Supporting Information

for

A far-red, photo- and bio-stable fluorescent marker selective to endoplasmic reticulum and its application to tunicamycin-treated HeLa cells

Hye-Ri Kim,[a,†] Rajesh Kumar,[b,†] Woori Kim,[a] Jae Hong Lee,[a] Myungkoo Suh,[a] Amit Sharma,[b] Chang Hoe Kim,[b] Chulhun Kang,[a,*] and Jong Seung Kim[b,*]

[a]*Department of Chemistry, Korea University, Seoul, 136-701, Korea*
[b]*The School of East-West Medical Science, Kyung Hee University, Yongin, 446-701, Korea*

Corresponding E-mails: jongskim@korea.ac.kr (J. S. Kim); kangch@khu.ac.kr (C. Kang)

List of the Contents

General Methods and Materials:

General materials. All the reactions were performed under an inert atmosphere in flame-dried reaction flasks. All reagents were purchased from Aldrich and were used as obtained. ^{1}H NMR spectra were recorded on a Varian (400 MHz); ^{13}C NMR spectra were recorded on a Varian 400 (100 MHz); The data for NMR spectra were reported as follows: chemical shifts (δ) were reported in ppm, and coupling constants (J) are in Hertz (Hz).

Spectroscopic measurements. Stock solutions of ERp were prepared in DMSO. For fluorescence spectra, excitation was carried out at 570 nm ~ 650 nm and both excitation and emission slit widths were 5 nm unless noted otherwise. Samples for all spectroscopic measurements were prepared in PBS buffer containing 1% (v/v) DMSO, pH 7.4. Absorption spectra were recorded on an V-560 (Jasco) spectrophotometer, and fluorescence spectra were recorded using an RF-5301 PC spectrofluorometer (Shimadzu) equipped with a xenon lamp at ambient temperature?.

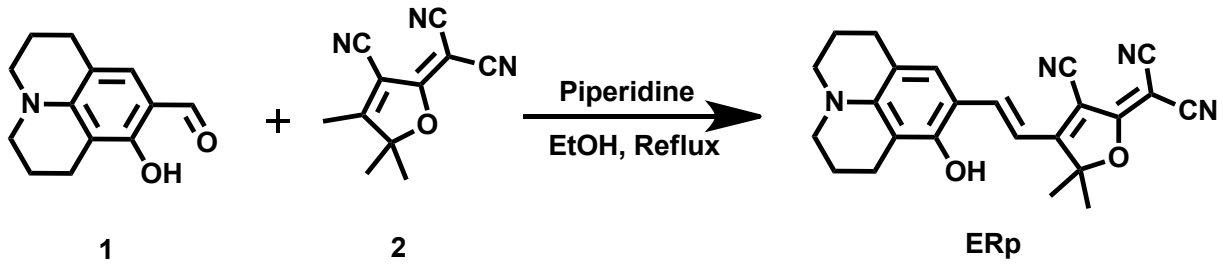
Cell Culture and Imaging. A human cervical cancer cell line (HeLa) was cultured in Dulbecco's Modified Eagle's Medium (DMEM), with 10% FBS (Gipco), penicillin (100 units/mL), and streptomycin (100 μg/mL). One day before imaging, the cells were placed on glass-bottomed dishes (SPL) which were incubated in a humidified atmosphere containing 5% (v/v) CO_2 at 37 °C. Cell images were obtained using a confocal microscope (Zeiss model LSM 510). All fluorescence images of **ERp** were obtained using an excitation wavelength of 543 nm and a long path (>650 nm) emission filter. Tracking dyes for co-localization experiments were bought from Invitrogen. Other information is available in the Figure captions.

pH titration/Chemicals. Samples were prepared in a universal buffer which contains 10 mM of citric acid, 10 mM monobasic potassium phosphate (KH_2PO_4) and 10 mM Tris base. pH of the samples was adjusted by adding HCl as the pH was monitored by using a Thermo Fisher pH electrode. 10 mM stock solutions of CTAB (cetyl trimethylammonium bromide) and SDS (sodium dodecyl sulfate) were prepared each in the universal buffer and diluted before the measurement. All reagents except tracking dyes were purchased from Sigma Aldrich.

MTT assay. Cell viability was assessed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Cells at 2×10^5/ml were treated with various concentrations of **ERp** in 96-well plates for 24 h at 37°C. Then, MTT working solution (5 mg/ml in serum free media) was added to each well and cells were incubated for 1 h. The water-insoluble formazan was formed during incubation and was solubilized by adding DMSO to each well. The amount of formazan was determined by measuring the absorbance at 570 nm using a multi-well plate reader (n=3).

Synthesis of probe ERp

Compound 2 was synthesized from reporting literature procedure in quantitative yield.[1]



Scheme S1. Synthesis of probe **ERp.**

In a flamed dried RBF, **1** (0.100 g, 0.460 mmol) and **2** (0.092 g, 0.460 mmol) were dissolved in absolute ethanol (20 ml). Piperidine (5uL, catalytic) was added and the mixture was refluxed under an inert atmosphere for 3 hrs. After TLC check, Reaction mixture was cooled to room temperature and green precipitates formed were filtered, washed with ethanol, diethyl ether, dried to furnish **ERp** (0.160 g, 87%) as green powder. ^{1}H NMR (DMSO-*d6*) δ (ppm): δ 1.65 (s, 6H), 1.81-1.86 (m, 4H), 2.53-2.58 (m, 4H), 2.63-2.66 (m, 4H), 6.68-6.72 (br s, 1H), 7.45 (s, 1H), 8.42- 8.45 (m, 1H), 9.80 (br s, 1H), 7.41 (s, 1H); ^{13}C NMR (DMSO-*d6*) δ (ppm): 20.71, 21.34, 21.75, 26.86, 27.42, 50.29, 51.09, 97.33, 97.35, 106.87, 106.89, 114.37, 114.62, 115.53, 118.37, 118.39, 151.85, 158.03, 178.33, HRMS: m/z = 270.0892 [M].

Absorption spectra of ERp in various pHs

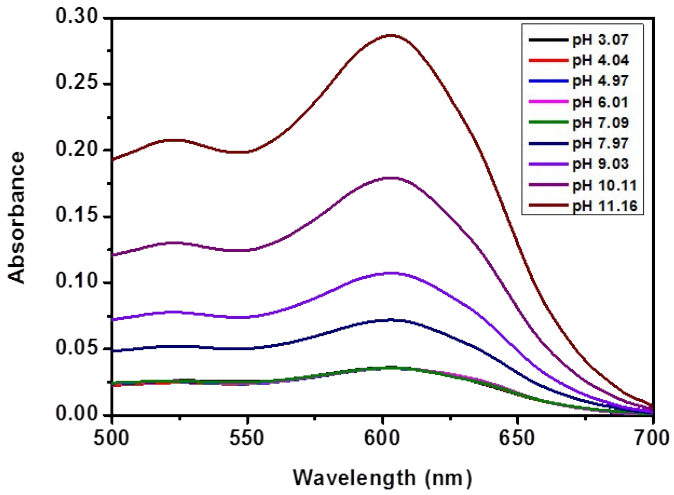


Figure S1. Absorption spectra of ERp (10 μM) in various pHs.

Spectral changes in the presence of micelles:

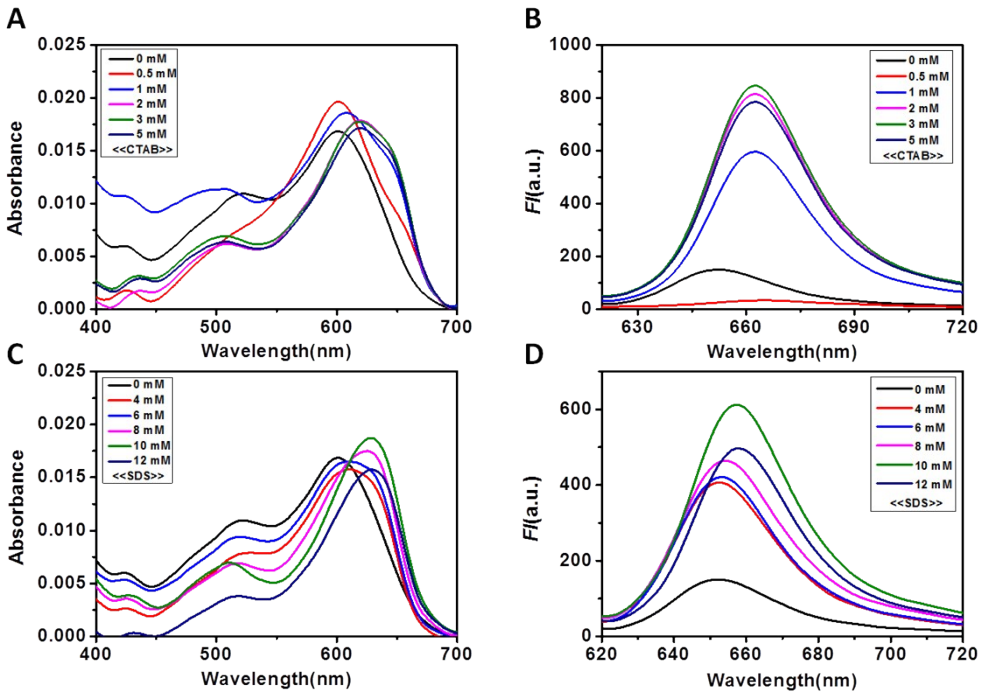


Figure S2. Absorption and fluorescence spectra of **ERp** (5μM) in the presence of CTAB micelles (A and B), or SDS micelles (C and D) at various concentrations. For fluorescence spectra, the samples were excited at 600nm.

3-Color imaging.

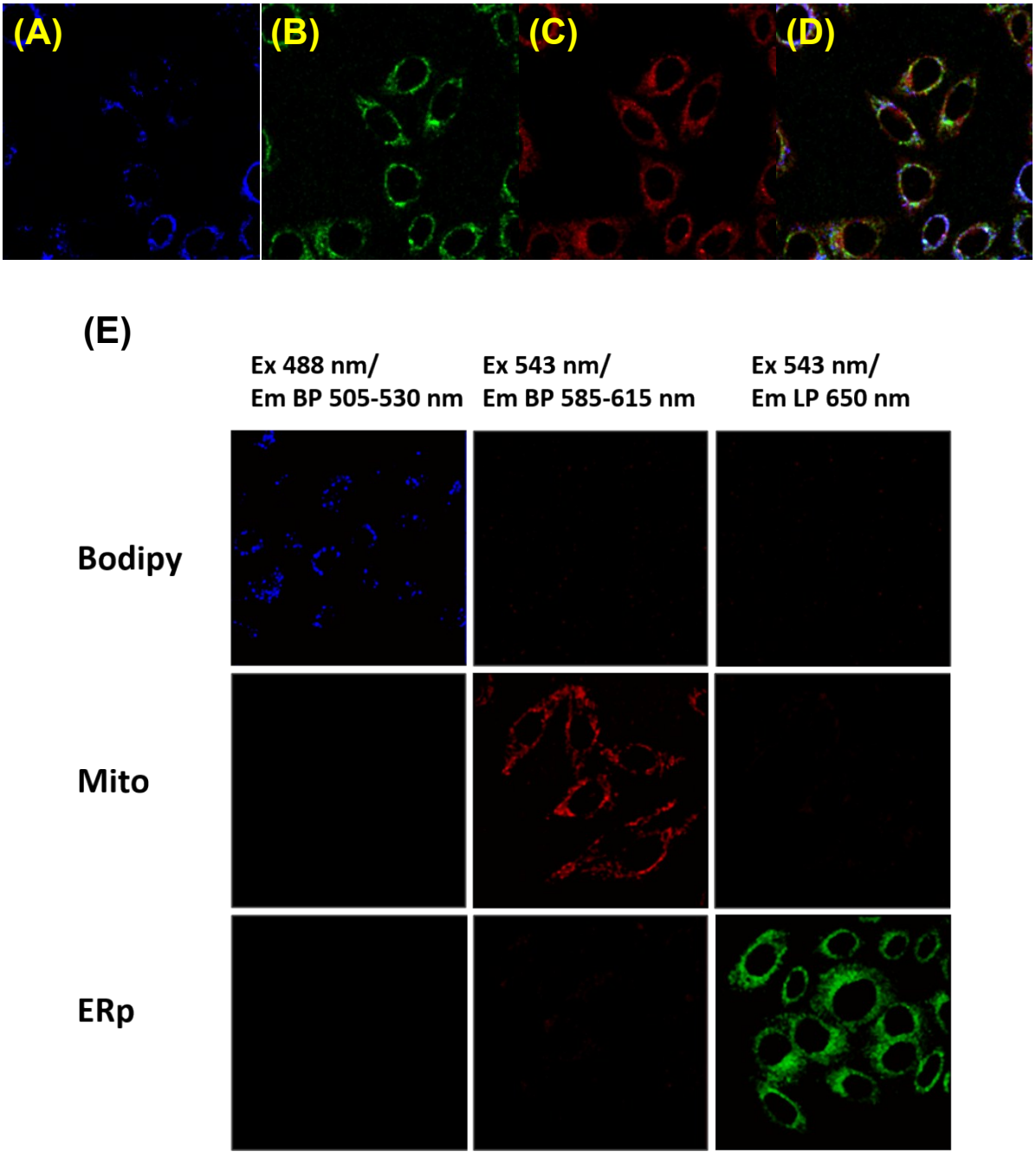


Figure S3. Fluorescence images of Hela cells using **ERp**. (2.5 μM) (B), BOIPY (0.5 μM) (A), and Mito-tracker (RED-CMXRos) (0.02 μM) (C). The cells were incubated with the probes for 15 min at 37°C. The images (A, B, C) were obtained upon excitation at 488 nm/with BP 505-530 nm filter, excitation 543 nm with LP 650 nm or BP 585-615 nm filter, respectively. The merged image is shown in the panel D. The panel (E) shows of the selectively acquisited image for each probe.

ER stress monitoring.

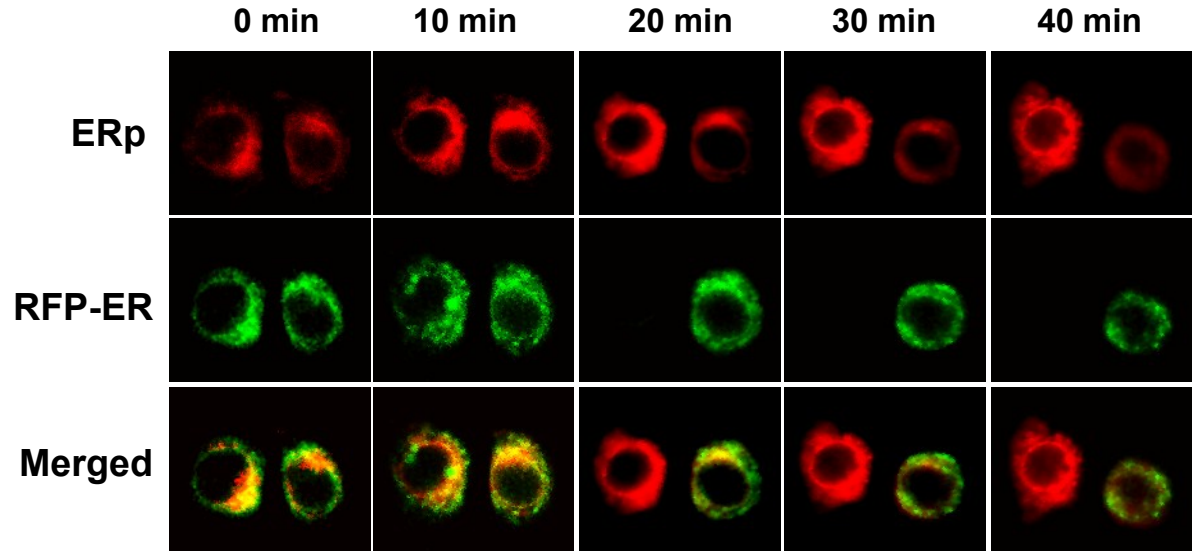


Figure S4. Fluorescence images of Hela cells using tunicamycin (40 μg/ml) treated for 1 hr before ERp (2.5 μM) for 20 min. RFP-ER (C-10591; Thermo) have to be incubated over night at 37°C before the experiment. Confocal analysis with ER-RFP of fluorescence was performed, excitation 543nm/ emission bp 585-615nm.

Photo-stability comparison upon UV irradiation

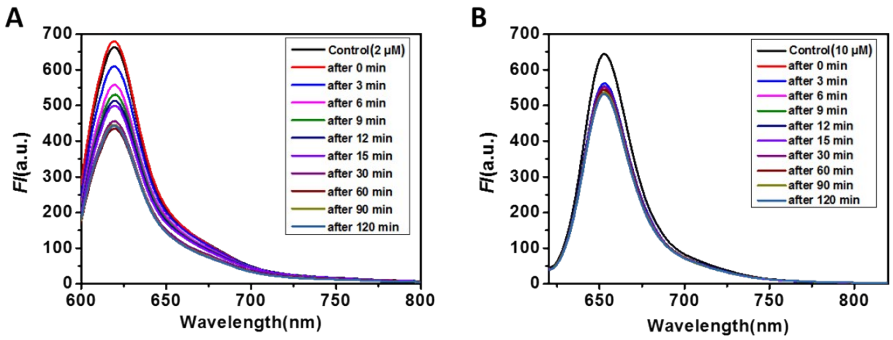


Figure S5. Fluorescence spectra of ER tracker red (Invitrogen; E34251, 2μM) (A), and 10μM **ERp** (B). Both samples were placed in quartz cuvettes upon continuous exposure to a UV lamp (FL20T10BLB, Lumiaction Co. Ltd., Taiwan). For aqusition of the spectra, the excitation at 580 nm and 600 nm were used for ER tracker red and **Erp**, respectively.

Cell viability

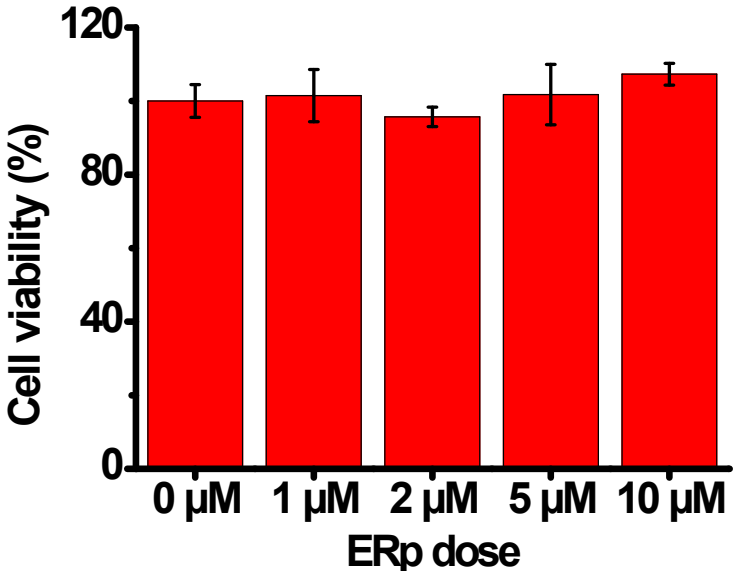


Figure S6. Cells at 2 × 10^5/ml were treated with various concentrations of **ERp** in 96-well plates for 24 h at 37°C. Then, MTT working solution (5 mg/ml in serum free media) was added to each well and cells were incubated for 1 h. The amount of formazan was determined by measuring the absorbance at 570 nm using a multi-well plate reader. (n=3)

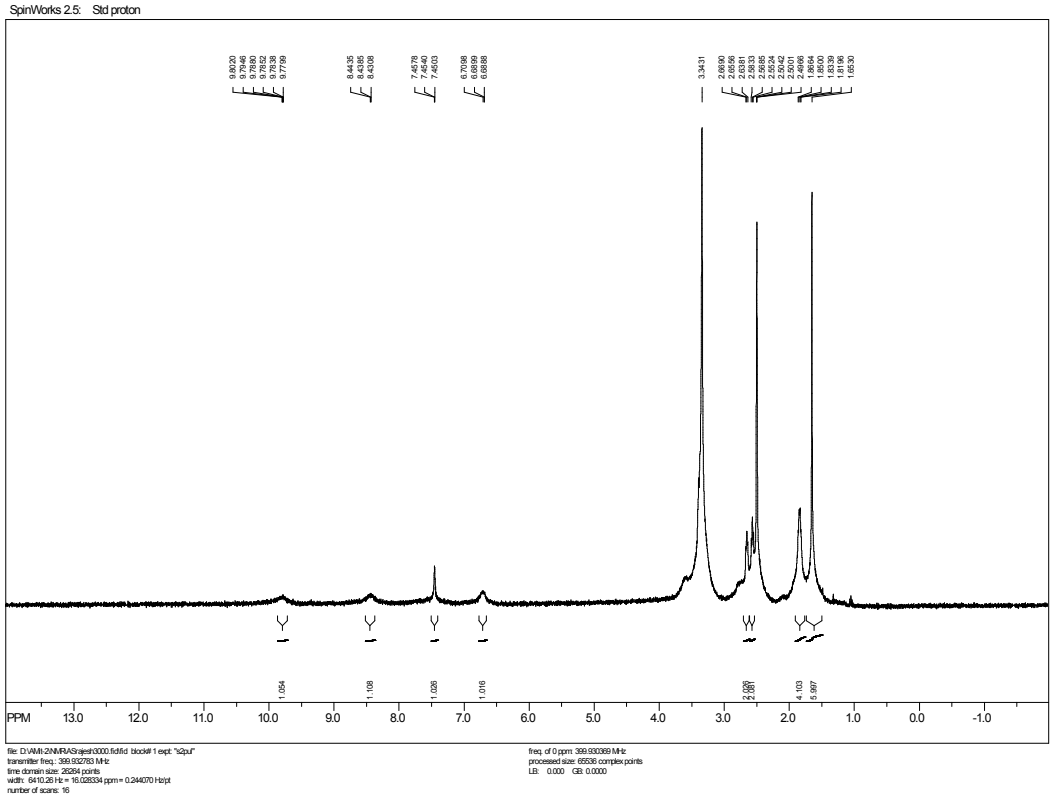


Figure S7. ^{1}H NMR spectra (400 MHz) of **ERp** in DMSO-*d6*

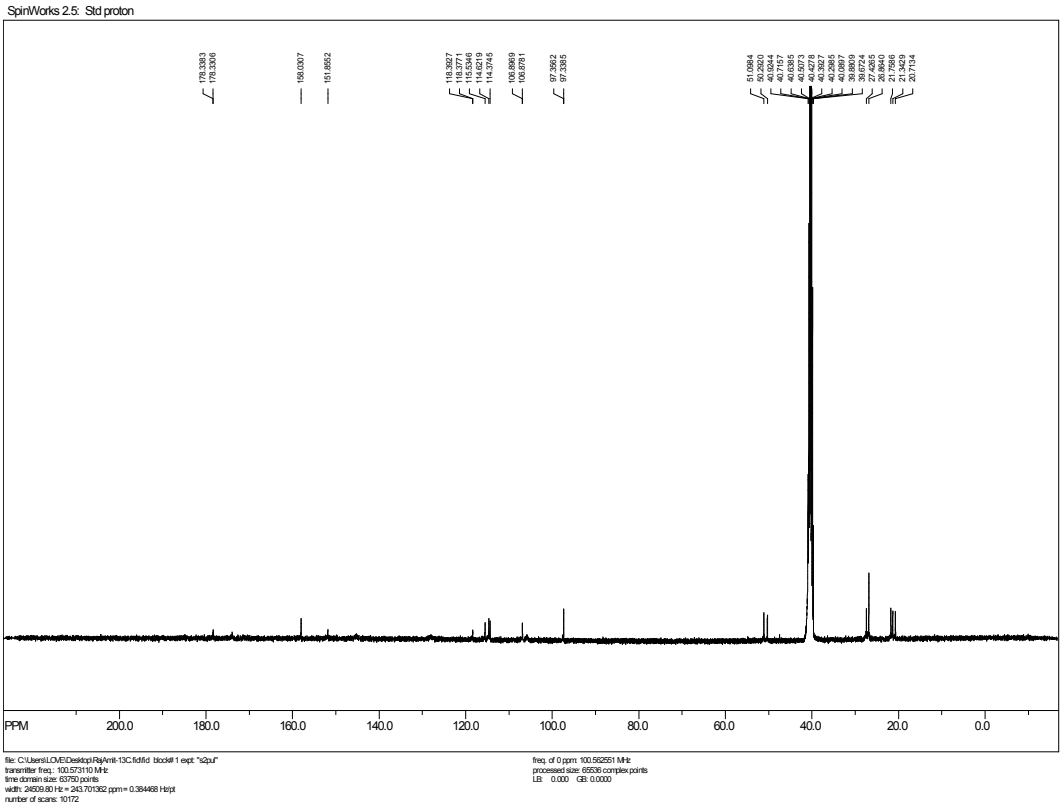


Figure S8. ^{13}C NMR spectra (100 MHz) of **ERp** in DMSO-*d6*

Reference

1. T. Zhang, K. P. Guo, L. Qiu, Y. Shen, *Synth. Commun*. **2006**, 36, 1367-1372