

Luminescent Iridium Complex-Peptide Hybrids (IPHs)  
for Therapeutics of Cancer: Design and Synthesis of  
IPHs for Detection of Cancer Cells and Induction of  
Their Necrosis-type Cell Death

*Abdullah-Al Masum,<sup>a</sup> Yosuke Hisamatsu,<sup>a</sup> Kenta Yokoi,<sup>a</sup> and Shin Aoki<sup>\*a,b</sup>*

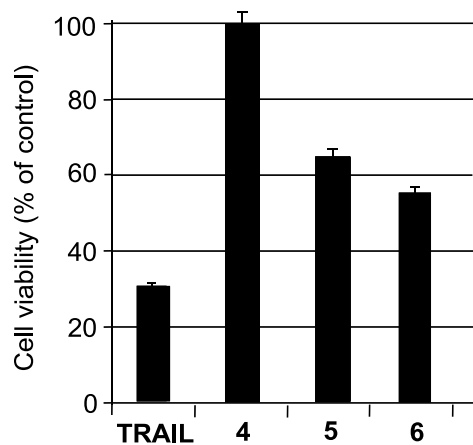
<sup>a</sup> Faculty of Pharmaceutical Sciences, Tokyo University of Science,  
2641 Yamazaki, Noda, Chiba 278-8510, Japan,

<sup>b</sup> Imaging Frontier Center, Tokyo University of Science  
2641 Yamazaki, Noda, Chiba 278-8510, Japan

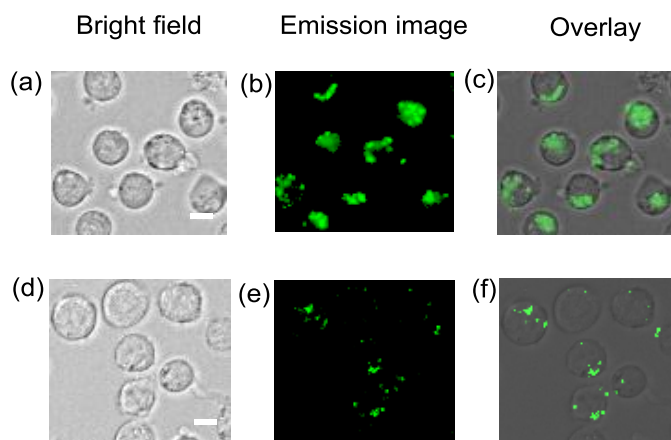
\*Corresponding author: E-mail, [shinaoki@rs.noda.tus.ac.jp](mailto:shinaoki@rs.noda.tus.ac.jp)

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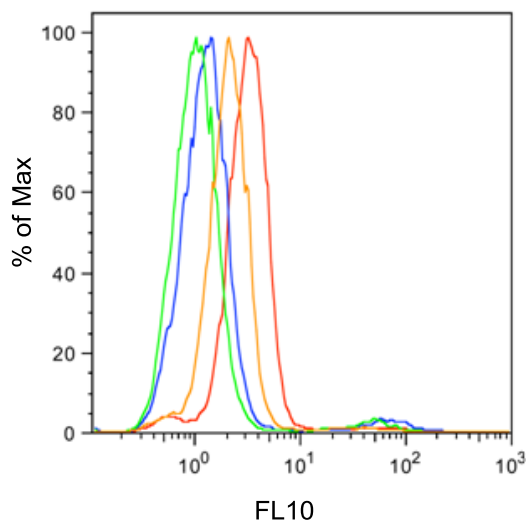
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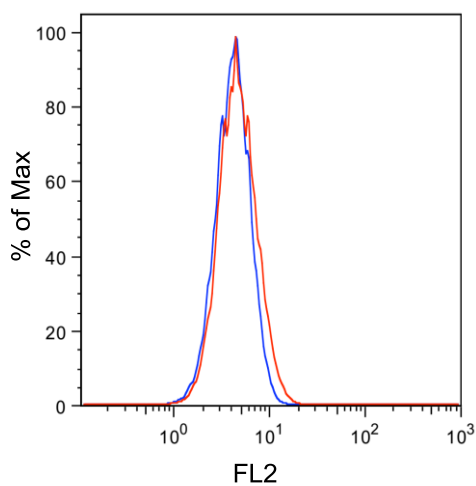
**Figure S1.** The results of MTT assay: cell viability of Jurkat cells (% of control at [Ir complex] = 0  $\mu\text{M}$ ) in presence of TRAIL (150 ng/mL), **4**, **5** and **6** (75  $\mu\text{M}$ ) after incubation at 37 °C for 16 h.



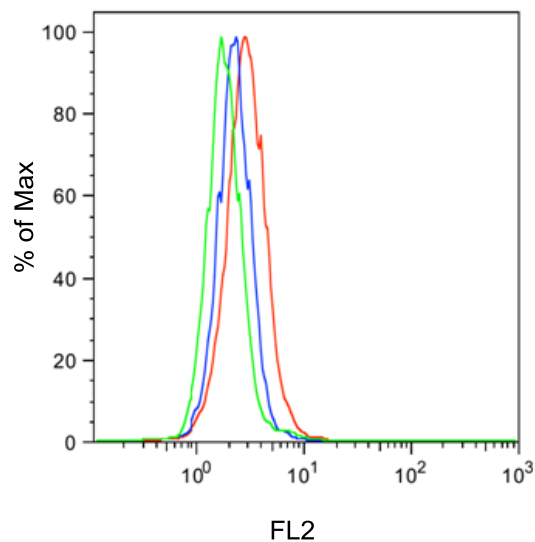
**Figure S2.** The results of competitive staining of Jurkat cells with **5** and **CP1** (luminescence microscopy images on Biorevo, BZ-9000, Keyence, x 40). (a-c) Jurkat cells were incubated with **5** (10  $\mu\text{M}$ ) at 37°C for 1 h. (d-f) Jurkat cells were incubated with **CP1** (100  $\mu\text{M}$ ) at 37°C for 1 h and then with **5** (10  $\mu\text{M}$ ) at 37°C for 1 h. Scale bar (white) = 10  $\mu\text{m}$ .



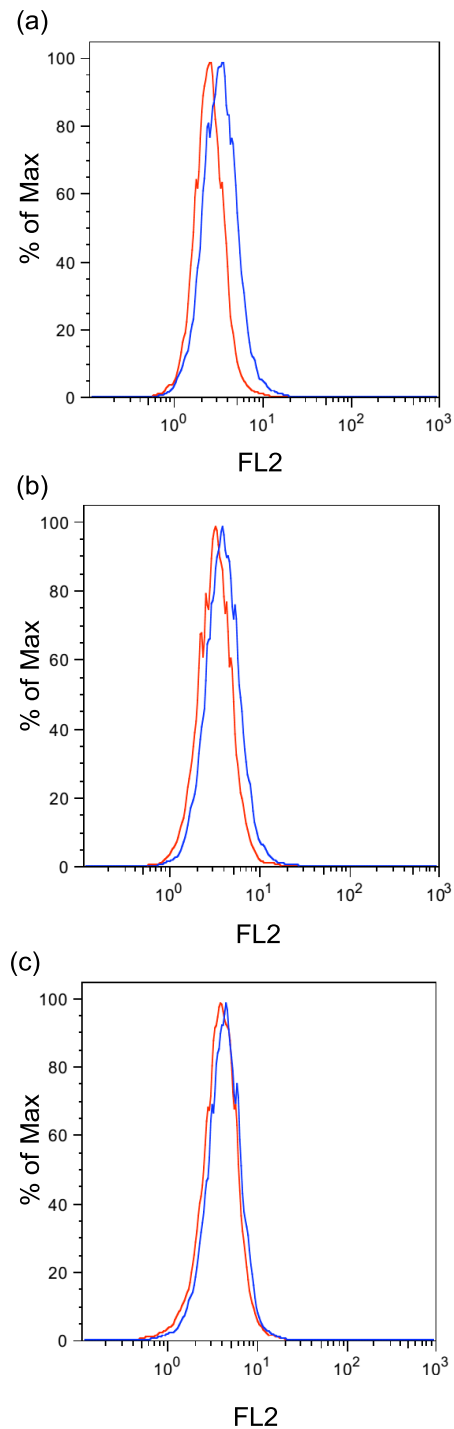
**Figure S3.** Flow cytometry analysis of competitive staining of Jurkat cells with **5** and **CP1**. The red histogram corresponds to Jurkat cells treated with **5** (5  $\mu$ M) at 37 °C for 1 h, the orange histogram corresponds to Jurkat cells treated with **CP1** (100  $\mu$ M) at 37 °C for 1 h and then **5** (5  $\mu$ M) at 37 °C for 1 h, the blue histogram corresponds to Jurkat cells treated with **5** (2  $\mu$ M) at 37 °C for 1 h and the green histogram corresponds to the cells treated with **CP1** (100  $\mu$ M) at 37 °C for 1 h and then **5** (2  $\mu$ M) at 37 °C for 1 h.



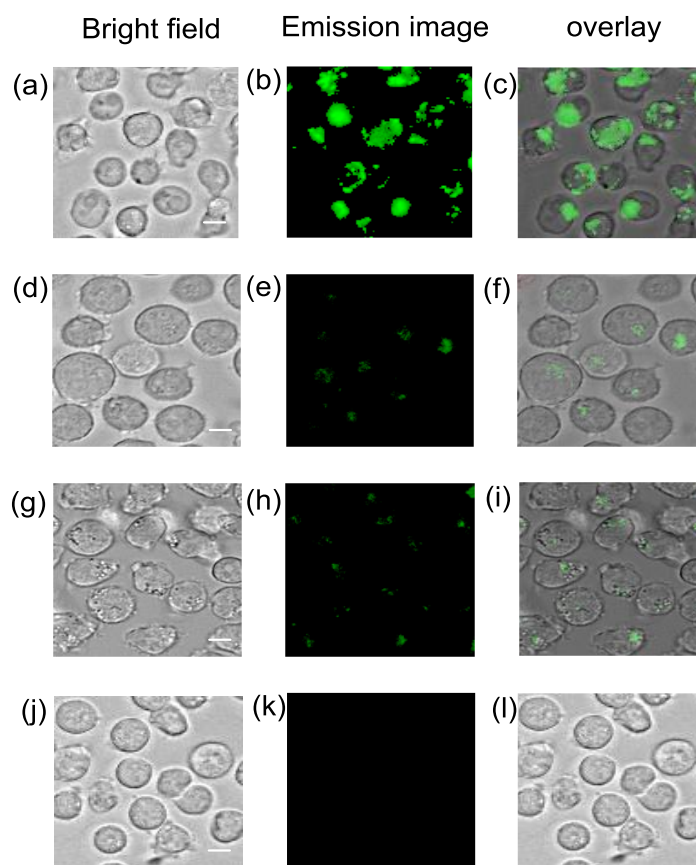
**Figure S4.** Flow cytometry analysis of co-staining assay of anti-DR5 antibody and **5**. The blue histogram corresponds to the cells treated with anti-DR5 antibody (15  $\mu$ g/mL) at 4 °C for 15 min, and the red histogram corresponds to the cells treated with anti-DR5 antibody (15  $\mu$ g/mL) at 4 °C for 15 min and then with **5** (5  $\mu$ M) at 37 °C for 1 h.



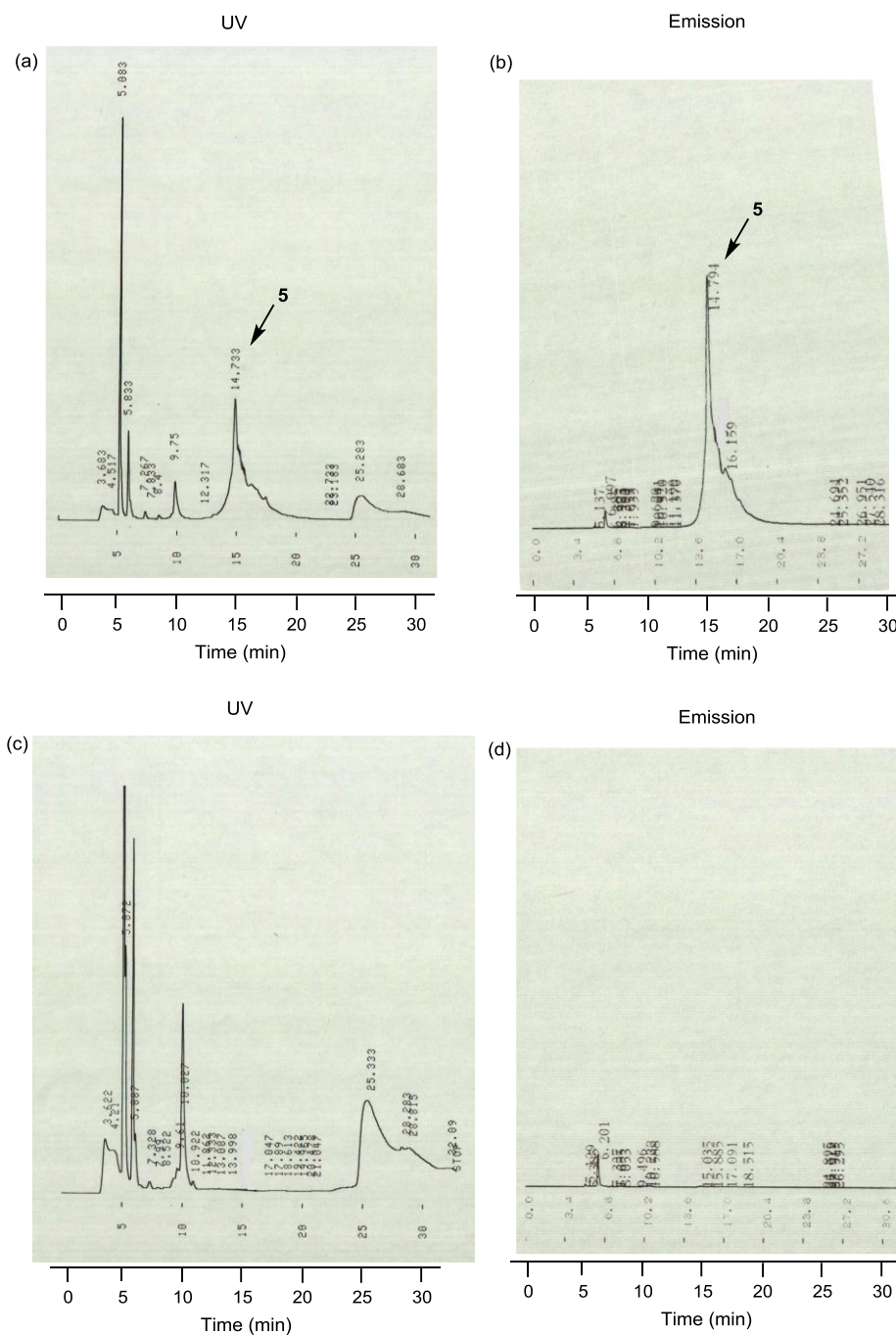
**Figure S5.** Flow cytometry analysis of co-staining of Jurkat cells with **5** and anti-DR5 antibody. The red histogram corresponds to the cells treated with only anti-DR5 antibody (15  $\mu\text{g/mL}$ ) at 4  $^{\circ}\text{C}$  for 15 min (Method a of Chart 5 in text), the blue histogram corresponds to the cells treated with **5** (5  $\mu\text{M}$ ) at 37  $^{\circ}\text{C}$  for 1 h and then anti-DR5 antibody (15  $\mu\text{g/mL}$ ) at 4  $^{\circ}\text{C}$  for 15 min (Method c of Chart 5 in text), and the green histogram corresponds to the cells treated with **5** (10  $\mu\text{M}$ ) at 37  $^{\circ}\text{C}$  for 1 h and then anti-DR5 antibody (15  $\mu\text{g/mL}$ ) at 4  $^{\circ}\text{C}$  for 15 min (Method c of Chart 5 in text).



**Figure S6.** Flow cytometry analysis of Jurkat cells incubated with **5** ( $5 \mu\text{M}$ ) and anti-DR5 antibody ( $15 \mu\text{g/mL}$ ). Red histograms correspond to the cells treated with **5** and anti-DR5 antibody, blue histograms correspond to the cells treated with only anti-DR5 antibody. (a) after 1 h incubation with **5** (Method c in Chart 5) (b) after 1 h incubation with **5** and then again 1 h incubation in fresh medium (Method d in Chart 5). (c) after 1 h incubation with **5** and then again 6 h incubation in fresh medium (Method d in Chart 5).

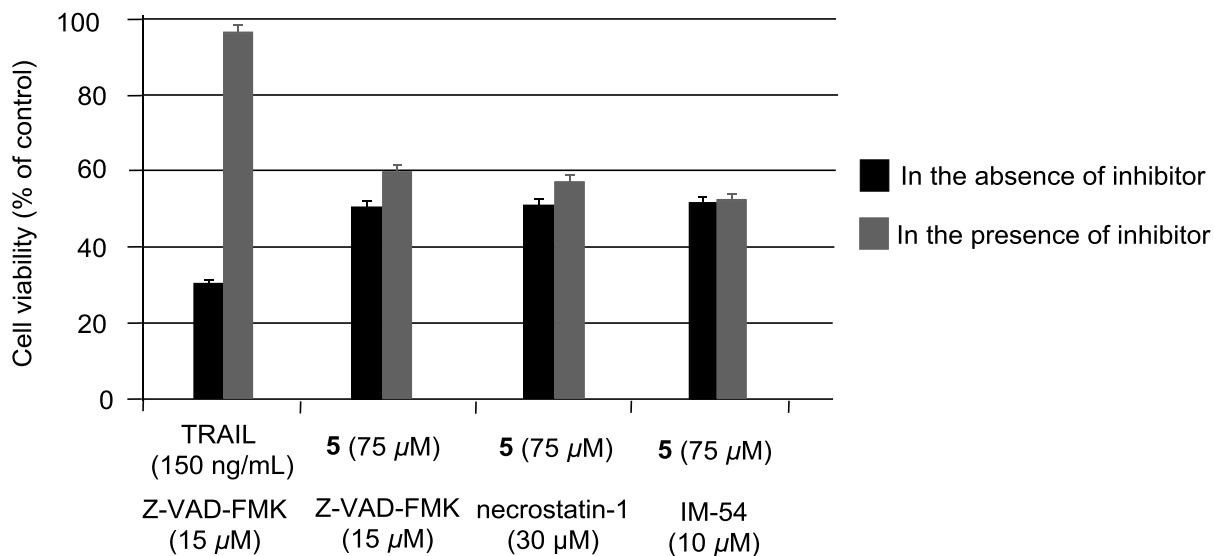


**Figure S7.** Luminescence microscopy images (Biorevo, BZ-9000, Keyence) of Jurkat cells, K562 cells and Molt-4 cells (x 40) stained with **5**. (a-c) Jurkat cells were incubated with **5** (10  $\mu$ M) at 37°C for 1 h. (d-f) K562 cells were incubated with **5** (10  $\mu$ M) at 37°C for 1 h. (g-i) Molt-4 cells were incubated with **5** (10  $\mu$ M) at 37°C for 1 h. (j-l) Jurkat cells were incubated with **9** (5  $\mu$ M) at 37°C for 1 h. Scale bar (white) = 10  $\mu$ m.

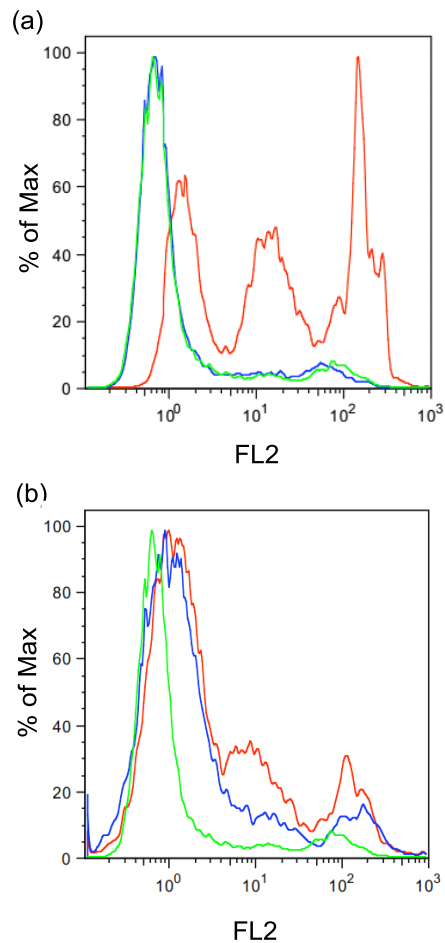


**Figure S8.** HPLC charts of (a,b) **5** after incubation at 37 °C in RPMI 1640 (MTG free) medium for 24 h. (c,d) only RPMI 1640 (MTG free) medium (without **5**) after incubation at 37 °C for 24 h. (Senshu Pak PEGASIL ODS 4.6  $\phi$  X 250 mm UV: 254 nm, Excitation: 366 nm, Emission: 520 nm, flow rate 1.0 mL/min with continuous gradient elution (20-50% solvent B, 0-30 min, linear) with solvent A (0.1% TFA in H<sub>2</sub>O) and solvent B (0.1% TFA in MeCN).

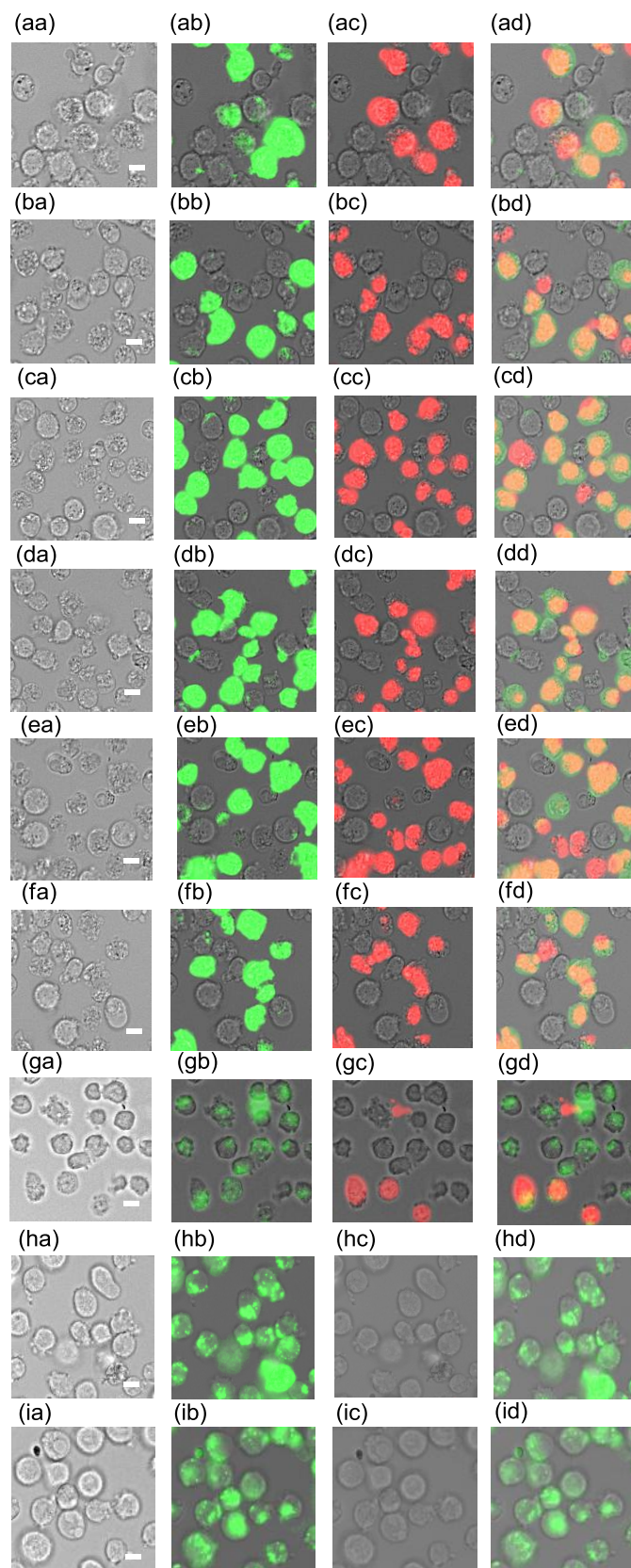




**Figure S9.** The results of MTT assay of TRAIL (150 ng/mL) and **5** (75 μM) in the presence and the absence of caspase inhibitor (Z-VAD-FMK), necroptosis inhibitor (Necrostatin-1) and oxidative stress induced necrosis inhibitor (IM-54): cell viability of Jurkat cells (% of control) after incubation at 37 °C for 24 h.

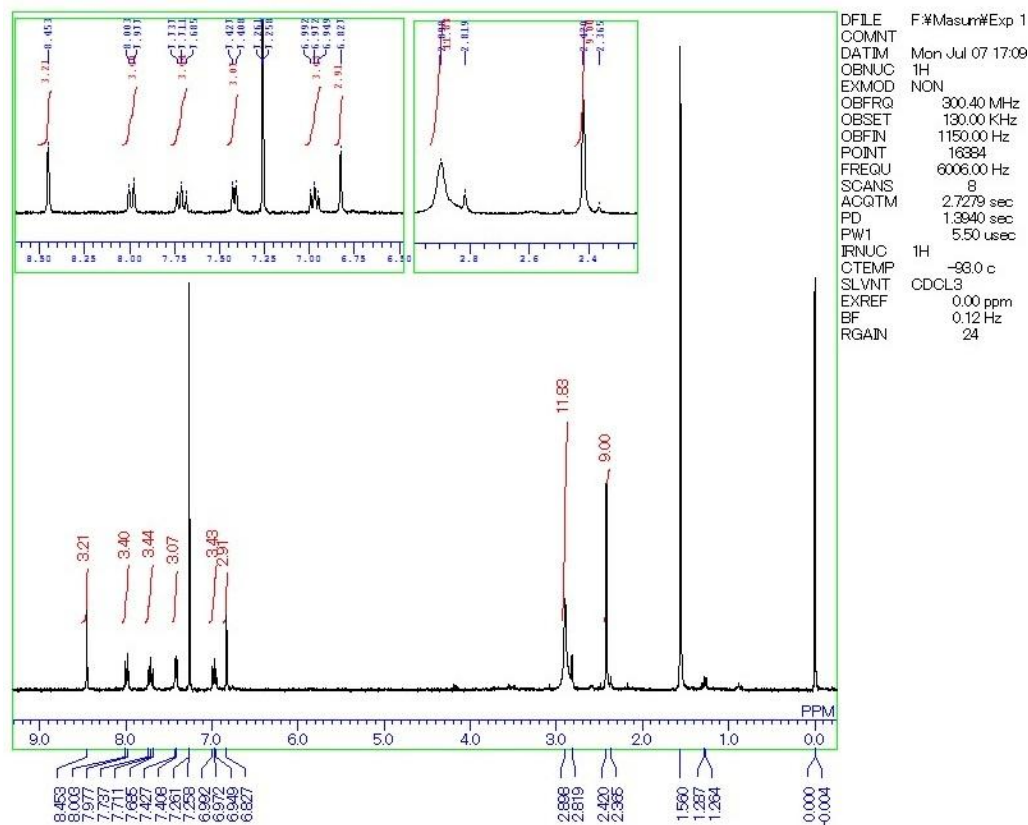


**Figure S10.** The results of flow cytometry assay of (a) TRAIL (150 ng/mL) and (b) **5** (75  $\mu$ M). The red histograms correspond to the Jurkat cells treated with TRAIL (150 ng/mL) or **5** (75  $\mu$ M) at 37 °C for 24 h and then with PI (30  $\mu$ M) at room temperature for 10-15 min. The blue histograms correspond to the Jurkat cells treated with caspase inhibitor (Z-VAD-FMK) (15  $\mu$ M) at 37 °C for 1h and then TRAIL (150 ng/mL) or **5** (75  $\mu$ M) at 37 °C for 24 h followed by the treatment with PI (30  $\mu$ M) at room temperature for 10-15 min. The green histograms correspond to the Jurkat cells not treated with TRAIL or **5**.

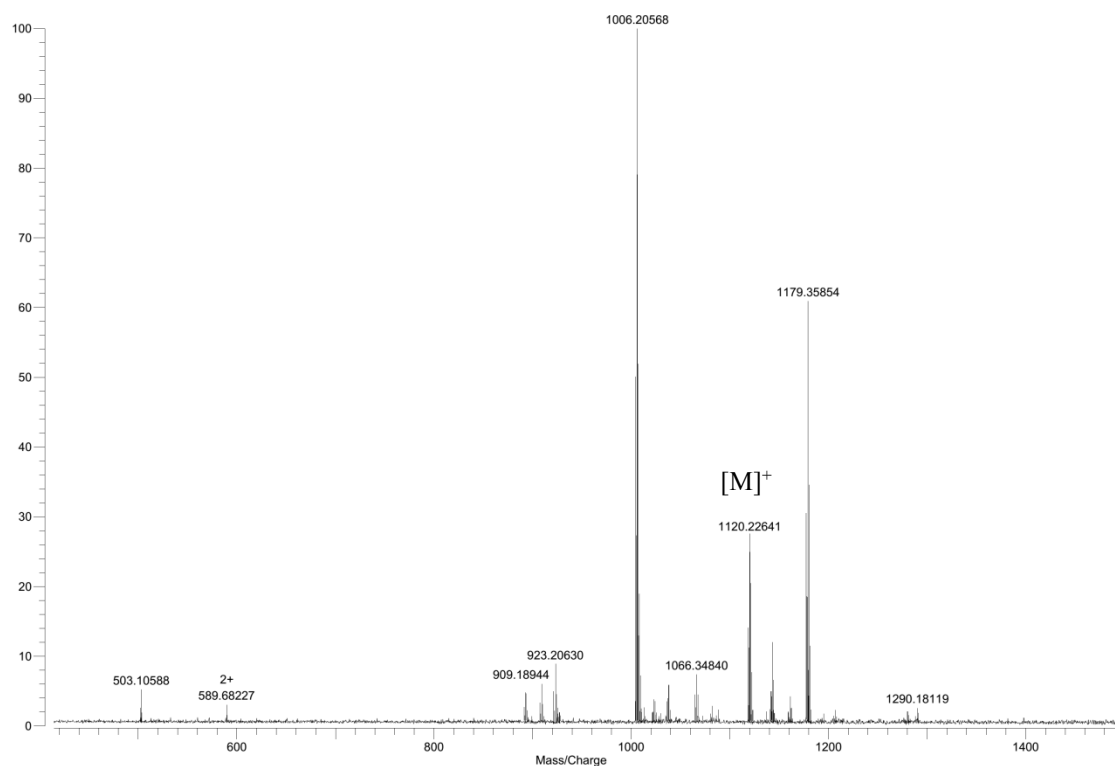


**Figure S11.** Luminescent microscopy images (Biorevo, BZ-9000, Keyence) of Jurkat cells (x 40) incubated with **5** ( $35 \mu\text{M}$ ) in presence of (aa-ad) 4-aminopyridine ( $10 \mu\text{M}$ ), (ba-bd) amiloride ( $100 \mu\text{M}$ ), (ca-cd) bafilomycin A1 ( $10 \mu\text{M}$ ), (da-dd) CCCP ( $40 \mu\text{M}$ ), (ea-ed) chloroquinine ( $10 \mu\text{M}$ ), (fa-fd)

phentolamine (10  $\mu\text{M}$ ), (ga-gd) quinidine (100  $\mu\text{M}$ ), (ha-hd) nicardipine (50  $\mu\text{M}$ ), and (ia-id) verapamil (20  $\mu\text{M}$ ) at 37  $^{\circ}\text{C}$  for 24 h. Scale bar (white) = 10  $\mu\text{m}$ .



**Figure S12:**  $^1\text{H}$  NMR chart of NHS ester of Ir complex **7**



**Figure S13:** ESI mass chart of NHS ester of Ir complex **7**

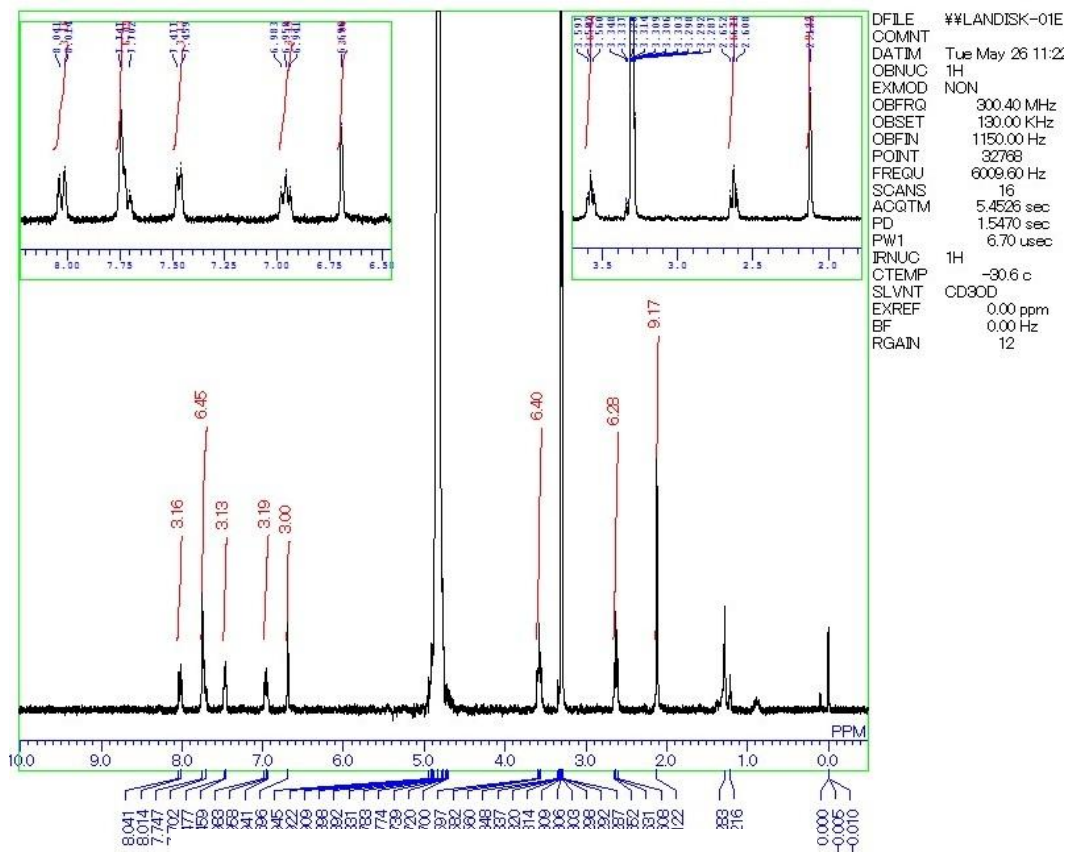


Figure S14:  $^1\text{H}$  NMR chart of Ir complex 8

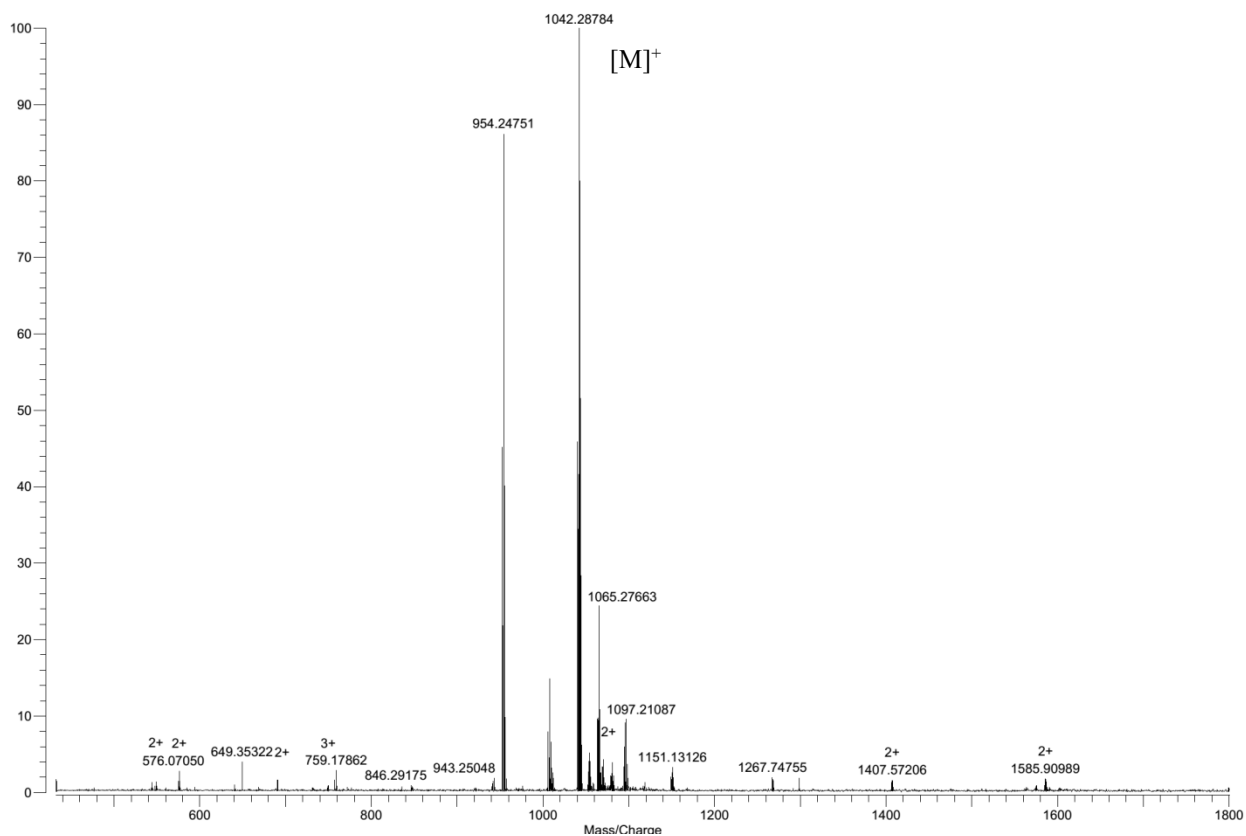


Figure S15: ESI mass chart of Ir complex 8

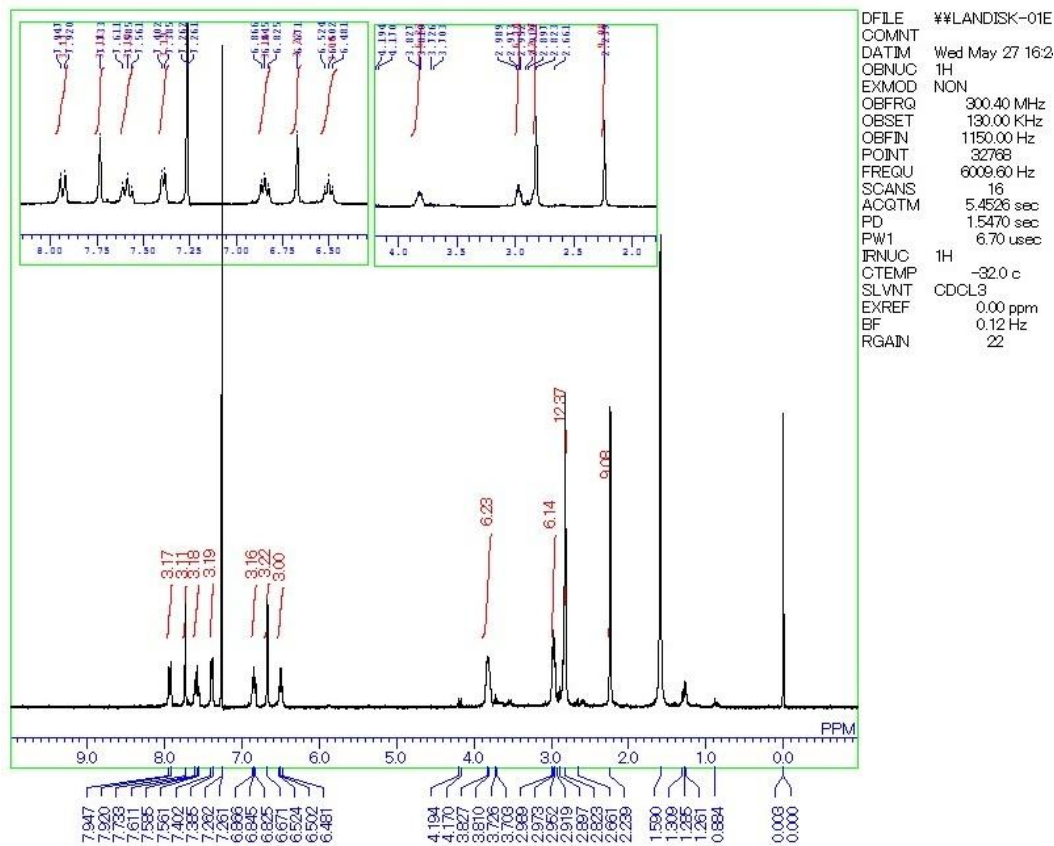


Figure S16:  $^1\text{H}$  NMR chart of NHS ester of Ir complex **8**

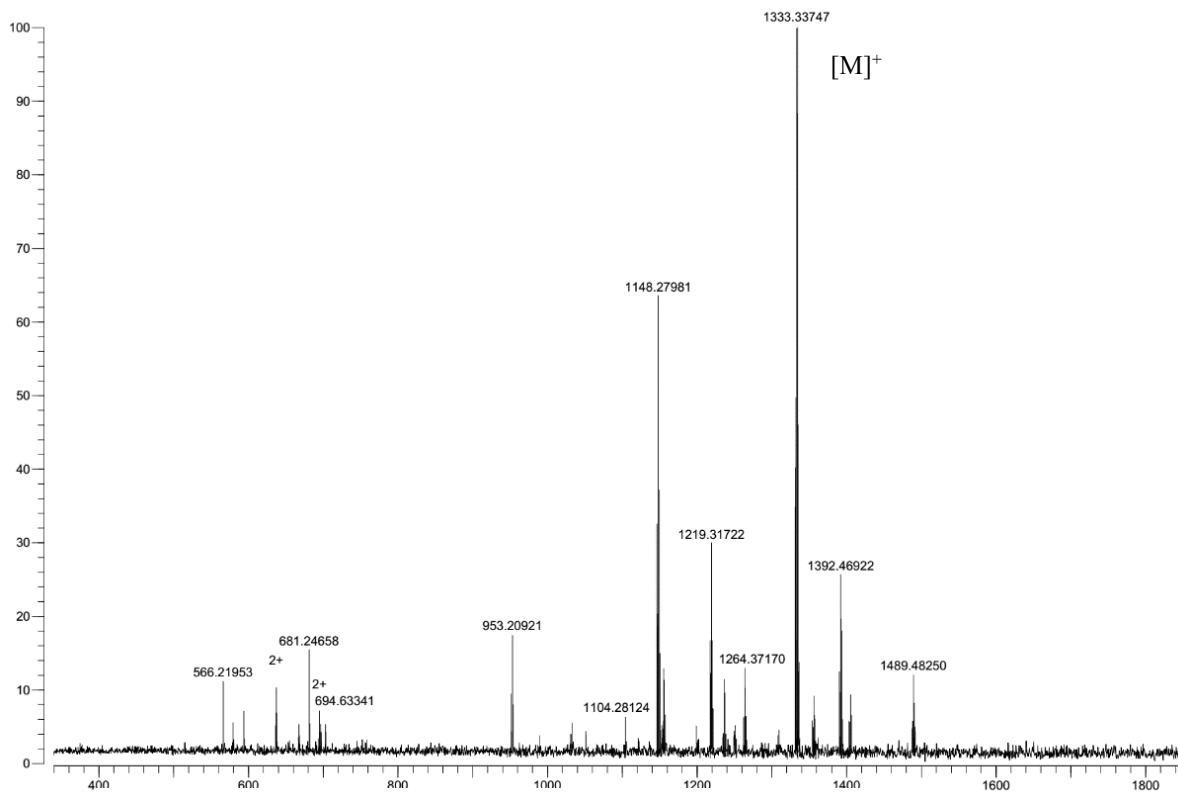
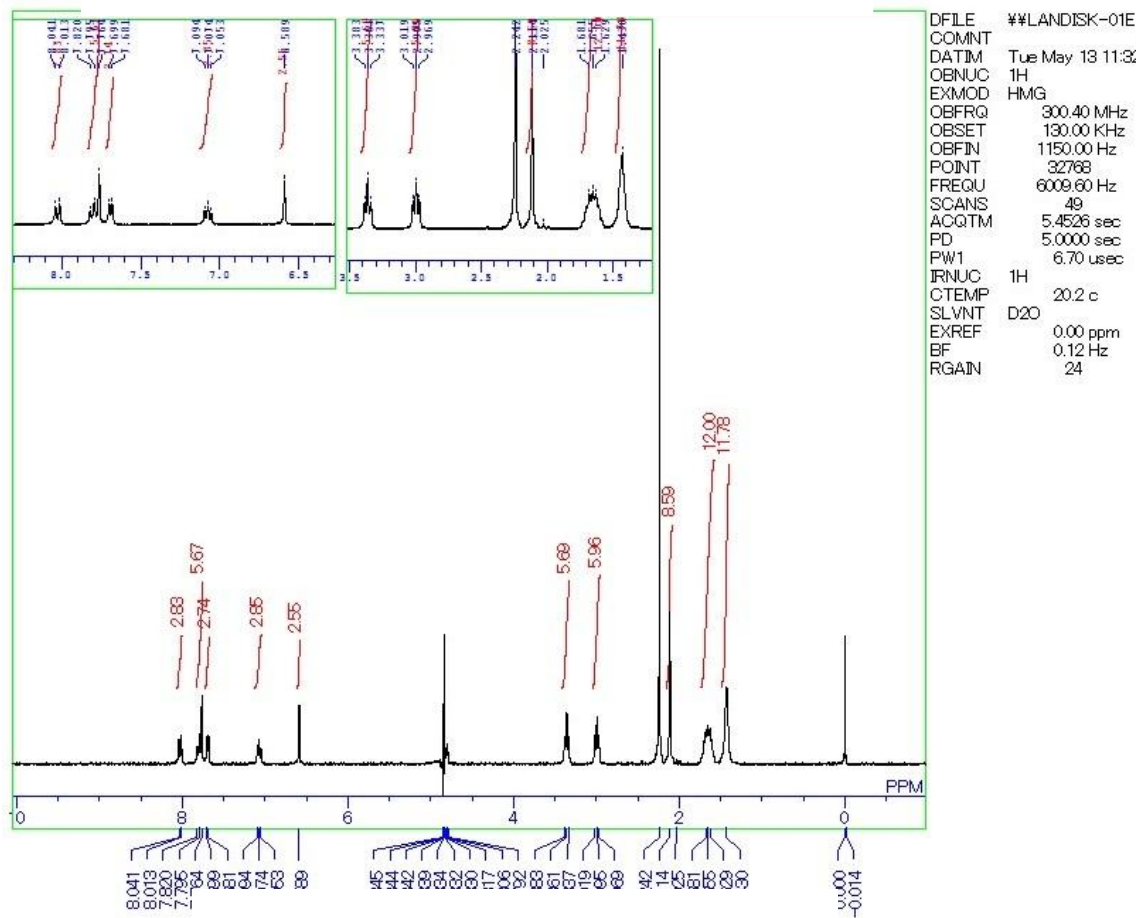
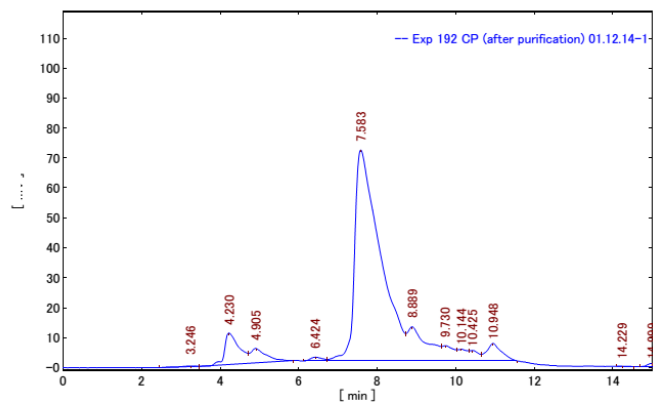


Figure S17: ESI mass chart of NHS ester of Ir complex **8**

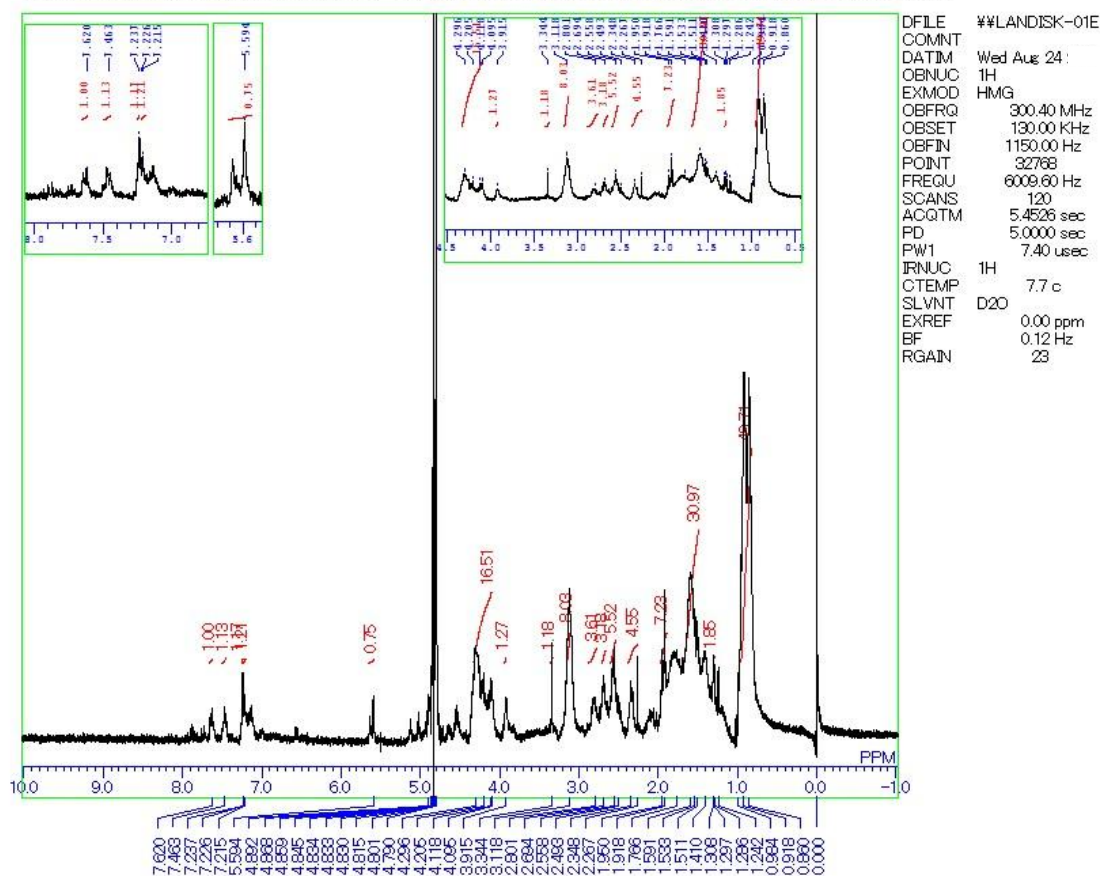




**Figure S20: HPLC analysis of CPI**

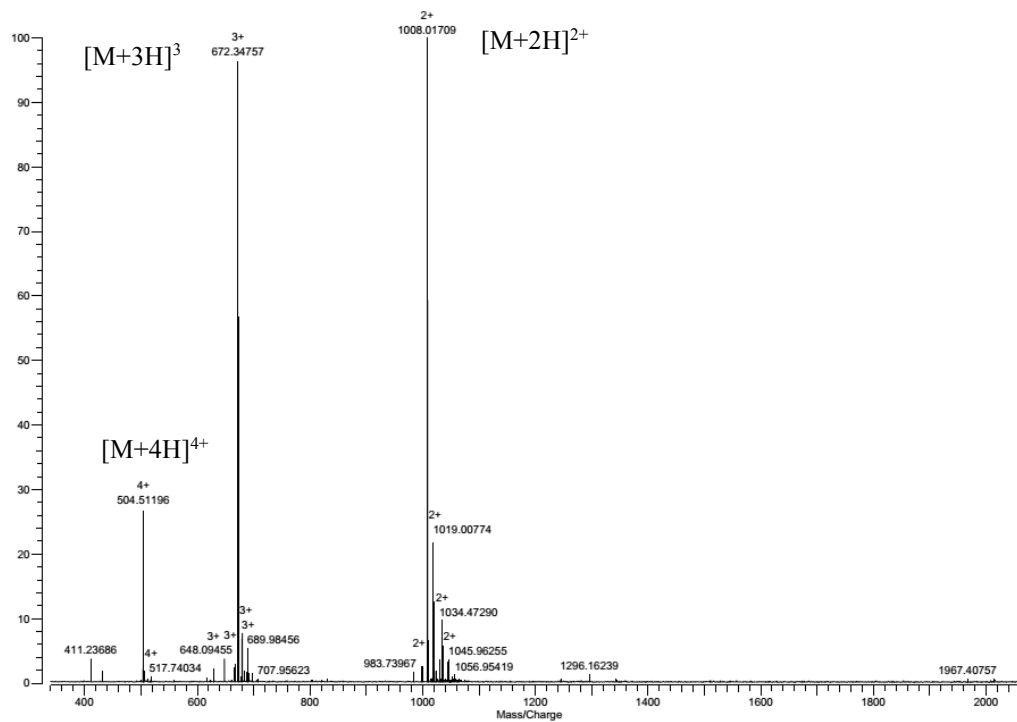
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 UV: 280 nm, flow rate 1.0 mL/min with  
 continuous gradient elution (20-50% solvent B,  
 0-30 min, linear) with solvent A (0.1% TFA in  
 H<sub>2</sub>O) and solvent B (0.1% TFA in MeCN).

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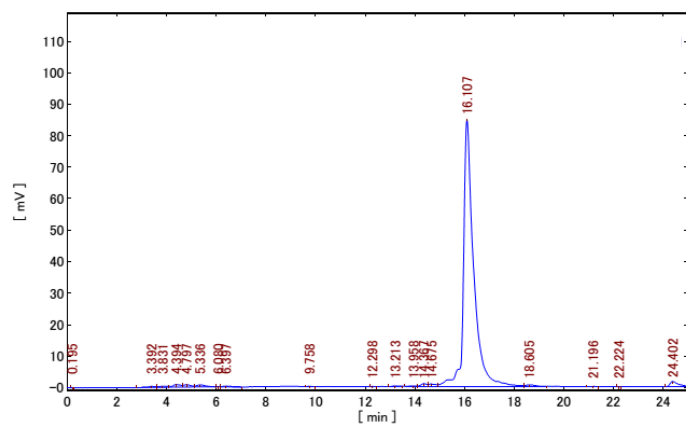


**Figure S21: <sup>1</sup>H NMR chart of CPI**





**Figure S22: ESI Mass chart of CP1**



Senshu Pak PEGASIL ODS 4.6  $\phi$  X 250 mm  
 UV: 280 nm, flow rate 1.0 mL/min with  
 continuous gradient elution (10-40% solvent B,  
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 H<sub>2</sub>O) and solvent B (0.1% TFA in MeCN).

**Figure S23: HPLC analysis of CP2**

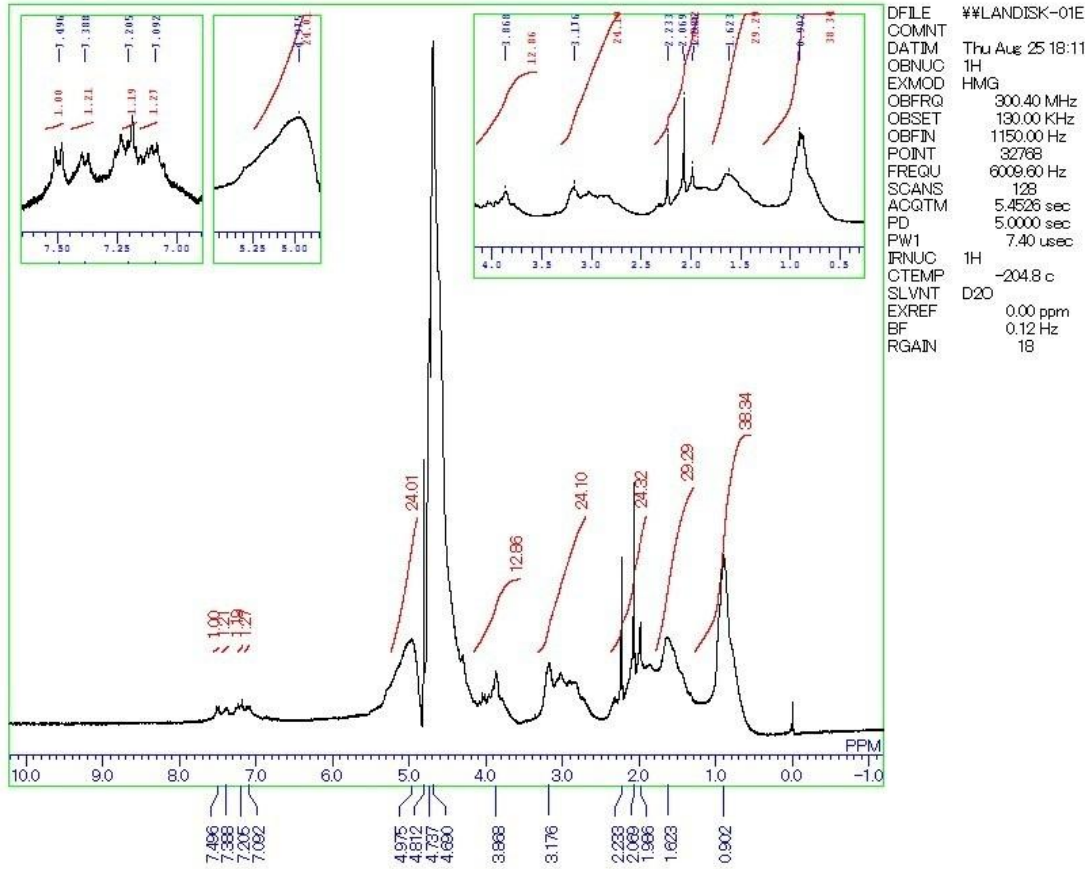


Figure S24: <sup>1</sup>H NMR chart of CP2

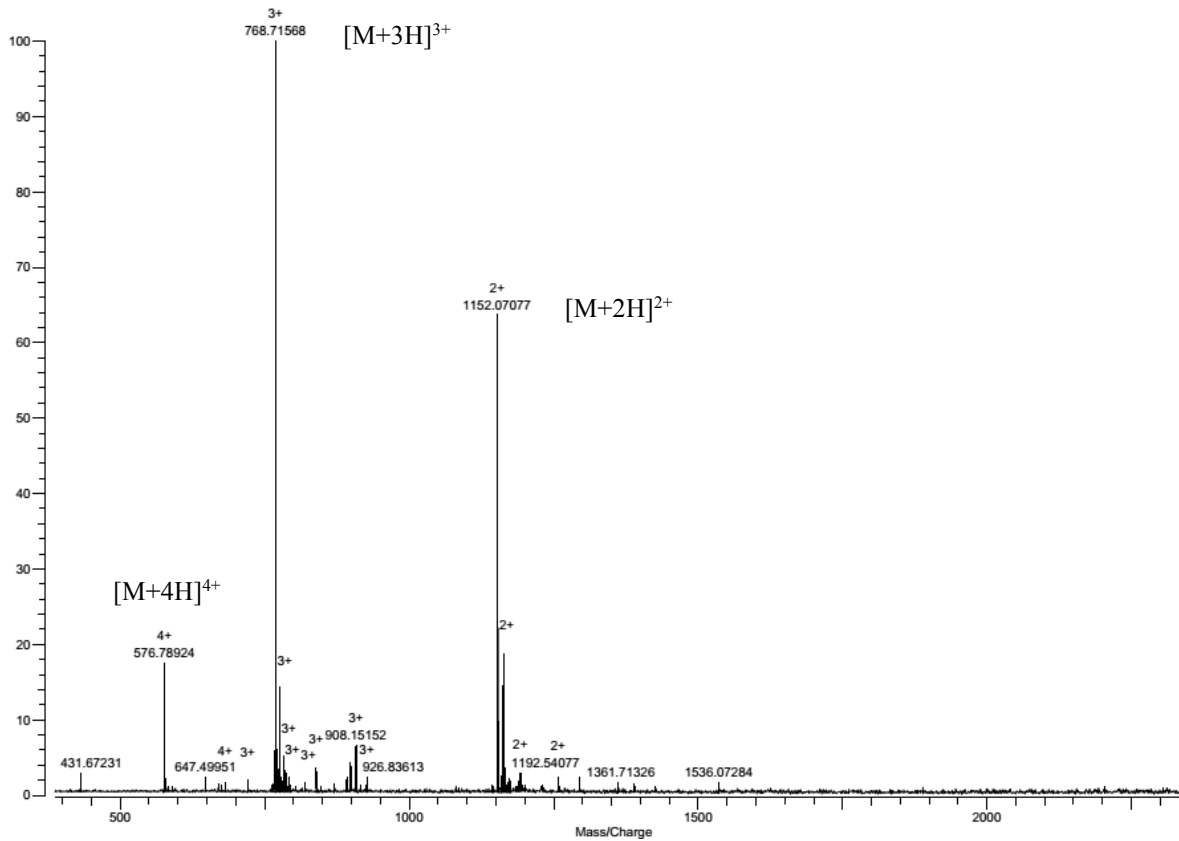
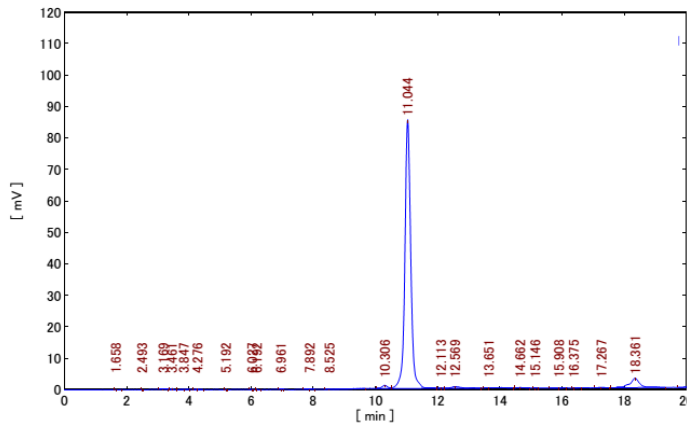


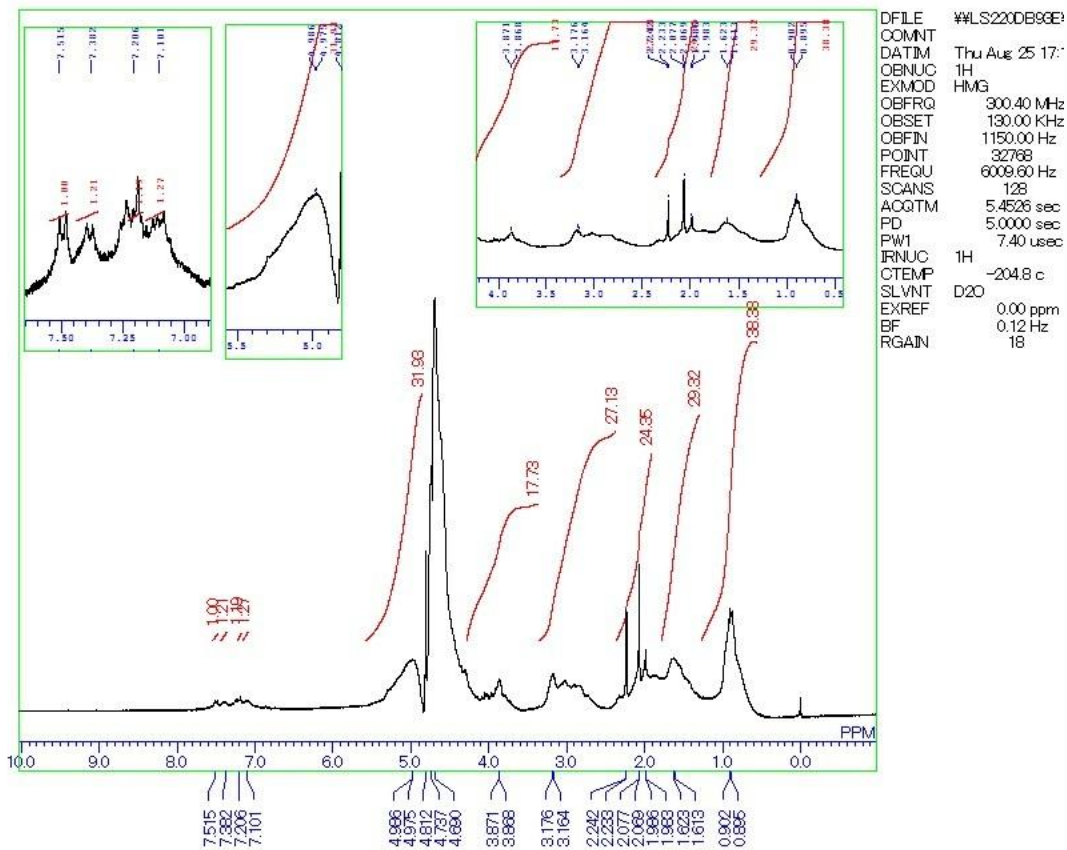
Figure S25: ESI Mass chart of CP2



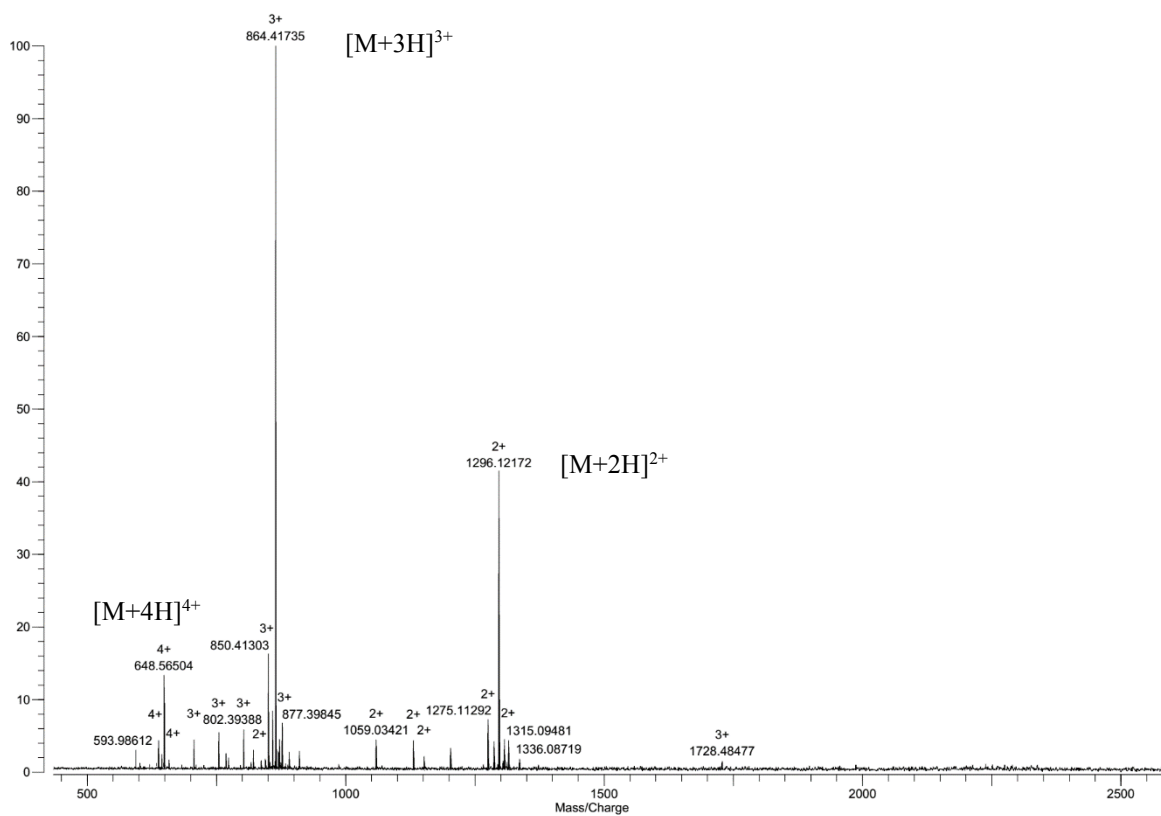
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 UV: 280 nm, flow rate 1.0 mL/min with  
 continuous gradient elution (10-40% solvent  
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**Figure S26: HPLC analysis of CP3**

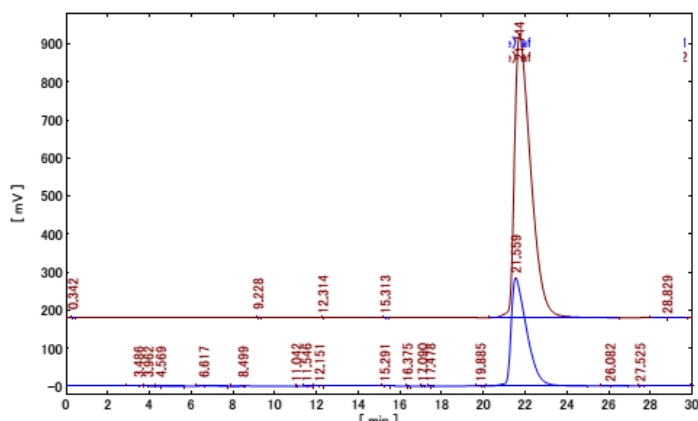
##LS220DB93E#share#LAN DISC#Students' Data(学生データ)#Abdullah-AI MASUM#NMR data#Cyclic Peptide 8.als



**Figure S27: <sup>1</sup>H NMR chart of CP3**



**Figure S28:** ESI Mass chart of CP3



Senshu Pak PEGASIL ODS 4.6  $\phi$  X 250 mm  
 UV: 254 nm, Ex.: 366 nm, Em.: 520 nm  
 flow rate 1.0 mL/min with continuous  
 gradient elution (20-50% solvent B, 0-30  
 min, linear) with solvent A (0.1% TFA in  
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**Figure S29:** HPLC analysis of Ir complex-peptide hybrid 4

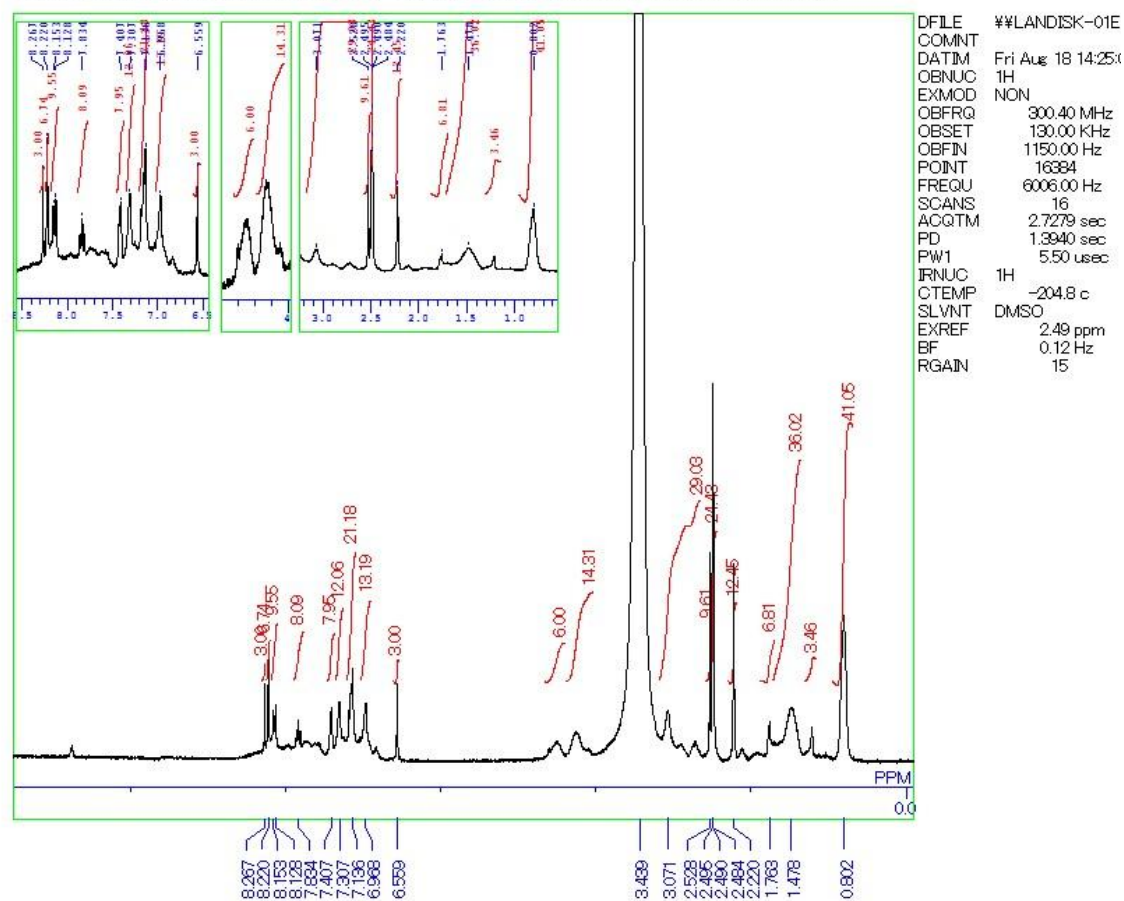


Figure S30:  $^1\text{H}$  NMR chart of Ir complex-peptide hybrid **4**

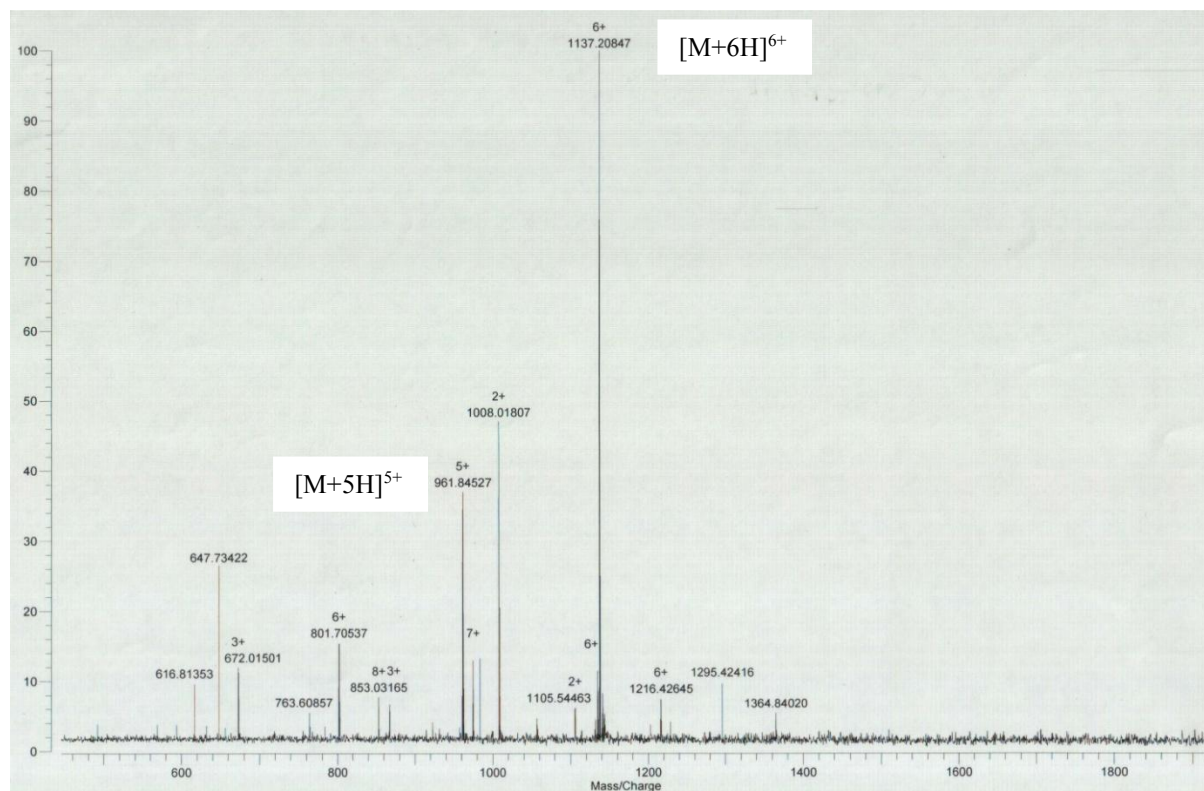
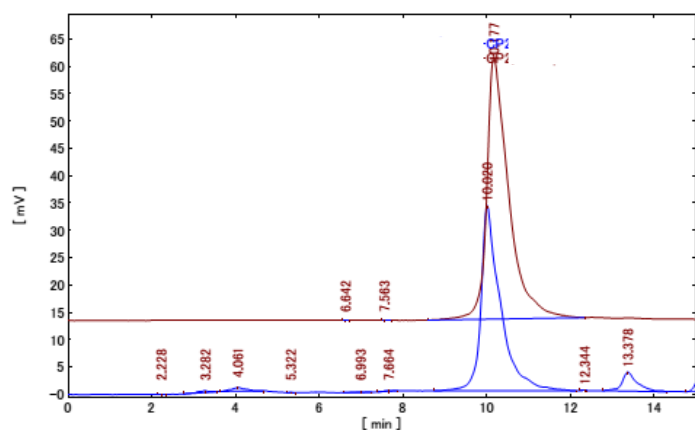
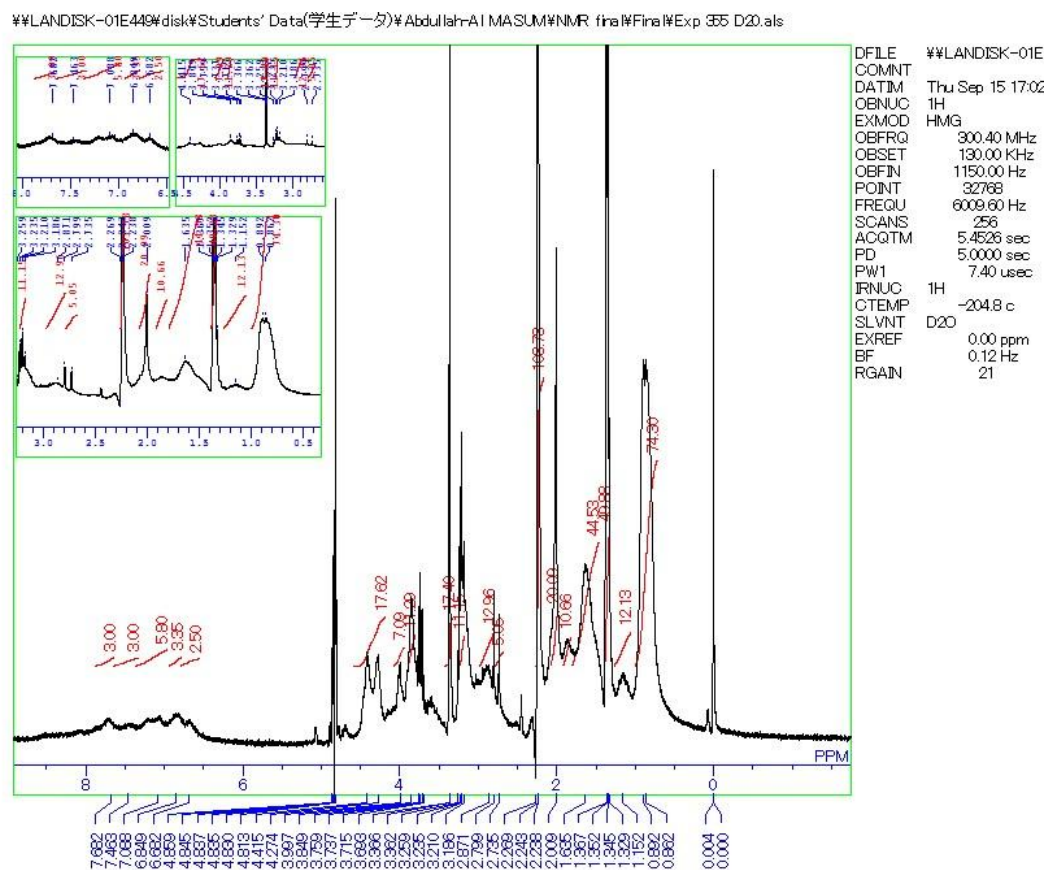


Figure S31: ESI Mass chart of Ir complex-peptide hybrid **4**

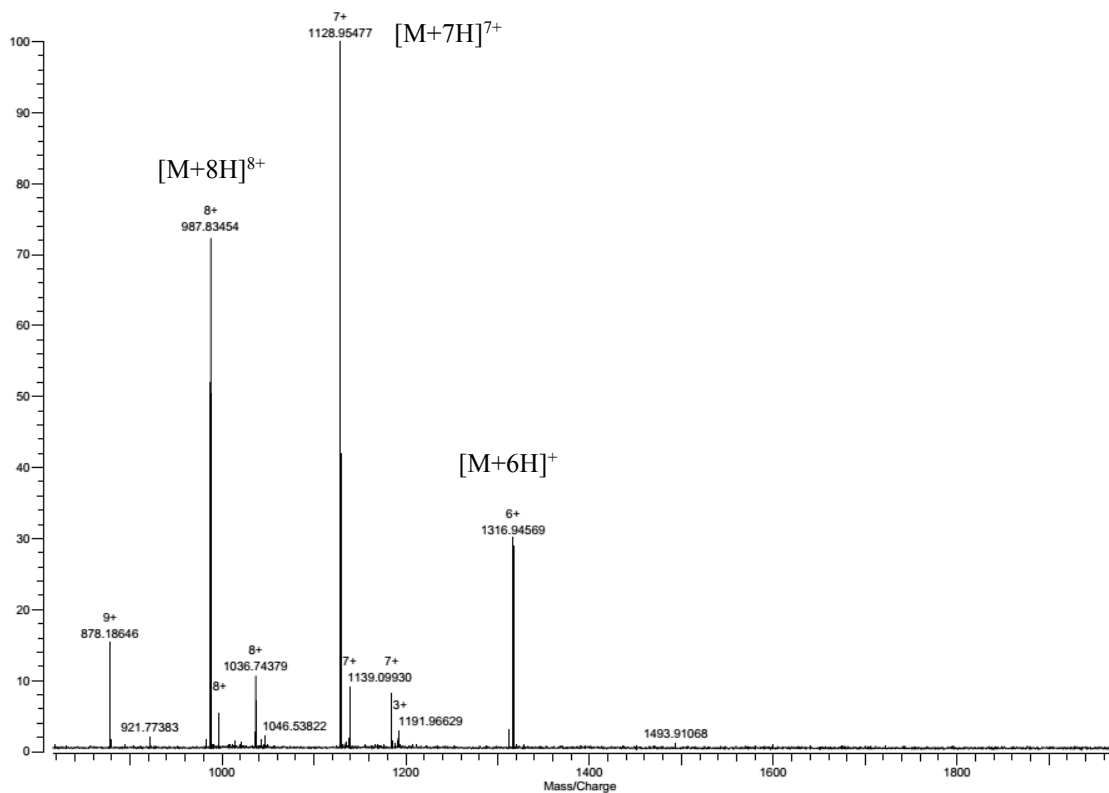


Senshu Pak PEGASIL ODS 4.6  $\phi$  X 250 mm  
 UV: 254 nm, Ex.: 366 nm, Em.: 520 nm  
 flow rate 1.0 mL/min with continuous  
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 min, linear) with solvent A (0.1% TFA in  
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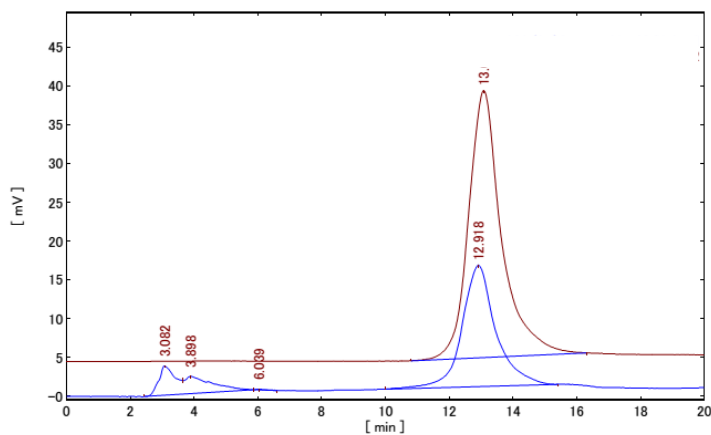
**Figure S32:** HPLC analysis of Ir complex-peptide hybrids **5**



**Figure S33:** <sup>1</sup>H NMR chart of Ir complex-peptide hybrids **5**



**Figure S34:** ESI Mass chart of Ir complex-peptide hybrid 5



Senshu Pak PEGASIL ODS 4.6  $\phi$  X 250 mm  
 UV: 254 nm, Ex.: 366 nm, Em.: 520 nm  
 flow rate 1.0 mL/min with continuous  
 gradient elution (10-40% solvent B, 0-30  
 min, linear) with solvent A (0.1% TFA in  
 H<sub>2</sub>O) and solvent B (0.1% TFA in MeCN).

**Figure S35:** HPLC analysis of Ir complex-peptide hybrids 6

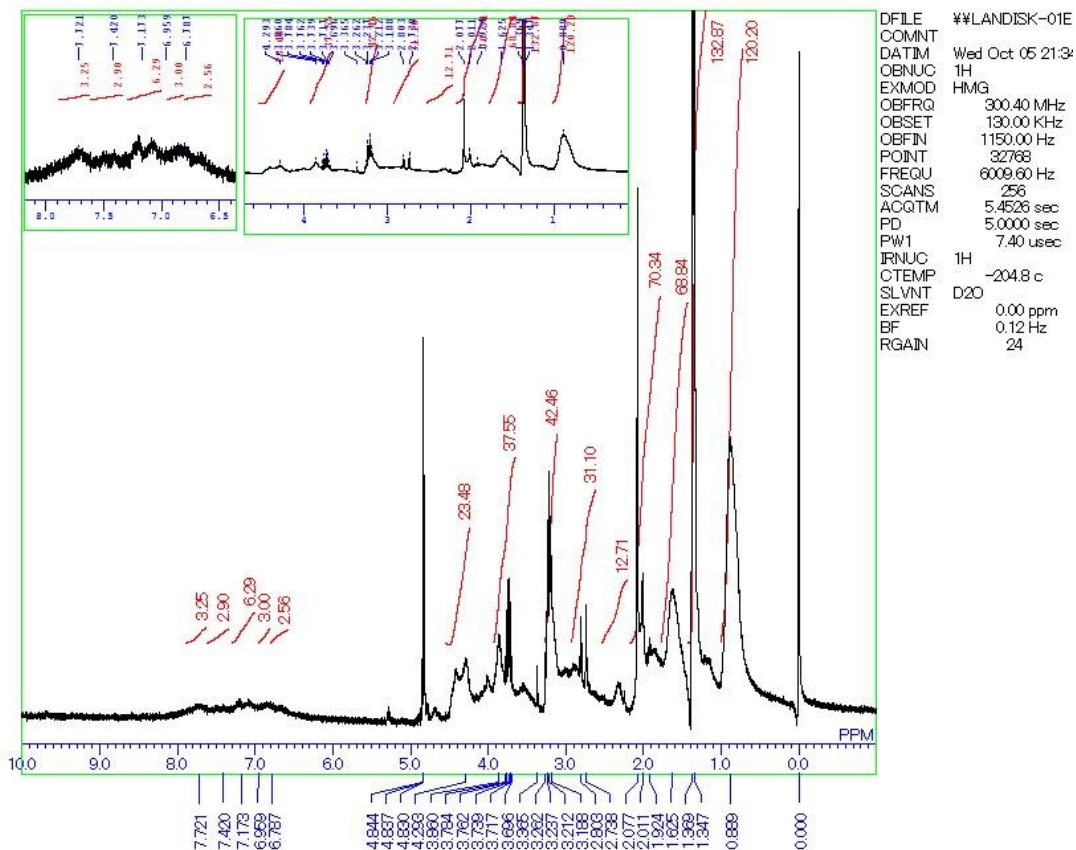


Figure S36: <sup>1</sup>H NMR chart of Ir complex-peptide hybrids 6

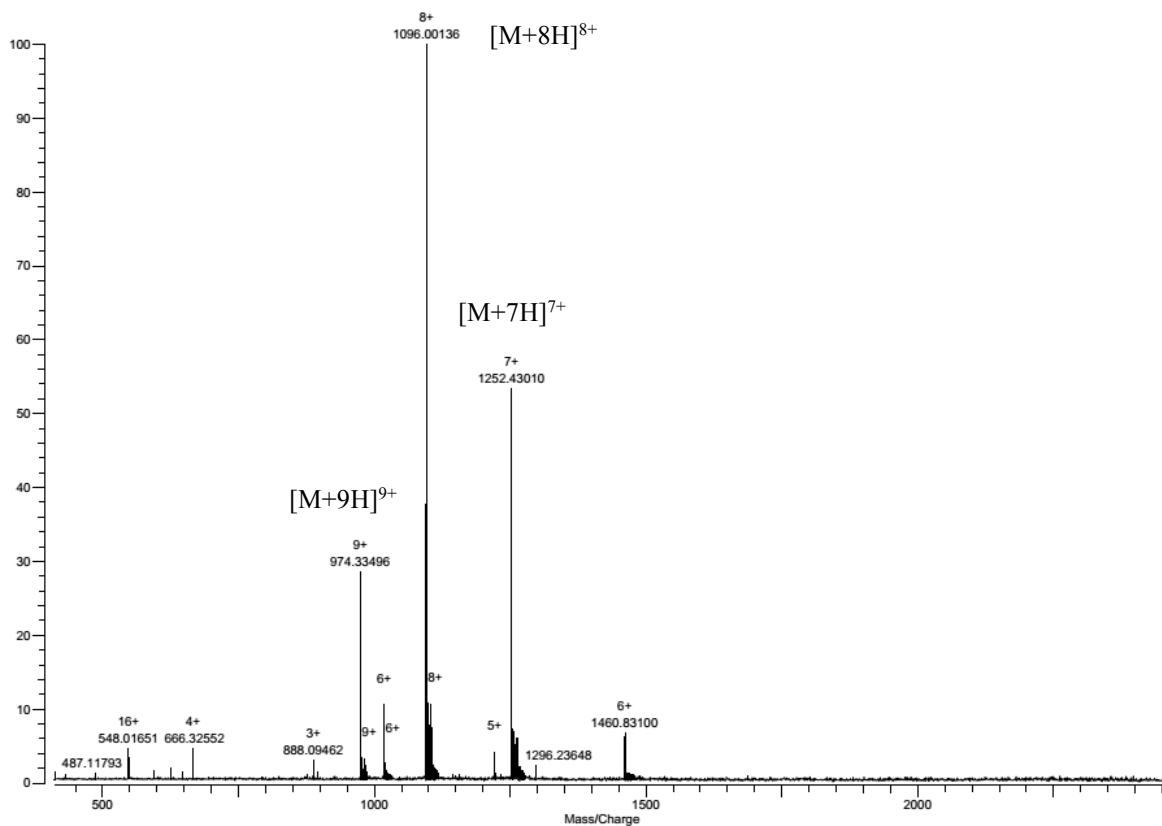


Figure S37: ESI Mass chart of Ir complex-peptide hybrid 6