Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting information for

Two-photon red-emissive fluorescent probe for imaging nitroxyl (HNO) in living cells and tissues

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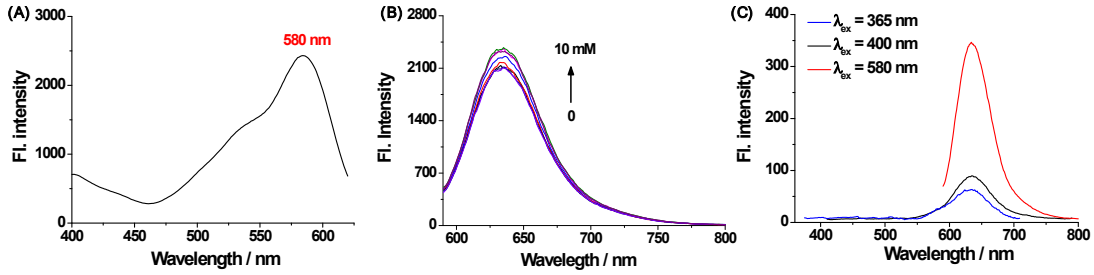

Fig. S1 (A) Excitation spectrum (λ_{em} = 638 nm) of 5 μM **RP** in presence of 100 μM AS in PBS (5% MeOH, 20 mM, pH = 7.4). (B) Fluorescence spectra of 5 μM **Rho** in presence of $NaClO_4$ with different concentrations (0-10 mM), λ_{ex} = 580 nm. (C) Fluorescence spectra of 5 μM **RP** in presence of 100 μM AS under excitation at 365 nm, 400 nm and 580 nm in PBS (5% MeOH, 20 mM, pH = 7.4).

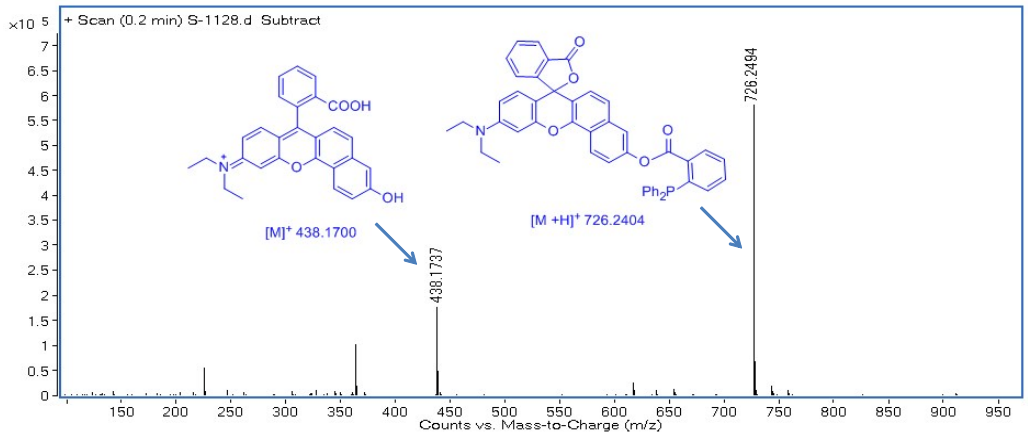

Fig. S2 HRMS study of the reaction product between **RP** and AS in PBS (5% MeOH, 20 mM, pH = 7.4)

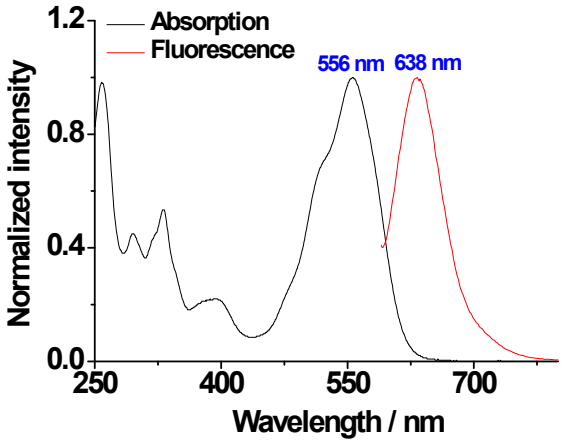

Fig. S3 Normalized absorption and fluorescence spectrum (λ_{ex} = 580 nm) of 5 μM **Rho** in PBS (5% MeOH, 20 mM, pH = 7.4). [1]

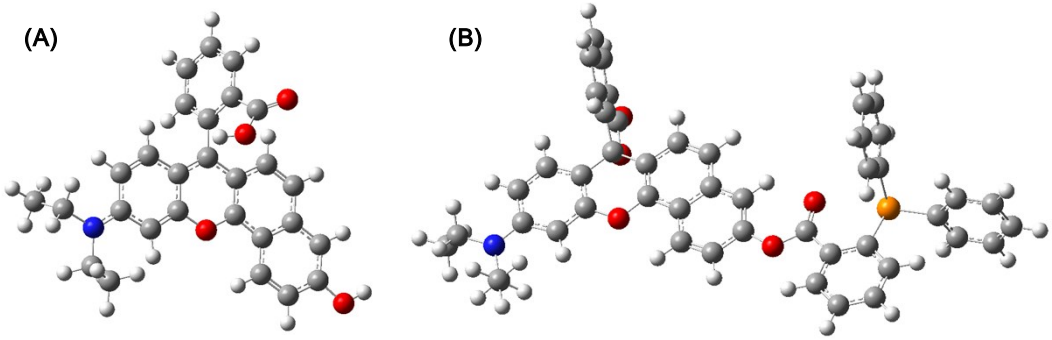

Fig. S4 DFT optimized structures of **Rho** (A) and **RP** (B). In the ball-and-stick representation, carbon, nitrogen, oxygen, and phosphorus atoms are colored in gray, blue, red, and orange, respectively.

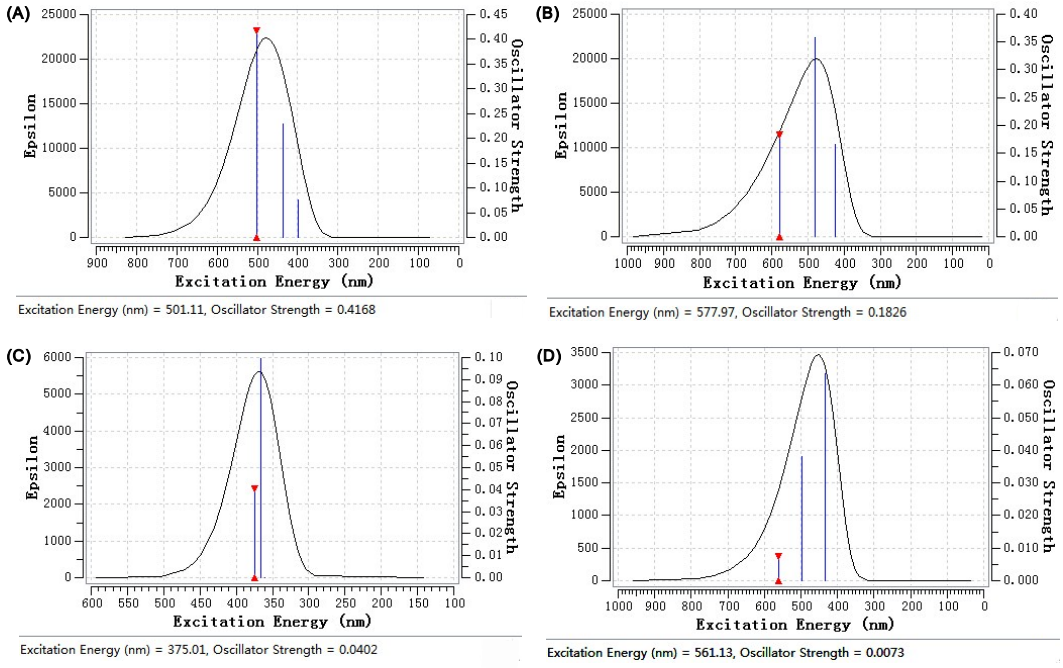

Fig. S5 TD-DFT calculated absorption (A) and fluorescence (B) spectra of **Rho**, and TD-DFT calculated absorption (C) and fluorescence (D) spectra of **RP**.

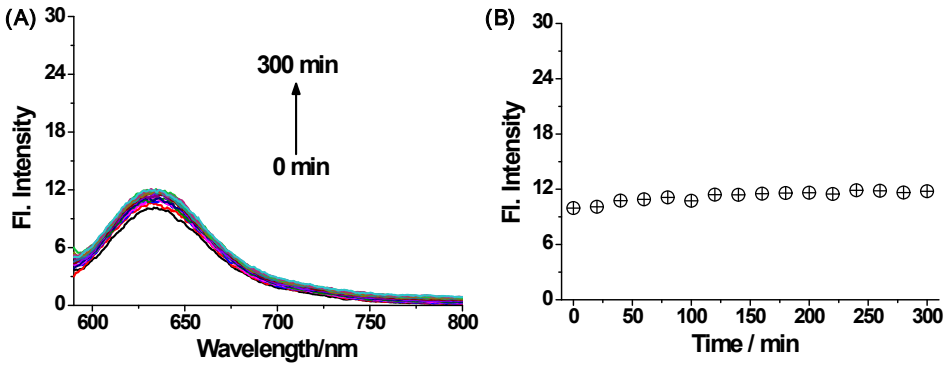

Fig. S6 (A) Time-dependent fluorescence spectra of 5 μM **RP** under excitation at 580 nm in PBS (5% MeOH, 20 mM, pH = 7.4). (B) Fluorescence intensity at 638 nm as a function of time.

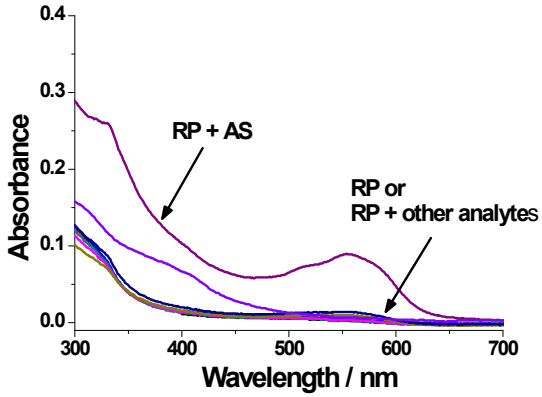

Fig. S7 UV-Vis absorption spectra of 5 μM **RP** in presence of various species in PBS buffer (5% MeOH, 20 mM, pH = 7.4).

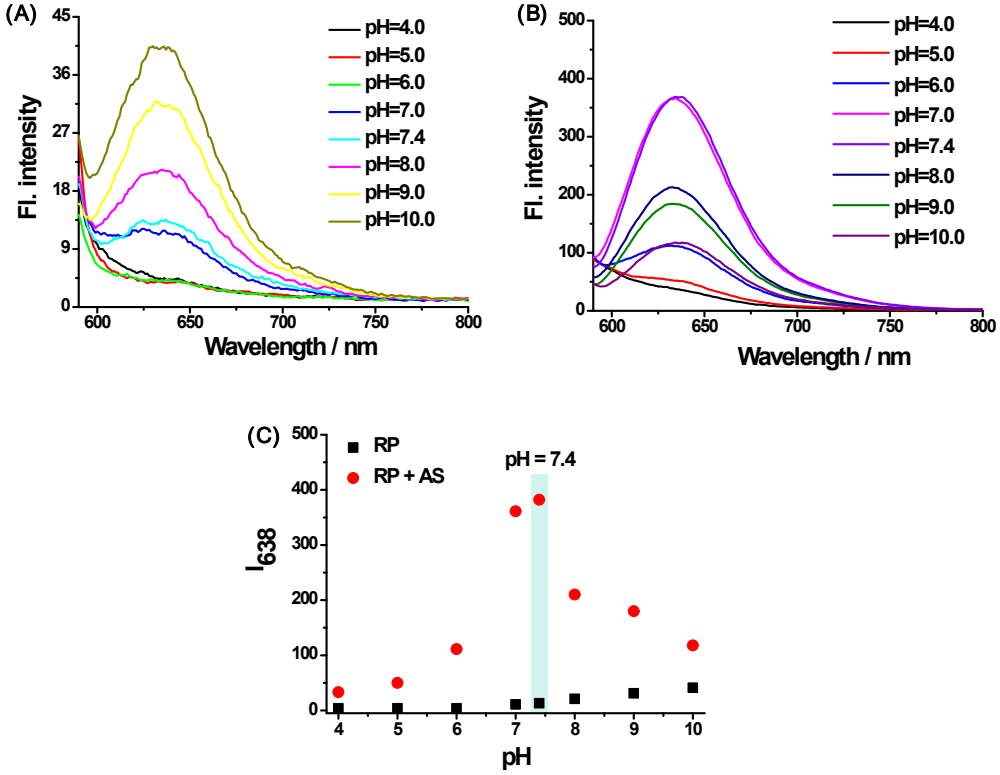

Fig. S8 (A) Fluorescence spectra of 5 μM **RP** at various pH. (B) Fluorescence spectra of 5 μM **RP** in presence of 100 μM AS at various pH. (C) Fluorescence intensity at 638 nm of 5 μM **RP** in absence and presence of 100 μM AS at various pH. λ_{ex} = 580 nm.

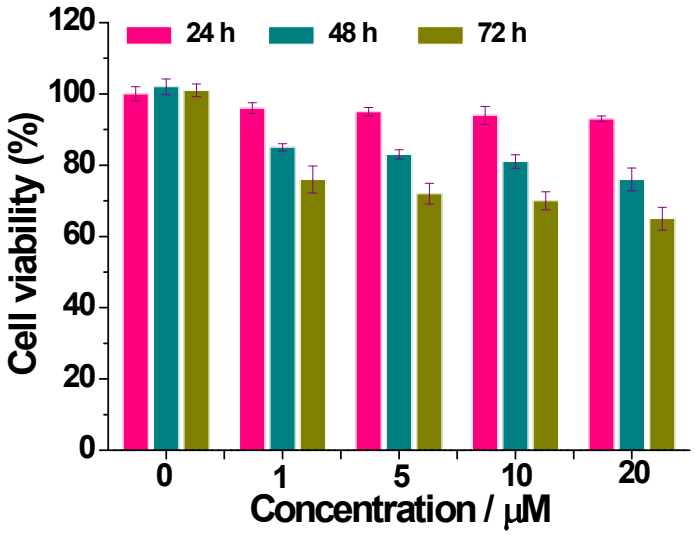

Fig. S9 Cytotoxicity assays of **RP** at various concentrations for HeLa cells in 24 h, 48 h and 72 h.

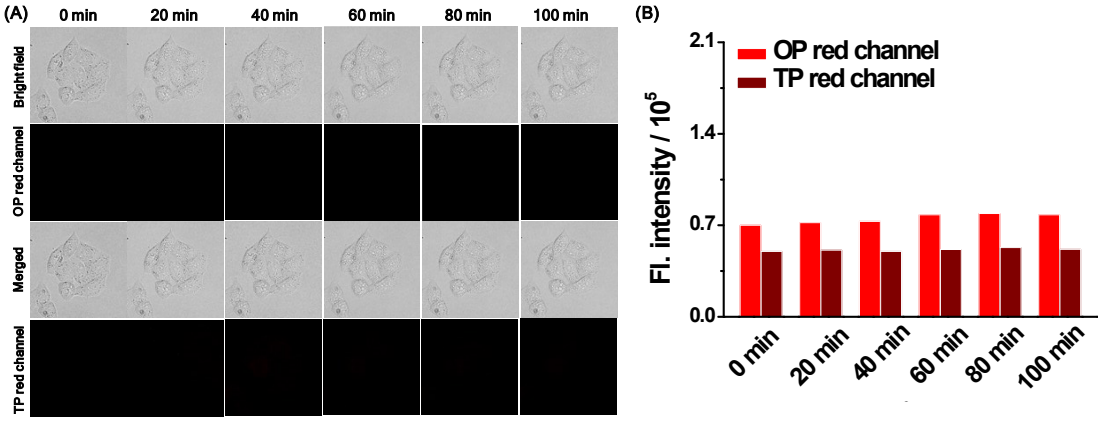

Fig. S10 (A) Fluorescence images of HeLa cells treated with 10 μM **RP** for various time. One-photon (OP) emission was collected at 570-620 nm with excitation at 561 nm. Two-photon (TP) emission was collected at 570-620 nm with excitation at 800 nm. Scale bar: 20 μm. (B) Quantified fluorescence intensity of single cell in one-photon (OP) and two-photon (TP) red channels analyzed by ImageJ software.

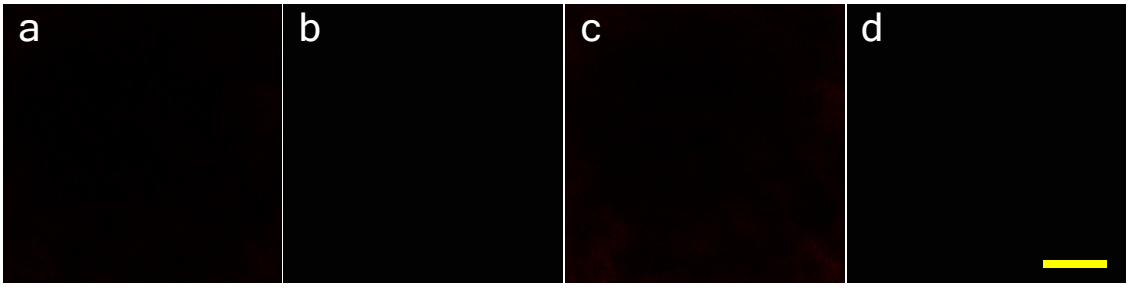

Fig. S11 Representative TP images of mouse liver tissues stained with 10 μM **RP** for 30 min at the depth of 0 μm (a), 40 μm (b), 80 μm (c) and 120 μm (d). λ_{ex} = 800 nm, λ_{em} = 570-620 nm, Scale bar: 25 μm

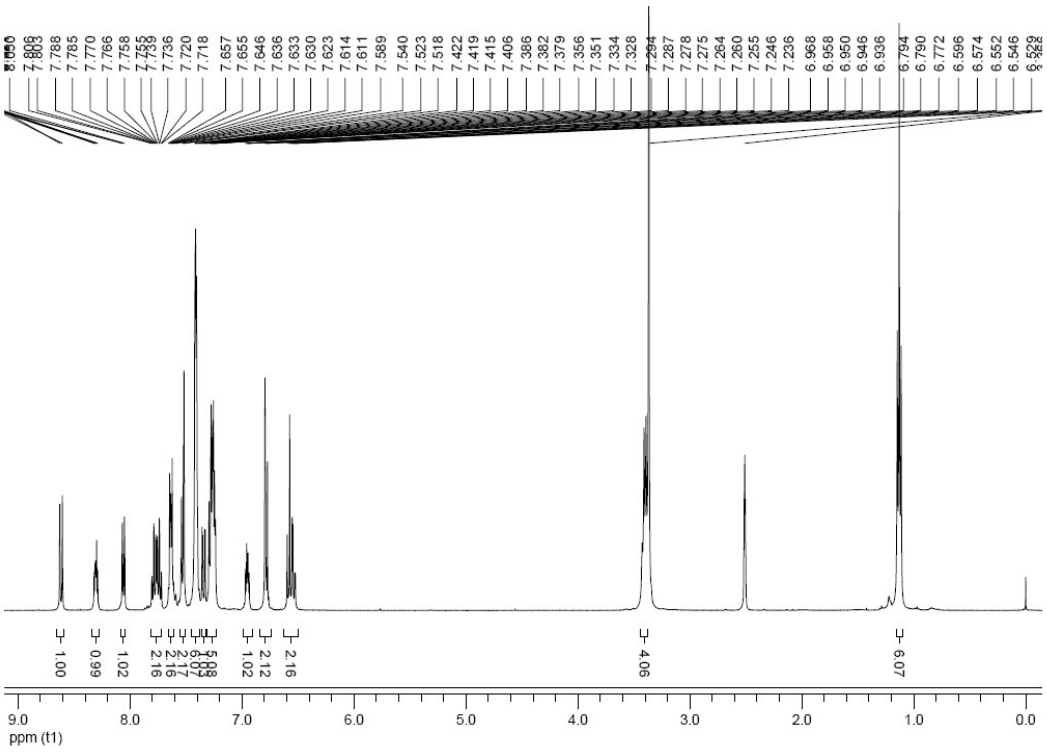

Fig. S12 ^{1}H NMR spectrum of **RP** (DMSO-d_6, 400 MHz)

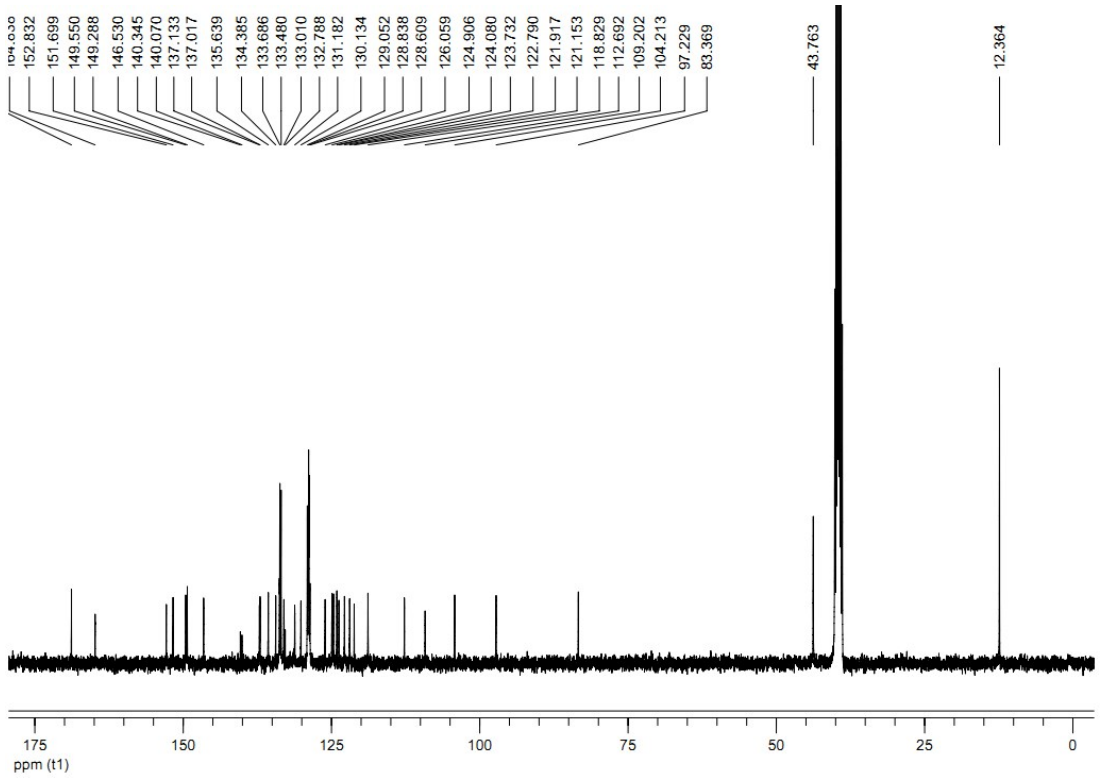

Fig. S13 ^{13}C NMR spectrum of **RP** (DMSO-d_6, 100 MHz)

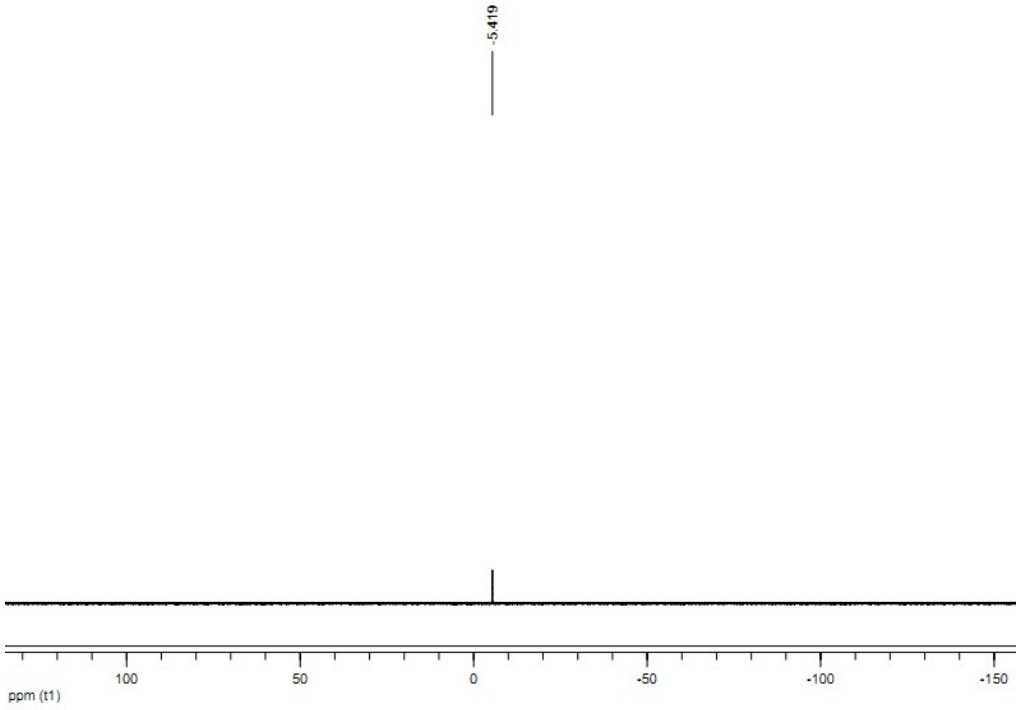

Fig. S14 ^{31}P NMR spectrum of **RP** (DMSO-d_6, 162 MHz)

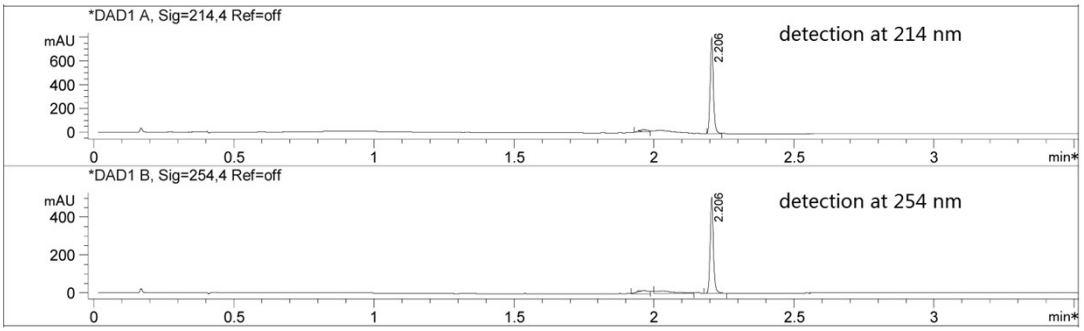

Fig. S15 HPLC data of **RP**

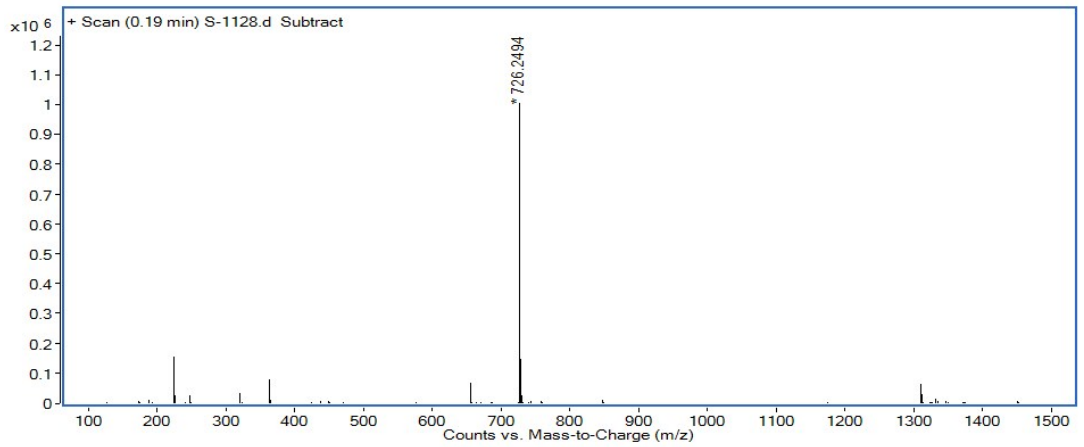

Fig. S16 HRMS data of **RP**

Reference:

1. X. Song, B. Dong, X. Kong, C. Wang, N. Zhang and W. Lin, *Anal. Methods*, 2017, 9, 1891-1896