated with **RRA** (2–0.1 μM) and **RRB** (10–0.5 μM) with different concentrations for 24 h, respectively. After the treatment, the media containing sensors were discarded, and the cells were washed with Hank's balanced salt solution (HBSS) twice and their viability was assessed using Cell Counting Kit-8 (CCK-8, Sigma-Aldrich, St Louis, USA). In brief, cells were cultured with fresh DMEM containing CCK-8 reagent (100 μL of media and 10 μL of CCK-8 reagent per well) for 2 h. The absorbance of the supernatant was read at 450 nm using SpectraMax M2 Microplate Reader (Molecular Devices, California, USA). Two independent experiments were performed in triplicate.

Cellular gold(III) ion sensing

HeLa cells were cultured in μ-Slide 8 well chambers with polymer coverslip bottom (ibidi GmbH, Munich, Germany) at the density of 1×10^4 cells/well. The cells were firstly treated with gold(III) ion (Au^{3+}) at different concentrations (20, 10 and 5 mM) for 24 h. After the treatment, the cells were gently washed with fresh DMEM twice, and the media containing **RRB** at the concentration of 2 μM were added to the well and incubated with cells for 2 h. Afterwards, the cells were washed with HBSS to remove the extra sensor and then stained with Hoechst 33342 for 15 min. The fluorescence in the cells were examined using Nikon Eclipse Ti microscope equipped with Nikon Intensilight C-HGFI (light sources). All the fluorescent images were acquired under same exposing conditions.

Synthesis



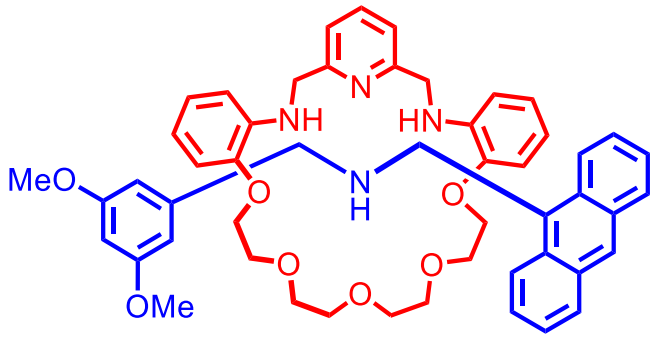
Synthesis of **RA**-H·PF_6:

The synthesis of **RA**-H·PF_6 was reported in literature.[1]

^{1}H NMR spectrum (400 MHz, CD_3CN) of **RA**-H·PF_6. δ 9.91 (br, 2H), 8.55 (dd, J = 8.9, 1.0 Hz, 2H), 8.35 (s, 1H), 7.92 (s, 2H), 7.80 (d, J = 8.4 Hz, 2H), 7.71 (t, J = 7.7 Hz, 1H), 7.34 – 7.21 (m, 4H), 7.19 – 7.11 (m, 2H), 7.08 – 6.98 (m, 4H), 6.83 (td, J = 7.6, 1.2 Hz, 2H), 6.52 (d, J = 2.3 Hz, 2H), 6.48 (dd, J = 7.8, 1.6 Hz, 2H), 6.11 (t, J = 2.3 Hz, 1H), 5.82 (t, J = 6.6 Hz, 2H), 5.13 (t, J = 6.8 Hz, 2H), 4.35 – 4.25 (m, 4H), 4.15 – 3.99 (m, 2H), 3.87 – 3.57 (m, 10H), 3.32 (s, 6H).

^{13}C NMR spectrum (101 MHz, CD_3CN) of **RA**-H·PF_6. δ 161.89, 157.96, 154.20, 153.98, 144.57, 140.38, 138.87, 133.03, 132.42, 131.19, 130.19, 129.81, 129.42, 127.87, 126.76, 126.03, 125.58, 122.46, 119.12, 112.85, 106.80, 99.73, 70.71, 67.73, 55.90, 54.63, 45.47.

HRMS (MALDI-TOF): calcd. for $C_{51}H_{53}N_4O_7$ $[M–PF_6]^+$ *m/z* 833.3914, found 833.3945.



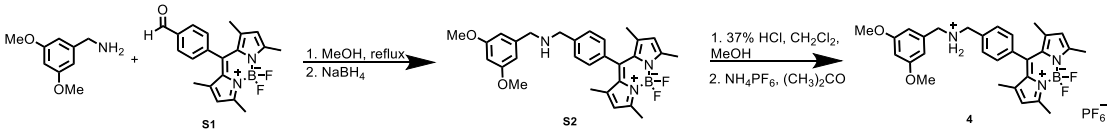
Synthesis of **RRA**:

A solution of **RA**-H·PF_6 (300 mg, 0.31 mmol) in $MeNO_2$ (150 mL) was added 1 M BH_3-THF (2 mL) at 0 ºC. The yellow mixture was stirred for overnight at room temperature. The excess of solvent was evaporated under reduced pressure. The mixture was dissolved in $CHCl_3$ (50 mL) and washed with 1 M HCl (20 mL), 1 M NaOH (20 mL). The organic layer was dried with anhydrous $MgSO_4$ and concentrated by evaporation. The product was purified by neutral aluminum oxide column chromatography with (1/3, *v*/*v*) CH_2Cl_2/*n*-hexane, followed by CH_2Cl_2 to afford **RRA** as a pale-yellow powder (210 mg, Yield: 70%). Single crystals suitable for X-ray analysis were obtained by slow evaporation in CH_2Cl_2/MeCN (CCDC code: 1942423).

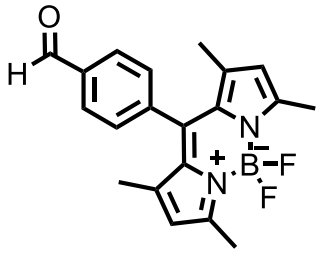
^{1}H NMR spectrum (400 MHz, CD_3CN) of **RRA**. δ 8.44 – 8.34 (m, 3H), 7.93 (d, J = 8.4 Hz, 2H), 7.34 (q, J = 7.6 Hz, 3H), 7.24 – 7.15 (m, 4H), 6.83 (t, J = 7.6 Hz, 2H), 6.79 – 6.70 (m, 4H), 6.63 – 6.52 (m, 4H), 6.23 (m, 2H), 6.03 (s, 1H), 4.61 (d, J = 7.2 Hz, 2H), 4.31 (dd, J = 12.8, 7.8 Hz, 2H), 4.11 (dd, J = 12.8, 4.2 Hz, 2H), 4.02 – 3.94 (m, 4H), 3.70 (d, J = 7.7 Hz, 2H), 3.47 (s, 6H), 3.45 – 3.36 (m, 2H), 2.98 (dd, J = 25.3, 9.8 Hz, 3H), 2.82 – 2.71 (m, 2H), 2.71 – 2.59 (m, 2H), 2.54 – 2.45 (m, 2H), 2.45 – 2.35 (m, 2H).

^{13}C NMR spectrum (101 MHz, CD_3CN) of **RRA**. δ 160.59, 159.40, 147.57, 139.70, 137.69, 132.47, 131.64, 129.34, 126.60, 126.26, 125.74, 123.31, 122.08, 116.94, 110.98, 110.32, 107.08, 99.31, 70.77, 70.61, 68.79, 55.63, 50.73.

HRMS (ESI): calcd. for $C_{51}H_{56}N_4O_7$ $[M+H]^+$ *m/z* 837.4222, found 837.4193.

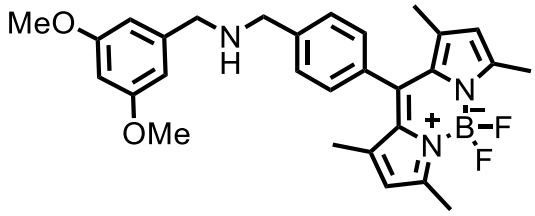


Scheme S1. Synthetic route of Axle **4**



Synthesis of compound **S1:**

Compound **S1** was synthesized according to literature reported procedure.[2]



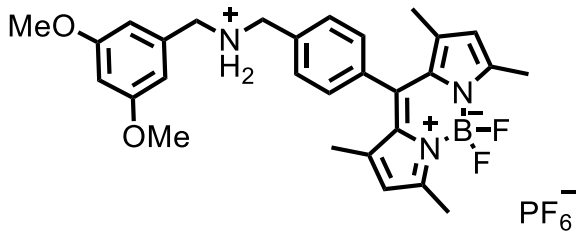
Synthesis of **S2**:

A solution of **S1** (300 mg, 0.85 mmol) in MeOH (150 mL) was added 3,5-dimethoxybenzylamine (142 mg, 0.85 mmol) at room temperature. The orange colour mixture was reflux for overnight. Then the mixture was cooled to 0 °C and $NaBH_4$ (0.10 g, 2.64 mmol) was added. The mixture was stirred for overnight and then quenched by water. The solvents were evaporated under reduced pressure. The mixture was dissolved in ethyl acetate (50 mL) and washed with water. The organic layer was dried with anhydrous $MgSO_4$ and concentrated by evaporation. The product was purified by silica gel column chromatography with (1/3, *v/v*) CH_2Cl_2/*n*-hexane, followed by CH_2Cl_2 to afford **S2** as an orange powder (110 mg, Yield: 26%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 8.0 Hz, 2H), 7.24 (d, *J* = 8.1 Hz, 2H), 6.52 (d, *J* = 2.2 Hz, 2H), 6.38 (t, *J* = 2.2 Hz, 1H), 5.97 (s, 2H), 3.89 (s, 2H), 3.80 (s, 6H), 3.76 (s, 2H), 2.55 (s, 6H), 1.38 (s, 6H). NH signal is missing.

^{13}C NMR (101 MHz, $CDCl_3$) δ 161.04, 155.48, 143.17, 142.25, 141.83, 140.98, 133.78, 131.59, 129.02, 128.11, 121.28, 106.26, 99.10, 55.43, 53.14, 52.62, 14.68, 14.54.

HRMS (ESI): calcd. for $C_{29}H_{32}BF_2N_3O_2$ $[M+H]^+$ *m/z* 504.2634, found 504.2617.



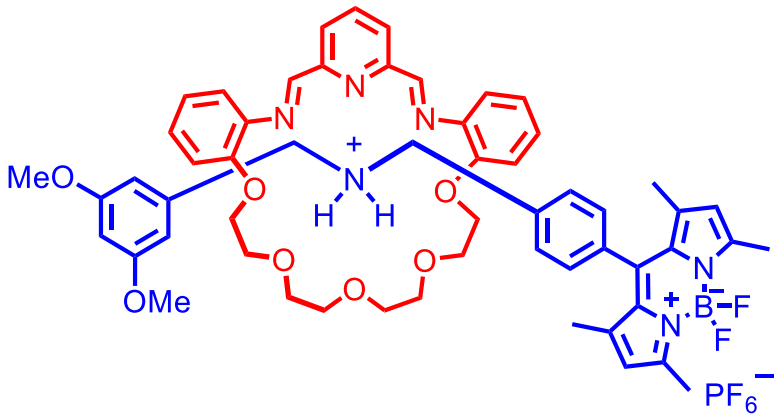
Synthesis of **4**:

A solution of **S2** (0.15 g, 0.30 mmol) in CH_2Cl_2/MeOH (10 mL) was added conc. HCl (2 mL). The mixture was stirred for 2 hours. Then the solvent was evaporated under reduced pressure. The mixture was dissolved in acetone (50 mL) and saturated NH_4PF_6 aqueous solution was added. The mixture was stirred for 2 hours. Then the organic solvent was evaporated under reduced pressure. The precipitate was filtered and washed with water to afford **Ar^{1}Ar^1**-H·PF_6 as a red solid (0.16 g, 83%).

^{1}H NMR (400 MHz, CD_3CN) δ 7.61 (d, J = 8.2 Hz, 2H), 7.45 (d, J = 8.3 Hz, 2H), 6.62 (d, J = 2.2 Hz, 2H), 6.57 (t, J = 2.2 Hz, 1H), 6.10 (s, 2H), 4.29 (s, 2H), 4.16 (s, 2H), 3.80 (s, 6H), 2.49 (s, 6H), 1.38 (s, 6H). NH_2^+ signal is missing.

^{13}C NMR (101 MHz, CD_3CN) δ 162.33, 156.68, 144.46, 142.22, 137.07, 133.32, 132.44, 132.13, 131.98, 129.85, 122.43, 108.91, 102.14, 56.24, 52.43, 51.91, 14.77, 14.75.

HRMS (ESI): calcd. for $C_{29}H_{33}BF_2N_3O_2$ $[M-PF_6]^+$ *m/z* 504.2628, found 504.2617.



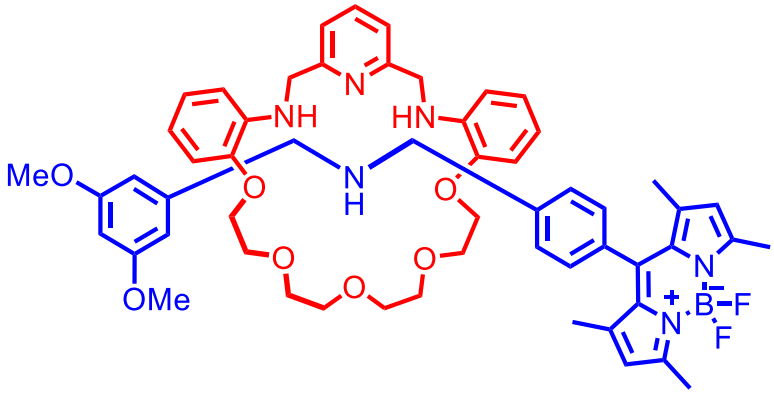
Synthesis of **RB**-H·PF_6:

A solution of thread **4** (71.3 mg, 0.11 mmol) in MeCN (15 mL) was added diamine **1** (41.4 mg, 0.11 mmol) and dialdehyde **2** (14.85 mg, 0.11 mmol). The red mixture was stirred for overnight at room temperature. The solvents were evaporated under reduced pressure. The mixture was purified by neutral alumina column chromatography with (1/3, *v/v*) CH_2Cl_2/*n*-hexane, followed by CH_2Cl_2 to afford **RB**-H·PF_6 as a red powder (90.0 mg, 83.3%).

^{1}H NMR (400 MHz, CD_3CN) δ 10.12 (br, 2H), 8.43 (s, 2H), 8.05 – 7.98 (m, 1H), 7.69 (d, J = 7.8 Hz, 2H), 7.41 – 7.34 (m, 2H), 7.27 (d, J = 8.2 Hz, 2H), 7.20 (dd, J = 8.4, 1.2 Hz, 2H), 7.04 (td, J = 7.6, 1.1 Hz, 2H), 6.99 – 6.93 (m, 4H), 6.52 (d, J = 2.3 Hz, 2H), 5.99 (t, J = 2.3 Hz, 1H), 5.95 (s, 2H), 4.80 – 4.72 (m, 2H), 4.63 – 4.51 (m, 4H), 4.47 – 4.40 (m, 2H), 3.98 – 3.87 (m, 4H), 3.62 – 3.50 (m, 8H), 3.38 (s, 6H), 2.41 (s, 6H), 0.97 (s, 6H).

^{13}C NMR (101 MHz, CD_3CN) δ 162.07, 161.09, 156.45, 152.98, 152.86, 144.30, 142.44, 141.00, 140.07, 135.76, 135.56, 134.80, 131.89, 130.82, 130.68, 129.94, 128.95, 122.68, 122.19, 121.92, 113.58, 108.90, 101.47, 71.63, 71.39, 70.15, 69.63, 55.60, 53.12, 52.53, 14.72, 14.66.

HRMS (ESI): calcd. for $C_{56}H_{62}BF_2N_6O_7$ $[M–PF_6]^+$ *m/z* 979.4736, found 979.4719.



Synthesis of **RRB**:

A solution of **RB**-H·PF_6 (40 mg, 0.04 mmol) in MeCN (150 mL) was added 1 M BH_3-THF (0.5 mL) at 0 ºC. The yellow mixture was stirred for overnight at room temperature. The solvents were evaporated under reduced pressure. The mixture was dissolved in $CHCl_3$ (50 mL) and washed with 1 M HCl (20 mL), 1 M NaOH(20 mL). The organic layer was dried with anhydrous $MgSO_4$ and concentrated by evaporation. The product was purified by neutral aluminum oxide column chromatography with (1/3, *v/v*) CH_2Cl_2/*n*-hexane, followed by CH_2Cl_2 to afford **RRB** as a red powder (30.0 mg, Yield: 75%).

^{1}H NMR (400 MHz, CD_3CN) δ 7.43 (d, J = 7.9 Hz, 2H), 7.35 (dd, J = 8.4, 6.8 Hz, 1H), 7.29 – 7.24 (m, 2H), 6.98 – 6.94 (m, 2H), 6.77 (td, J = 7.6, 1.3 Hz, 2H), 6.69 (dt, J = 8.0, 1.2 Hz, 4H), 6.55 (td, J = 7.7, 1.6 Hz, 2H), 6.51 (d, J = 2.3 Hz, 2H), 6.04 (t, J = 2.3 Hz, 1H), 6.02 – 5.97 (m, 2H), 4.29 – 4.14 (m, 4H), 4.07 – 3.94 (m, 4H), 3.57 (s, 6H), 3.57 – 3.51 (m, 4H), 3.49 – 3.43 (m, 2H), 3.40 – 3.31 (m, 4H), 3.31 – 3.20 (m, 6H), 2.44 (s, 6H), 1.27 (s, 6H). NH singals are missing.

^{13}C NMR (101 MHz, CD_3CN) δ 161.05, 159.76, 156.02, 147.46, 144.56, 143.97, 139.50, 137.82, 132.74, 132.25, 129.29, 127.83, 123.33, 121.91, 117.10, 110.86, 110.69, 107.85, 99.15, 71.91, 71.52, 70.78, 68.50, 55.64, 54.61, 53.53, 50.79, 30.62, 15.10, 14.69.

HRMS (MAIDI-TOF): calcd. for $C_{56}H_{65}BF_2N_6O_7$ $[M+H]^+$ *m/z* 983.5054, found 983.5035.

Additional spectra

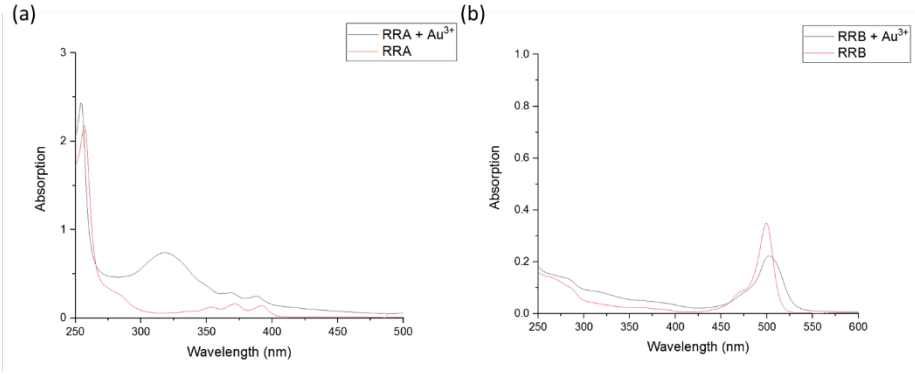


Figure S1. (a) Absorption spectrum of **RRA** (20 μM) upon addition of 10 equiv of Au^{3+} ion in THF/MeCN/H_2O solution (2:48:50, *v/v*). (b) Absorption spectrum of **RRB** (5 μM) upon addition of 10 equiv of Au^{3+} in MeCN/H_2O solution (50:50, *v/v*).

The calculation of binding constants (K_a) of **RRA** and **RRB** were determined by the Benesi–Hildebrand equation: $K_a=1/(m(I_{max} - I_o))$.[3] Where m is the slope of the graph $1/(I - I_o)$ against $1/[Au^{3+}]$.

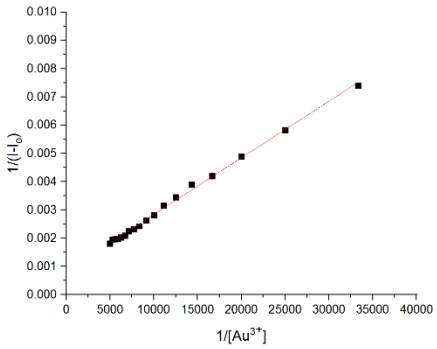


Figure S2. Benesi–Hildebrand Plot of **RRA** (20 μM) upon addition of Au^{3+}. Slope = 2.00×10^{-7}.

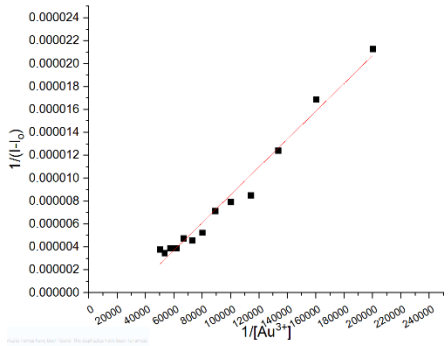


Figure S3. Benesi–Hildebrand Plot of **RRB** (20 μM) upon addition of Au^{3+}. Slope = 1.21×10^{-10}.

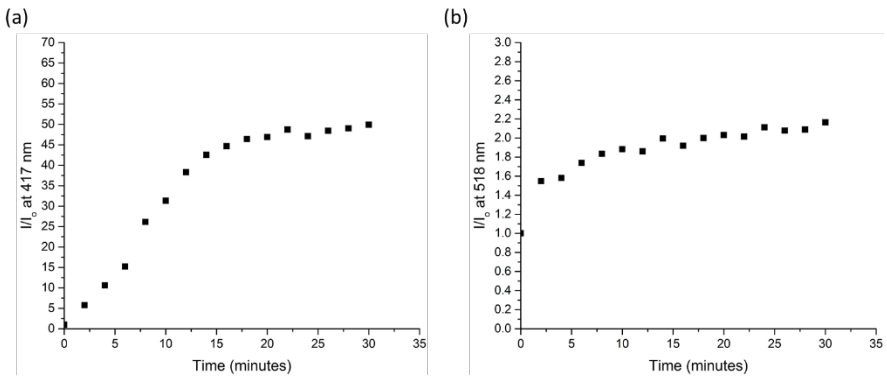


Figure S4. (a) Time related fluorescence response of **RRA** (20 μM, λ_{ex} = 370 nm) at 417 nm upon addition of 10 equiv of Au^{3+} in THF/MeCN/H_2O solution (2:48:50, *v/v/v*). (b) Time related fluorescence response of **RRB** (5 μM, λ_{ex} = 500 nm) at 518 nm upon addition of 10 equiv of Au^{3+} in MeCN/H_2O solution (50:50, *v/v*).

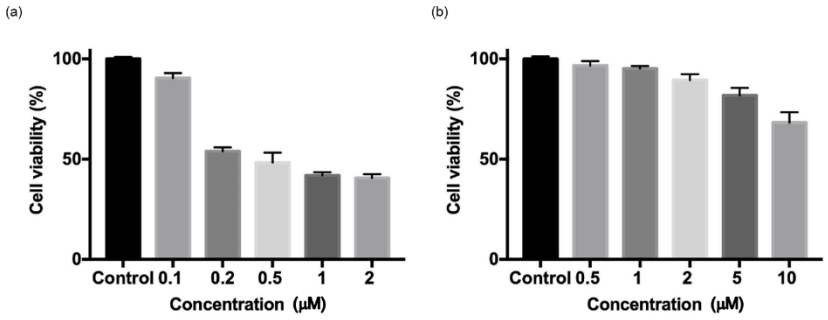


Figure S5. The cell viability of HeLa cells treated with (a) **RRA** and (b) **RRB** for 24 h was assessed using CCK-8.

NMR spectra

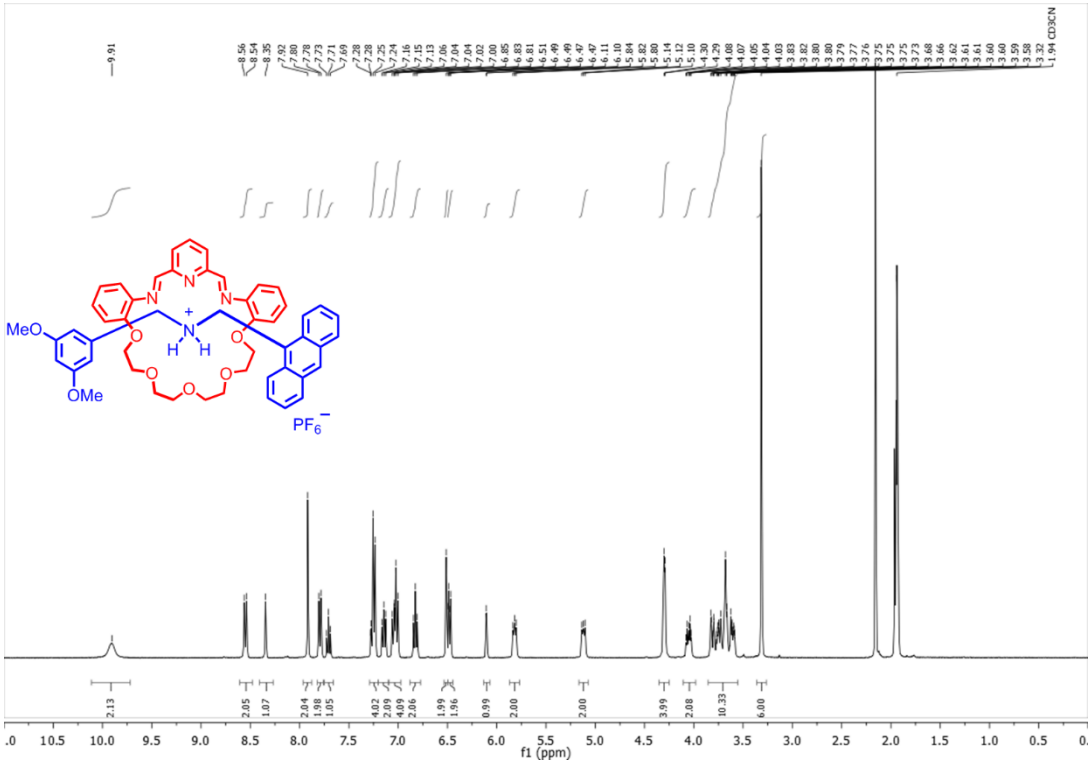


Figure S6. ^{1}H NMR spectrum (400 MHz, CD_3CN) of **RA**-H·PF_6.

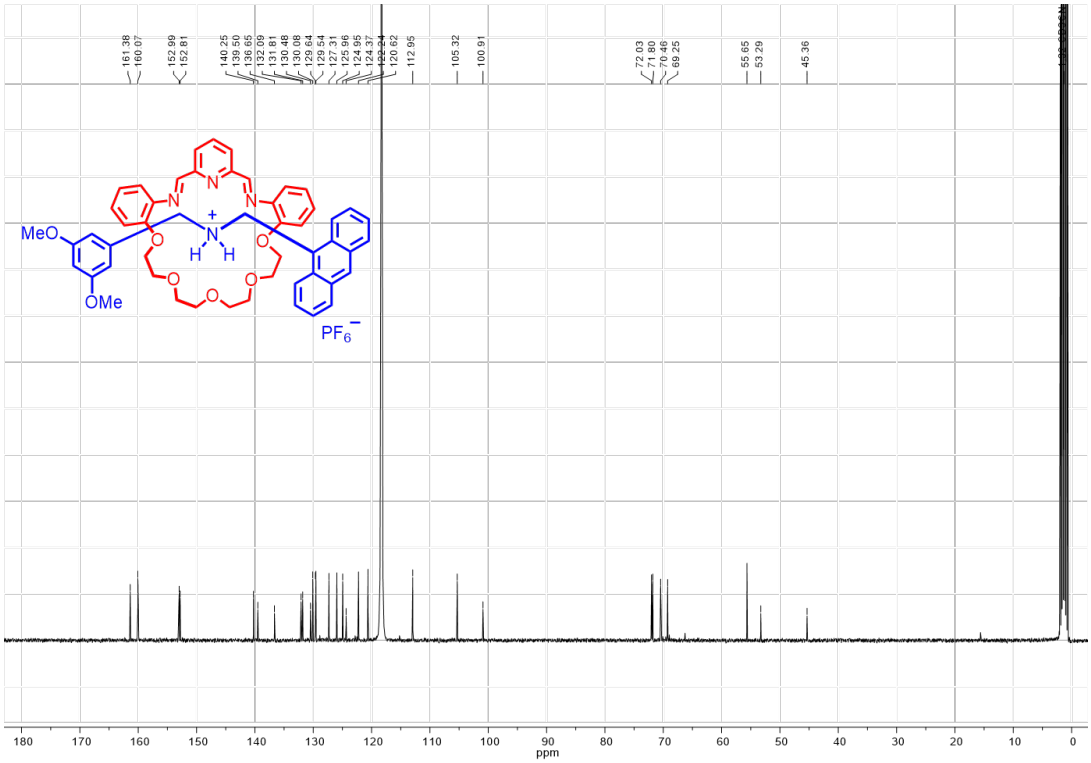


Figure S7. ^{13}C NMR spectrum (101 MHz, CD_3CN) of **RA**-H·PF_6.

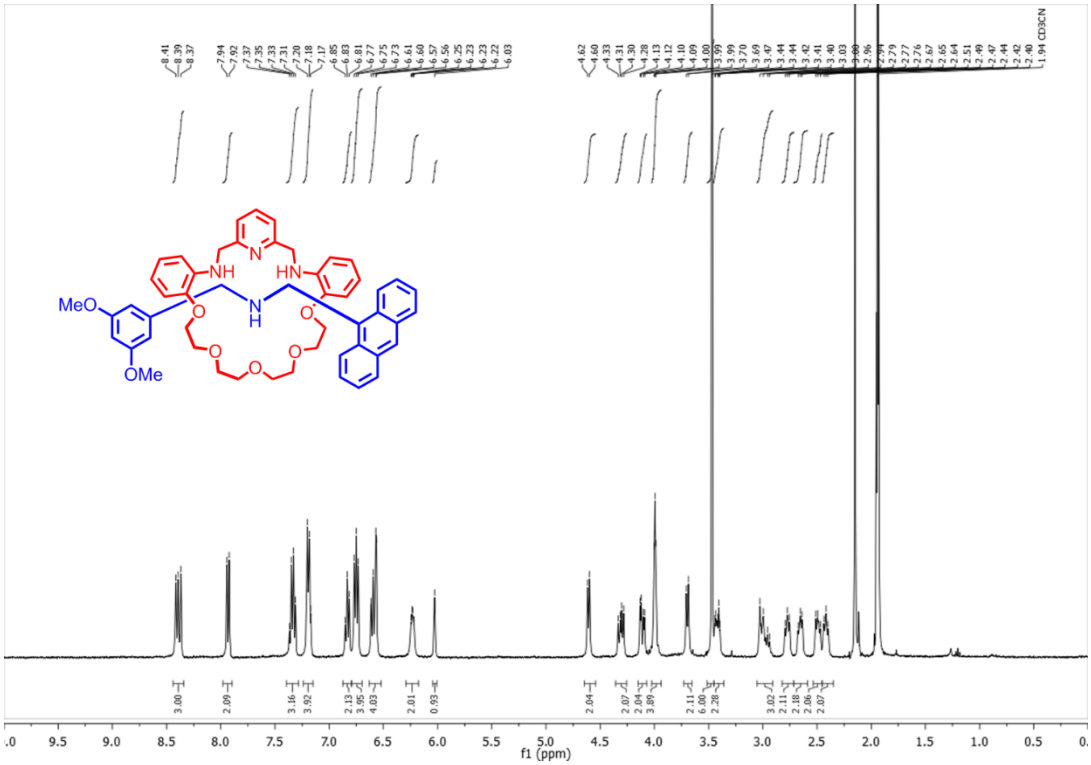


Figure S8. ^{1}H NMR spectrum (400 MHz, CD_3CN) of **RRA**.

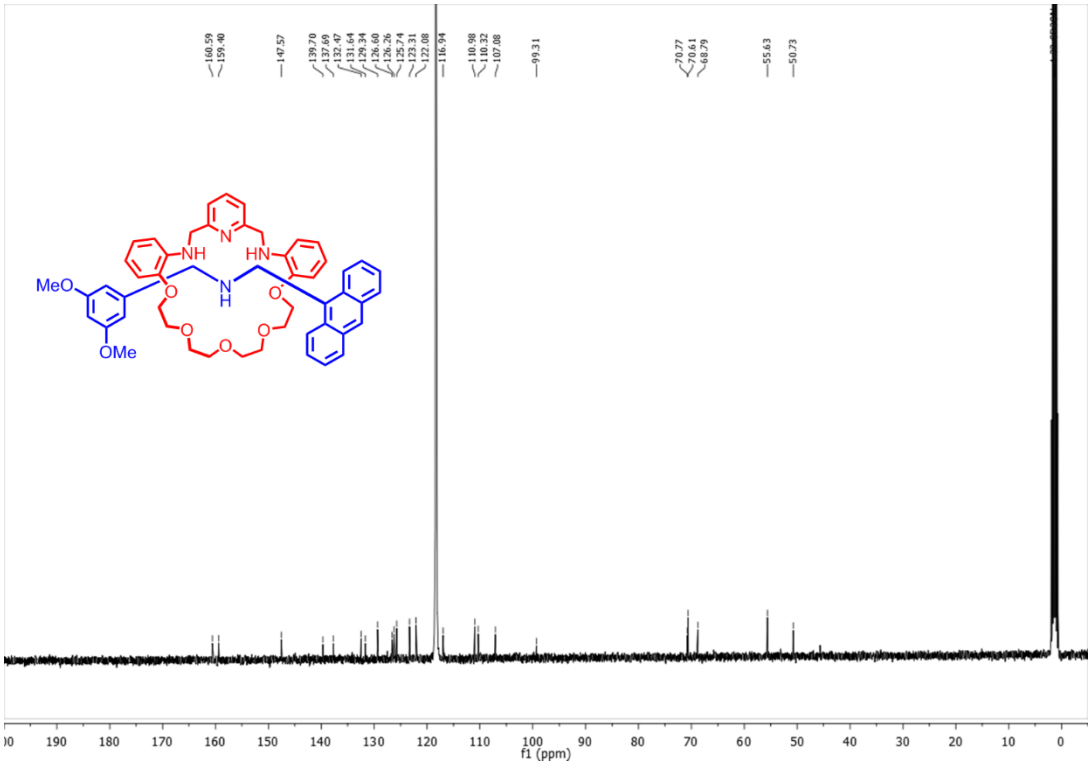


Figure S9. ^{13}C NMR spectrum (101 MHz, CD_3CN) of **RRA**.

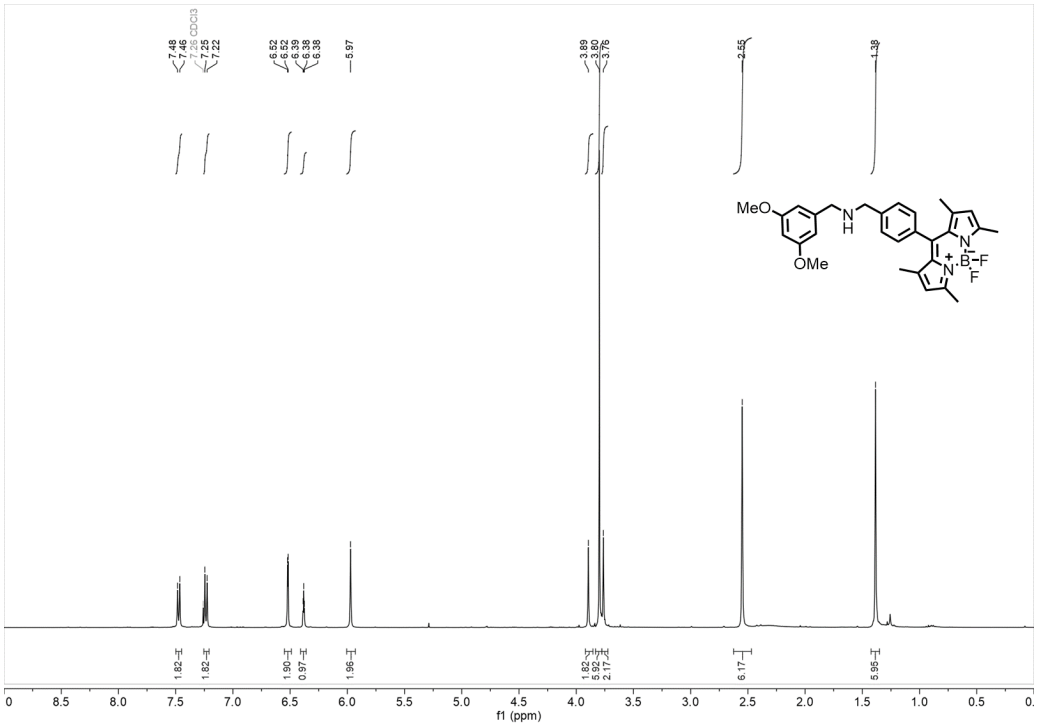


Figure S10. ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **S2**.

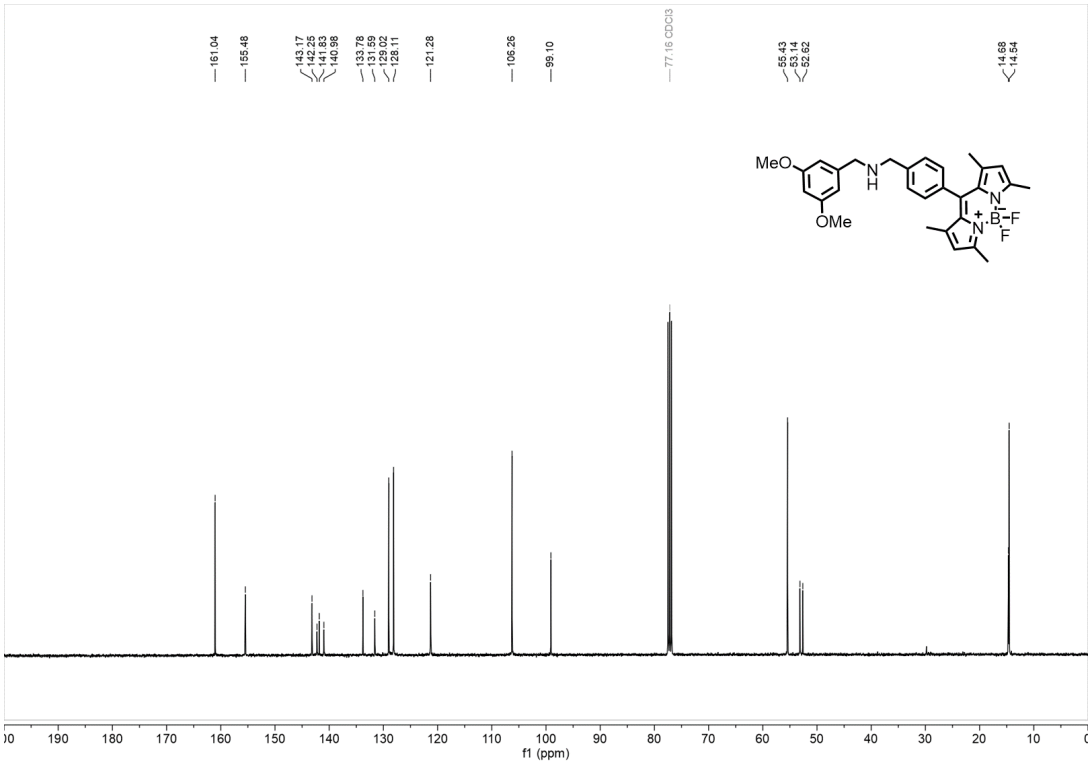


Figure S11. ^{13}C NMR spectrum (101 MHz, CD_3CN) of **S2.**

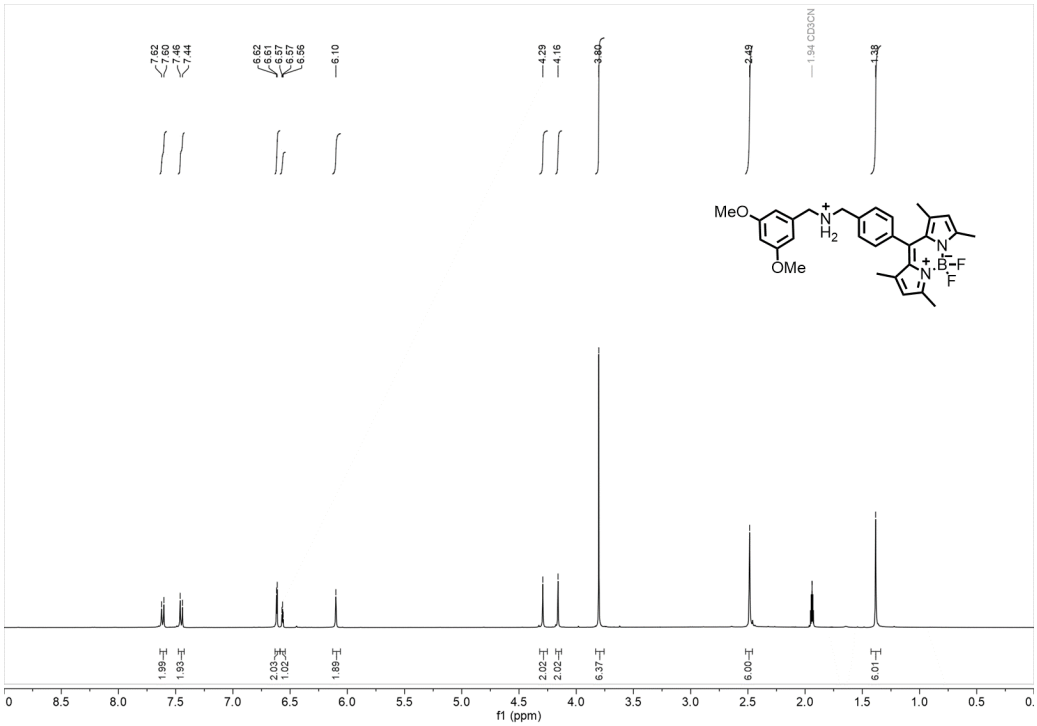


Figure S12. ^{1}H NMR spectrum (400 MHz, CD_3CN) of **4**.

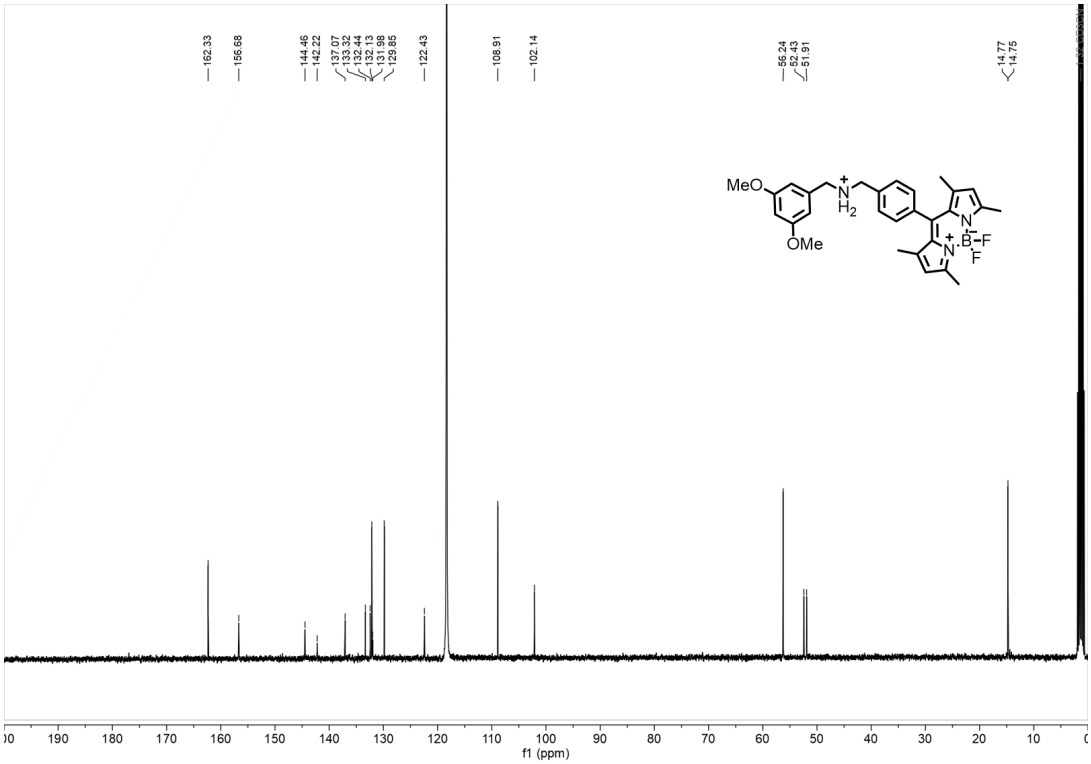


Figure S13. ^{13}C NMR spectrum (101 MHz, CD_3CN) of **4**.

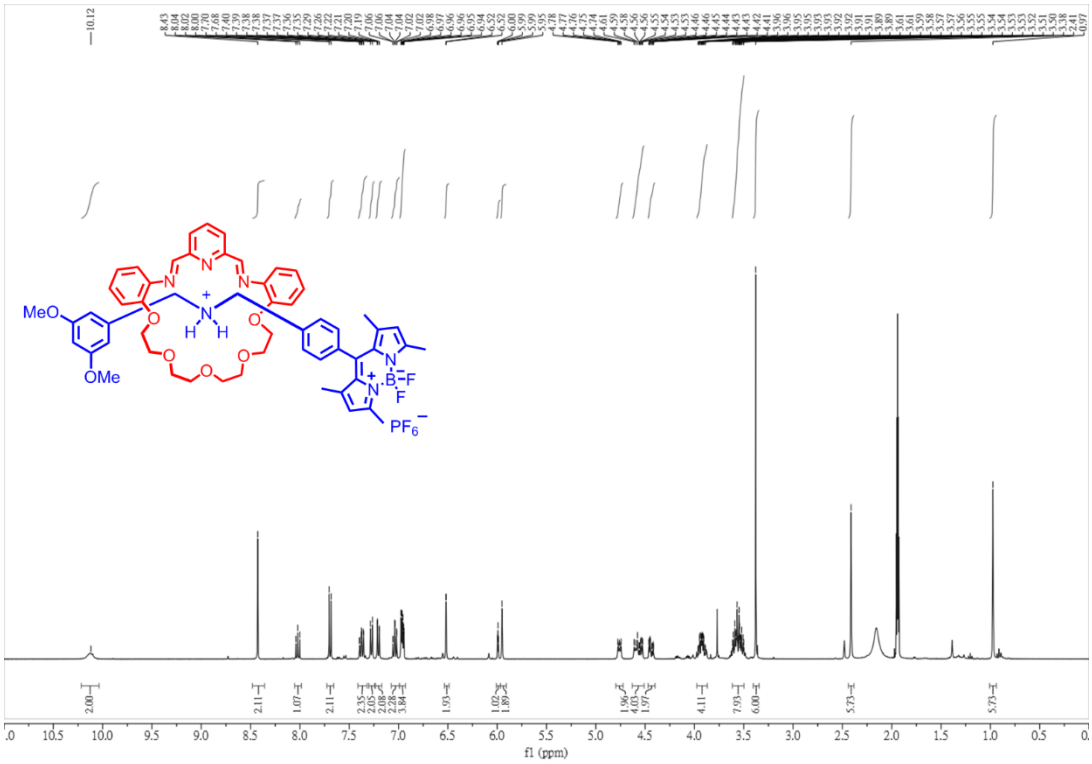


Figure S14. ^{1}H NMR spectrum (400 MHz, CD_3CN) of **RB**-H·PF_6.

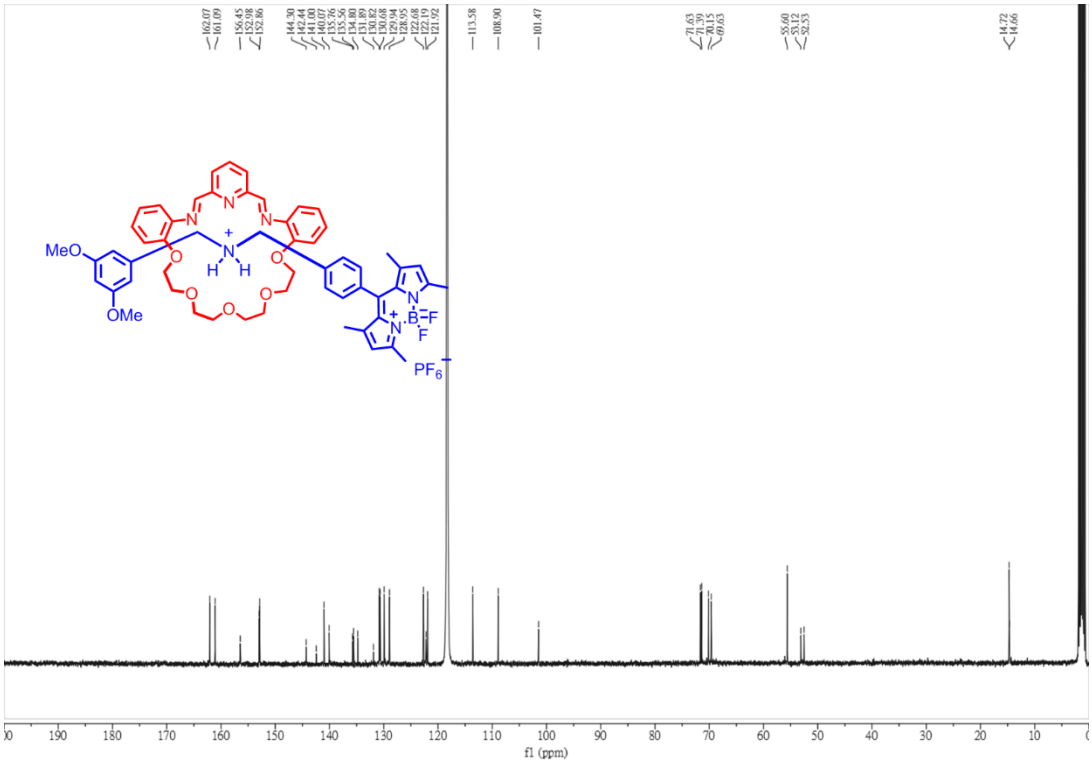


Figure S15. ^{13}C NMR spectrum (101 MHz, CD_3CN) of **RB**-H·PF_6.

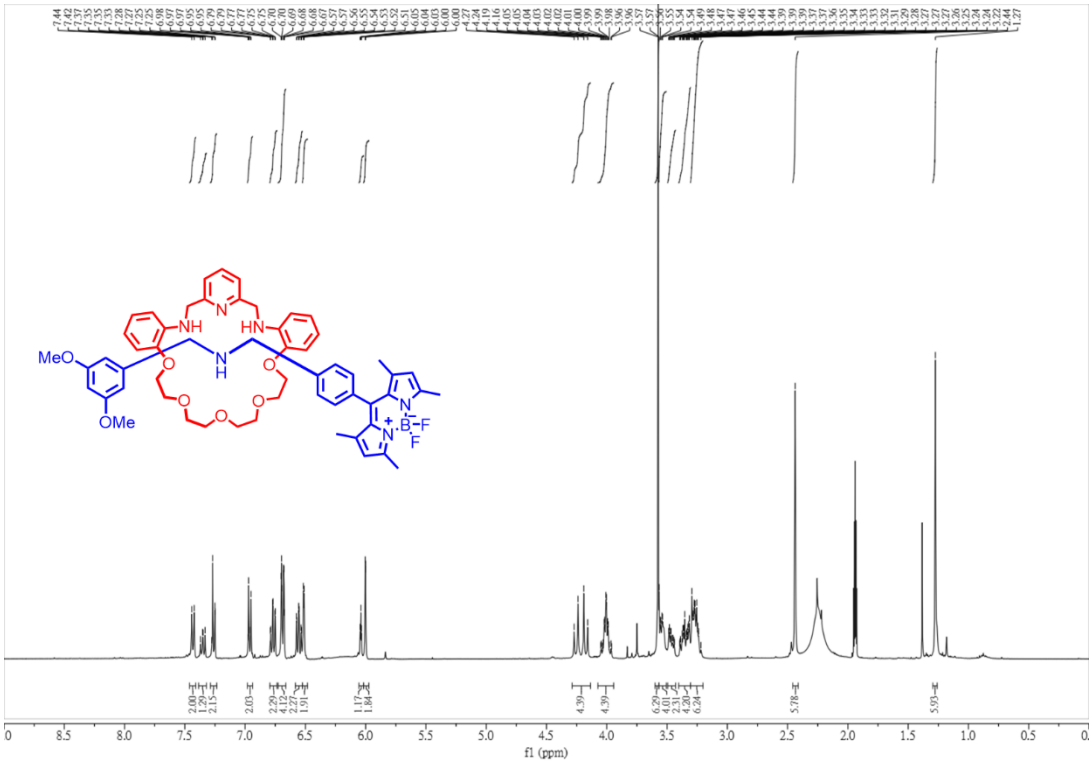


Figure S16. ^{1}H NMR spectrum (400 MHz, CD_3CN) of **RRB**.

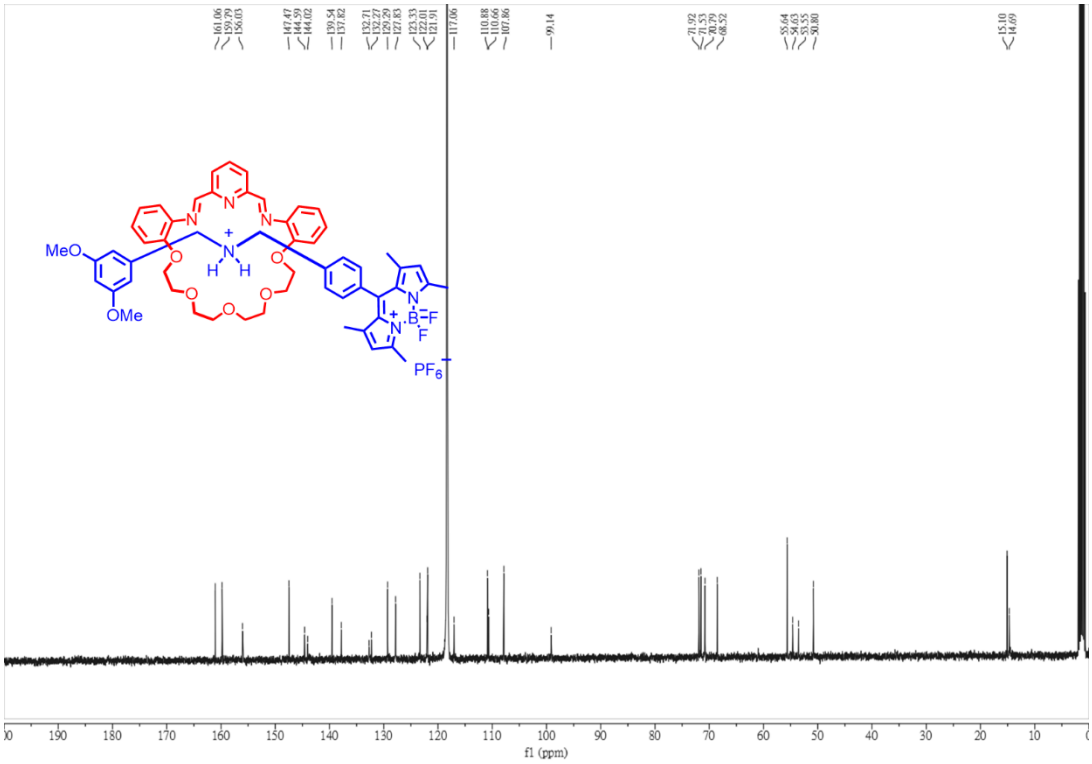


Figure S17. ^{13}C NMR spectrum (101 MHz, CD_3CN) of **RRB**.

Mass spectra

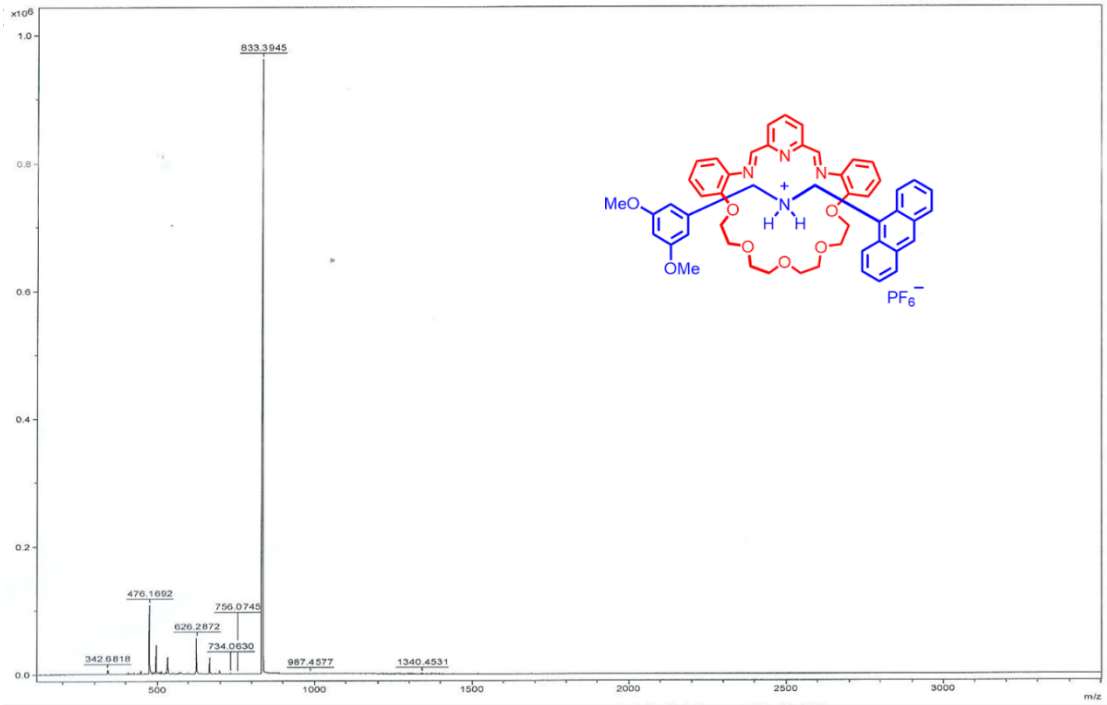


Figure S18. HRMS (MADLI-TOF) of **RA**-H·PF_6.

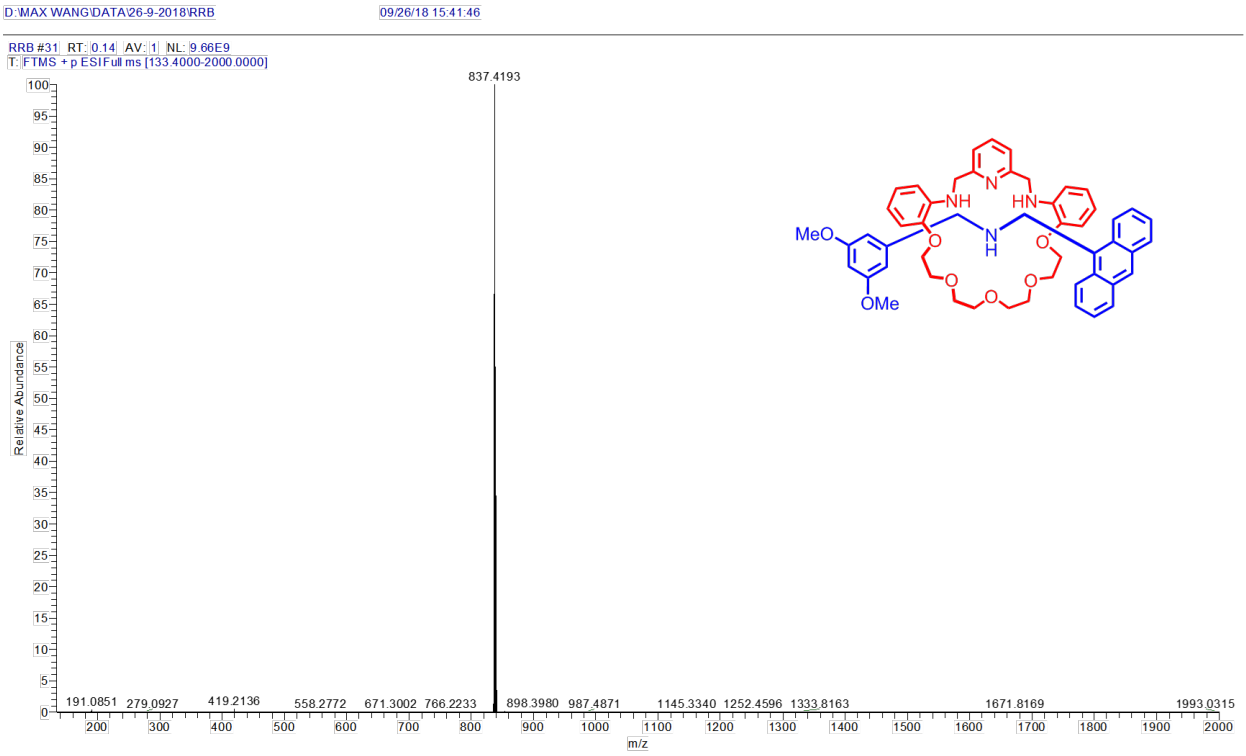


Figure S19. HRMS (ESI) of **RRA**.

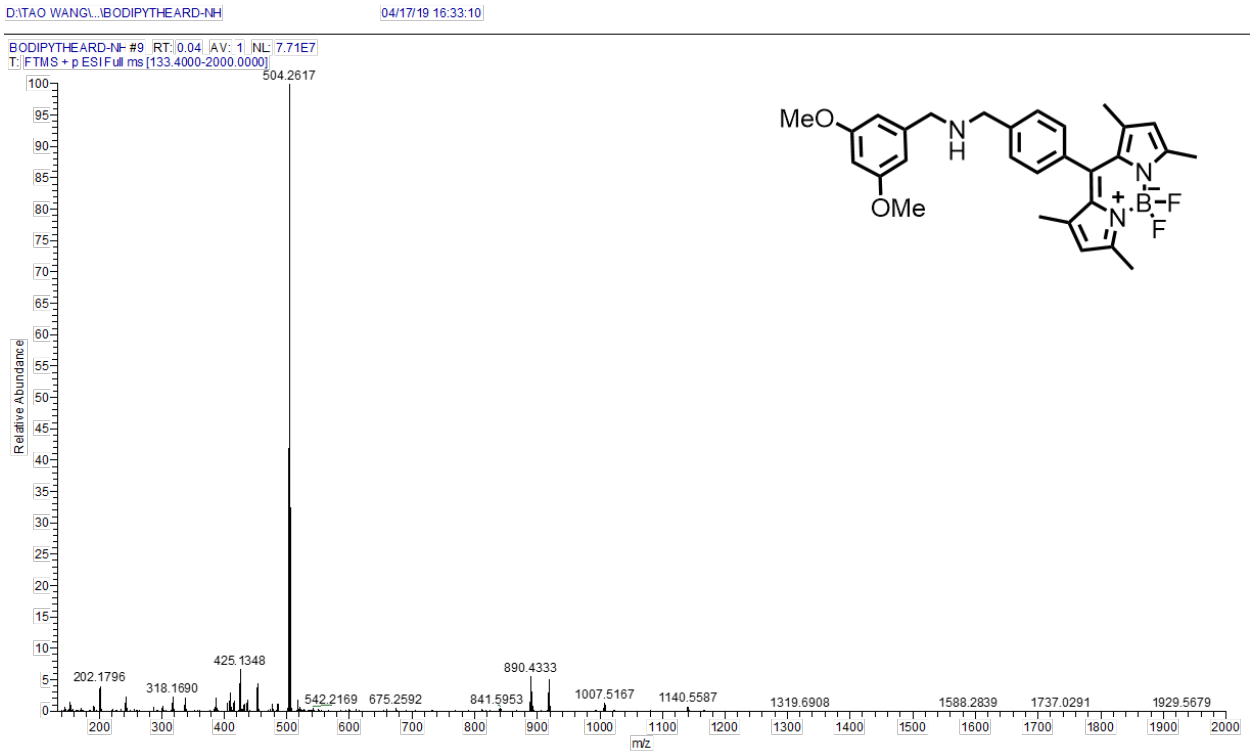


Figure S20. HRMS (ESI) of **S2**.

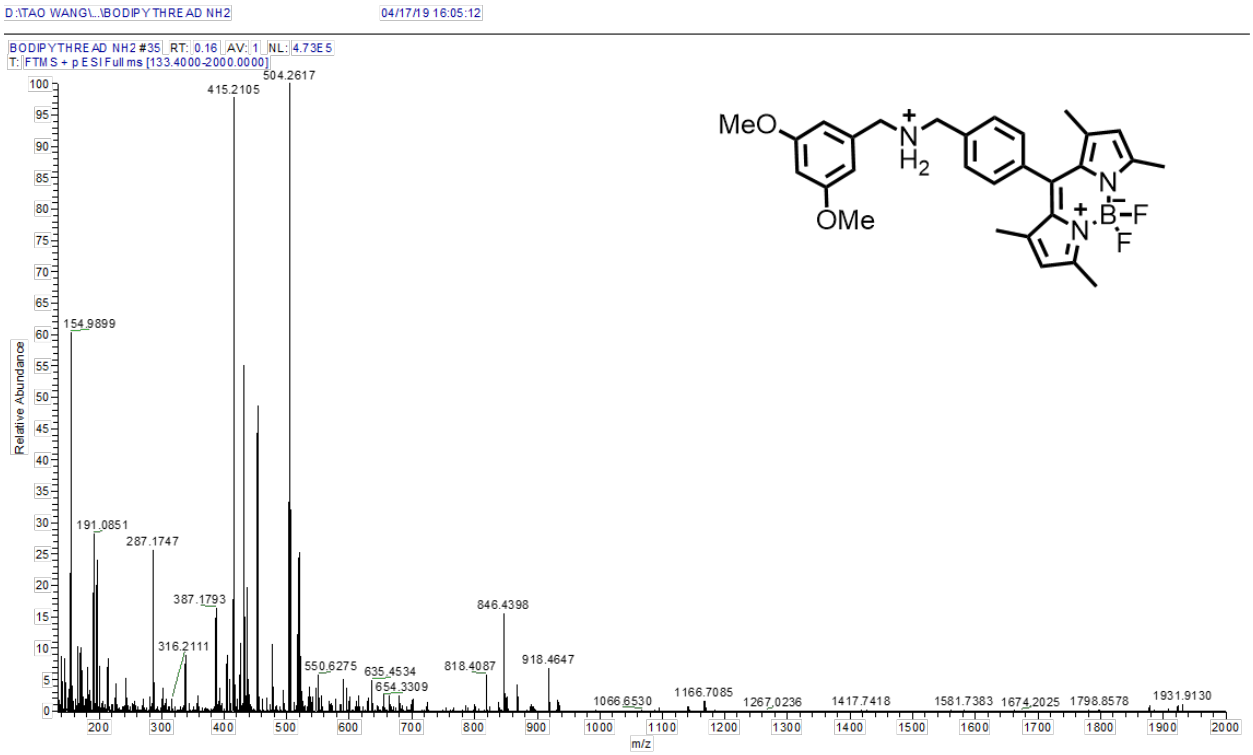


Figure S21. HRMS (ESI) of **4**.

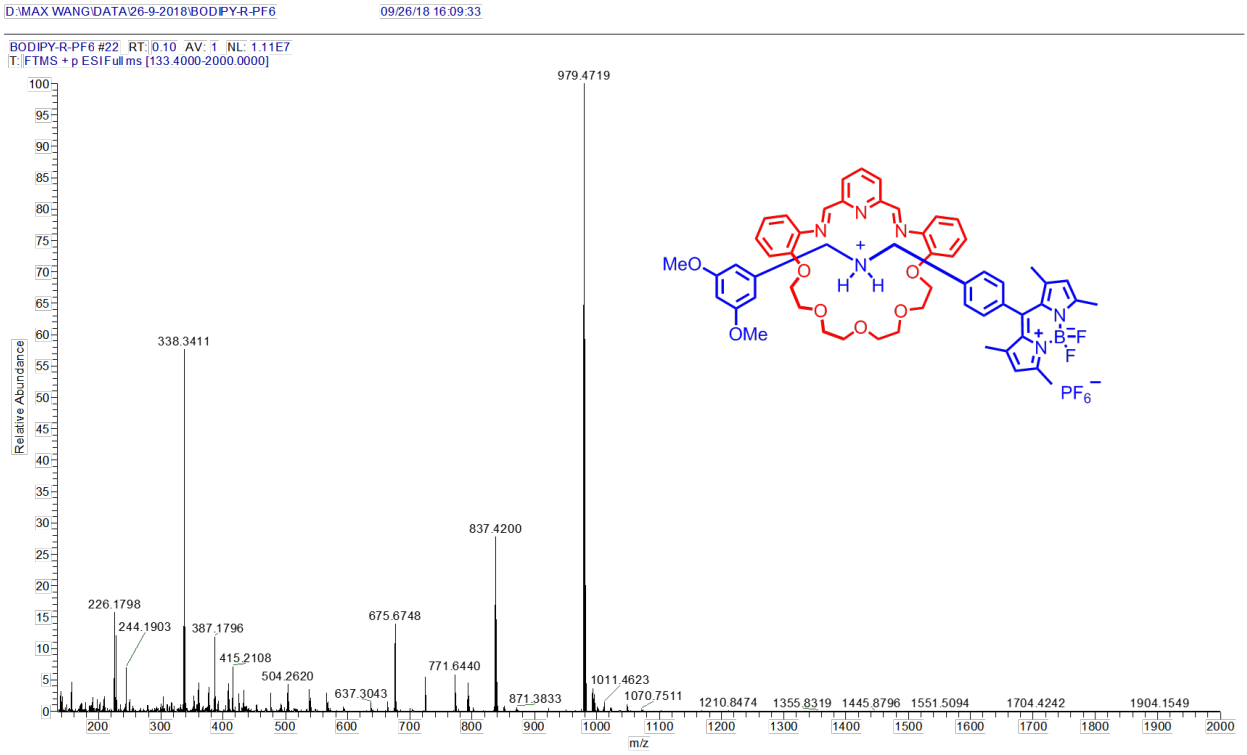


Figure S22. HRMS (ESI) of **RB**-H·PF_6.

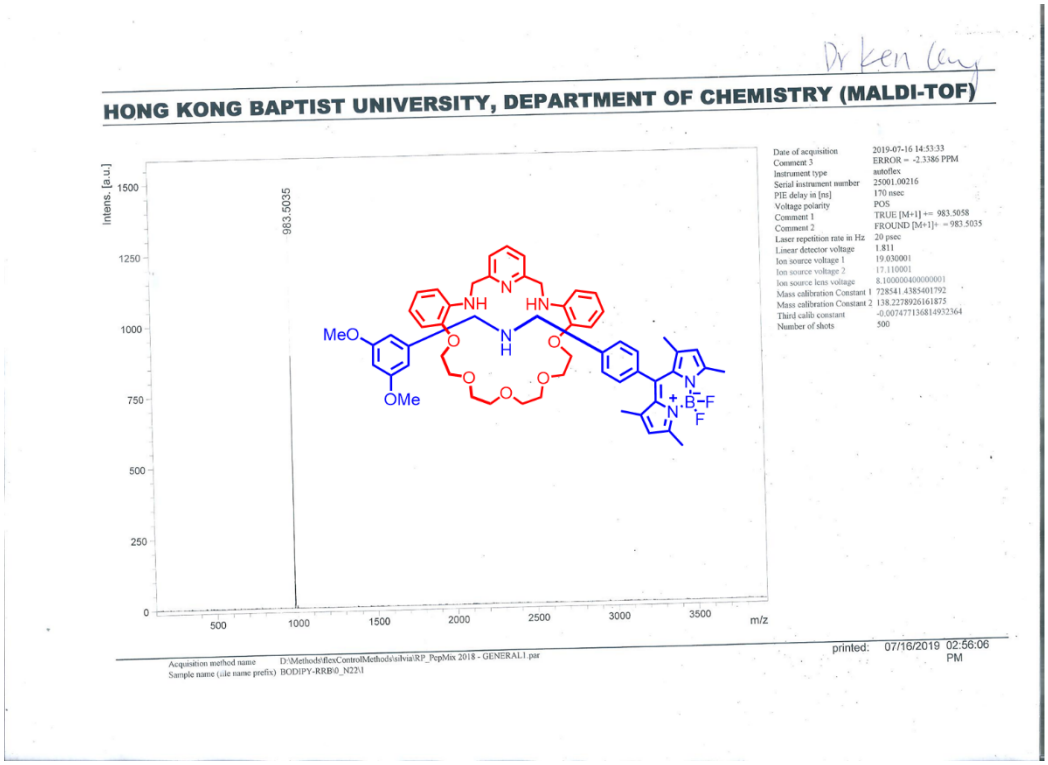


Figure S23. HRMS (MALDI-TOF) of **RRB**.

Table S1 Crystal data and structure refinement for RRA (CCDC code: 1942423).

Identification code	SAM020
Empirical formula	$C_{52}H_{57.5}N_{4.5}O_7$
Formula weight	857.52
Temperature/K	173
Crystal system	monoclinic
Space group	$P2_1/c$
a/Å	15.215(5)
b/Å	14.040(5)
c/Å	23.048(8)
α/°	90
β/°	100.849(6)
γ/°	90
Volume/Å^3	4835(3)
Z	4
ρ_{calc}g/cm^3	1.178
μ/mm^{-1}	0.079
F(000)	1828.0
Crystal size/mm^3	0.5 × 0.4 × 0.2
Radiation	MoKα (λ = 0.71073) 2Θ range for data collection/°
2.726 to 52.744	
Index ranges	-19 ≤ h ≤ 19, -17 ≤ k ≤ 17, -28 ≤ l ≤ 28
Reflections collected	96415
Independent reflections	9868 [R_{int}
= 0.0385, R_{sigma} = 0.0184]	
Data/restraints/parameters	9868/2/584
Goodness-of-fit on F^2	1.063
Final R indexes [I>=2σ (I)]	R_1
= 0.0717, wR_2 = 0.2199	Final R
indexes [all data]	R_1 = 0.0866,

wR_2 = 0.2441 Largest diff. peak/hole / e $Å^{-3}$ 1.28/-0.70

References

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