Supplementary Information

A senolysis-based theragnostic prodrug strategy towards chronic renal failure†

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†Electronic supplementary information (ESI) available: Experimental section, additional figures, synthetic procedures, and original spectra of new compounds.

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1. Materials and methods

Unless otherwise noted, all chemical reagents, biological reagents and solvents were purchased from commercial suppliers, including YEASEN, Sigma-Aldrich, Aladdin, Energy Chemical, etc., as analytical grade and no further purification is required. β-galactosidase (β-gal) Staining Kit and *E.coli* β-gal (G5635) was purchased from Beyotime Biotechnology and Sigma Aldrich, respectively. A549 (Human non-small cell lung cancer cell) and NRK-52E (Rat renal tubular epithelial cells) were acquired from Procell Life Science&Technology Co., Ltd.. Mitomycin C (MitoC) was purchased from Aladdin. Ham's F12K (Kaighn's) medium was purchased from Servicebio. Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco. Penicillin/streptomycin was purchased from Yeasen. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AMX-400 and AMX-600 spectrometers. The chemical shift in the front field of tetramethylsilane (TMS) is reported to be parts per million (PPM, δ). The proton coupling modes are described as single (s), double (d), three-wire (t), four-wire (q), multi-wire (m), and wide-wire (br) states. A Waters LCT Premier XE spectrometer was used to obtain high resolution mass spectrometry (HRMS) data by electroionization (EI) and electrospray ionization (ESI). High performance liquid chromatography (HPLC) analysis were carried out using an Agilent 1200 series and SHIMADZU LC-20A series.

Optical properties of TSPD

TSPD and compound **3** were prepared in DMSO to obtain the stock solutions (10 mM, respectively) and then diluted to final concentration (10 μM, respectively) in PBS buffer (pH = 7.4, 10 mM) for measurements. *E. coli* β-gal was dissolved in PBS buffer to make 100 U/mL stock solutions and then diluted to final concentration (0.1-5 U/mL, respectively) in PBS buffer for measurements.

For the selectivity tests, the biological analytes used in the selectivity experiment, including Na^{+}, K^{+}, Mg^{2+}, Al^{3+}, HCO^{3-}, NO^{2-}, H_2O_2, L-cysteine, lysozyme, carboxylesterase, pepsase, trypsase and cellulase were dissolved in PBS buffer to obtain stock solutions (100 U/mL or 10 mM, respectively) , the final concentration of all enzymes was 1 U/mL, and the final concentration of other analytes was 1 mM.

For the enzyme kinetics assay, **TSPD** incubated with *E. coli* β-gal (1 U/mL) at 37 °C for 30 min. After incubation, the fluorescence intensity of the mixtures was measured for quantification analyses. The initial reaction velocity was calculated and plotted against the concentration of **TSPD**. The kinetic parameters were calculated by direct fits of the data to the Michaelis-Menten Equation:

$$V = V_{max}\,[S]/(K_m + [S])$$

where V is initial velocity, and [S] is substrate concentration.

Ultraviolet-visible spectra were determined by U-2910 spectrometer (Hitachi). Fluorescence spectra were collected by F-4700 fluorescence spectrometer (Hitachi) with a 10-mm quartz cuvette.

Cell culture conditions and treatments

A549 cells were cultured in Ham's F12K (Kaighn's) medium supplemented with 10% FBS and 1% penicillin/streptomycin and maintained at 37 °C with 5% CO_2. NRK-52E cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin and maintained at 37 °C with 5% CO_2. The senescent cell model was established by treating cells (A549 cells and NRK-52E cells) with MitoC. Briefly, A549 cells were seeded at 8×10^4 cells/mL, and allowed to adhere for 24 h. Then the cells were treated with 0.5 μM MitoC for 48 h. After that, the medium was replaced with fresh Ham's F12K (Kaighn's) medium and incubated for another 48 h. At this time, A549 cells became enlarged and flattened morphologically, which are classic phenotypes of senescent cells. The senescent NRK-52E cells were induced the same way as A549 cells

***LacZ* gene transient transfection**

The target plasmid (pORF-*LacZ*) was constructed by Shanghai Yuxiu Biotech Co., Ltd.. A549 cells were seeded in a 12-well plate at a density of 2.5×10^4 cells per well and cultured for 24 h. According to the Lipofectamine® 3000 (Invitrogen, L3000015) manufacturer's instructions, 2.5 μg of the pORF-*LacZ* vector and 5 μL of P3000 were added to 125 μL of Opti-MEM culture medium in a tube. Next, 3.75 μL of liP3000 was added to 125 μL of Opti-MEM culture medium in another tube, and the two tubes were fully mixed and incubated at room temperature for 20 min. The mixture was diluted in fresh penicillin/streptomycin-free Ham's F12K (Kaighn's) medium, then incubated with the cells for 6 h. After replacing the medium with fresh Ham's F12K (Kaighn's) medium, the cells were cultured for another 48 h before using.

X-gal staining assay

Uninduced (control) cells and MitoC-induced senescent (sct) cells were plated on a 12-well plate for SA-β-gal staining according to the staining manufacturer's instructions provided by Beyotime Biotechnology (C0605). The cells were washed with PBS buffer and fixed in 4% formaldehyde at room temperature for 15 min. Then, the cells were incubated with fresh staining solution (1 mg/mL X-gal, pH = 6.0) overnight (without CO_2) at 37 °C. After that, the images were collected by using Nikon Eclipse Ti2 microscope with 10x magnification lenses.

Cell viability assay

The cell viability was assessed by using a cell counting kit 8 (CCK-8) assay. Briefly, Uninduced (control) cells and MitoC-induced senescent (sct) cells were seeded into a 96-well plate at 5000 cells per well respectively and allowed to adhere overnight, then the cells were treated with various concentrations of **TSPD** or gemcitabine (A549 cells for 72 h and NRK-52E for 48 h). After that, the culture medium was replaced with fresh medium containing 10% CCK-8 and incubated for another 1.5 h. The absorbance of each well at 450 nm was recorded using a Microplate Reader (Bio-Tek Instruments, Synergy H1). The cell viability was evaluated by measuring the absorbance at 450 nm and calculated by the formula (cell viability = ($OD_{positive} - OD_{control}$) / ($OD_{negative} - OD_{control}$)). All experiments were performed in triplicate.

Fluorescence imaging of cells

Uninduced (control) cells and MitoC-induced senescent (sct) cells were plated in a 12-well plate at a density of 2.5×10^4 cells per dish and incubated for 24 h. Then, the medium was replaced with serum-free medium containing **TSPD** (20 μM). The cells were incubated for another 4 h at 37 °C and then washed 3 times with PBS buffer before fluorescence imaging. Images were collected by using Nikon Eclipse Ti2 microscope with 20x magnification lenses.

Western blot analysis

Cells cultured on 10 cm dishes were collected, rinsed three times with PBS buffer, and then were lysed in RIPA lysis buffer (strong) (YEASEN, 20101ES60) supplemented with PMSF (1 mM). The protein concentrations were measured by using an enhanced BCA Protein Quantification Kit (YEASEN, 20201ES76). Equal amounts of each sample were mixed with 4×SDS-PAGE Protein Loading Buffer (YEASEN, 20315ES05), denatured by heating at 95 °C for 10 min, then the protein was separated through SDS-PAGE gel electrophoresis and transferred onto the 0.22 μm NC membranes (Millipore). The membrane was blocked by using 5% nonfat dry milk for 1 h at room temperature, followed by overnight incubation at 4 °C with primary antibodies against target proteins: *E. coli* β-gal (Abcam, ab4761, 1/1000 dilution), p53 (Santa Cruz, sc-126, 1/1000 dilution), p21 (Santa Cruz, sc-6246, 1/500 dilution) or GAPDH (ABclonal, AC033, 1/50000 dilution). Next, the membrane was incubated with species-specific horseradish peroxidase-conjugated secondary antibody (Yeasen, 33201ES60, 1:10000 dilution and Yeasen, 33101ES60, 1:10000 dilution). The protein bands were visualized by using a chemiluminescence imaging system (Tanon4600SF). ImageJ 1.50 software was used to quantify the band intensity.

Animals

Male C57BL/6 mice (7 weeks, weighing 20-22 g) were purchased from Shanghai Jiesijie Laboratory Animal Co., Ltd. and maintained under control temperature (22-23 °C) and light (12:12-hours light-dark cycle) at the animal center, approved by the Institutional Animal Care

and Use Committees of Tongji University in compliance with Chinese law for experimental animals with an approval number of SYXK (Shanghai) 2020-0002. As previously described[1, 2], renal unilateral ischemia/reperfusion injury (UIRI) was induced in mice through unilateral pedicle clamping. Briefly, mice were anesthetized with isoflurane and kept on a heating pad to maintain body temperature during surgery. The left renal pedicle was isolated and fixed with a nontraumatic vascular clamp for 35 min. The right kidney was left in situ. After removal of the clip, the kidney was observed to ensure blood reflow. Sham-operated mice were subjected to the exact same surgical procedure as UIRI, except the placement of clamp. After drug treatment and euthanization, kidneys were paraffin-embedded for immunohistology, frozen in Optimal Cutting Temperature Compound (OCT, Leica) solution for cryosectioning and SA-β-gal staining.

Drug treatment

One week after the surgery, the UIRI model mice were randomly divided into five groups: UIRI group, UIRI + Gem (1 mg/kg) group, UIRI + Gem (5 mg/kg) group, UIRI + **TSPD** (1 mg/kg) group and UIRI + **TSPD** (5 mg/kg) group. The sham-operated mice was set as sham group. All drugs were mixed with 2% DMSO (Greagent), 90% PBS, 4% Tween-80 (Greagent), and 4% polyethylene glycol (PEG) (Greagent) and were administered to mice by intraperitoneal (i.p.) injection twice a week for three weeks[3]. Four weeks after the surgery, the blood was taken via jugular vein, and then the mice were sacrificed and the kidneys were harvested and analyzed. Kidneys were weighed at the time of sacrifice.

Renal function assay

The renal function was evaluated by determining the levels of blood urea nitrogen (BUN) and serum creatinine (SCr) in the serum samples. Plasma samples from each group were collected and spuned for 20 min at 3000 rpm. The supernatants were collected and sent to KingMed Diagnostics, Shanghai, China, for BUN and SCr measurement.

X-gal staining assay of frozen sections

For endogenous acidic β-gal staining, the kidney samples were collected and embedded in OCT (Leica) solution, frozen in liquid nitrogen (N_2), and were cut into 20 μm thick sections. The kidney sections were dried at 37 °C for 20–30 min and then fixed in 4% formaldehyde at room temperature for 15 min, according to the staining manufacturer's instructions provided by Beyotime Biotechnology (C0605). The frozen sections were washed three times with PBS buffer and incubated with fresh staining solution (1 mg/mL X-gal, pH = 6.0) overnight (without CO_2) at 37 °C. Images were collected by using Nikon Eclipse Ti2 microscope with 20x magnification lenses.

Sirius red staining assay

Paraffin-embedded kidney tissue sections (5 μM) were deparaffinized, rehydratedand incubated in the picrosirius red solution for 8 min. The sections were then dehydrated and mounted. Images were collected by using Nikon Eclipse Ti2 microscope with 20x magnification lenses.

Immunofluorescence assay

Paraffin-embedded sections (5 μm thick) were deparaffinized and rehydrated. The sections were prepared for antigen retrieval in EDTA solution at 120 °C for 20 min. Then the sections were washed three times with PBS buffer and treated with blocking buffer at room temperature for 1 h. After adding Ki67 antibody (Cell Signaling, 12202, 1/500 dilution), Lamin B1 antibody (abcam, ab229025, 1/1000 dilution) or KIM-1 antibody (Bio-Techne, MAB1817, 1/200 dilution) overnight at 4 °C, the sections were washed three times with PBS buffer and then incubated with secondary antibody (abcam, ab150077, 1/500 dilution and Invitrogen A-21434, 1/500 dilution) for 2 h at room temperature. After washing with PBS buffer, 4′,6-diamidine-2′-phenylindole dihydrochloride (DAPI) (Servicebio, G10121, 1/1000 dilution) was added to stain the nucleus. All images were acquired using Nikon Eclipse Ti2 microscope with 40x magnification lenses.

Subacute toxicity experiment

Male and female ICR mice (8 weeks, weighing 20-24 g) were purchased from Zhejiang Vital River Laboratory Animal Technology Co., Ltd.. and maintained under control temperature (22-23 °C) and light (12:12-hours light-dark cycle) at the animal center, approved by the Institutional Animal Care and Use Committees of Tongji University in compliance with Chinese law for experimental animals with an approval number of SYXK (Shanghai) 2020-0002. These mice were randomly divided into three groups: control group, **TSPD**-5mg/kg group, **TSPD**-25mg/kg group. **TSPD** was mixed with 2% DMSO (Greagent), 90% PBS, 4% Tween-80 (Greagent), and 4% polyethylene glycol (PEG) (Greagent) and were administered to mice by intraperitoneal (i.p.) injection for two weeks. After two weeks, the blood was taken via jugular vein, then the mice were sacrificed and the heart, liver, spleen, lung and kidney were harvested and weighed at the time of sacrifice. Plasma samples from each group were collected and spuned for 20 min at 3000 rpm. Then the supernatants were collected and sent to Shanghai Jinji Medical Laboratory Co., Ltd., for AST and ALT measurement. These organs were paraffin-embedded for hematoxylin-eosin (HE) staining.

2. Additional data

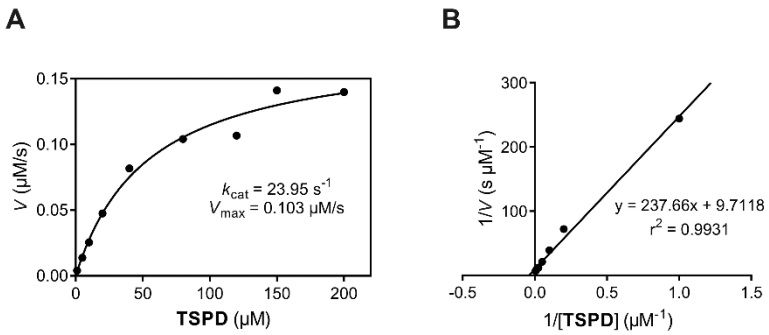


Fig. S1 (A) Non-linear regression analysis. Plot of initial velocity (*V*) of *E. coli* β-gal-catalyzed hydrolysis upon incubation with increasing concentrations (1, 5, 10, 20, 40, 80, 120, 150, 200 μM) of **TSPD**. (B) Linear regression analysis of (A).

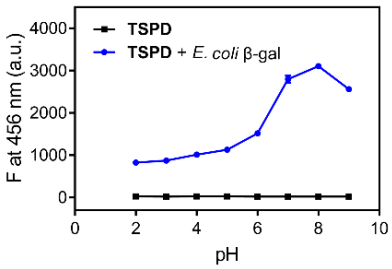


Fig. S2 Fluorescent intensity changes of **TSPD** (10 μM) and **TSPD** (10 μM) incubation with *E. coli* β-gal (1 U/mL) at different pH values (2.0−9.0). All experiments were performed at 37 °C for 15 min, λ_{ex} = 360 nm. Data are represented as mean ± SD (n = 3).

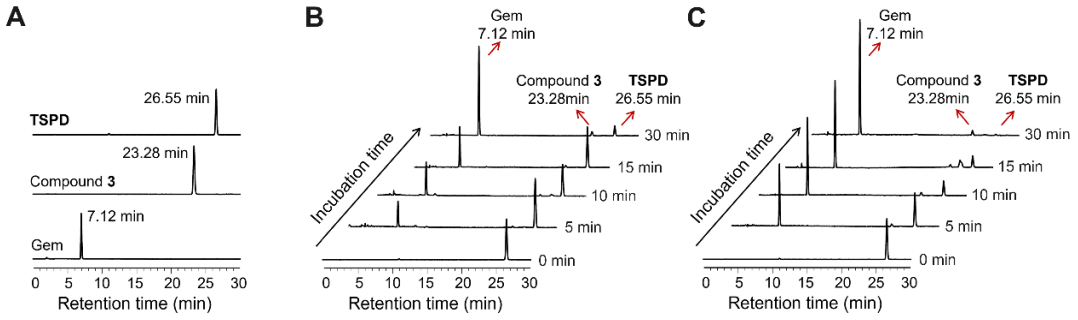


Fig. S3 (A) HPLC chromatogram of **TSPD**, compound **3** and gemcitabine. **TSPD** (50 μM) was incubated with 0.2 U/mL (B) or 0.5 U/mL (C) *E. coli* β-gal at 37 °C for the indicated times, and the reaction mixture was analyzed with HPLC using a UV detector (270 nm). Eluent A, MeOH; eluent B, water; 0-30 min, A/B = 10/90-80/20; Flow rate = 1.0 mL/min. The HPLC chromatogram were carried out using Agilent 1200 series.

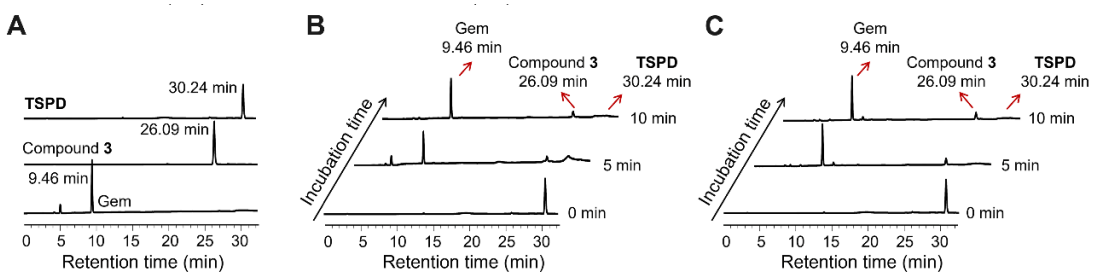


Fig. S4 (A) HPLC chromatogram of **TSPD**, compound **3** and gemcitabine. **TSPD** (50 μM) was incubated with 5 U/mL (B) or 25 U/mL (C) *E. coli* β-gal at 37 °C for the indicated times, and the reaction mixture was analyzed with HPLC using a UV detector (270 nm). Eluent A, MeOH; eluent B, water; 0-30 min, A/B = 10/90-80/20; Flow rate = 1.0 mL/min. The HPLC chromatogram were carried out using SHIMADZU LC-20A series.

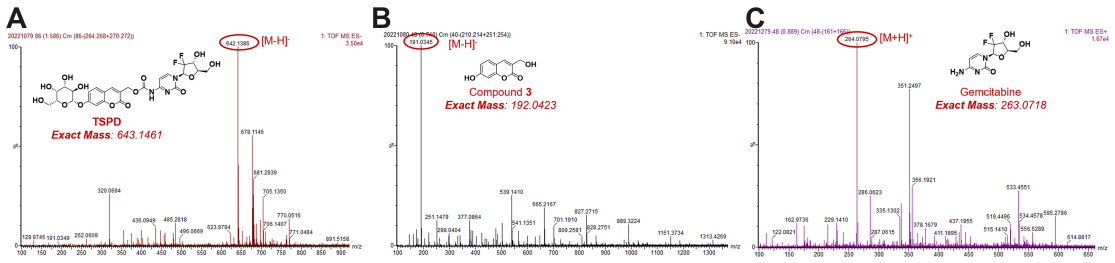


Fig. S5 (A) HRMS spectra of **TSPD** (10 μM). (B and C) HRMS spectra of the mixture of **TSPD** (10 μM) incubation with 1 U/mL of *E. coli* β-gal at 37 °C for 15 min.

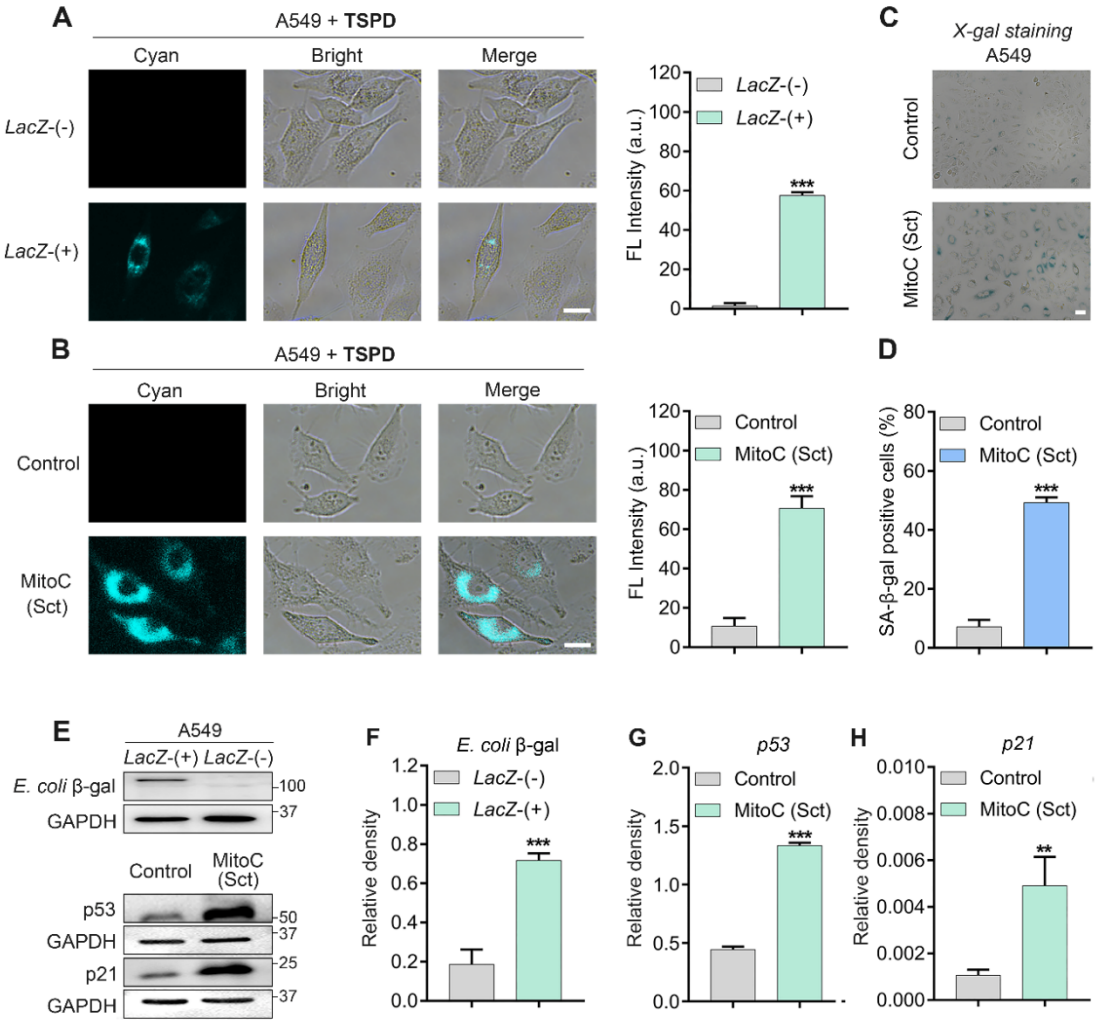


Fig. S6 (A) Fluorescence images of *LacZ*-(-) A549 cells and *LacZ*-(+) A549 cells after incubation with **TSPD** (20 μM) for 4 h. *LacZ*-(-) cells could not overexpress *E. coli* β-gal and *LacZ*-(+) cells could overexpress *E. coli* β-gal. Quantification of relative fluorescence intensity of (A) is on the right. Scale bar = 20 μm. (B) Fluorescence images of uninduced A549 cells (control) and MitoC-induced senescent (sct) A549 cells after incubation with **TSPD** (20 μM) for 4 h. Quantification of relative fluorescence intensity of (B) is on the right. Scale bar = 20 μm. (C) X-gal staining images of uninduced A549 cells and MitoC-induced senescent A549 cells. (D) The average percentage of SA-β-gal positive cells of (C). (E) Western blotting analysis of *E. coli* β-gal in A549 cells with or without *LacZ* gene and western blotting analysis of p53 and p21 in uninduced A549 cells and MitoC-induced senescent A549 cells. GAPDH was chosen as an internal reference. (F) Quantification of western blotting analysis of *E. coli* β-gal in A549 cells transfected with or without *LacZ* gene. And quantification of western blotting analysis of p53 (G) and p21 (H) in uninduced A549 cells (control) or in MitoC-induced senescent (sct) A549 cells. GAPDH was chosen as an internal reference. Significant

differences (**$P < 0.01$, ***$P < 0.001$) are analyzed with *t*-test. Data are represented as mean ± SD (n = 3).

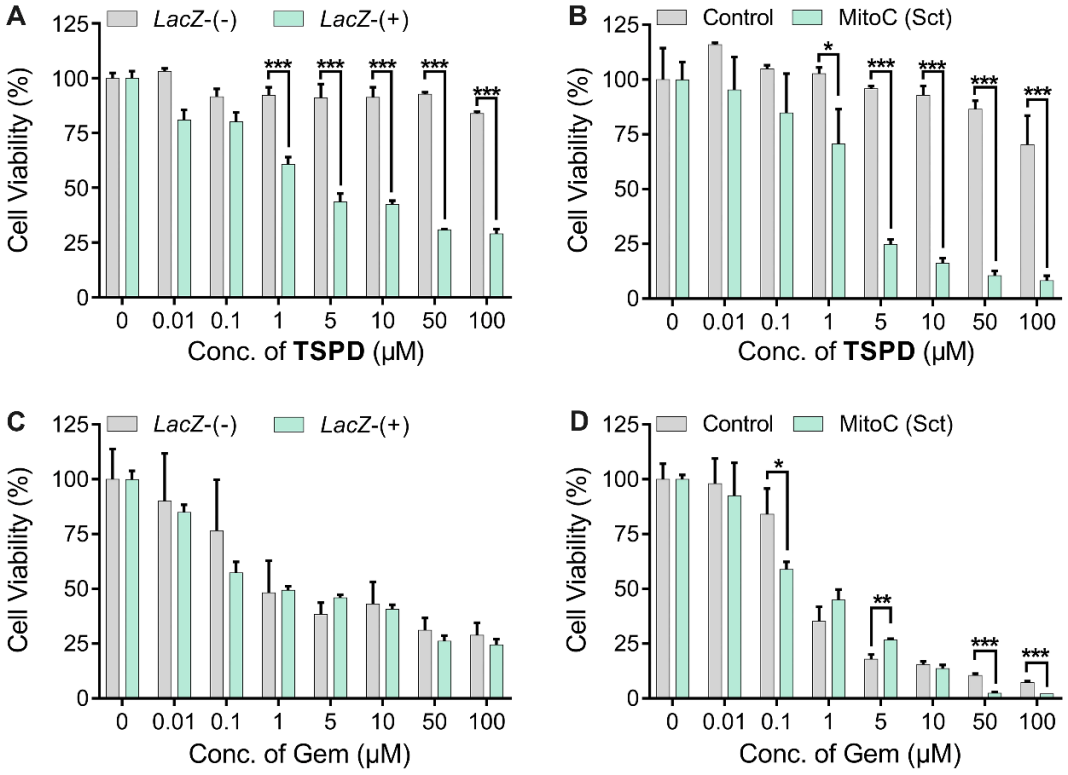


Fig. S7 (A) Quantification of cell viability of *LacZ*-(-) and *LacZ*-(+) A549 cells incubated with increasing concentrations of **TSPD** for 3 days. (B) Quantification of cell viability of uninduced A549 cells and MitoC-induced senescent A549 cells incubated with increasing concentrations of **TSPD** for 3 days. (C) Quantification of cell viability of *LacZ*-(-) and *LacZ*-(+) A549 cells incubated with increasing concentrations of gemcitabine for 3 days. (D) Quantification of cell viability of uninduced A549 cells and MitoC-induced senescent A549 cells incubated with increasing concentrations of gemcitabine for 3 days. Significant differences (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) are analyzed with *t*-test. Data are represented as mean ± SD (n = 3).

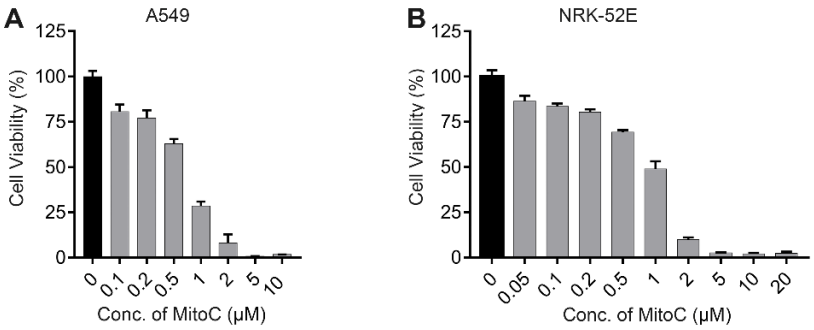


Fig. S8 (A) Cytotoxicity of MitoC on A549 cells. A549 cells were treated with increasing concentrations of MitoC for 48 h, then the cell viability was determined by CCK-8 assay. (B) Cytotoxicity of MitoC on NRK-52E cells. NRK-52E cells were treated with increasing concentrations of MitoC for 48 h, then the cell viability was determined by CCK-8 assay. Data are represented as mean ± SD (n = 3).

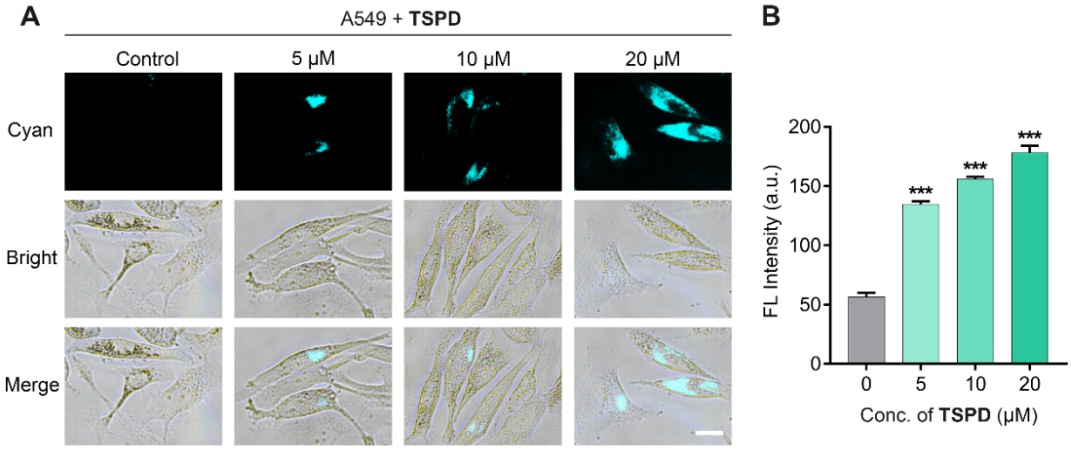


Fig. S9 (A) Fluorescent images of senescent A549 cells treated with **TSPD**. Senescent A549 cells were incubated with various concentrations of **TSPD** for 4 h and then the fluorescence images were acqured. (B) Fluorescence intensities output of (A). Scale bar = 20 μm. Significant differences (***$P < 0.001$) are analyzed with *t*-test. Data are represented as mean ± SD (n = 3).

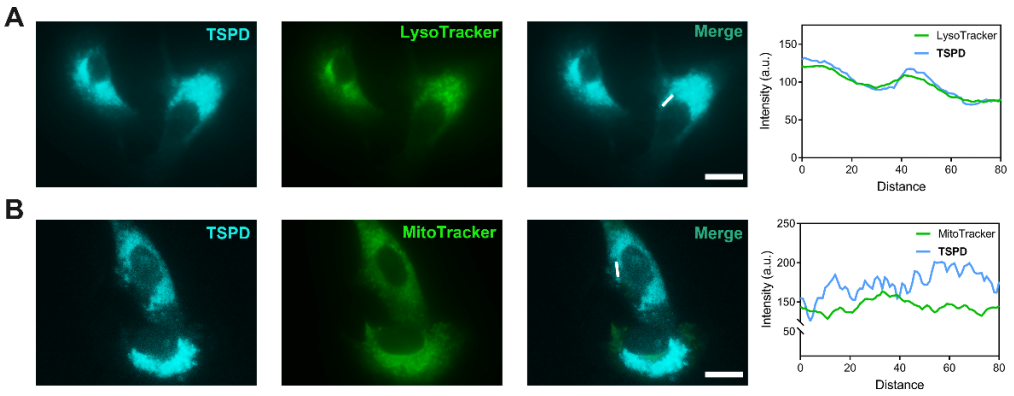


Fig. S10 Intracellular localization of **TSPD** in MitoC-induced senescent A549 cells. Cells were incubated with **TSPD** (20 μM) for 4 h and then co-stained with 1 μM LysoTracker (LysoGreen) for 30 min (A), or 200 nM MitoTracker Green for 30 min. (B) Cross-sectional analysis along the white line were on the right. Scale bar = 10 μm.

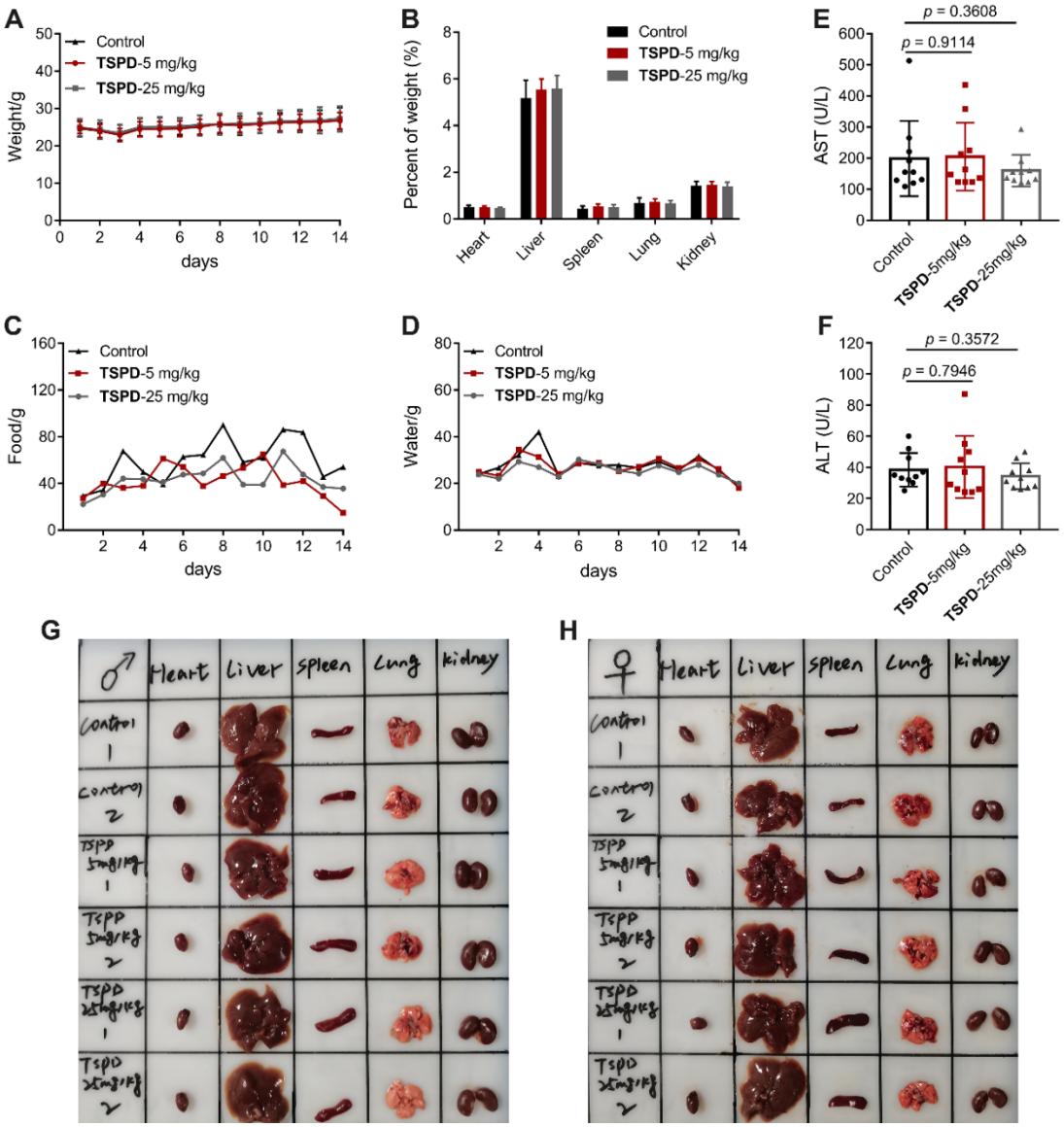


Fig. S11 Subacute toxicity experiment**.** (A) Effect of **TSPD** on mice body weight (n = 12). (B) Effect of **TSPD** on organ percent of weight in mice (n = 12). (C and D) Effect of **TSPD** on mice food intake and water intake. (E and F) Serum biochemical test. The levels of AST and ALT (n = 10). (G and H) Representative images of hearts, livers, spleens, lungs and kidneys.

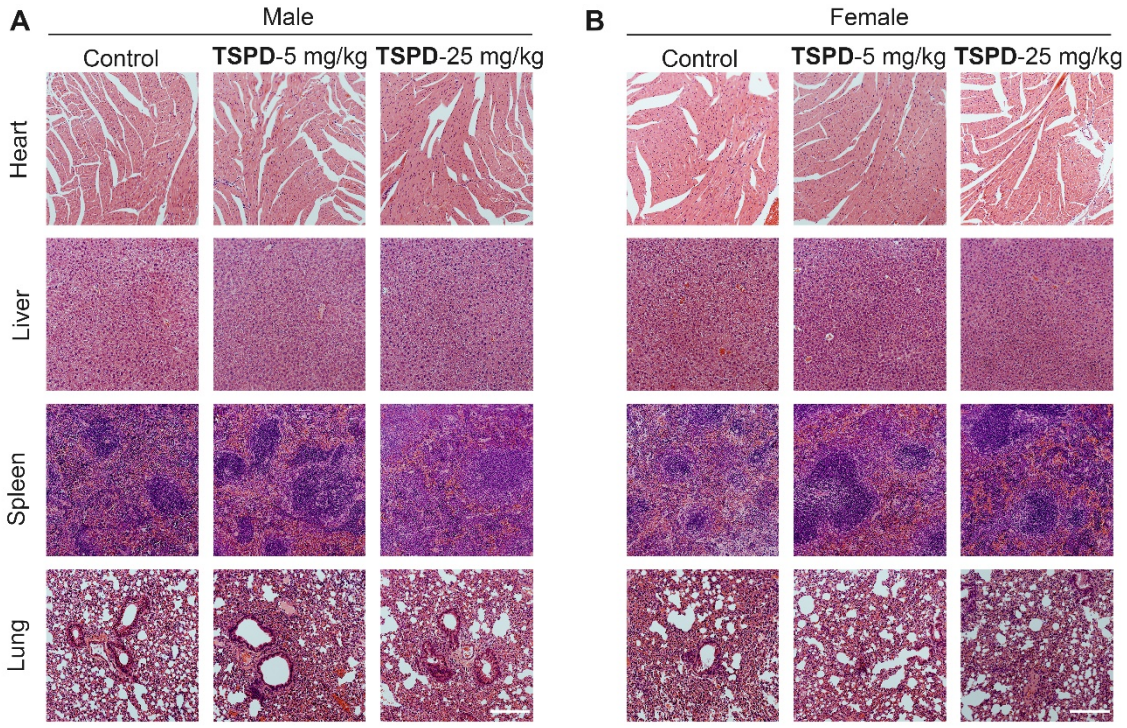


Fig. S12 (A and B) Hematoxylin-eosin (HE) staining of vital organs including heart, liver, spleen and lung in the mice of subacute toxicity experiment. Scale bar = 50 μm.

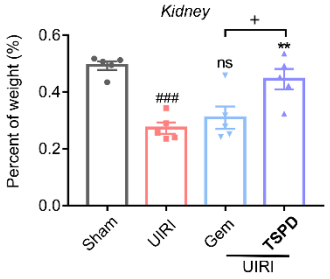
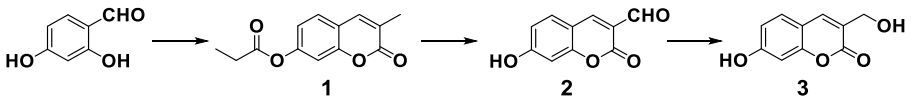


Fig. S13 The ratio of left kidney weight to body weight from each group. Significant differences ($^{###}P < 0.001$, compared with Sham group, $^{**}P < 0.01$, compared with UIRI group, $^{+}P < 0.05$, compared with UIRI + Gem group) are analyzed with *t*-test. Data are represented as mean ± SD (n = 5).

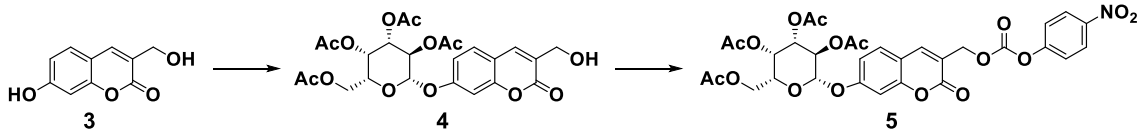
3. Chemical synthesis and structural characterization



Compound **1** and Compound **2** were synthesized according to previously published methods[4, 5].

Synthesis of compound 3. Compound **2** (1.90 mg, 10 mmol) was dissolved in 100 mL of dry CH_3OH and cooled to 0 °C. Then $NaBH_4$ (0.60 g, 16.2 mmol) was added portion-wise to the mixture. After that, the reaction mixture was removed to room temperature and stirred for 2 h. After completion of the reaction, the reaction mixture was quenched with saturated ammonium chloride solution and diluted with EtOAc (100 mL), and then washed twice with brine. The organic layer was seperated, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography on silica gel to give compound **3** in 57.29% yield as a yellow solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.44 (s, 1H), 7.85 (s, 1H), 7.57 (d, J = 8.4 Hz, 1H), 6.78 (dd, J = 8.5, 2.3 Hz, 1H), 6.72 (d, J = 2.2 Hz, 1H), 5.32 (t, J = 5.7 Hz, 1H), 4.30 (d, J = 3.9 Hz, 2H). ^{13}C NMR (151 MHz, Acetone-d_6) δ 162.16, 161.96, 156.73, 139.22, 131.01, 126.90, 114.71, 114.15, 103.96, 60.78. HR-MS (EI): calcd for $C_{10}H_8O_4$ $(M + Na)^+$ 215.0320, found: 215.0.

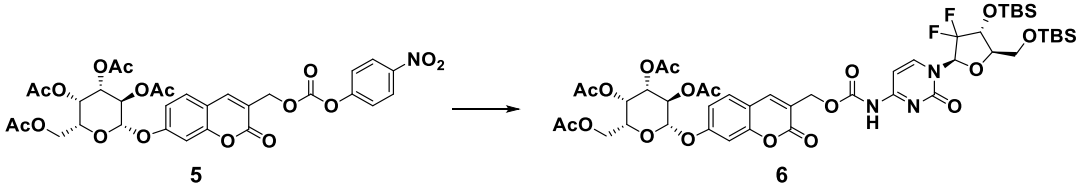


Synthesis of compound 4. A solution of 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTTA) (1.80 mL, 6.3 mmol) and Ag_2CO_3 (9 g, 33.3 mmol) in anhydrous CH_3CN (13.50 mL) was stirred at room temperature for 2 h. Then compound **3** (1.73 g, 9 mmol) and solid 2,3,4,6-tetra-*O*-acetyl-α-*D*-galactopyranosyl bromide (11.10 g, 27 mmol) was added at 0 °C, and the solution mixture was stirred for 4 hours at room temperature. After filtration over a pad of celite, the filtrate was washed with 1N HCl and extracted with EtOAc (3 x 50 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography on silica gel to give compound **4** in 38.50% yield as a white solid. Because of its unstable chemical properties, it is directly used for the next step of synthesis.

Synthesis of compound 5. Compound **4** (522 mg, 1 mmol) was dissolved in dry THF (15 mL) under N_2 atmosphere and cooled to 0 °C. DIPEA (0.72 mL, 4 mmol) and 4-Nitrophenyl chloroformate (0.62 g, 3 mmol) were added, followed by pyridine (25 μL, 0.3 mmol). The solution mixture was stirred for 1 h. The reaction mixture was diluted with EtOAc then washed

twice with saturated NH_4Cl solution and once with brine. The organic phase was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography on silica gel to give compound **5** in 43.67% yield as a white solid.

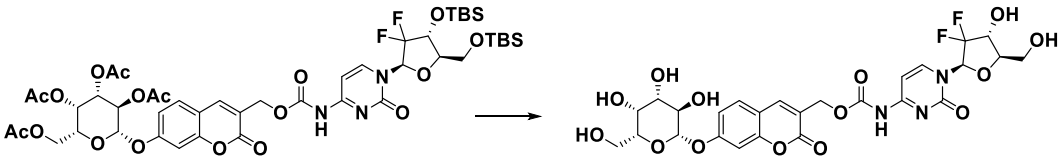
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33 (d, J = 9.1 Hz, 2H), 8.21 (s, 1H), 7.79 (d, J = 8.6 Hz, 1H), 7.63–7.57 (m, 2H), 7.11 (d, J = 2.3 Hz, 1H), 7.01 (dd, J = 8.7, 2.4 Hz, 1H), 5.68 (d, J = 6.3 Hz, 1H), 5.38 (s, 1H), 5.26 (t, J = 6.2 Hz, 2H), 5.17 (s, 2H), 4.52 (t, J = 6.4 Hz, 1H), 4.12 (d, J = 6.3 Hz, 2H), 2.15 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.96 (s, 3H). ^{13}C NMR (151 MHz, Acetone-d_6) δ 171.68, 171.46, 171.15, 170.73, 161.80, 161.37, 157.58, 157.10, 147.46, 143.99, 131.76, 127.05, 124.10 (2C), 121.77 (2C), 115.92, 105.30, 100.09, 73.18, 72.41, 70.16, 69.11, 67.61, 63.22, 21.53, 21.51, 21.46, 21.41. HR-MS(EI): calcd for $C_{31}H_{29}NO_{17}$ $(M + Na)^+$ 710.1333, found:710.1.



Synthesis of compound 6. TBSGem was synthesized according to previously published methods[6].

TBSGem (491 mg,1 mmol) was dissolved in dry THF (35 mL) under N_2 atmosphere and cooled to -78 °C. 1 M LiHMDS was added dropwise. The mixed solution was removed to room temperature and stirred for 0.5 h. Compound **5** (450 mg, 0.655mmol) was dissolved in dry THF (10 mL) and added to the reaction mixture. After completion of the reaction, the reaction mixture was concentrated in vacuo. The residue was purified by column chromatography on silica gel to give compound **6** in 55.82% yield as a white solid.

^{1}H NMR (400 MHz, Chloroform-d) δ 8.03 (dd, J = 9.0, 1.6 Hz, 2H), 7.78 (s, 1H), 7.40 (d, J = 8.5 Hz, 1H), 7.19 (s, 1H), 6.91 (d, J = 2.2 Hz, 1H), 6.89–6.85 (m, 2H), 6.23 (d, J = 10.1 Hz, 1H), 5.44 (dd, J = 10.4, 7.9 Hz, 1H), 5.41 (d, J = 3.5 Hz, 1H), 5.12–5.06 (m, 3H), 4.26 (td, J = 11.6, 8.0 Hz, 1H), 4.18–4.10 (m, 2H), 4.08–4.04 (m, 1H), 3.95 (d, J = 12.0 Hz, 1H), 3.89 (d, J = 8.2 Hz, 1H), 3.73 (dd, J = 11.9, 1.9 Hz, 1H), 2.11 (s, 3H), 2.03 (s, 3H), 2.00 (s, 3H), 1.95 (s, 3H), 0.87 (s, 9H), 0.82 (s, 9H), 0.06–0.02 (m, 12H). ^{13}C NMR (151 MHz, Acetone-d_6) δ 176.54, 176.32, 176.02, 175.59, 170.09, 166.40, 166.10, 161.64, 150.34, 147.07, 136.30, 132.66, 129.20, 127.69, 122.34, 120.92, 120.74, 110.19, 105.00, 101.31, 87.79, 78.04, 77.29, 76.49, 76.33, 75.04, 73.99, 69.02, 68.09, 66.94, 32.08, 31.67, 26.41, 26.39, 26.33, 26.28, 24.73 (3C), 24.26 (3C), 1.19, 0.83, 0.52, 0.50. HR-MS(ESI): calcd for $C_{46}H_{63}F_2N_3O_{18}Si_2$ $(M + Na)^+$ 1062.3511, found: 1062.3512.



Synthesis of TSPD. Compound **6** (550 mg,0.53 mmol) was dissolved in dry THF (30 mL) under N_2 atmosphere and 1.32 mL TBAF (1 M in THF, 1.32 mmol) was added dropwise. The solution mixture was stirred for 0.5 hour. The reaction mixture was diluted with DCM (200 mL) then washed with water (5 x 100 mL) The organic phase was dried over anhydrous Na_2SO_4 and concentrated in vacuo to give the crude intermediate product.

The intermediate was dissolved in dry CH_3OH (14 mL) under N_2 atmosphere and 0.4 mL CH_3ONa (5 M in CH_3OH, 2 mmol) was added dropwise. The solution mixture was stirred for 0.5 h. After completion of the reaction, the solution pH was neutralized with acetic acid. The reaction mixture was concentrated in vacuo. The residue was purified by column chromatography on silica gel to give **TSPD** in 62.75% yield as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.05 (s, 1H), 8.24 (d, J = 7.6 Hz, 1H), 8.13 (s, 1H), 7.68 (d, J = 8.6 Hz, 1H), 7.12–7.03 (m, 3H), 6.38 (s, 1H), 6.17 (t, J = 7.4 Hz, 1H), 5.32 (d, J = 26.4 Hz, 2H), 5.07–4.94 (m, 4H), 4.71 (s, 1H), 4.63–4.56 (m, 1H), 4.20 (td, J = 12.7, 8.2 Hz, 1H), 3.89 (dt, J = 8.6, 3.0 Hz, 1H), 3.81 (d, J = 12.6 Hz, 1H), 3.71 (d, J = 5.3 Hz, 1H), 3.68 (d, J = 5.9 Hz, 1H), 3.65–3.56 (m, 2H), 3.56–3.46 (m, 2H), 3.43 (dd, J = 9.6, 3.2 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 163.86, 160.91, 160.26, 155.00, 154.49, 144.82, 141.98, 130.07, 125.16, 123.44, 121.73, 120.16, 114.49, 113.45, 103.45, 101.08, 95.55, 81.47, 76.19, 73.68, 70.56, 68.67 (t, J = 22.4 Hz, 1C), 62.64, 60.85, 59.25. HR-MS(ESI): calcd for $C_{26}H_{27}F_2N_3O_{14}$ $(M + Na)^+$ 666.1359, found: 666.1357.

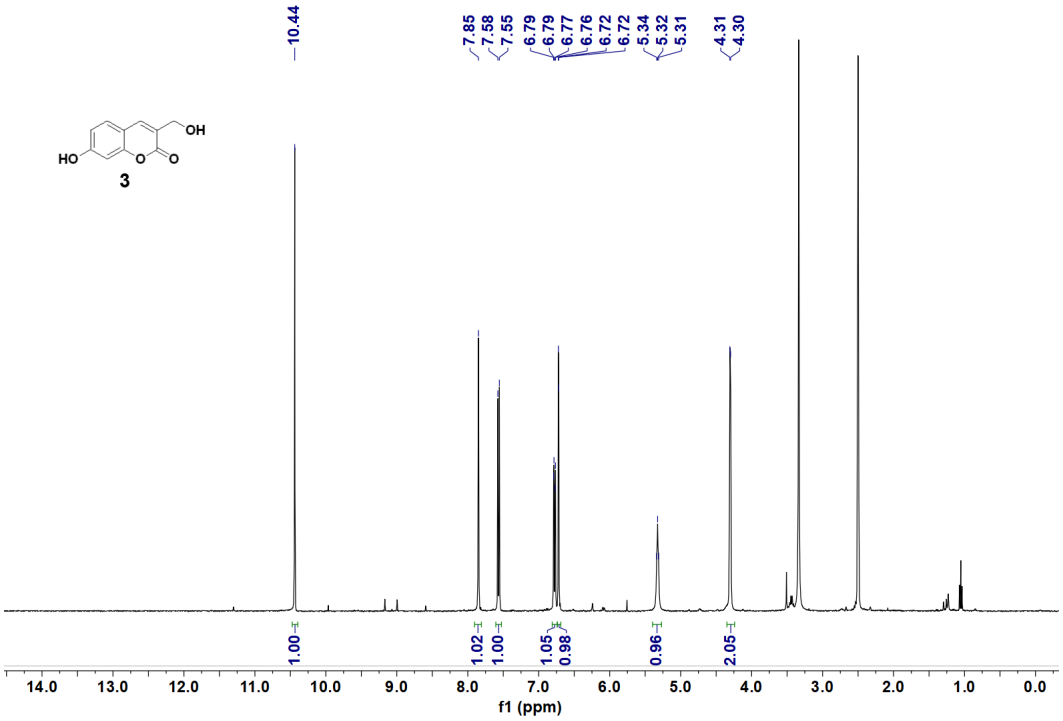


Fig. S14 The ^{1}H-NMR of compound **3** in DMSO

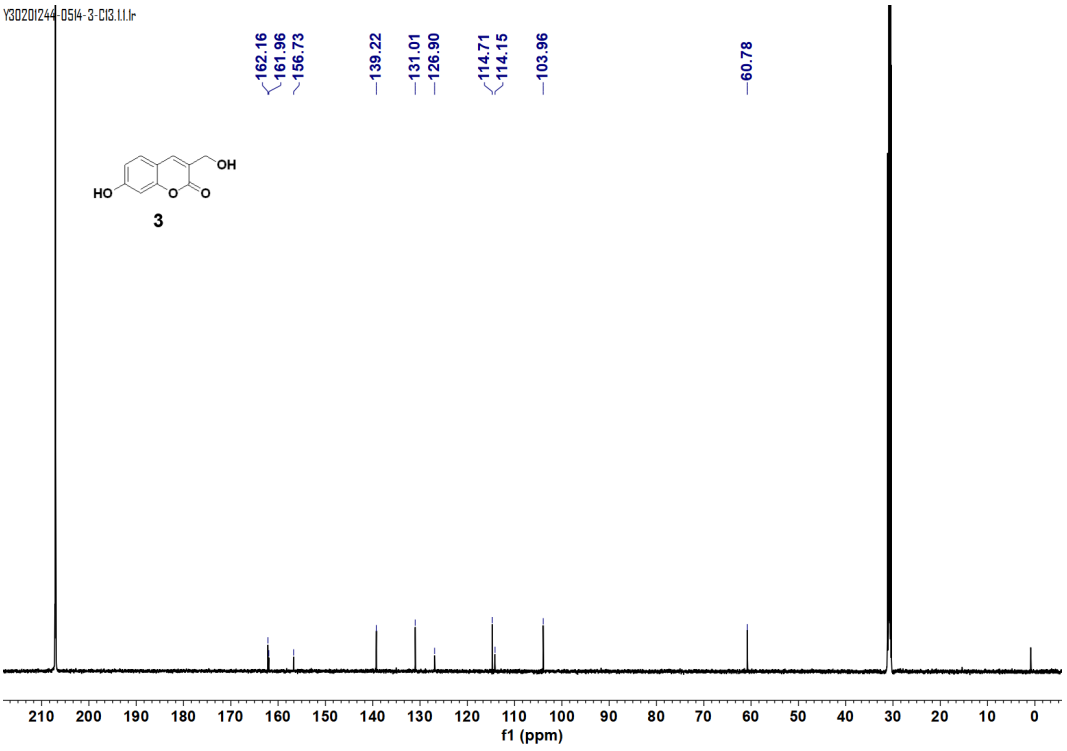


Fig. S15 The ^{13}C-NMR of compound **3** in acetone

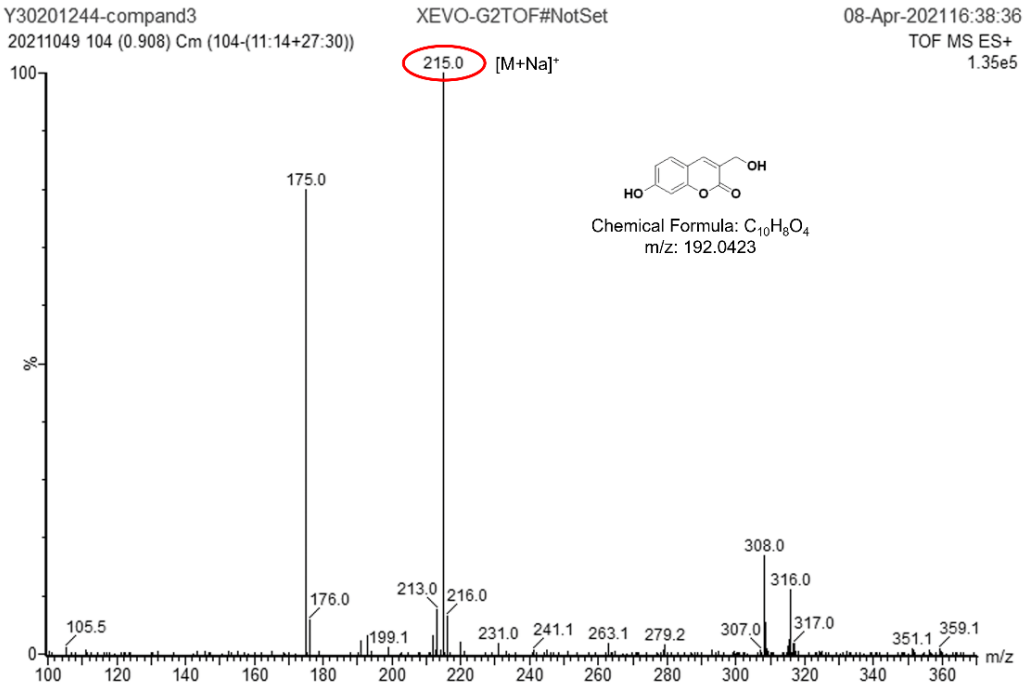


Fig. S16 The HRMS of compound **3**

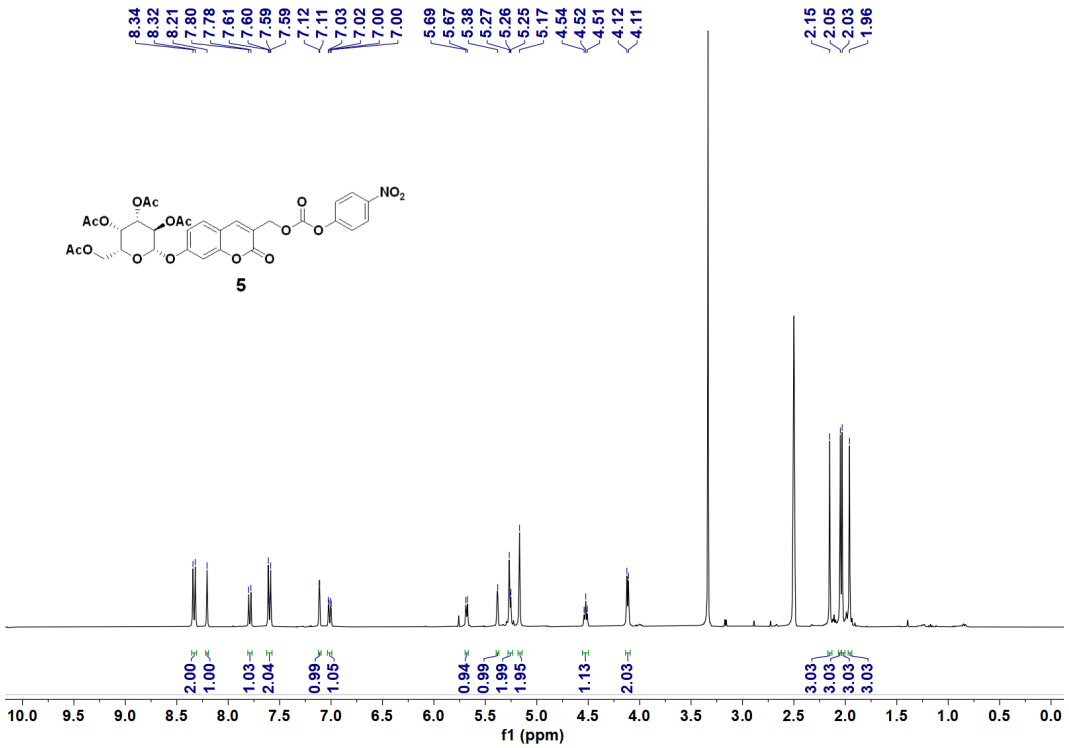


Fig. S17 The ^{1}H-NMR of compound **5** in DMSO

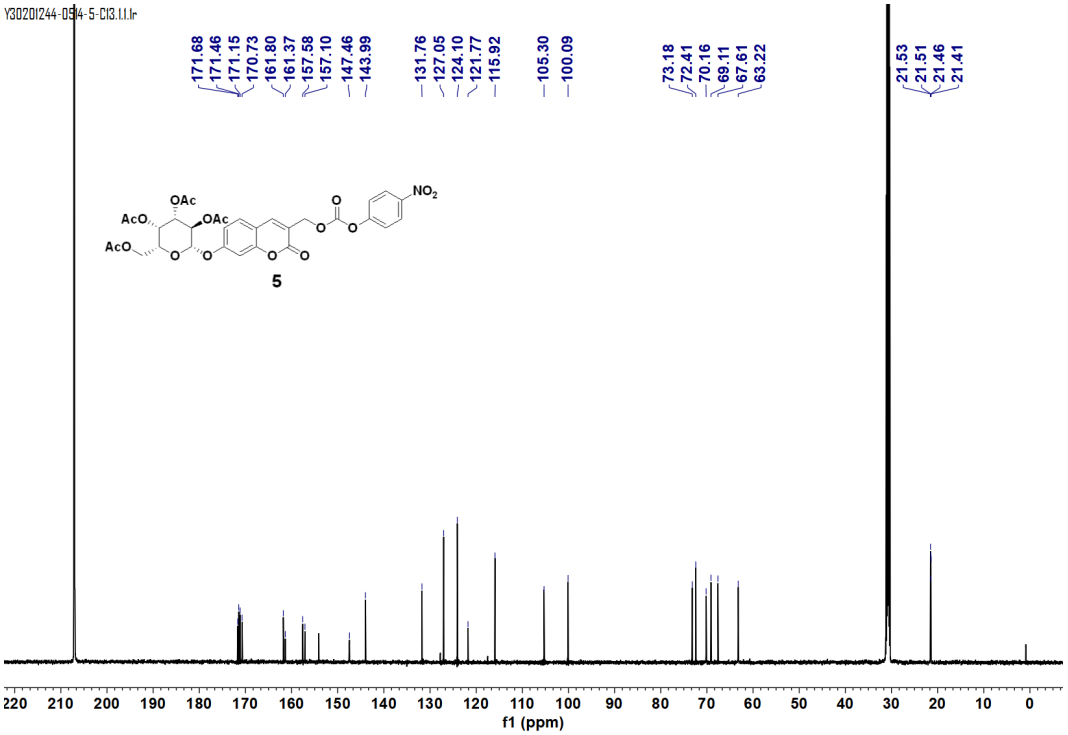


Fig. S18 The ^{13}C-NMR of compound **5** in acetone

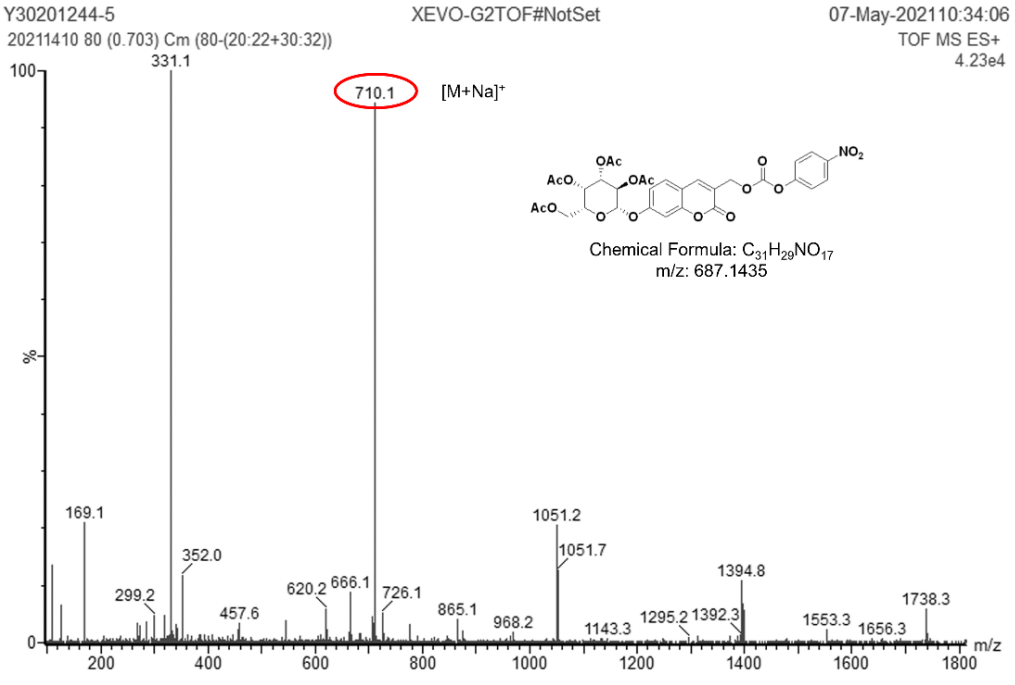


Fig. S19 The HRMS of compound **5**

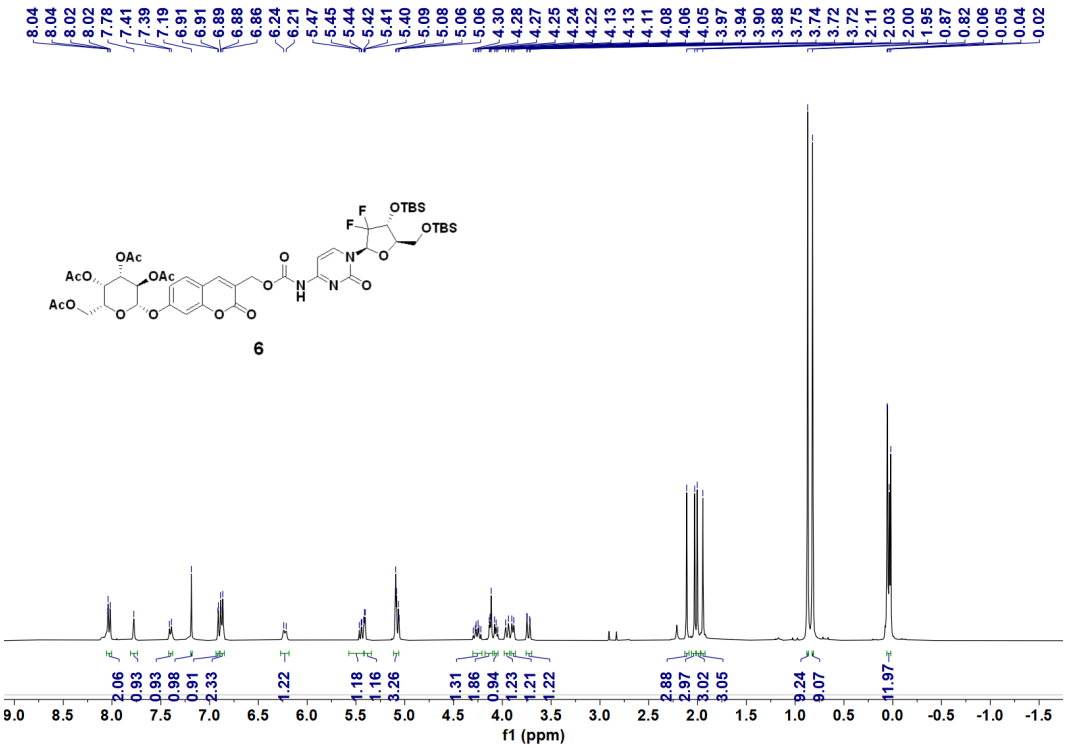


Fig. S20 The ^{1}H-NMR of compound **6** in $CDCl_3$

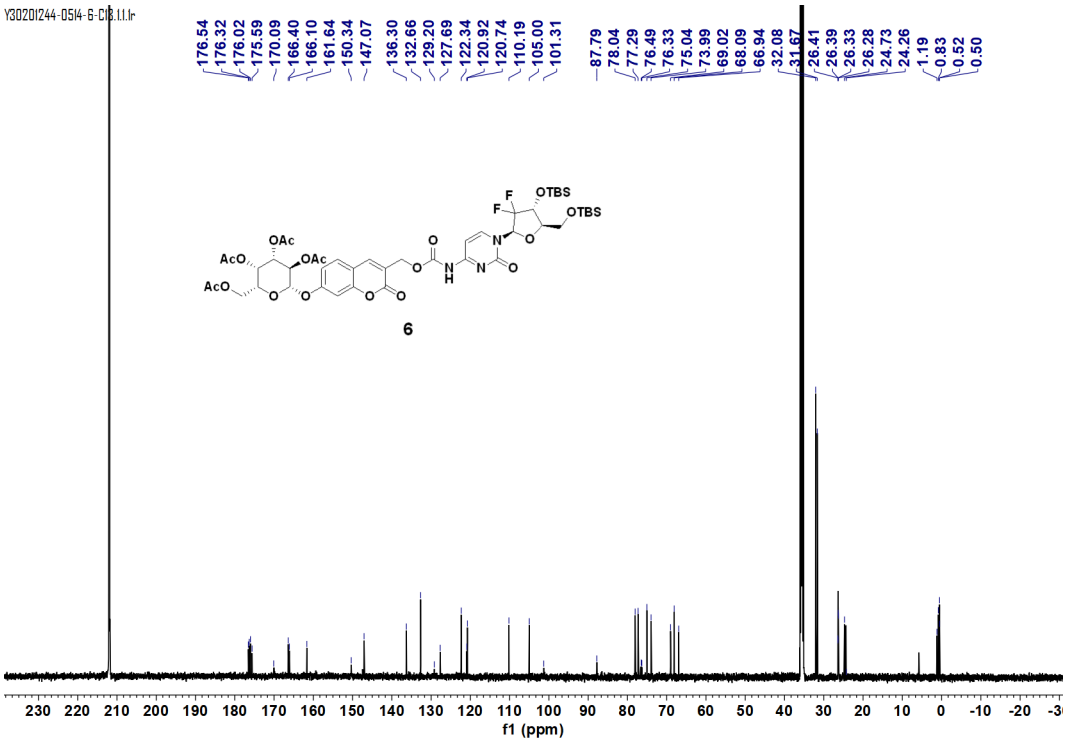


Fig. S21 The ^{13}C-NMR of compound **6** in acetone

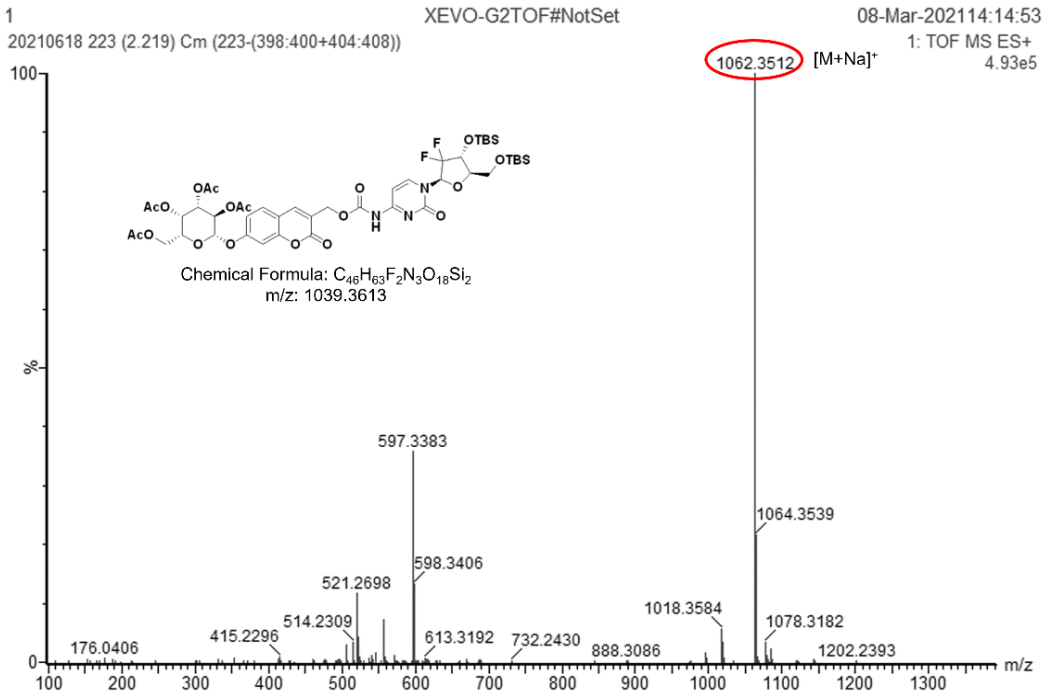


Fig. S22 The HRMS of compound **6**

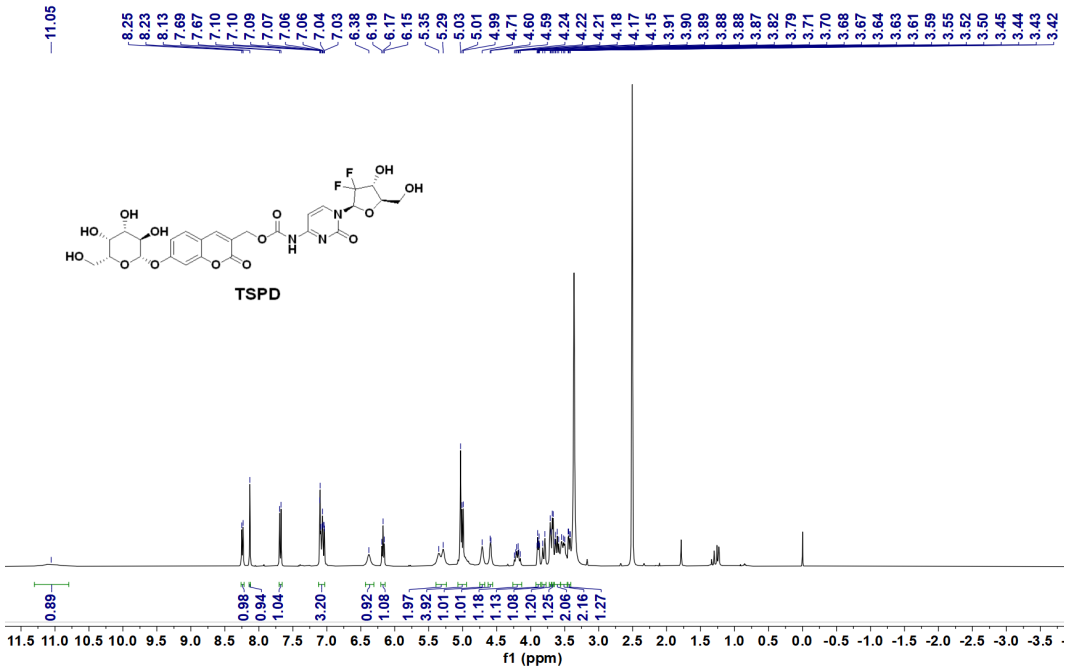


Fig. S23 The ^{1}H-NMR of **TSPD** in DMSO

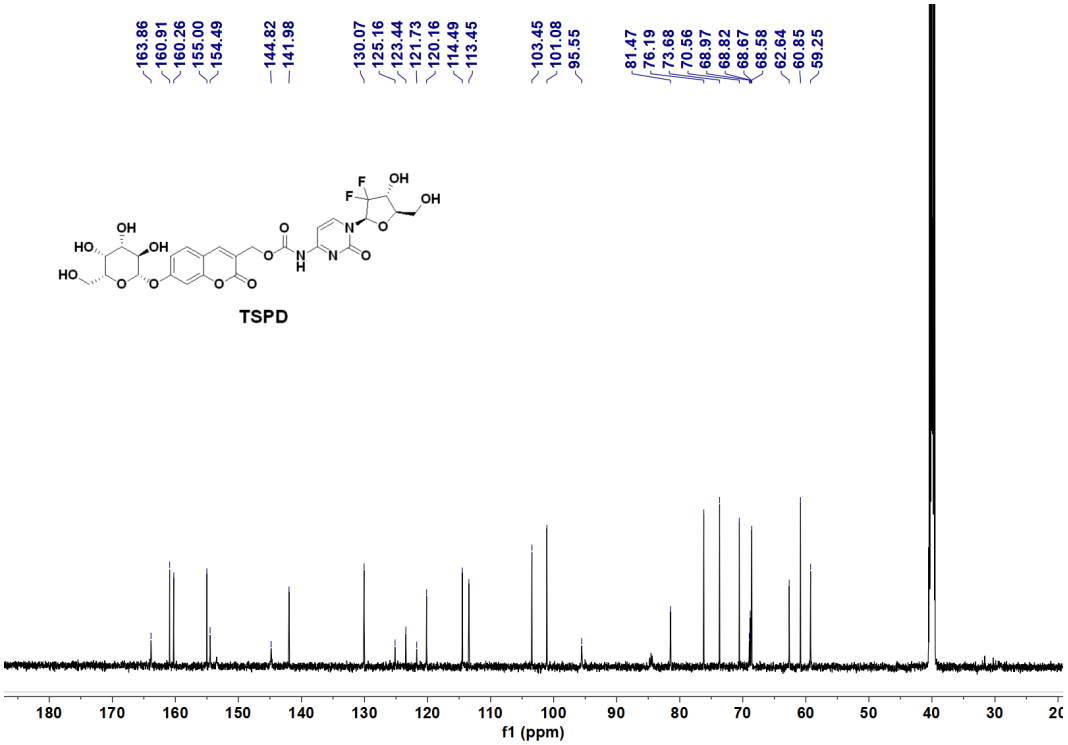


Fig. S24 The ^{13}C-NMR of **TSPD** in DMSO

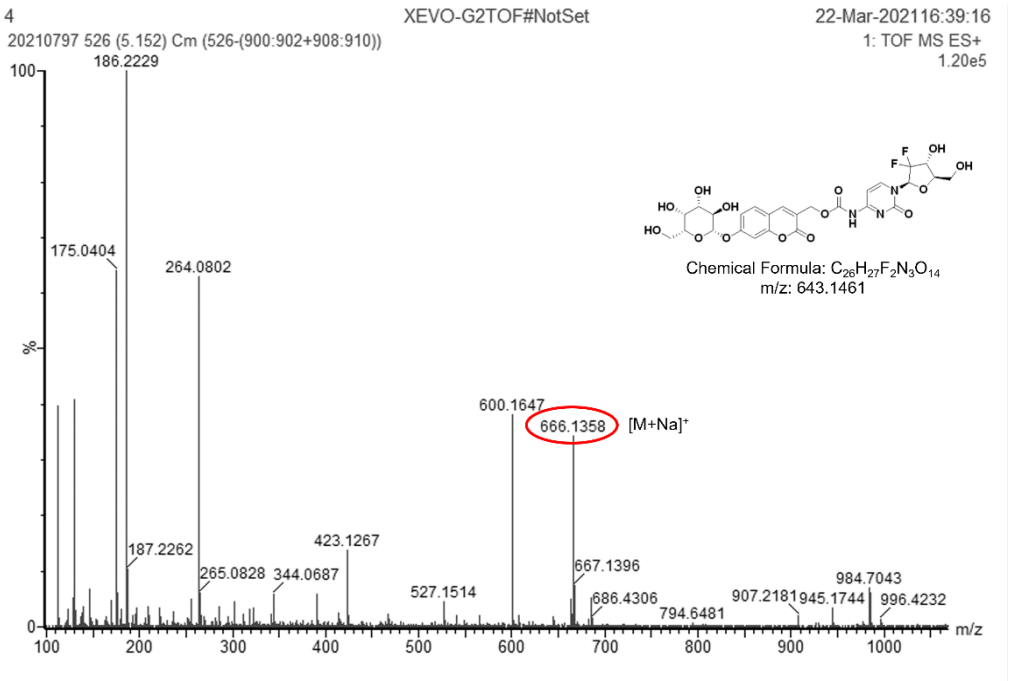


Fig. S25 The HRMS of **TSPD**

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