$\alpha_v\beta_3$-isoform specific erbium complexes for bladder cancer imaging and photodynamic therapy

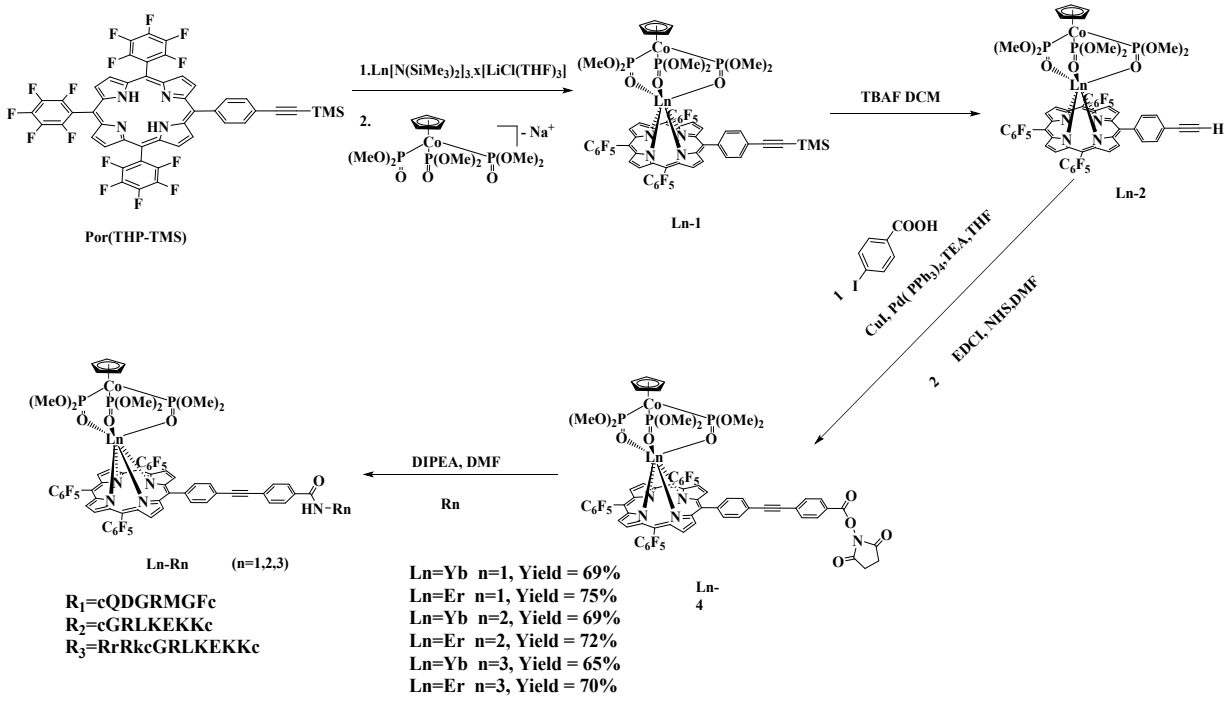
Supporting Information

Table of Content

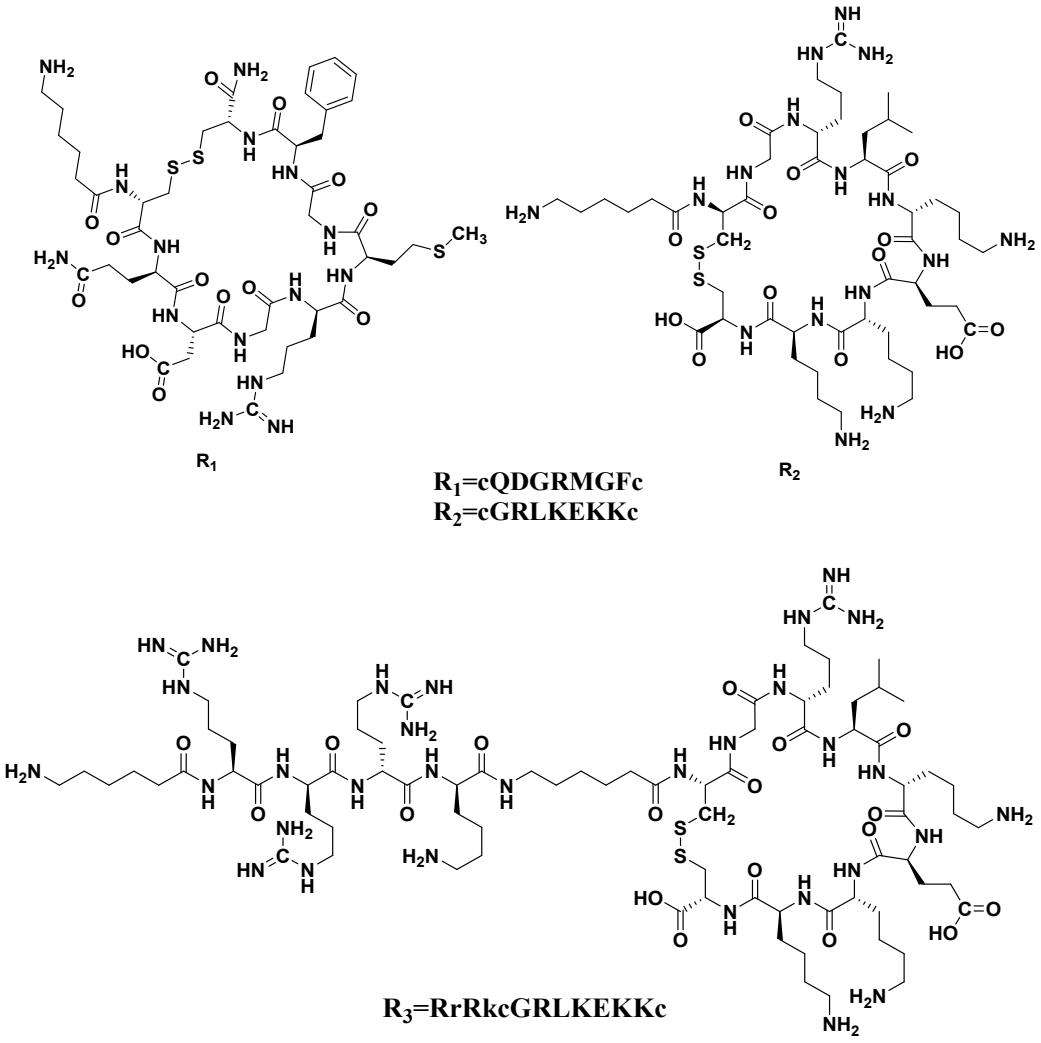
Experimental

General Information about the compound synthesis.

All chemicals used were of reagent - grade and were purchased from Sigma - Aldrich and used without further purification. All analytical - grade solvents were dried by standard procedures, distilled and deaerated before use. NMR spectra were recorded on a Bruker Ultra shield 400 Plus NMR spectrometer. The ^{1}H NMR chemical shifts were referenced to tetramethylsilane, TMS (d = 0.00). High - resolution mass spectra, reported as m/z, were obtained on a Bruker Autoflex MALDI - TOF mass spectrometer. The synthetic Route of intermediates and Ln-Rn (Ln=Yb,Er, n=1,2,3) were shown in Scheme S1 .



Scheme S1. The synthetic scheme of Ln-Rn .(Ln=Yb,Er, n=1,2,3)



Scheme S2. The molecular structures of bladder cancer peptides.

Synthesis of the intermediates and Ln-R_n （Ln=Yb,Er, n=1,2,3）

Preparation of compound Por(THP-TMS)

Pyrrole (280uL, 4.0mmol), pentafluorobenzaldehyde (588mg, 3.0 mmol) and 4-[2-(trimethylsilyl)ethynyl]benzaldehyde 6 (202 mg, 1.0 mmol) were dissolved in 410 mL dry CH_2Cl_2 under an argon atmosphere. After 10 min $BF_3.O(Et)_2$ (0.60 mL of 2.65 M stock solution, 1.32 mmol) was added *via* syringe with vigorous stirring. After addition was complete, the reaction was left to stir for 1 h at room temperature. DDQ (0.68 g, 3.0 mmol) was added and after 1 h stirring at room temperature the solvent was removed in vacuo. The crude reaction mixture was passed through a short silica column (hexanes-CH_2Cl_2 (9:1)). and then remove the solvent under reduced pressure to obtain the pure product.
5,10,15-Tris(pentafluorophenyl)-20-[4-{2-(trimethylsilyl)ethynyl}phenylporphyrin]
gave a pink/purple solid (238 mg, 22.8%); ^{1}HNMR ($CDCl_3$): -2.87 (2 H, s, NH), 7.91 (2 H, d,

J 8.1 Hz, Ar-H),8.17 (2 H, d, *J* 8.1 Hz, Ar-H), 8.89 (2 H, d, *J4.7* Hz, P-pyrrole), 8.93(4 H, s, P-pyrrole), 8.94(2 H, d, *J* 4.7 Hz, P-pyrrole);0.387（9H,s） MS (MALDI) calcd. for $C_{49}H_{23}F_{15}N_4Si$ $[M+H]^+$ 981.1513,found. 981.1523.

Preparation of compound Ln-1

$Ln[N(SiMe_3)_2]_3 \cdot x[LiCl(THF)_3]$: $HN(SiMe_3)_2$ (10.8 ml 0.050 mol) was dissolved 20 ml of dry THF in ice bath, then n-BuLi (1.6 M in hexane) was added slowly over 30-min period. The resulting solution was magnetically stirred for 12 hours until a clear pale yellow solution was obtained. Then the solution was transferred slowly to a Schlenk flask with $LnCl_3$ (4.74 g, 0.017 mol) suspended in 20 ml THF. The resulting mixture was magnetically stirred for 24 h until all of the solid $LnCl_3$ was disappeared. The resultant solution $Ln[N(SiMe_3)_2]_3x[Li(THF)_3Cl]$ (x = 3 ~ 5) was referred to as solution A for Ln =Yb and solution B for Ln = Er.

Yb-1: Solution A (2.5 ml, 0.52 mmol of Yb) prepared above was transferred to a Schlenk flask and the solvent was removed under vacuum. Then 10 ml CH_2Cl_2 was added, for the precipitation of LiCl. The mixture was centrifuged and the clear layer was transferred to another Schlenk flask with dry porphyrin free base (0.1 g, 0.16mmol) dissolved in 15 ml toluene. The resulting solution was refluxed until most of the free base coordinated with the metal ion. Dry NaL_{OMe} (0.1 g, 0.22 mmol)[L_{OMe}- ((cyclopentadienyl)tris(dimethylphosphito)-cobaltate, an anionic tripodalligand] was then added and magnetically stirred for another 12 hours before the reaction solution was cooled down to room temperature. Upon completion of the reaction, the solvent was removed in vacuum and the residue dissolved in $CHCl_3$, filtered and chromatographed on silica gel using $CHCl_3$/petroleum ether (V/V 1:1) as eluent. The product was dissolved in CH_2Cl_2 (5 ml) and the solution was filtered.

Yb-1: Yield: 81%; ^{1}HNMR ($CDCl_3$): δ -4.95(s, 5H), 0.92 (s, 9H), 6.34 (s,18H), 8.69 (s, 1H), 8.97 (d, *J* = 4.96 Hz, 1H), 10.86 (s, 1H), 14.61 (s, 2H), 14.84(s, 2H), 15.13 (s, 2H), 15.52 (s, 2H), 17.34 (s, 1H); MALDI-TOF MS: calcd. for $C_{60}H_{44}CoF_{15}N_4O_9P_3SiYb$ $[M]^+$ 1603.0571, found: 1603.0512.

Er-1: The same procedure with Yb-1, replace solution A with Solution B; Yield: 80%. ^{1}HNMR ($CDCl_3$): δ -35.54 (s, 5H), 3.48 (s, 9H), 13.50(s, 1H), 14.09 (s, 1H), 20.73 (s, 18H), 21.16 (s, 1H), 31.22 (s, 2H), 32.94 (s, 2H),36.38 (s, 2H), 37.77 (s, 2H), 46.77 (s,1H); MALDI-TOF MS: calcd. for $C_{60}H_{44}CoErF_{15}N_4O_9P_3Si$ $[M]^+$ 1597.1878, found 1597.2927.

General procedure for the preparation of Ln-2

Yb-2: TBAF (1.0 M in THF, 0.2 mL, 0.2 mmol) was added to a solution of Yb-1 (0.1mmol 160.31mg,) in 10 ml CH_2Cl_2, and the solution was stirred for 30min. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was passed through a short of silica gel column, Elution with CH_2Cl_2, After removal of solvent, pure product was obtained.

Yb-2: Yield: 92%; ^{1}HNMR ($CDCl_3$): δ -4.82 (s, 5H), 4.13 (s, 1H), 6.30 (s,18H), 8.63(s, 1H), 8.95 (d, J = 4.44 Hz, 1H), 10.83 (s, 1H), 14.51 (s, 2H), 14.90(s, 2H), 15.08 (s, 2H), 15.45 (s, 2H), 17.21 (s, 1H); MALDI-TOF MS: calcd. for $C_{57}H_{36}CoF_{15}N_4O_9P_3Yb$ $[M]^+$ 1530.8176, found 1530.9929.

Er-2: The same procedure with Yb-2, replace Yb-1 with Er-1; Yield: 92%;^{1}HNMR ($CDCl_3$): δ -35.05 (s, 5H), 13.19(s, 1H), 13.94 (s, 1H), 20.56 (s, 18H), 21.02 (s, 1H), 30.97 (s, 2H), 32.77 (s, 2H),36.44 (s, 2H), 37.36 (s, 2H), 46.20 (s,1H); MALDI-TOF MS: calcd. for $C_{57}H_{36}CoErF_{15}N_4O_9P_3$ $[M]^+$: 1525.0067, found: 1525.0143.

General procedure for the preparation of Ln-4

Yb-4 :$Pd(PPh_3)_4$ (23.11 mg 0.02 mmol), CuI (1.90 mg, 0.01 mmol), Yb-2 (153.12mg, 0.1mmol)and 4-iodobenzoic acid (124.01mg, 0.5mmol) were placed in a dried flask and under nitrogen. Dry THF (15 mL) and NEt_3 (2 mL) were added and the reaction mixture degassed with nitrogen. The reaction mixture was stirred at 45 °C for 12 h. After that, the solvent was removed under reduced pressure. The residue was purified by chromatography. Elution with CH_2Cl_2/Methanol(12:1) afforded Yb-3. Yb-3(104.24mg,0.06mmol), EDCI(23.02mg,0.12mmol), NHS(13.81mg,0.12mmol) were placed in a dried flask and under nitrogen,10mL dry DMF was added. stirred at room temperature for 48h. then remove the solvent , The residue was recrystallized by diethyl ether and dried to give the title product.

Yb-4: Yield: 72%; ^{1}HNMR ($CDCl_3$): δ -4.82 (s, 5H), 6.34 (s,18H), 8.45 (s, 2H), 8.47 (s, 2H), 8.59 (s, 1H), 8.61 (s, 1H) , 10.91 (s, 1H), 14.53 (s, 2H), 14.80 (s, 2H), 15.12 (s, 2H), 15.51 (s, 2H), 17. 29 (s, 1H); MALDI-TOF MS: calcd. for $C_{68}H_{43}CoF_{15}N_5O_{13}P_3Yb$ $[M]^+$1748.0176,found 1748.0460. HPLC characterization: retention time = 7.02 min.

Er-4: The same procedure with Yb-4, only replace Yb-2, Yb-3 with Er-2, Er-3; Yield: 80%; ^{1}HNMR ($CDCl_3$): δ -36.64 (s, 5H), 3.61(s,2H), 4.03(s ,2H), 5.49 (s, 1H), 8.77 (d, J = 7.96 Hz, 2H),10.89 (d, J = 5.12 Hz, 2H), 13.20 (s, 1H), 13. 84 (s, 1H), 20.93 (s, 18 H), 21.07 (s,1H), 31.22 (s, 2H), 33.28(s, 2H), 36.82 (s, 2H), 38.04 (s, 2H), 46.89 (s, 1H);MALDI-TOF MS: calcd. For $C_{68}H_{43}CoErF_{15}N_5O_{13}P_3$ $[M+Cl^-]$:1777.2035,found:1777.4591.HPLC characterization: retention time = 7.12 min.

General procedure for the preparation of Ln-Rn

Yb-R_1 : A stirred solution of Yb-4(50mg, 0.029mmol) in anhydrous 5mL DMF was mixed with N,N'-diisopropylethylamine (DIPEA) 0.1mL . the mixture solution was added into a vial which containing peptide R_1(42.45mg, 0.038mmol). It was then reacted at RT overnight, after that, the solvent was removed under vacuum to get the dry compound. The residue was recrystallized by diethyl ether three times and dried to give the titled product.

Yb-R_1 : Yield: 69%. MALDI-TOF MS: calcd. for $C_{109}H_{109}CoF_{15}N_{19}O_{23}P_3S_3Yb$ $[M+H]^+$: 2760.4878 found: 2760.6458. HPLC characterization: retention time = 10.00 min.

Yb-R_2: The same procedure with Yb-R_1, peptide R_2 was used; Yield: 69% MALDI-TOF MS: calcd. for $C_{113}H_{129}CoF_{15}N_{21}O_{22}P_3S_2Yb$ $[M+H]^+$ 2808.6835 found: 2808.6715. HPLC characterization: retention time = 10.21 min.

Yb-R_3: The same procedure with Yb-R_1, peptide R_3 was used; Yield: 65% MALDI-TOF MS: calcd. for $C_{143}H_{187}CoF_{15}N_{35}O_{28}P_3S_2Yb$ $[M+H]^+$ 3520.2985 found: 3520.2543. HPLC characterization: retention time = 10.05 min.

Er-R_1 : The same procedure with Yb-R_1, replace Yb-4 with Er-4; Yield: 75% MALDI-TOF MS: calcd. for $C_{109}H_{109}CoErF_{15}N_{19}O_{23}P_3S_3$ $[M+K]^+$: 2791.4826. found: 2791.3747. HPLC characterization: retention time = 9.66min.

Er-R_2 : The same procedure with Yb-R_1, replace Yb-4 with Er-4;Yield: 72% MALDI-TOF MS: calcd. for $C_{113}H_{129}CoErF_{15}N_{21}O_{22}P_3S_2$ $[M+K]^+$: 2839.6015 found: 2839.2967. HPLC characterization: retention time =10.09 min.

Er-R_3 : The same procedure with Yb-R_1, replace Yb-4 with Er-4;Yield: 70% MALDI-TOF MS: calcd. for $C_{143}H_{187}CoErF_{15}N_{35}O_{28}P_3S_2$ $[M]^+$: 3511.4955 found: 3511.5162. HPLC characterization: retention time = 9.80 min.

Cell culture

Human bladder carcinoma (T24) and (5637) cells were cultured in RPMI 1640 medium (Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco) and antibiotics (penicillin, 50 $ugmL^{-1}$; streptomycin, 50 $ugmL^{-1}$). Human cervical carcinoma (HeLa) cells were cultured in DMEM (Gibco) supplemented with 10% FBS (Gibco) and antibiotics (penicillin, 50 $ugmL^{-1}$; streptomycin, 50 $ugmL^{-1}$). Human normal lung fibroblast (MRC-5) cells were maintained in minimum essential medium (MEM) supplemented with 10 % FBS and 1% 50 $ugmL^{-1}$ penicillin; 50 $ugmL^{-1}$ streptomycin. All cells were incubated at 37^{o}C in a humidified environment with 5% CO_2.

Dark cytotoxicity

T24, HeLa and MRC-5 cells (1 x 10^5) were treated with **Er-R_n** and **Yb-R_n** porphyrin complexes for 24 h at six concentrations (1, 5, 10, 50, 100, 500 uM). The cell monolayers were rinsed once with phosphate-buffered saline (PBS) and incubated with 500 $ugmL^{-1}$ 3-(4, 5-dimethylthiazol-2-yl)-2 and 5-diphenyltetrazolium bromide (MTT) solution. The cellular inhibitory potency of the complexes was examined by treating the cells with MTT for 3 hours to allow formazan production during cell metabolism. After that, the formazan crystals were fully dissolved in DMSO with oscillation. Finally, the absorbance of solution was measured with Biotek PowerWave XS microplate reader at the wavelengths of 570 and 690 nm.

Photo-cytotoxicity

T24, HeLa and MRC-5 cells (1 x 10^5) were treated with **Er-R_n** and **Yb-R_n** porphyrin complexes for 24 hours at four concentrations (1, 5, 10, 50 uM). Then, the cells were irradiated at 6 $mWcm^{-2}$ (equipped with 550 nm long pass filter) for about 27 minutes and further incubated for 24 hours. The cells were then treated according to the same protocol as the previous MTT assay.

In vitro confocal microscopy

To investigate the suitability of the obtained complexes as bioprobes, T24, 5637, HeLa and MRC-5 cells (1 x 10^5) were imaged. After incubation with the **Er-R_n** and **Yb-R_n** porphyrin complexes at 5 uM for 24 hours, the cells were washed with PBS for three times before imaging. LysoTracker Green DND-26 was used as costaining dye. Images were acquired on a Leica TCS SPE confocal laser-scanning microscope. The samples and LysoTracker were excited at wavelength of 561 and 488 nm respectively.

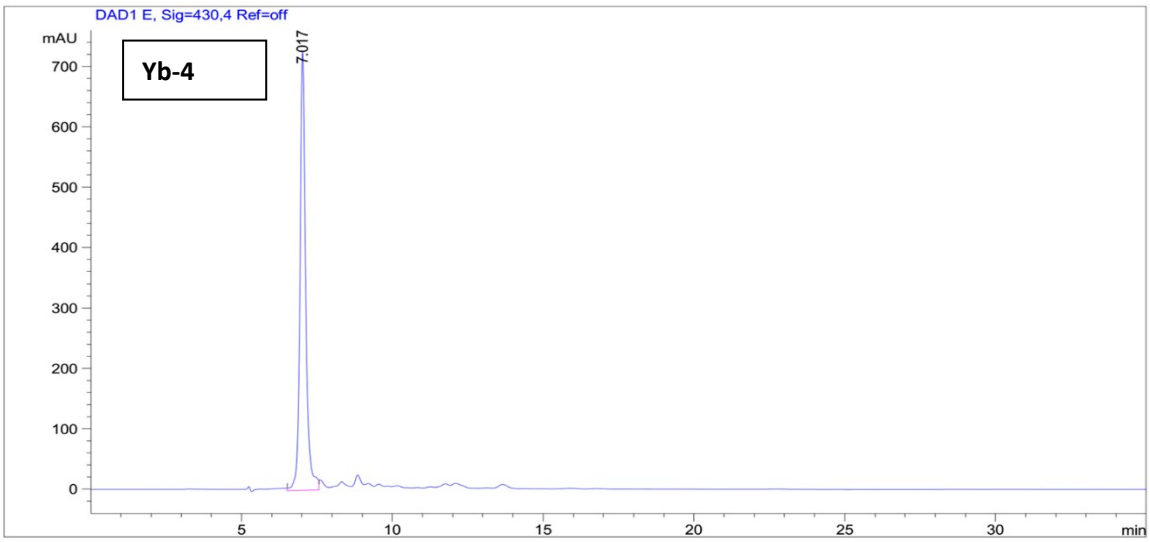
Flow cytometry measurements of cellular uptake

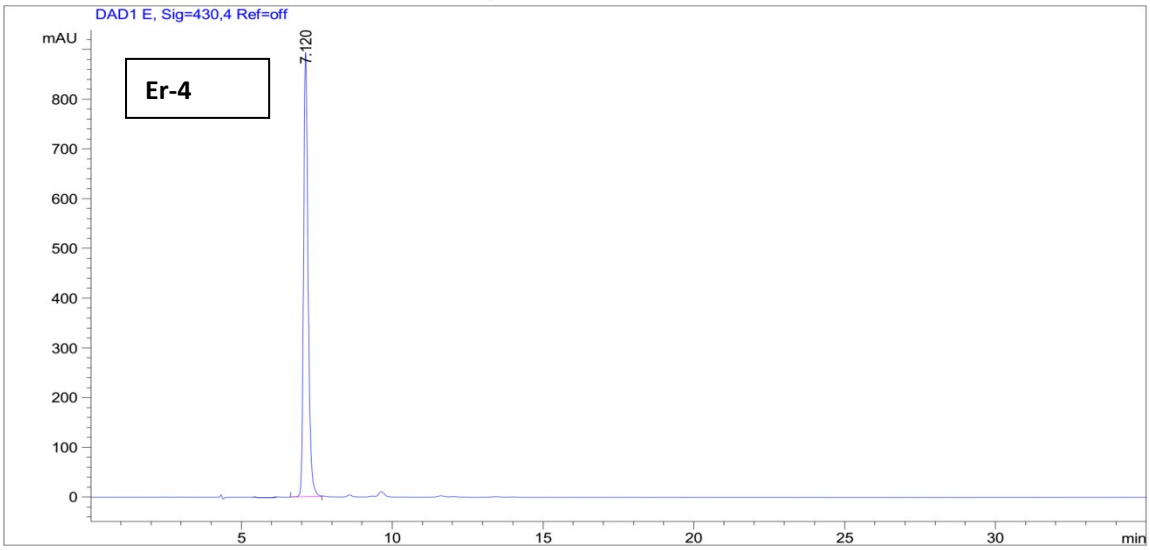
5637, T24, HeLa and MRC-5 cells (1 x 10^5 per sample) were seeded onto 35 mm Petri dishes and incubated overnight. Then the cells were incubated with the **Er-R_n** and **Yb-R_n** porphyrin complexes (5 uM) for 3, 6 and 24 hours. Cells were harvested with trypsin and washed twice with PBS. The uptake of the complexes by the T24, HeLa and MRC-5 cells was analyzed by flow cytometry. The cells were excited with a 488 nm argon laser and emission was collected in the FL-3 channel (with a 650 nm long- pass filter); 10000 events were analyzed.

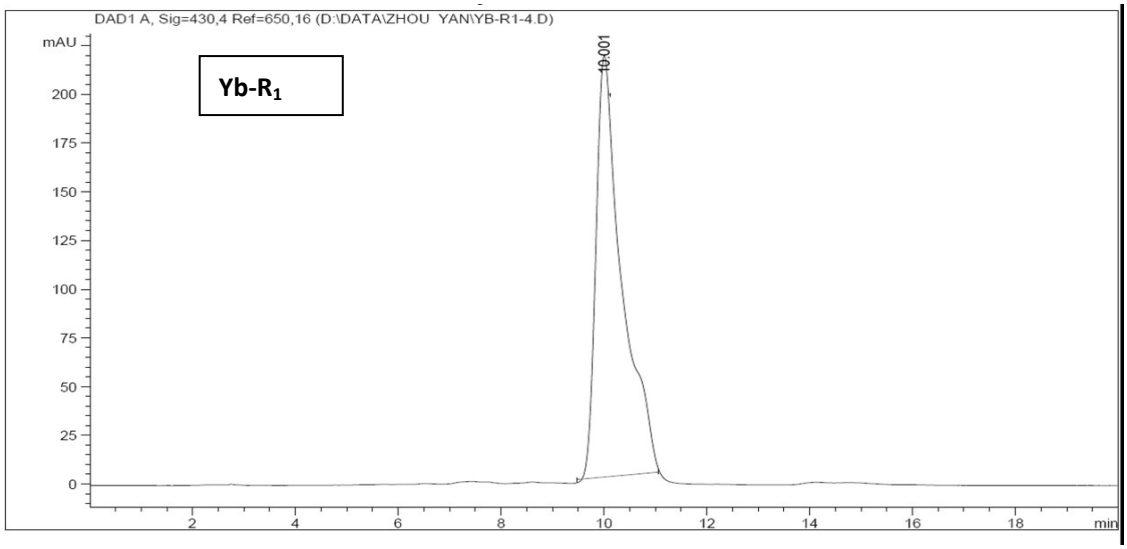
HPLC characterization of the complexes.

Table S1. Solvent gradient for HPLC

Time /min	0.05% TFA in water /%	MeOH /%
0	50	50
5	20	80
20	0	100



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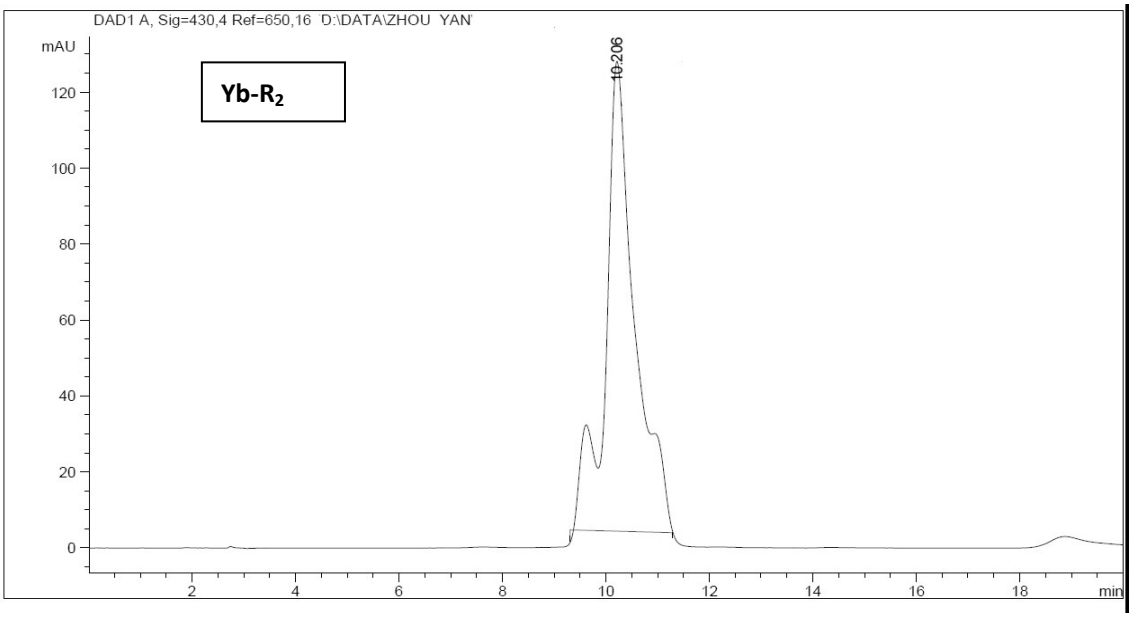
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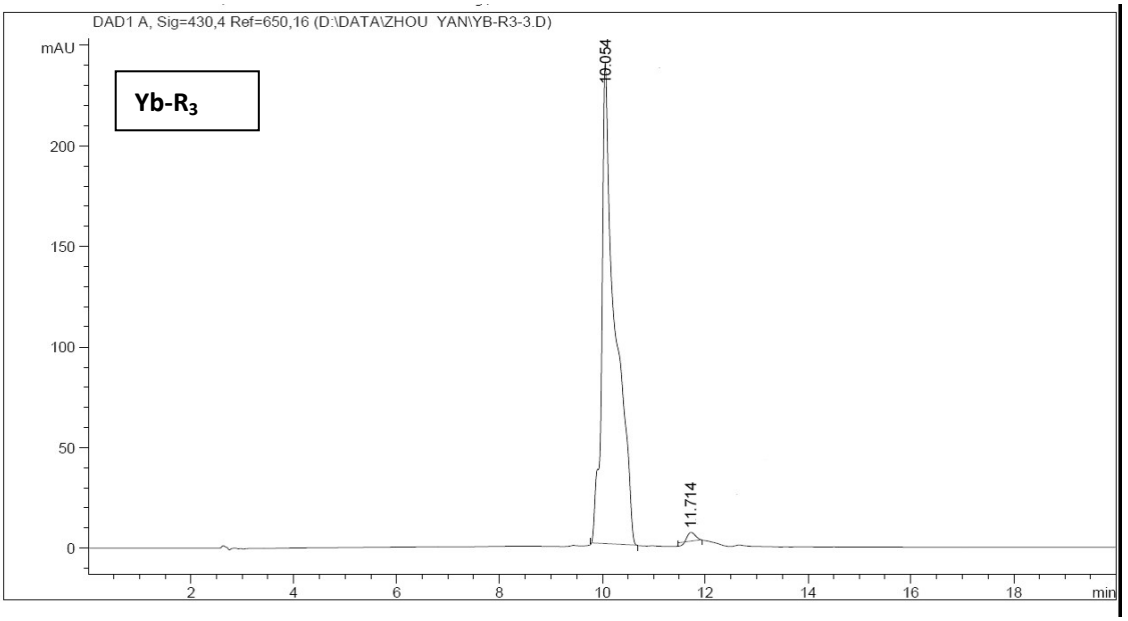
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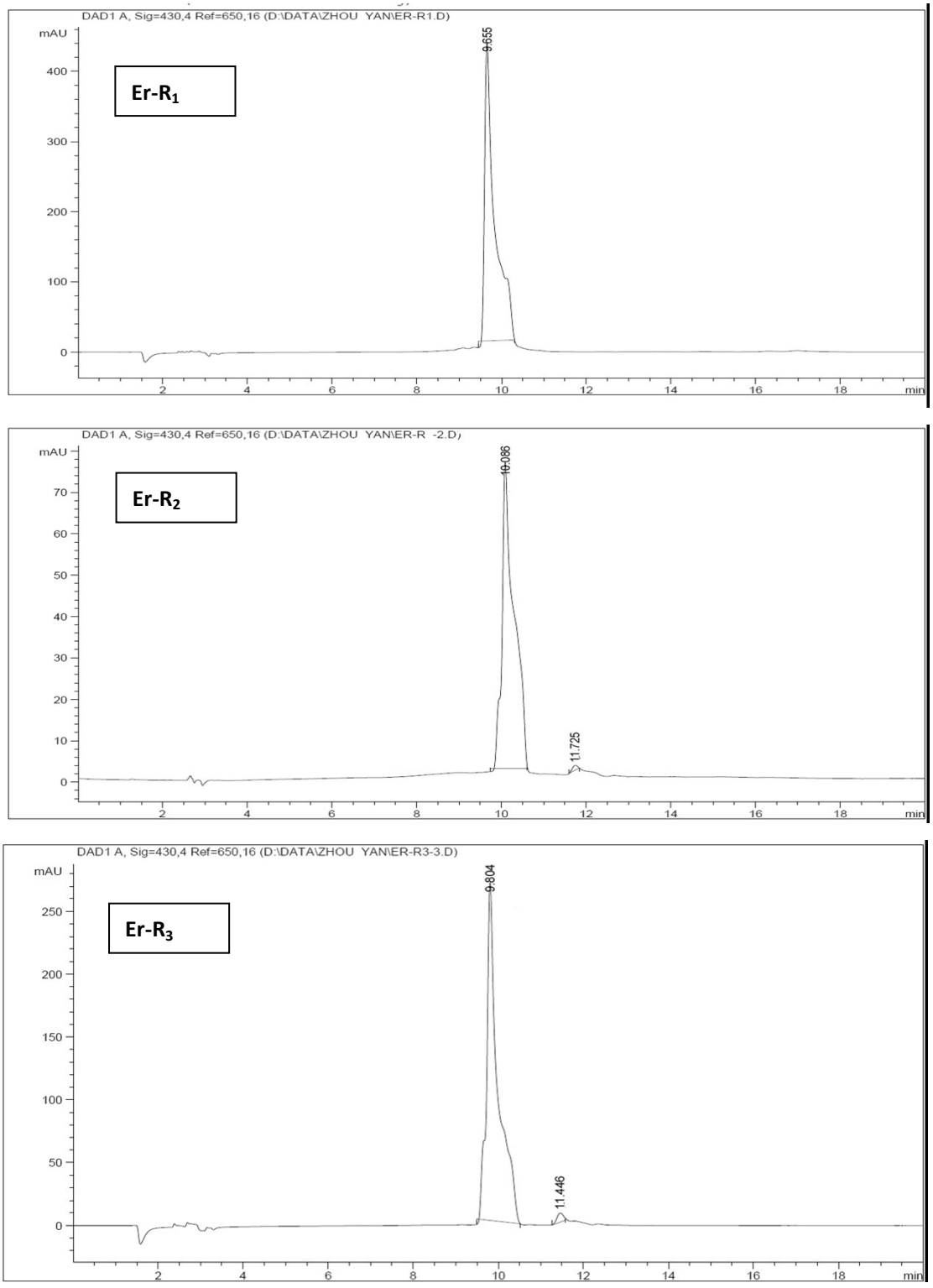

Figure. S1. HPLC chromatogram of the complexes. Elution conditions: column, Agilent ZORBAXSB-C18 (4.6 X 150 mm, particle size 5 ; flow rate, 1.0 mL/min; gradient elution; detection wavelength, 430 nm. Retention time: **Yb-4**, 7.02 min; **Er-4**, 7.12 min; **Yb-R$_1$**, 10.00 min; **Yb-R$_2$**, 10.21min; **Yb-R$_3$**, 10.05 min; **Er-R$_1$**, 9.66min; **Er-R$_2$**, 10.09min;and **Er-R$_3$**, 9.80min.

400 MHz-^{1}H-NMR and MALDI-TOF spectrum

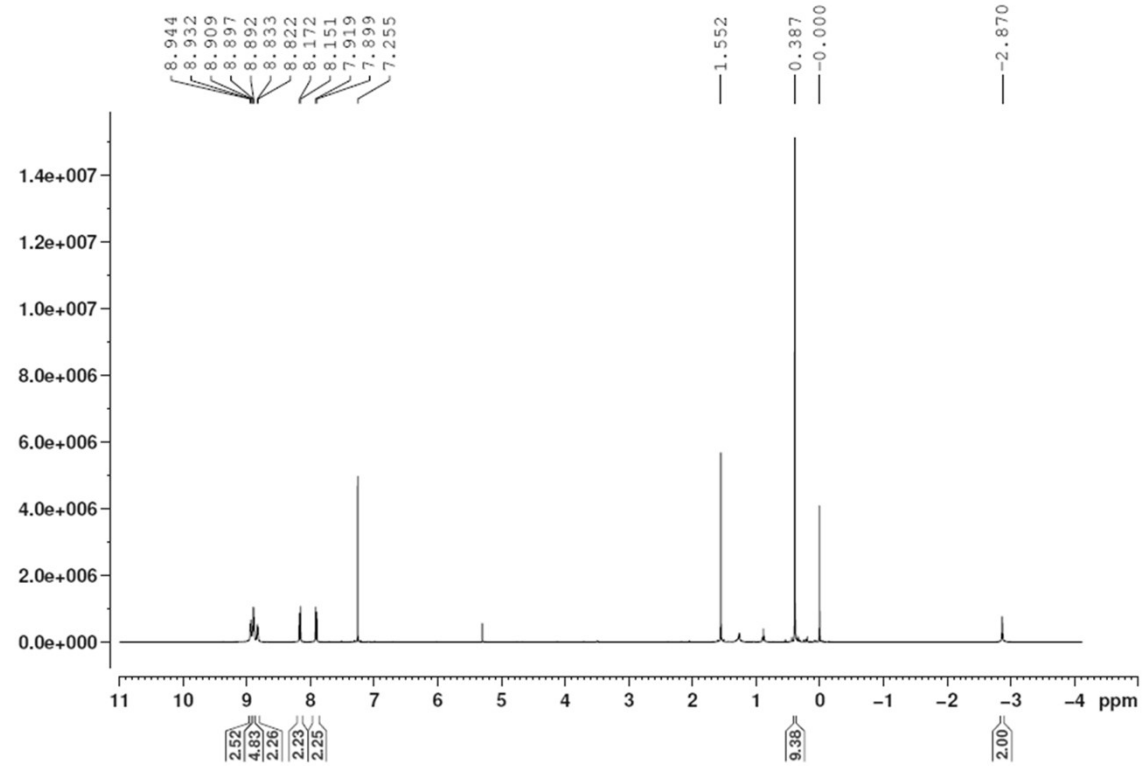

Figure S2. 400 MHz-^{1}H-NMR ($CDCl_3$) spectrum of **Por(THP-TMS)**

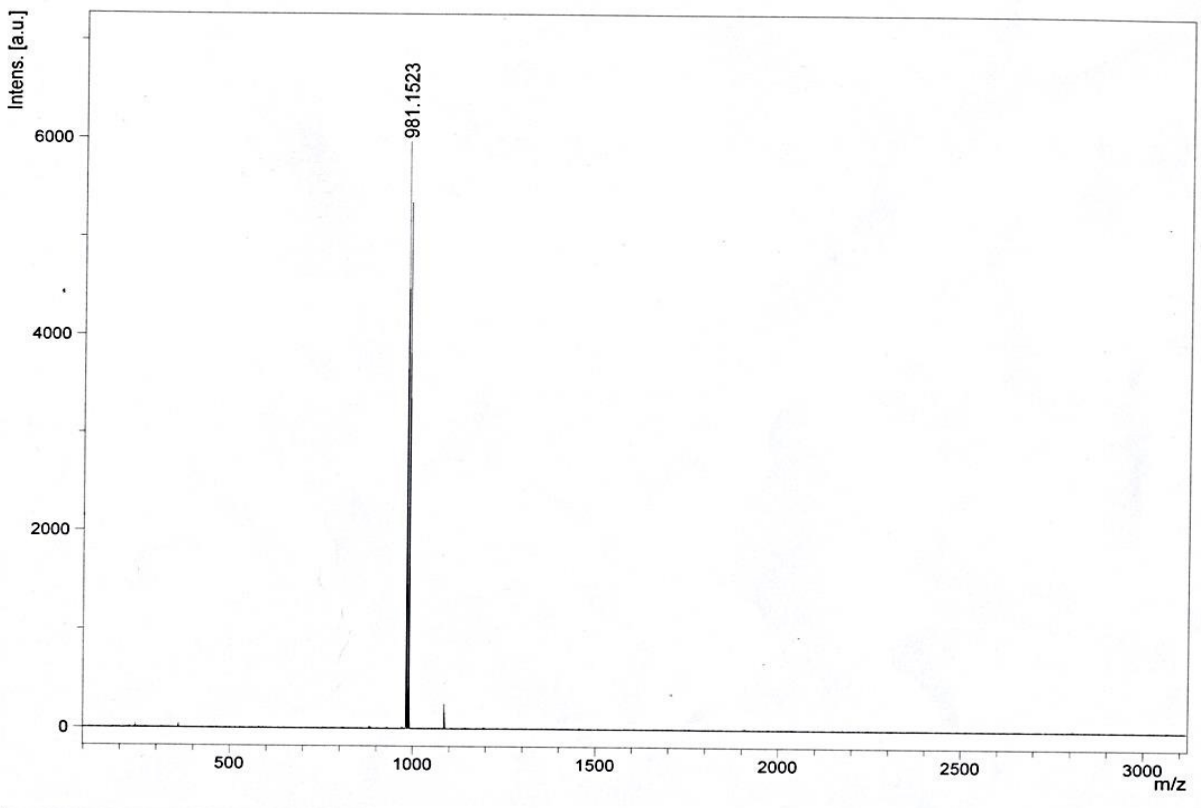

Figure S3. MALDI-TOF spectrum of **Por(THP-TMS)**

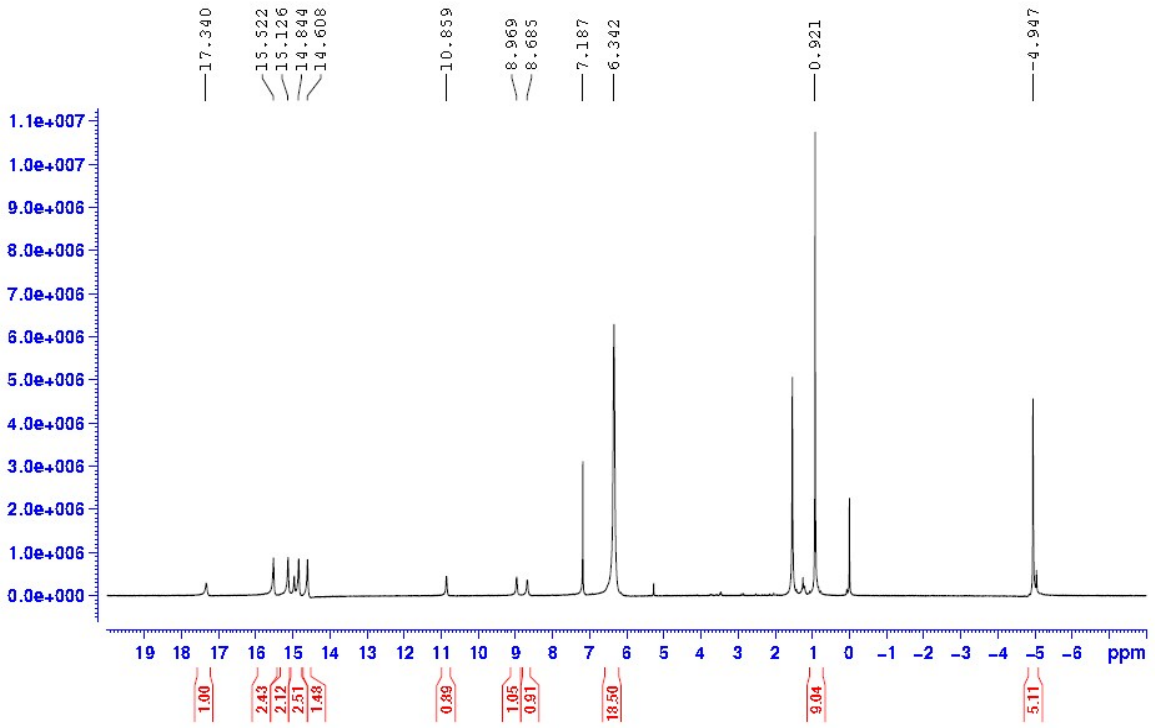

Figure S4. 400 MHz-^{1}H-NMR ($CDCl_3$) spectrum of **Yb-1**.

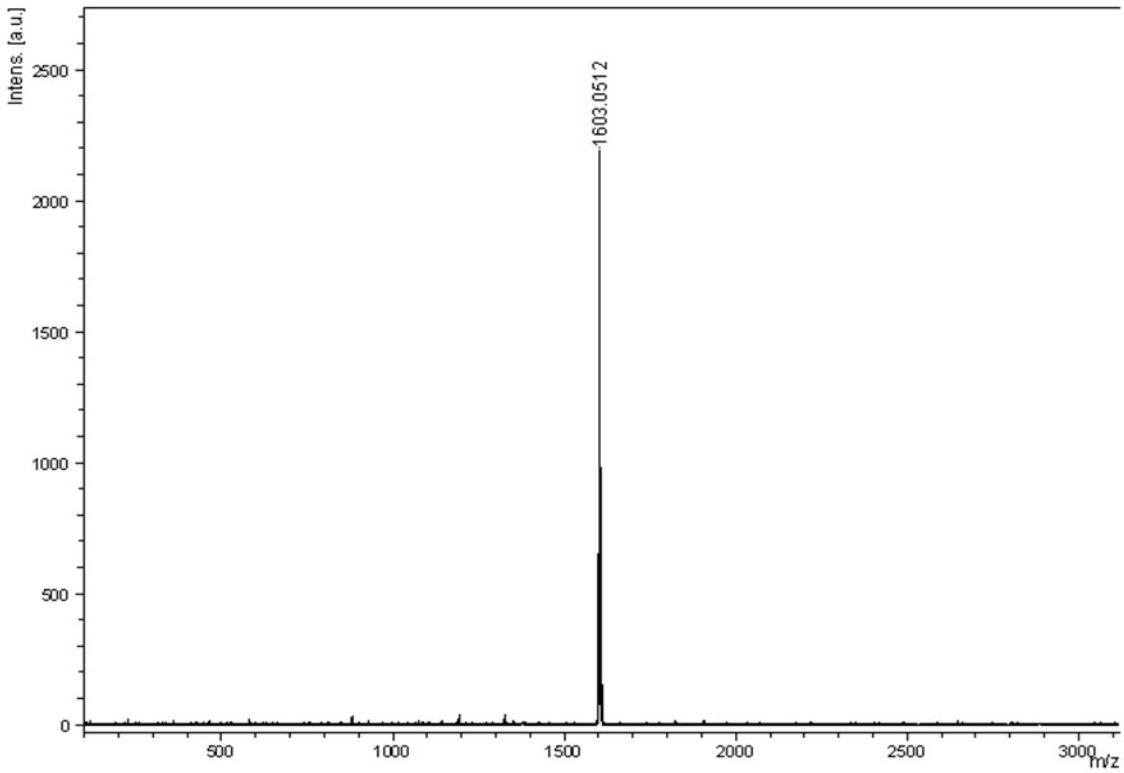

Figure S5. MALDI-TOF spectrum of **Yb-1** .

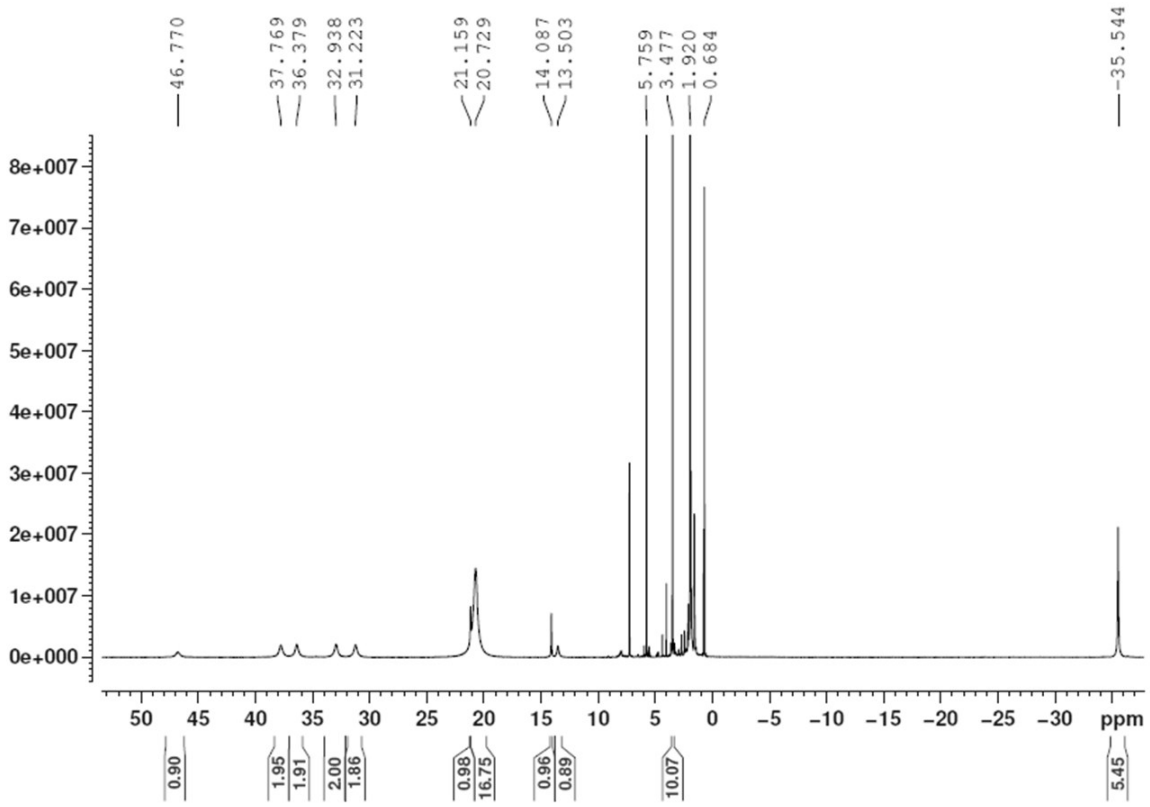

Figure S6. 400 MHz-^{1}H-NMR ($CDCl_3$) spectrum of **Er-1.**

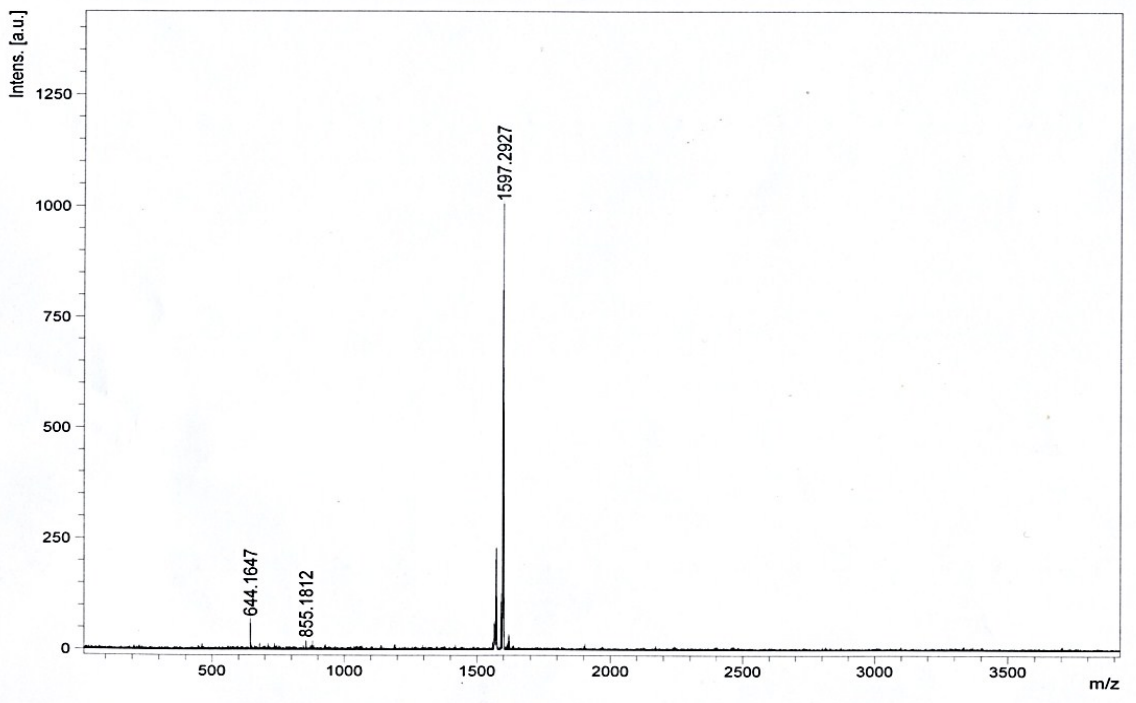

Figure S7. MALDI-TOF spectrum of **Er-1**

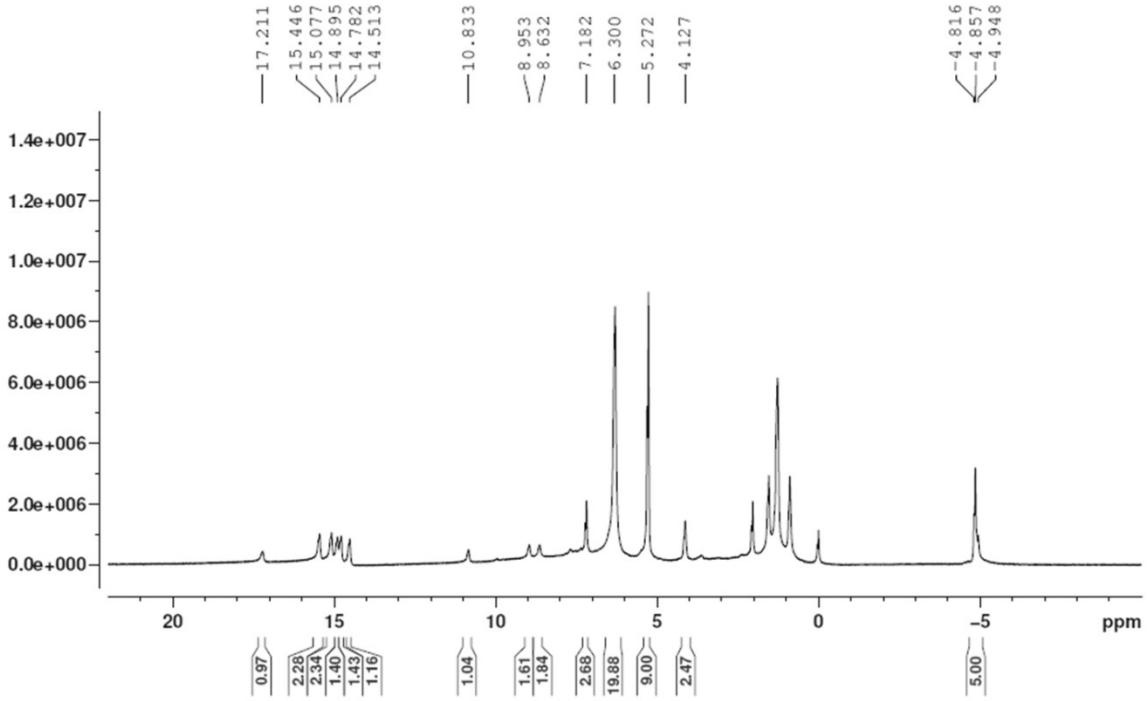

Figure S8. 400 MHz-^{1}H-NMR ($CDCl_3$) spectrum of **Yb-2**.

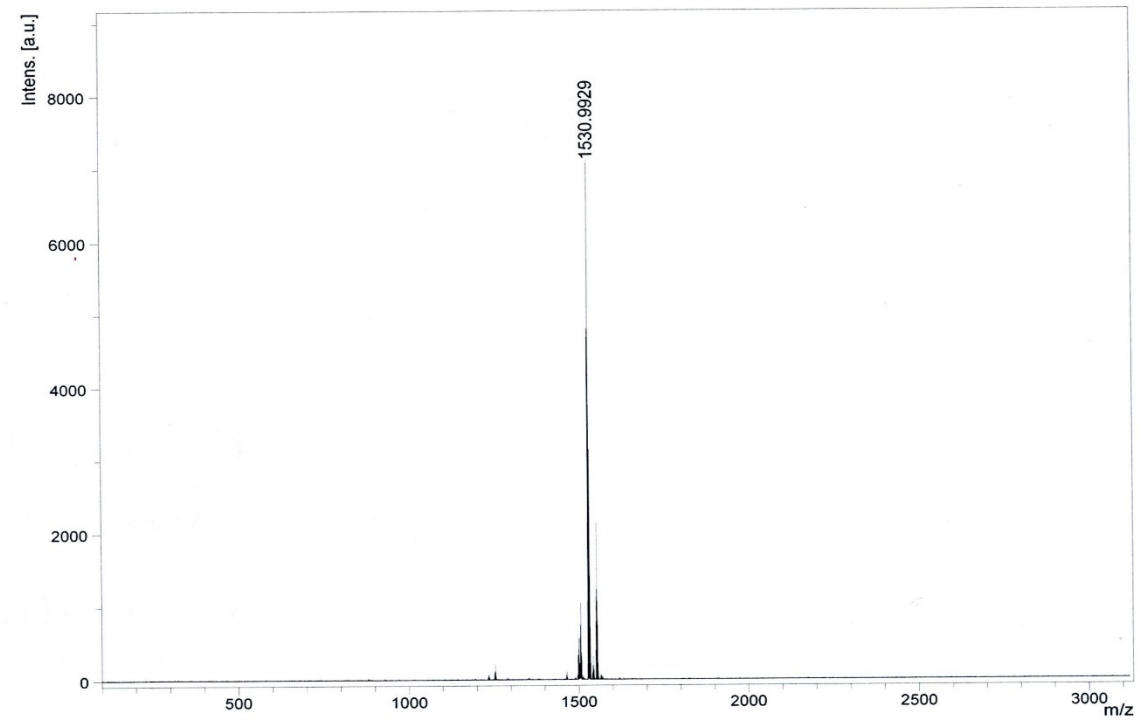

Figure S9. MALDI-TOF spectrum of **Yb-2**.

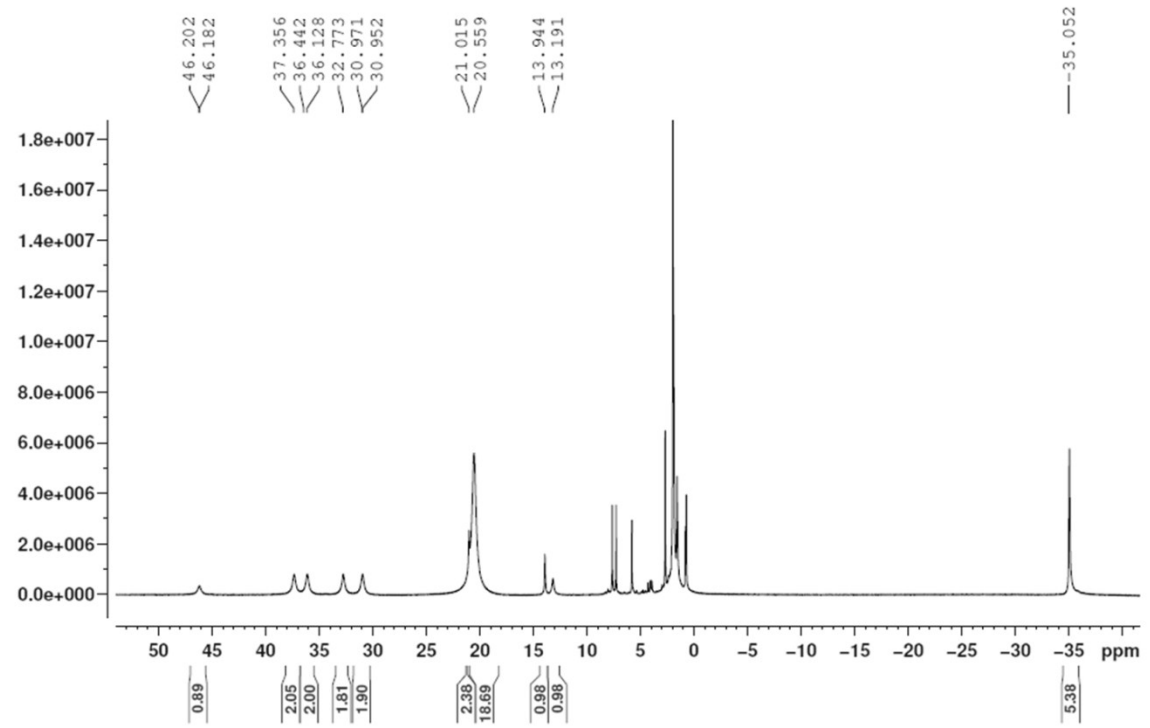

Figure S10. 400 MHz-^{1}H-NMR ($CDCl_3$) spectrum of **Er-2.**

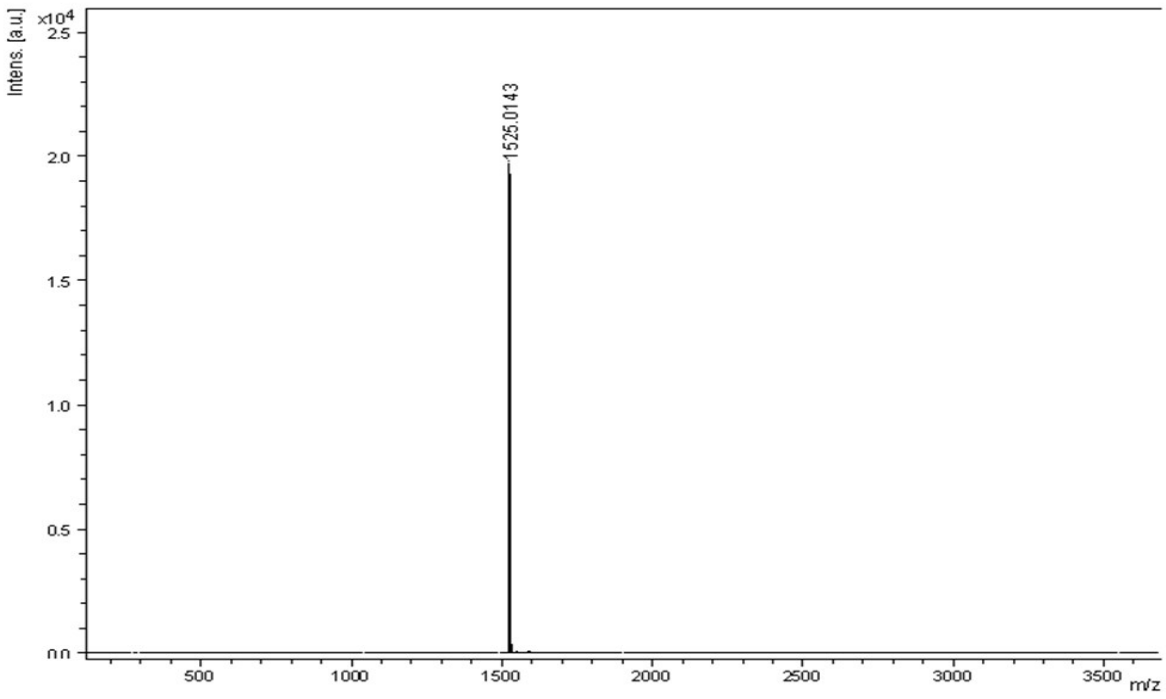

Figure S11. MALDI-TOF spectrum of **Er-2.**

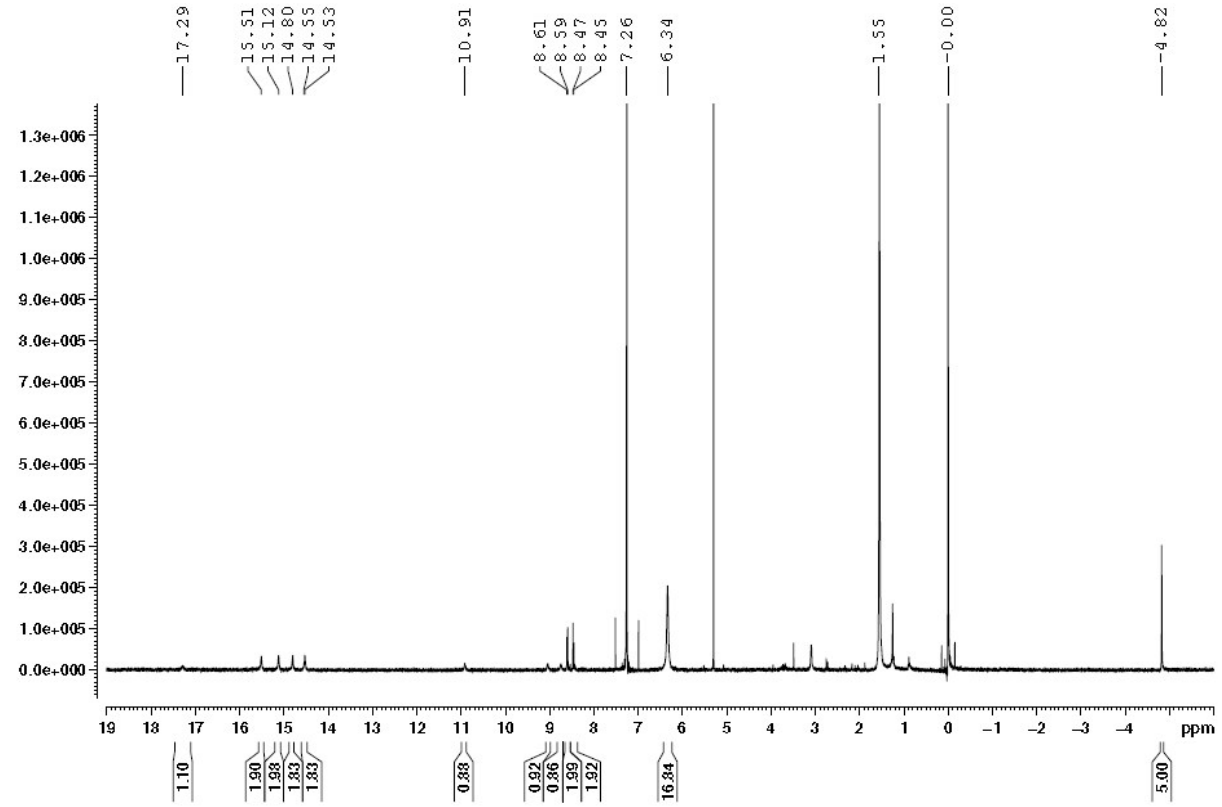

Figure S12. 400 MHz-^{1}H-NMR ($CDCl_3$) spectrum of **Yb-4.**

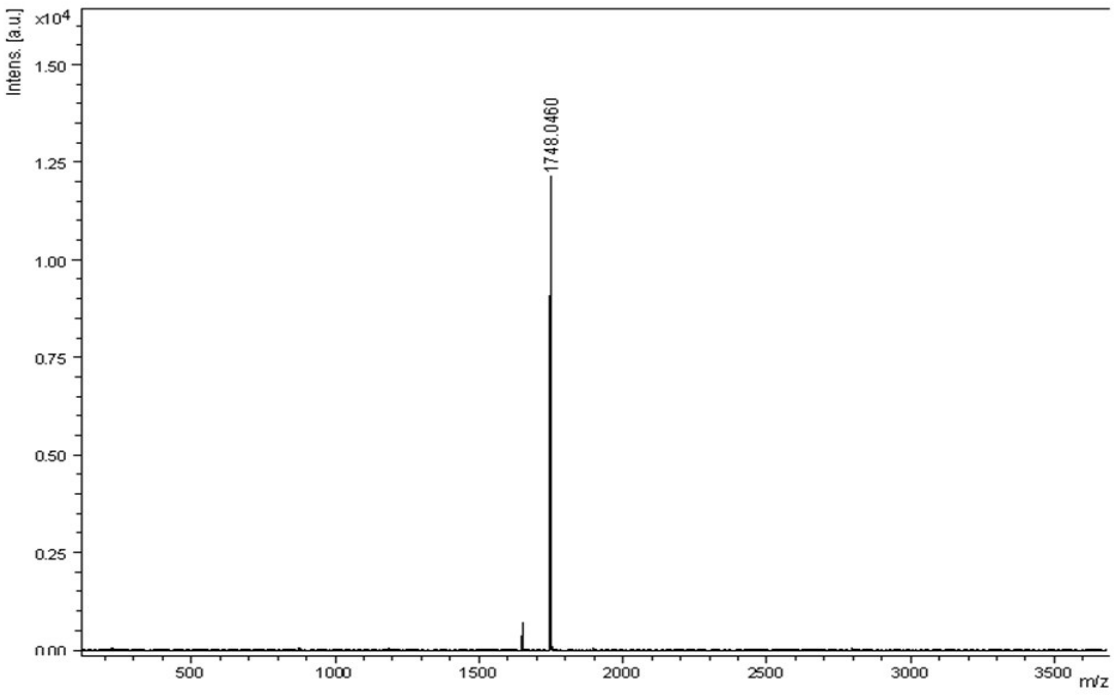

Figure S13. MALDI-TOF spectrum of **Yb-4.**

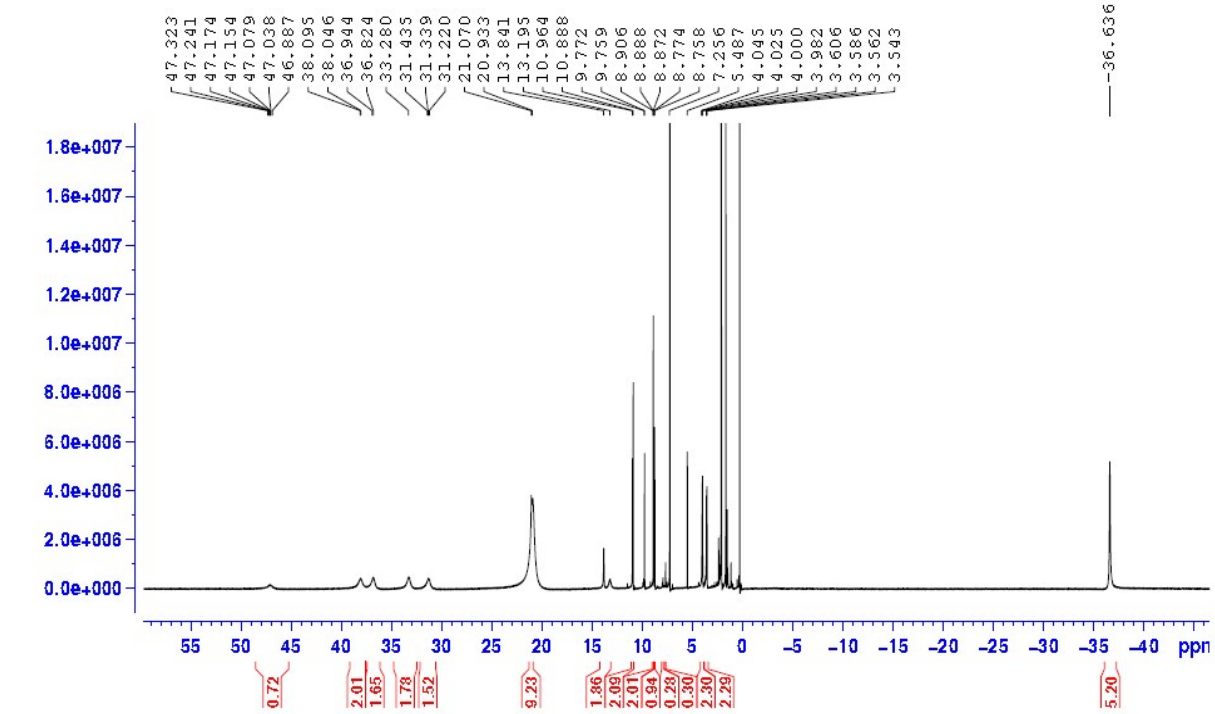

Figure S14. 400 MHz-^{1}H-NMR ($CDCl_3$) spectrum of **Er-4.**

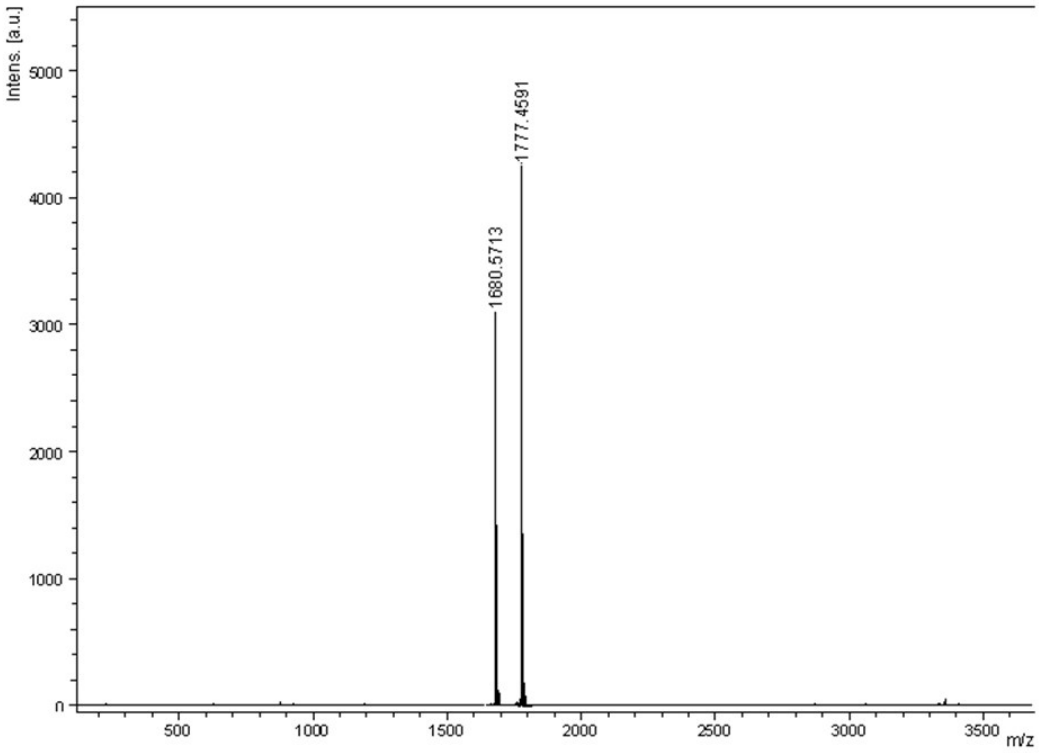

Figure S15. MALDI-TOF spectrum of **Er-4.**

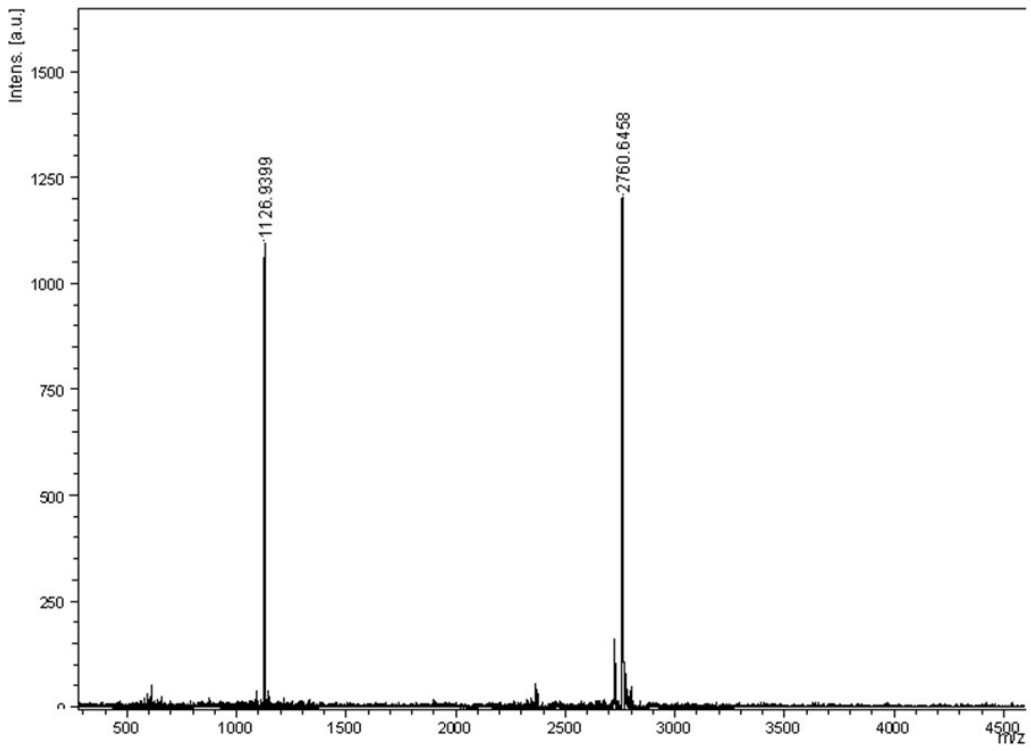

Figure S16. MALDI-TOF spectrum of **Yb-R$_1$**.

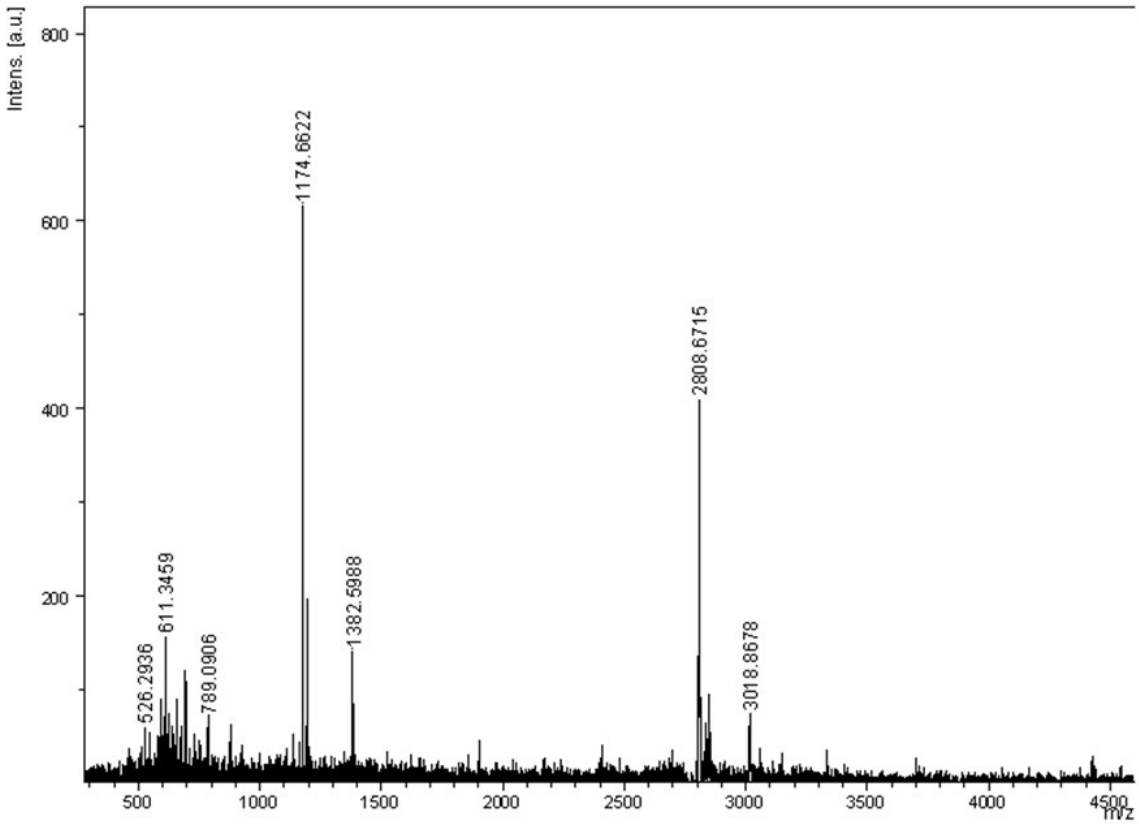

Figure S17. MALDI-TOF spectrum of **Yb-R$_2$**.

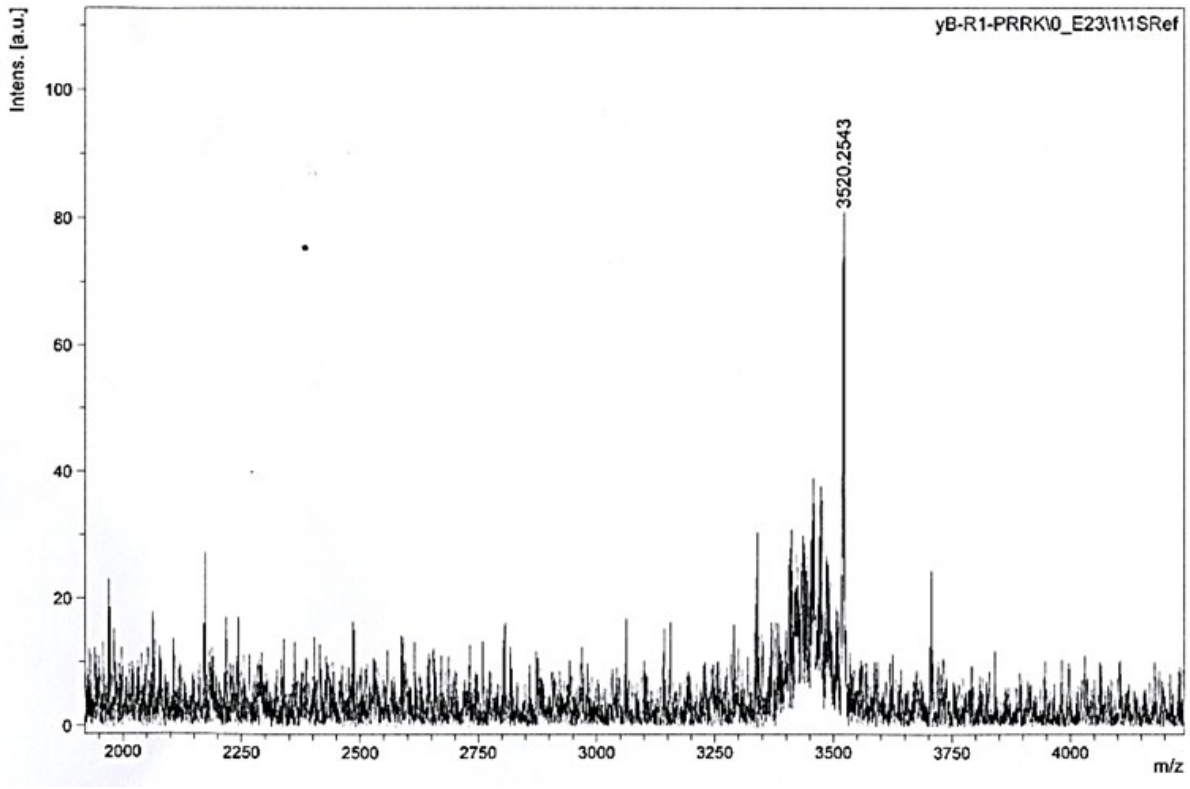

Figure S18. MALDI-TOF spectrum of **Yb-R$_3$**.

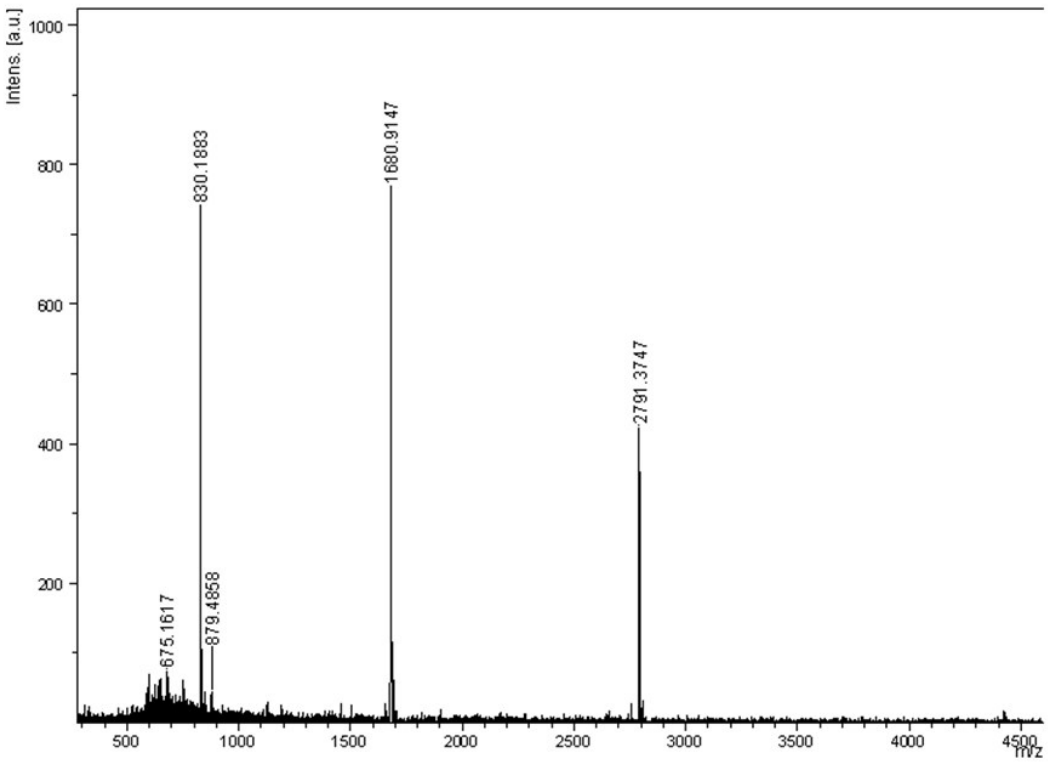

Figure S19. MALDI-TOF spectrum of **Er-R$_1$**

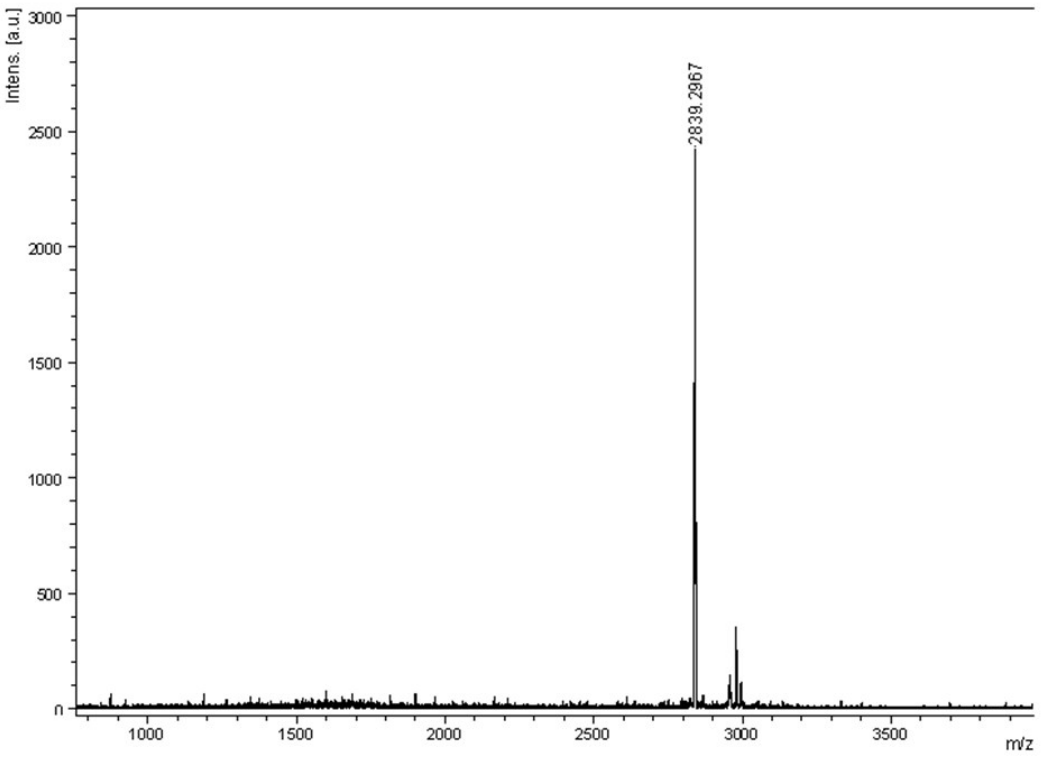

Figure S20. MALDI-TOF spectrum of **Er-R_2**

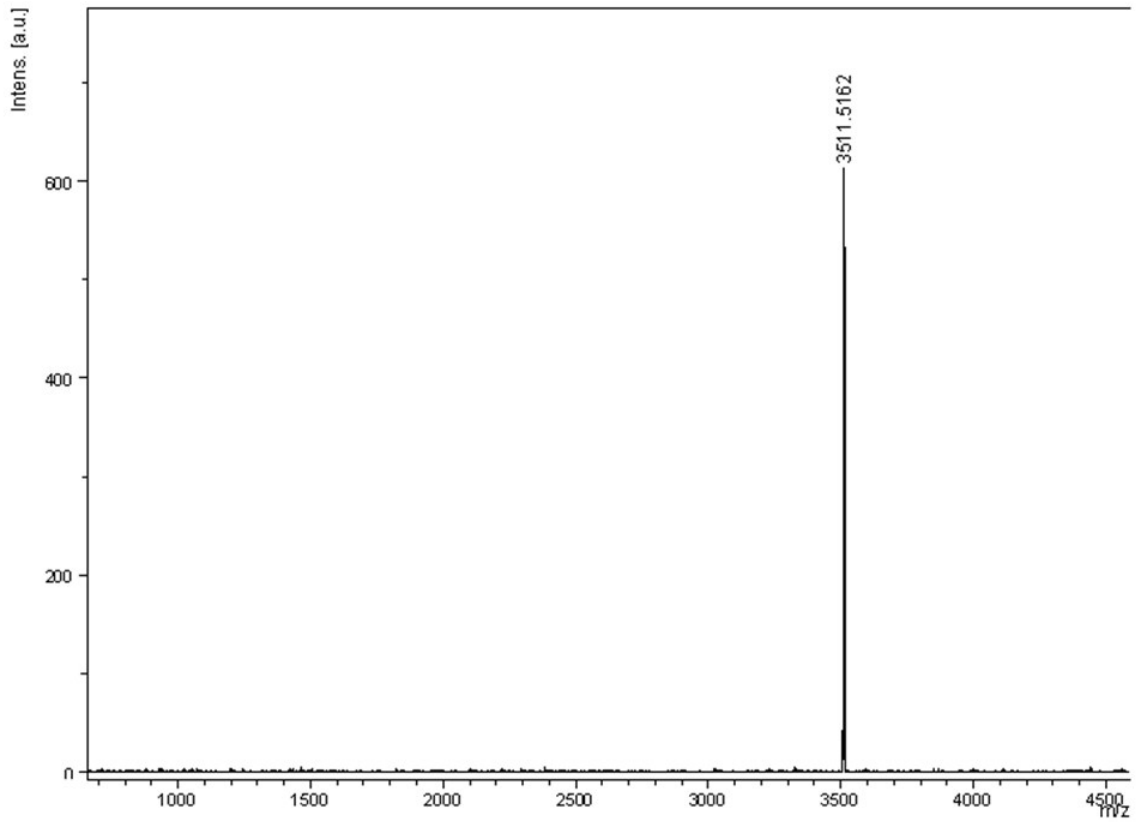

Figure S21. MALDI-TOF spectrum of **Er-R_3**

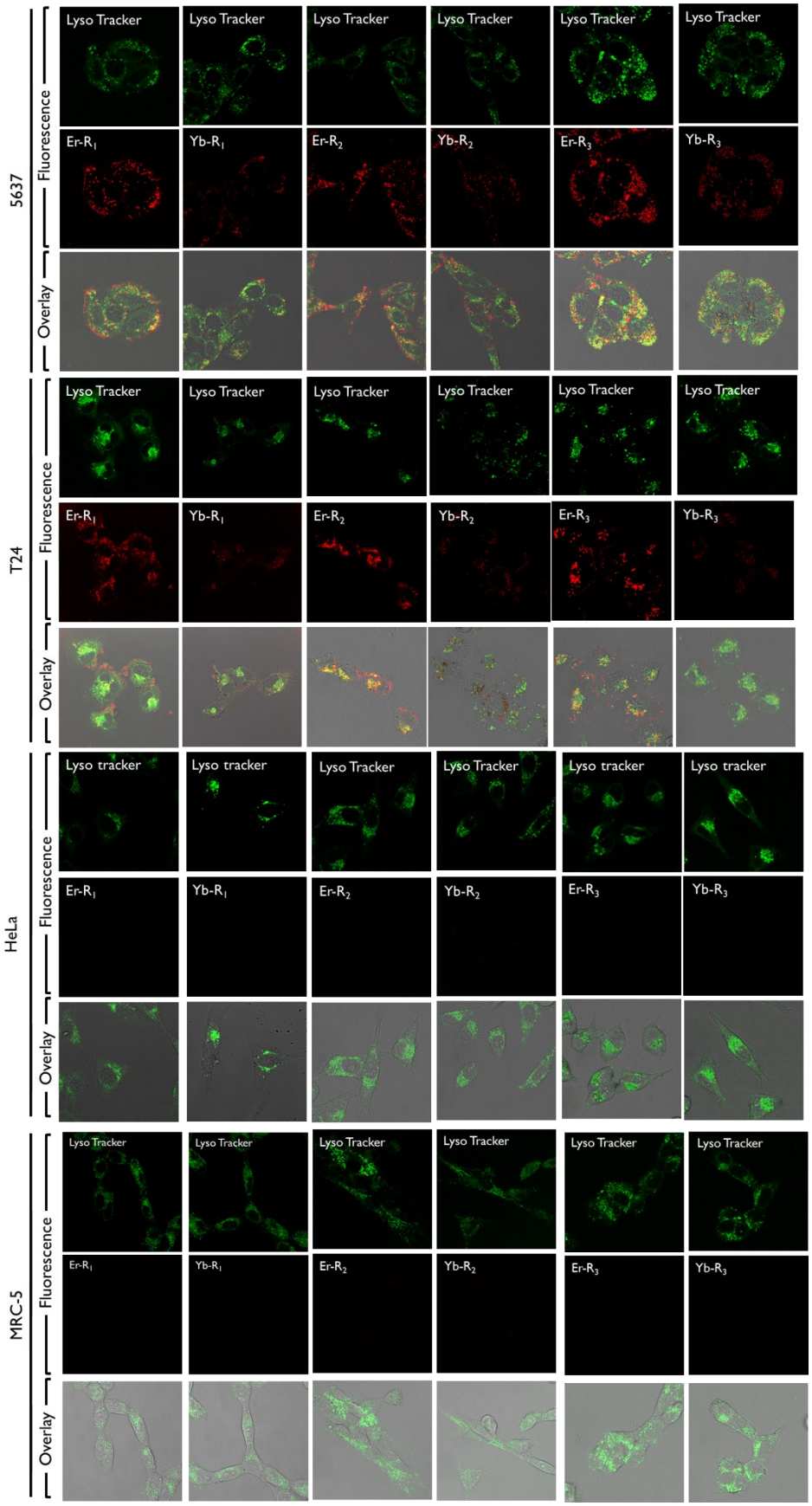

Figure S22. Subcellular localization of **Er-R$_n$** and **Yb-R$_n$** porphyrin complexes by staining with Lyso Tracker green in 5637, T24, HeLa and MRC-5 cells.

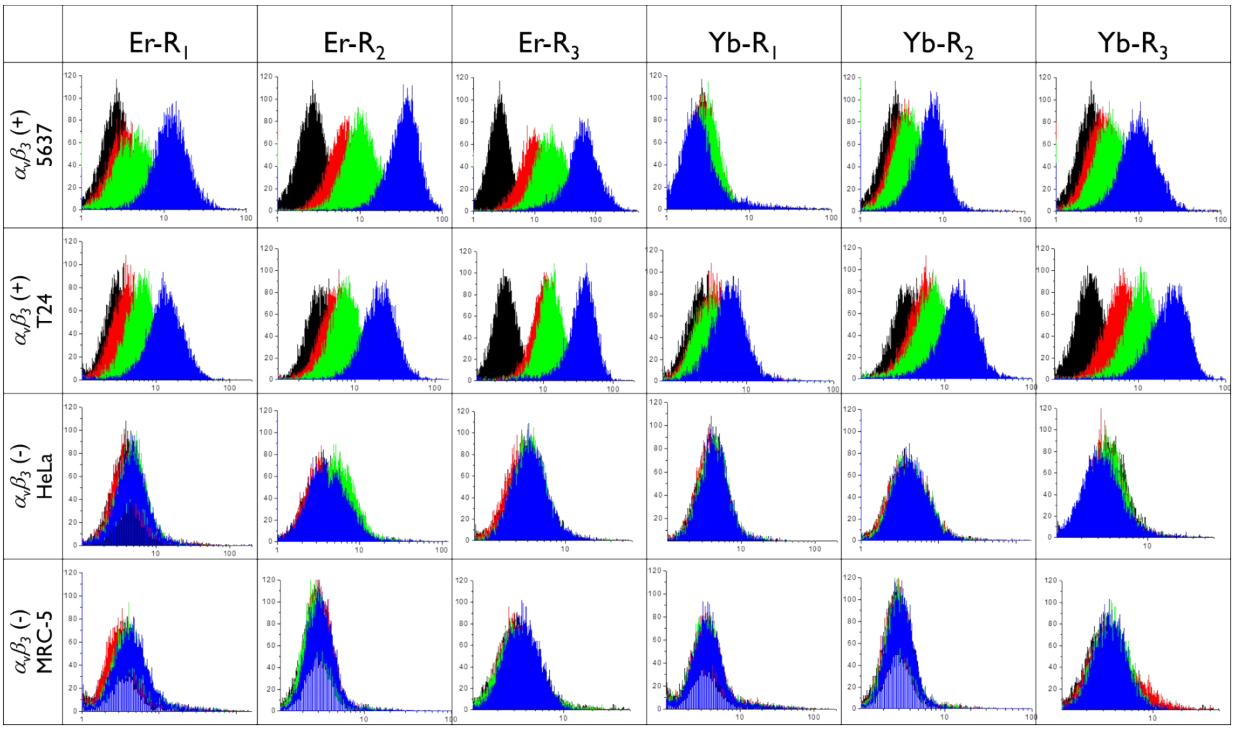

Figure S23. Cellular uptake kinetics analyzed by flow cytometry of **Er-R$_n$** and **Yb-R$_n$** porphyrin complexes in 5637, T24, HeLa and MRC-5 cells incubated for 3 (red histogram), 6 (green histogram), and 24 hours (blue histogram). The y-axis and x-axis are corresponding to cell counts and fluorescence intensity in FL3 channel (wavelength >650 nm) respectively.

Median Fluorescence Intensity	5637	T24	HeLa	MRC-5
Er-R$_1$	12.3	11.80	4.86	4.07
Yb-R$_1$	2.29	6.68	4.31	3.56
Er-R$_2$	35.55	21.21	4.11	3.09
Yb-R$_2$	7.23	14.07	4.21	3.02
Er-R$_3$	64.36	40.52	4.13	3.59
Yb-R$_3$	10	26.07	3.36	3.33

Table S2. Median fluorescence intensity from **Er-R$_n$** and **Yb-R$_n$** porphyrin complexes after incubation for 24 hours in 5637, T24, HeLa and MRC-5 cells.

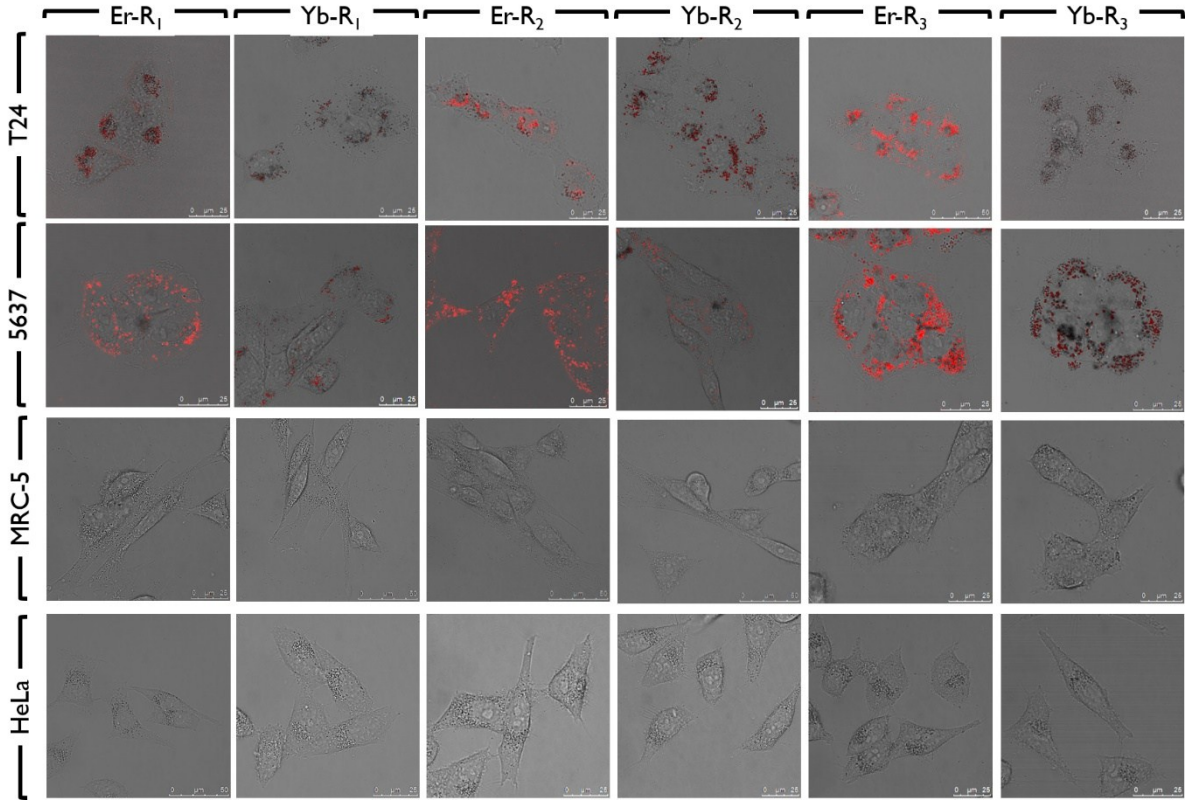

Figure S24. Subcellular localization of **Er-R$_n$** and **Yb-R$_n$** porphyrin complexes in human bladder carcinoma (T24 and 5637) cells, normal lung fibroblast (MRC-5) cells, and Human cervical carcinoma (HeLa) cells.