Supplementary Information for

Interlocked Supramolecular Glycoconjugated Polymers for Receptor-Targeting Theranostics

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Contents list:

S1. Additional figures

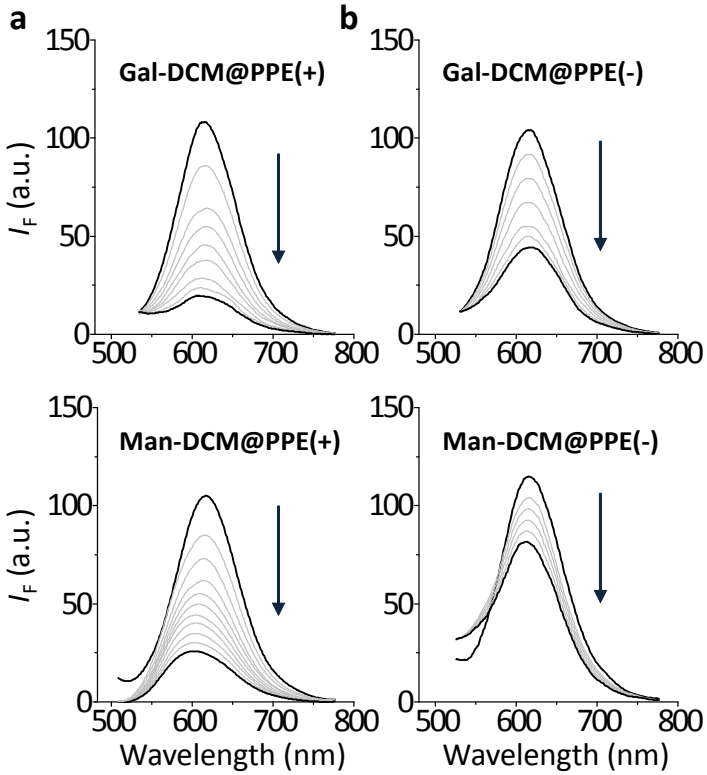


Figure S1. Fluorescence titration of (a) **Gal-DCM** (1 μM) with increasing **PPE(+)** (0-28 μM) and **PPE(-)** (0-29 μM), and (b) **Man-DCM** (1 μM) with increasing **PPE(+)** (0-50 μM) and **PPE(-)** (0-50 μM). All assays were carried out in Tris-HCl (0.01 M, pH 7.4) with an excitation wavelength of 465 nm.

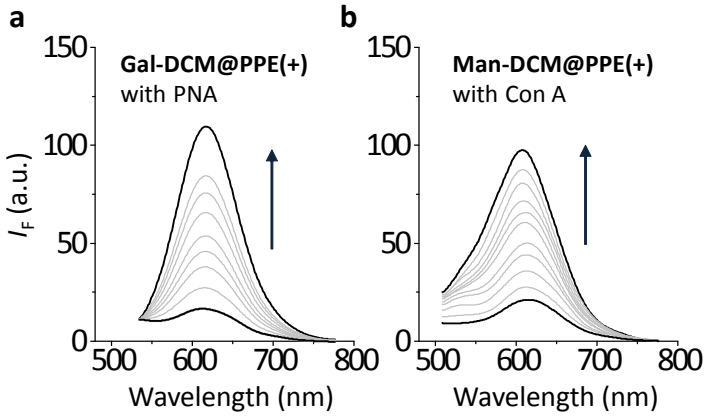


Figure S2. Fluorescence titration of (a) **Gal-DCM@PPE(+)** (1 μM/28 μM) with increasing PNA (0-20 μM), and (b) **Man-DCM@PPE(+)** (1 μM/50 μM) with increasing Con A (0-18 μM). All assays were carried out in Tris-HCl (0.01 M, pH 7.4) with an excitation wavelength of 465 nm.

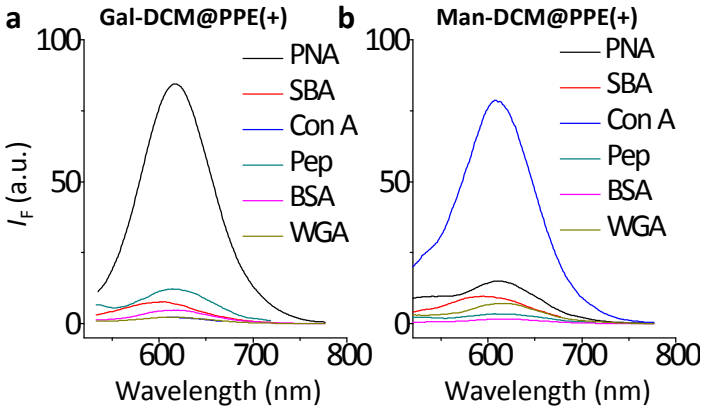


Figure S3. Fluorescence change of **Gal-DCM@PPE(+)** (1 μM/28 μM, 20 μM protein) and **Man-DCM@PPE(+)** (1 μM/50 μM, 20 μM protein) with different proteins (PNA = peanut agglutinin, SBA = soybean agglutinin, Con A = concanavalin A, Pep = pepsin, BSA = bovineserum albumin, WGA = wheat germ agglutinin). All assays were carried out in Tris-HCl (0.01 M, pH 7.4) with an excitation wavelength of 465 nm.

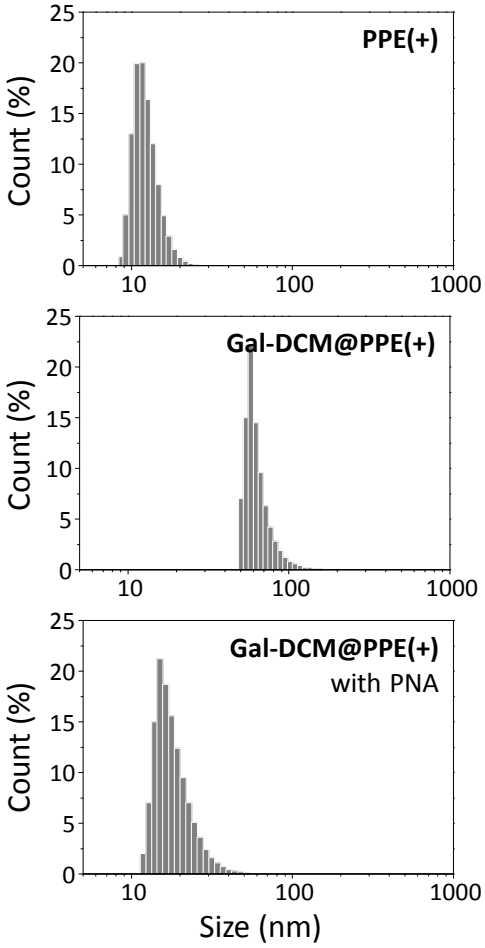


Figure S4. Dynamic light scattering of **PPE(+)** (28 μM), **Gal-DCM@PPE(+)** (1/28 μM) and **Gal-DCM@PPE(+)** with PNA (1/28/20 μM).

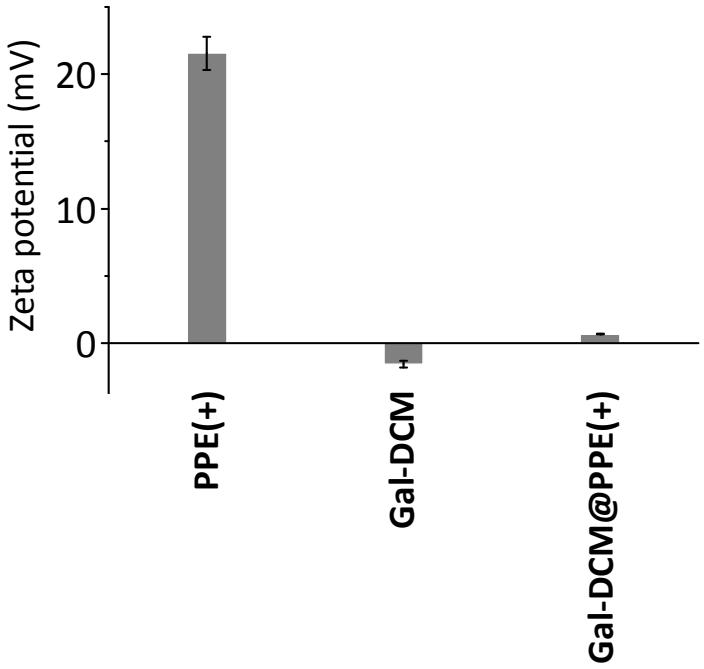


Figure S5. Zeta potential of **PPE(+)** (28 μM), **Gal-DCM** (1 μM) and **Gal-DCM@PPE(+)** (1/28 μM).

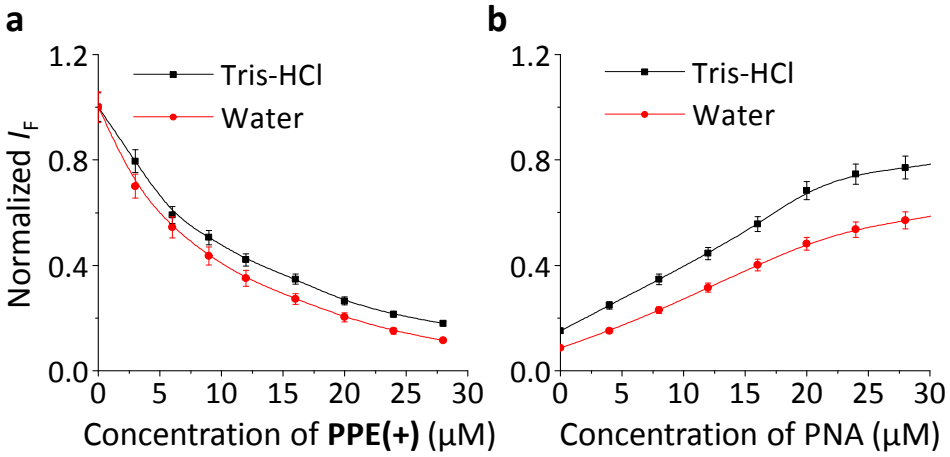


Figure S6. Fluorescence titration of (a) **Gal-DCM** (1 μM) with increasing **PPE(+)**, and (b) **Gal-DCM@PPE(+)** (1/28 μM) with increasing PNA in Tris-HCl (0.01 M, pH 7.4) or pure deionized water, with an excitation wavelength of 465 nm.

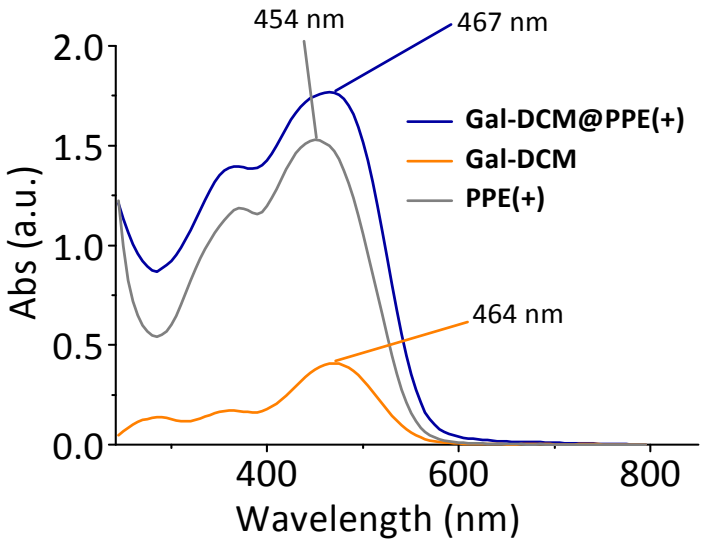


Figure S7. UV-vis absorbance **Gal-DCM** (1 μM), **PPE(+)** (28 μM) and **Gal-DCM@PPE(+)** (1/28 μM) in Tris-HCl (0.01 M, pH 7.4).

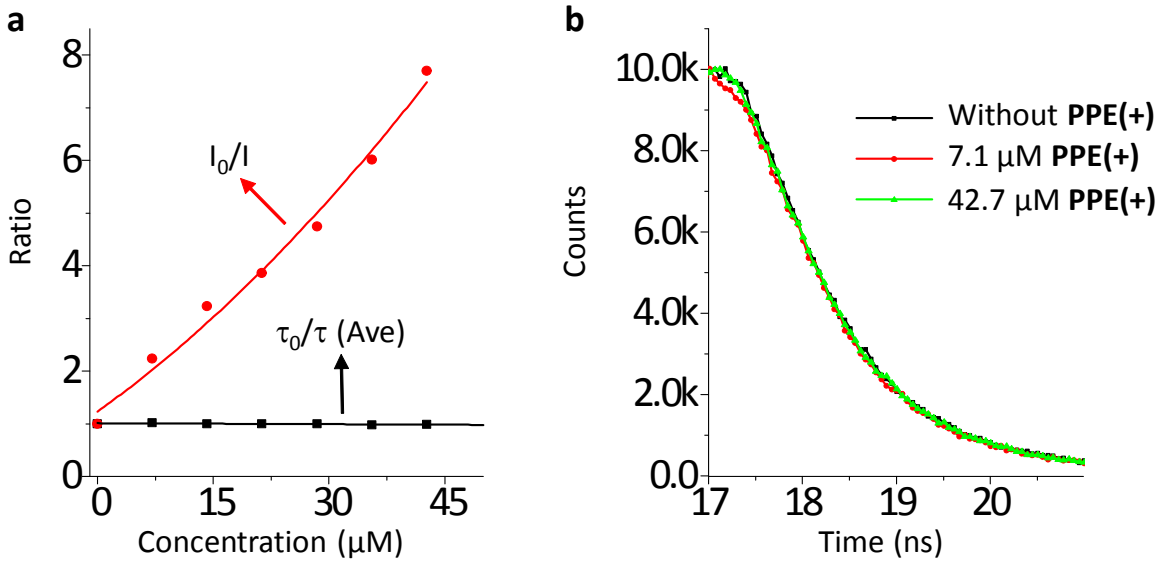


Figure S8. (a) Steady state and time-resolved fluorescence quenching Stern-Volmer plots of **Gal-DCM** with or without **PPE(+)**. (b) Time resolved fluorescence decay (615 nm) of **Gal-DCM@PPE(+)** in Tris-HCl (20 mM, pH 7.0). The fluorescence decays of **Gal-DCM** at 615 nm did not change obviously, suggesting a static pathway in the aqueous solution.

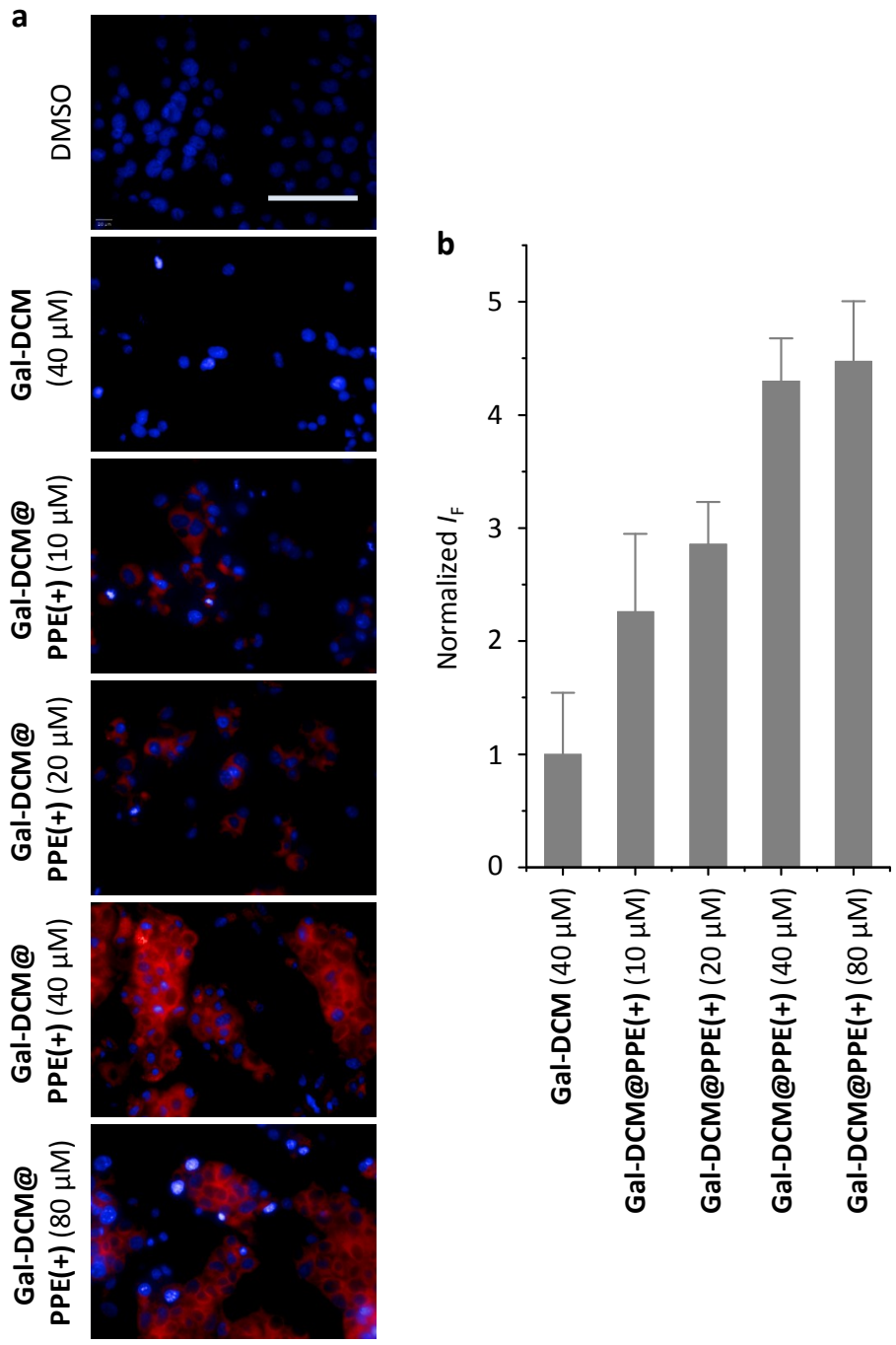
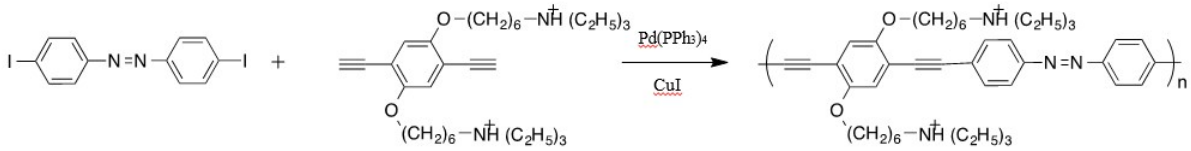


Figure S9. Fluorescence imaging (a) and quantification (c) of Hep-G2 cells treated with **Gal-DCM** in the absence and presence of increasing **PPE(+)** (excitation channel: 520-550 nm, emission: 580-650 nm, scale bar: 100 μm).

S2. Experimental section

*Synthesis of **PPE(+)**.* A solution of 5,5'-((2,5-diethynyl-1,4-phenylene)bis(oxy))bis(*N*,*N*,*N*-triethyl-pentan-1-aminium) (249 mg, 0.5 mmol) and 1,2-bis(4-iodophenyl) diazene (217 mg, 0.5 mmol) in 20 mL of dry THF/Et_3N (v/v = 2/1) fitted with a condenser were degassed with argon for 5 min. Then, 17.4 mg of $Pd(PPh_3)_4$ (15 μmol) and 8 mg of CuI (15 μmol) were added under argon. The reaction mixture was stirred at 60 ºC for 20 h. The resulting reaction solution was poured into 300 mL of methanol, and then the precipitation was purified by dissolution in THF and precipitation into methanol twice to obtain a dark red solid (359 mg, 85%) (Scheme below; synthesis of **PPE(-)** has been described in our previous study).[1]

^{1}H NMR (400 MHz, DMSO-d_6) 7.98 (br, d, 4H), 7.76 (br, s, 4H), 7.29 (br, s, 2H), 4.12 (br, d, 4H), 3.21 (br, d, 12H), 3.11 (br, s, 4H), 1.83 (br, s, 4H), 1.59 (br, s, 8H), 1.43 (br, s, 4H), 1.14 (br, t, 18H). Mw: 1.73×10^4, PDI: 1.55.



Fluorescence assays in solution. In a typical fluorescence quenching assay, the glycoprobe was incubated with **PPE** of different concentrations in Tris-HCl (0.01 M, pH 7.4) for 30 s, and then the fluorescence was measured on a Varian Cary Eclipsefluorescence spectrophotometer with an excitation of 465 nm. In a typical fluorescence recovery assay, proteins of different concentrations were added to the mixture of glycoprobe and **PPE**. The resulting mixture was incubated at 25 °C for 10 min. Then, the fluorescence measurements were carried out at room temperature with an excitation of 465 nm. Both steady state and time-resolved fluorescence spectra were recorded on a SPEX Flurolog 3-TCSPC instrument. Excitation and emission slits were set to be 5 nm, and the integration time was 0.1 s. In time-resolved fluorescence spectra measurements, 461 nm NanoLed was used as the excitation light source. Fluorescence decays were analyzed using Data Station developed by Horiba Jobin Yvon.

Solution-based determination of reactive oxygen species (ROS). In a typical solution-based ROS fluorescence assay, **PPE(+)** dissolved in a Tris-HCl buffer (0.01 M, pH 7.4) was mixed with protoporphyrin IX (PpIX) and dihydrorhodamine-123 (DHR123) under dark condition for 30 min. Oxidation of DHR123 by ROS resulted in the formation of highly fluorescent Rhodamine 123 (R123). Then, the fluorescence was measured on a Varian Cary Eclipse

fluorescence spectrophotometer upon white light (40 mV cm^{-2}) irradiation (0–25 min) with an excitation of 485 nm.

Cell culture. Hep-G2 and 293T cells were maintained in a Dulbecco's Modified Eagle's Medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Gibco, Gland Island, NY, USA) in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C and splited when the cells reached 90% confluency.

Establishment of the Hep-G2 knockdown stable cell line. Plasmids encoding ASGP-R1 specific shRNA or scramble shRNA were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Lentiviral particles were generated according to the manufacturer's instructions. Briefly, 293T cells were seeded in a six-well tissue culture plate and were grown to 80-90% confluency in antibiotic-free normal growth medium supplemented with FBS. 3 μg shRNA plasmid (shRNA of ASGP-R1 or control) was cotransfected with 1.8 μg pCAG-VSVG and 2.7 μg PAX2 into 293T cells using 15 μL lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). After 6 h, the medium was changed to fresh DMEM with 10% FBS. After 72 h, the lentivirus-containing supernatant were collected, filtered, and then employed for analysis.

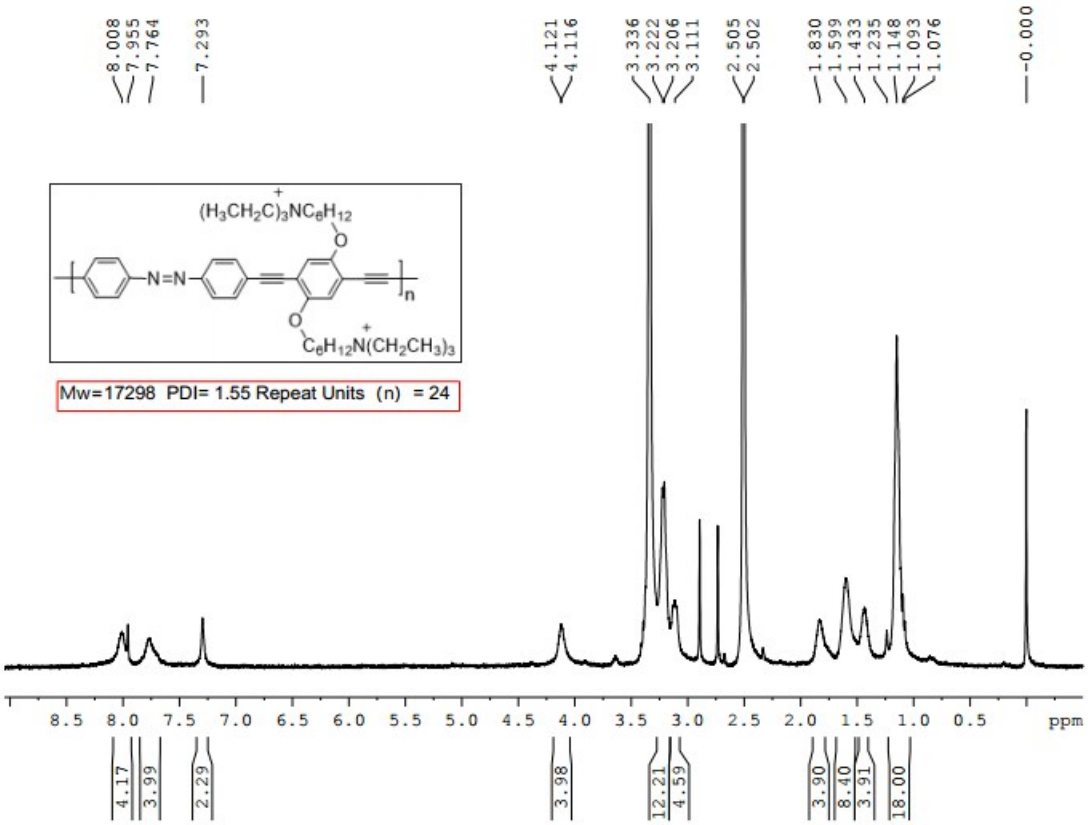
Generation of shASGP-R1 and control shRNA-Hep-G2 stable cell lines infected with lentivirus. Hep-G2 cells were plated in a 12-well plate 24 h prior to viral infection. The cells at approximately 50% confluency were infected with the lentiviral particles prepared as described above. The plates were incubated overnight and the medium was then changed to fresh complete medium. Two days after infection, the cells were splited at 1:5 and incubated for another 24 h in complete medium. Then puromycin dihydrochloride was added to select the stable clones expressing the shRNA. Medium was replaced with fresh puromycin containing medium every 3-4 days until resistant colonies can be identified. Several colonies were picked, expanded, and then assayed for stable shRNA expression by evaluating level of ASGP-R1 mRNA via real-time quantitative polymerase chain reaction (qPCR).

Cell-based determination of reactive oxygen species (ROS). Hep-G2, sh-ASGPr and HeLa were cultured in DMEM-HG supplemented with 10% FBS, and A549 were cultured in Ham's F-12 supplemented with 10% FBS. Then, cells (8.0×10^4 per well) were seeded on a black 96-well microplate with optically clear bottom (Greiner bio-one, Germany) overnight. Dihydrorhodamine-123 (DHR123, nonfluorescent) was used as a universal ROS tracking

agent. Oxidation of DHR123 by ROS resulted in the formation of fluorescent Rhodamine 123 (R123). The cells were preincubated with **PPE(+)** and **Gal-DCM@ PPE(+)** with different concentration for 15 min .Then, cells were gently washed with PBS once. The mixture (final concentrations of 10 μM DHR123 and 10 μM PpIX) was subsequently added to the cells under dark condition. Samples (n = 3) in a total volume of 100 μL in 96-well plates was irradiated by a broadband light source for different time durations. The fluorescence measurements were using an M5 microplate reader (Molecular Device, USA) at an excitation wavelength of 485 nm and an emission wavelength of 538 nm.

Cell viability assay. Cells were plated overnight on 96-well plates at 8000 cells per well in growth medium. After seeding, cells were treated with **Gal-DCM@PPE(+)** at different concentrations for 15 min. Then, cells were gently washed with PBS once. Samples (n = 3) in a total volume of 100 μL in 96-well plates was irradiated by a broadband light source for 30 min. After 48-h exposure,10 μL per well of MTS/PMS (20:1, Promega Corp) solution was added to each well containing 100 μL of serum-free HG-DMEM, followed by a gentle shake. After 2-h incubation at 37 °C under 5% CO_2, 80 μL of the mixture was transferred to another 96-well plate. The absorbance of the mixture solutions was measured at 490 nm with 650 nm as a reference, using an M5 microplate reader (Molecular Device, USA). The optical density of the result in MTS assay was directly proportional to the number of viable cells.

S3. Original NMR copy of new compound



^{1}H NMR spectrum of **PPE (+)**

S4. Additional reference

1. J. Wu, *et al. Chem. Commun.* **2013**, *49*, 11379-11381.