Experimental Supporting Information

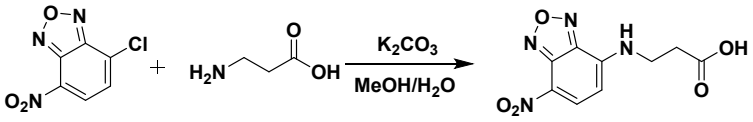
Materials and general methods:

Chemicals and materials: Fmoc-amino acids were obtained from GL Biochem (Shanghai). 4-Chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) was purchased from Aladdin Chemistry CO. Ltd. Pentafluorophenol and β-D- (+)-Glucose pentaacetate were brought from Meryer Chemical Technology CO. Ltd (Shanghai). 2-Cl-trityl chloride resin was obtained from Nankai Resin Co. Ltd. (Tianjin). β-Galactosidase (12.1 units/mg solid) was obtained from Sigma-Aldrich (Shanghai). All the other starting materials were obtained from Alfa. Commercially available reagents were used without further purification, unless noted otherwise. Nanopure water was used for all experiments. All other chemicals were reagent grade or better.

General methods: The synthesized compounds were characterized using ^{1}H NMR (Bruker ARX 400). ESI-MS were performed at the Thermo Finnigan LCQ AD System. HPLC was conducted at LUMTECH HPLC (Germany) system using a C18 RP column with MeOH (0.1% of TFA) and water (0.1% of TFA) as the eluents. Fluorescence spectrum was recorded on a BioTek Synergy™ 4 Hybrid Microplate Reader. Circular dichroism (CD) spectrum was obtained by a BioLogic Synergy 4. Dynamic light scattering (DLS) was performed on a laser light scattering spectrometer (BI-200SM) fixing the angle at 90° under room temperature (22-25 °C). Reology (TA instrument) test was done on an AR 2000ex system, 25 mm parallel plates were used during the experiment at the gap of 500 μm. Fluorescence spectra of the plates were recorded on a BioTek Synergy 4 Hybrid microplate reader. CLSM

tracking images were taken by a confocal laser scanning microscopy (Leica TSC SP8, Germany). LC-MS was conducted at the Shimadzu LCMS-20AD (Japan) system.

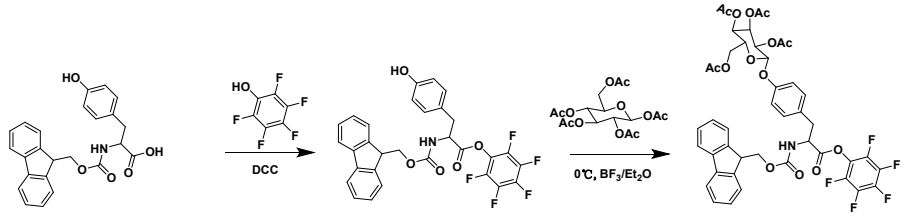
Preparation of NBD-β-Alanine



Scheme S1. The synthetic route for NBD-β-Alanine

To a 15 mL water solution containing 980 mg of β-Alanine (1.1 equiv., 11 mmol) and 4.14 g of K_2CO_3 (3 equiv.), 2 g of 4-Chloro-7-nitrobenzo-2-oxa-1, 3-diazole (1 equiv., 10 mmol) in 20 mL of CH_3OH was added slowly in the N_2 atmosphere. The reaction was completed 5 hours later at room temperature. The reaction mixture was concentrated by a rotary evaporator to remove the MeOH, The obtained aqueous solution was acidified to around pH 3 by HCl (2 mol/L). The aqueous mixture was extracted with ether (30 mL*2) and dichloromethane (20 mL*2). The combined organic solutions were dried over $MgSO_4$ and then concentrated by a rotary evaporator. The resulting yellowish solid (NBD-β-Alanine) was directly used for solid phase peptide synthesis.

Preparation of Fmoc-Tyr-[β-D-Glc(OAc)$_4$]-OPfp



Scheme S2. The synthetic route for Fmoc-Tyr- [β-D-Glc(OAc)$_4$]-OPfp

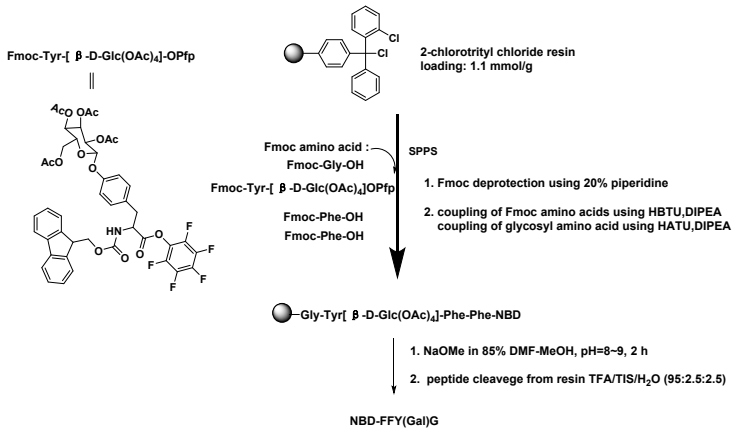
Synthesis of Fmoc-Tyr-OPfp: Pentafluorophenol (l.38g, 7.5 mmol) was dissolved in THF (50 cm^3). The solution was cooled to -20 ℃ on a solid CO_2-bath and DCC (1.14 g, 5.5 mmol) was added to the magnetically stirred solution. After 30 min, Fmoc-Tyr-

OH was added and the mixture was stirred overnight at -20 ℃. The precipitate was removed by filtration and the solution was concentrated to give an oil. Crystals formed by crystallization from acetonitrile contained an impurity (presumably dicyclohexylurea). Therefore, they were redissolved in THF, diluted with diethyl ether, evaporated to a small volume, diluted with diethyl ether, and then applied to a dry VLC column and eluted with EtOAc-light petroleum (1:5) to yield Fmoc-Tyr-OPfp.

Fmoc-Tyr-OPfp (0.5 mmol, 1 equiv), β-D-Glc(OAc)$_5$ (1.17 g, 3mmol, 6 equiv) and CH_2Cl_2 (10 mL) were placed in a round bottom flask and N_2 gas was bubbled for 5 min. The mixture was stirred for 30 min at room temperature under N_2. After being cooled down to 0 ℃, boron trifluoride ether complex (390 μL, 3 mmol, 6 equiv) was then added and N_2 gas bubbling continued for another 5 min. The reaction was stirred at 0 ℃ for 4 h. After the TLC showed the completion of the reaction, the mixture was filtered and the filtrate was washed with saturated $NaHCO_3$. The organic layer was rotavapored to produce a crude residue which was purified by silica gel column chromatography (light petroleum:EtOAc: = 3:2 by volume) to provide the final product.

Peptide synthesis: The O-glycopeptide derivative was synthesized by solid phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin, the corresponding N-Fmoc protected amino acids with side chains properly protected by different group. Firstly the C-terminal of the first amino acid was conjugated on the resin. Anhydrous N,N'-dimethyl formamide(DMF) containing 20% piperidine was used to remove Fmoc protected group. To couple the next amino acid to the free amino group, O-

(Benzotriazol-1-yl)-N,N,N’,N’-tetramethyluronium hexafluorophosphate (HBTU) was used as coupling reagent. O-glycosylated Fmoc amino acid were introduced using more reactive2-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU, 2.0 equiv). The growth of the peptide chain was according to the established Fmoc SPPS protocol. At the final step, NBD-β-Alanine was used to produce NBD-peptides. After the last coupling step, excessive reagents were removed by a single DMF wash for 5 minutes (5 mL per gram of resin), followed by five steps of washing using DCM for 2 min (5 mL per gram of resin). At the end of stepwise solid phase synthesis, OAc-protection of the glucose was removed with NaOMe in 85% DMF–MeOH (pH=8~9). The peptide derivative was cleaved using 95% of trifluoroacetic acid with 2.5% of TIS and 2.5% of H_2O for 30 minutes. Twenty mL per gram of resin of ice-cold diethylether was then added to cleavage reagent. The resulting precipitate was centrifuged for 10 min at 4 °C at 10 000 rpm. Afterward the supernatant was decanted, and the resulting solid was dissolved in DMSO for HPLC separation using CH_3OH and H_2O containing 0.1% of TFA as eluents. The yellow powder was obtained after freeze-drying. The synthetic process of glycopeptide was showed in Scheme S3.



Scheme S3. Solid-phase synthesis of NBD-FFY(Gal)G

Characterization of Fmoc-Tyr-[β-D-Glc(OAc)$_4$]-Opfp

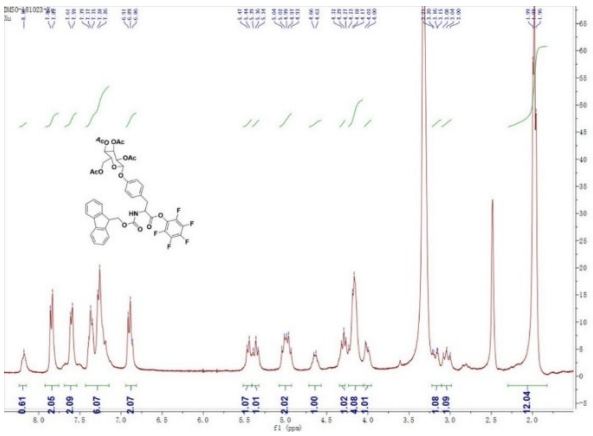


Figure S1. ^{1}H NMR spectrum of Fmoc-Tyr-[β-D-Glc(OAc)4]-Opfp

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.18 (s, 1H), 7.85 (d, J = 7.3 Hz, 2H), 7.60 (d, J = 6.9 Hz, 2H), 7.43 – 7.14 (m, 6H), 6.89 (t, J = 8.1 Hz, 2H), 5.46 (d, J = 8.5 Hz, 1H), 5.41 – 5.32 (m, 1H), 5.08 – 4.93 (m, 2H), 4.72 – 4.56 (m, 1H), 4.31 (d, J = 9.0 Hz, 1H), 4.24 – 4.06 (m, 4H), 4.01 (d, J = 8.7 Hz, 1H), 3.22 – 3.10 (m, 1H), 3.11 – 2.99 (m, 1H), 2.30 – 1.82 (m, 12H). M^+ = 899.2212, obsvd. $(M+NH_4)^+$ = 917.2552.

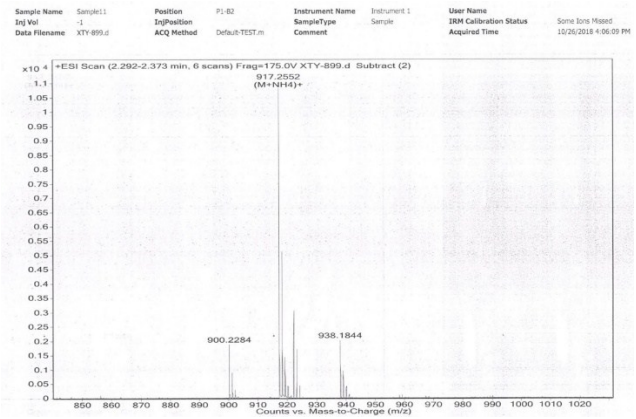


Figure S2. HR-MS spectrum of Fmoc-Tyr-[β-D-Glc(OAc)4]-Opfp

Characterization of Compound NBD-FFY(Gal)G:

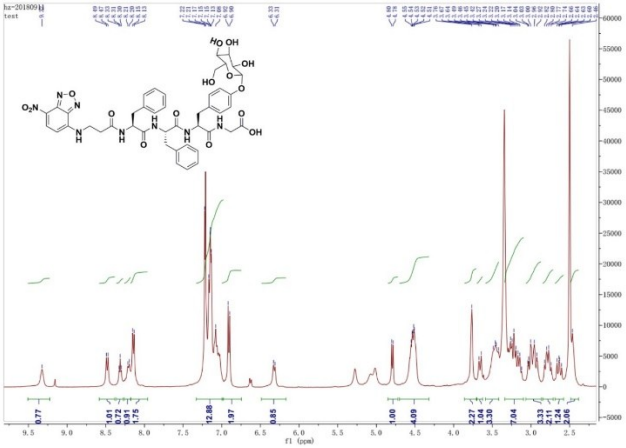


Figure S3. ^{1}H NMR spectrum of NBD-FFY(Gal)G

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.59 (s, 1H), 9.32 (s, 1H), 8.48 (d, J = 8.8 Hz, 1H), 8.31 (t, J = 5.6 Hz, 1H), 8.21 (d, J = 7.8 Hz, 1H), 8.14 (d, J = 8.0 Hz, 2H), 7.33 – 6.98 (m, 12H), 6.91 (d, J = 8.5 Hz, 2H), 6.32 (d, J = 8.4 Hz, 1H), 4.79 (d, J = 7.3 Hz, 1H), 4.70 – 4.32 (m, 4H), 3.86 – 3.71 (m, 2H), 3.66 (d, J = 11.2 Hz, 1H), 3.59 – 3.42 (m, 3H), 3.34 – 3.10 (m, 7H), 3.07 – 2.87 (m, 3H), 2.85 – 2.71 (m, 2H), 2.63 (dd, J = 13.6, 10.5 Hz, 1H), 2.49 – 2.38 (m, 2H). HR-MS: calcd. M^+ = 928.3239, obsvd. $(M+H)^+$ = 929.3313.

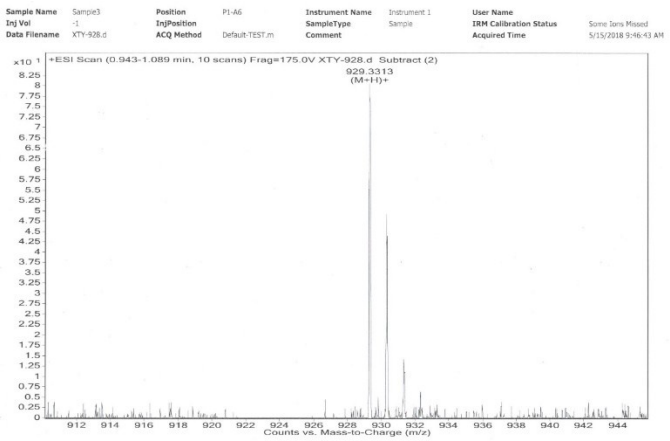


Figure S4. HR-MS spectrum of NBD-FFY(Gal)G

Characterization of Compound NBD-FFYG:

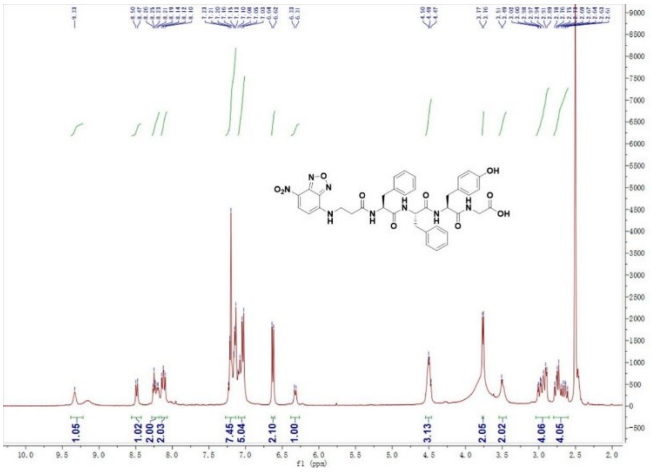


Figure S5. ^{1}H NMR spectrum of NBD-FFYG

^{1}H NMR (400 MHz, DMSO-d6) δ 9.33 (s, 1H), 8.49 (d, J = 8.8 Hz, 1H), 8.28 – 8.18 (m, 2H), 8.15 – 8.07 (m, 2H), 7.27 – 7.13 (m, 7H), 7.10 – 7.01 (m, 5H), 6.63 (d, J = 8.4 Hz, 2H), 6.32 (d, J = 8.5 Hz, 1H), 4.54 – 4.46 (m, 3H), 3.76 (d, J = 5.8 Hz, 2H), 3.50 (d, J = 5.8 Hz, 2H), 3.04 – 2.86 (m, 4H), 2.80 – 2.60 (m, 4H). HR-MS: calcd. M+ = 766.2711, obsvd. $(M–H)^{-}$ = 765.2673

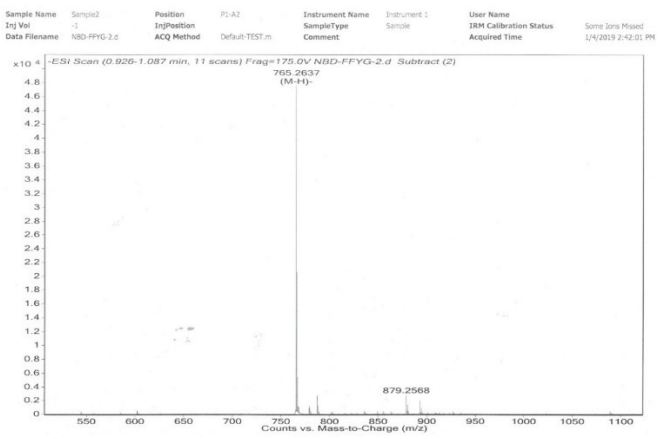


Figure S6. HR-MS spectrum of NBD-FFYG

Characterization of Compound NBD-FFY(p)G:

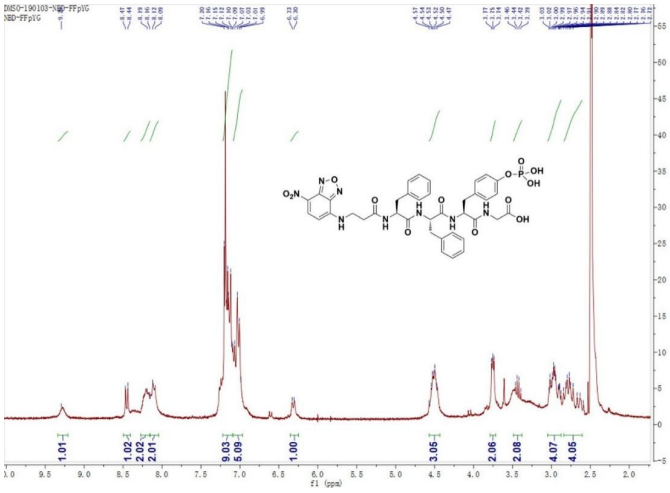


Figure S7. ^{1}H NMR spectrum of NBD-FFY(p)G

^{1}H NMR (300 MHz, DMSO-d_6) δ 9.29 (s, 1H), 8.46 (d, J = 9.1 Hz, 1H), 8.17 (d, J = 9.4 Hz, 2H), 8.10 (d, J = 9.6 Hz, 2H), 7.22 – 7.10 (m, 9H), 7.02 (q, J = 8.0, 5.8 Hz, 5H), 6.32 (d, J = 7.7 Hz, 1H), 4.57 – 4.43 (m, 3H), 3.79 – 3.72 (m, 2H), 3.43 (dd, J = 13.7, 7.0 Hz, 2H), 3.05 – 2.88 (m, 4H), 2.84 – 2.60 (m, 4H). HR-MS: calcd. M^{+} = 846.2374, obsvd. $(M–H)^{–}$ = 845.2295

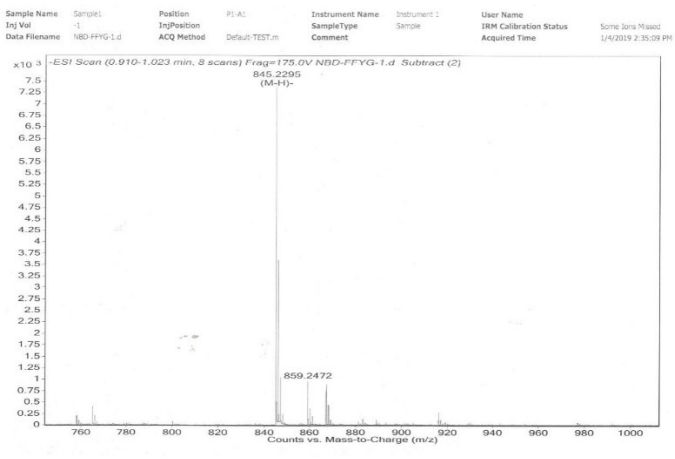


Figure S8. HR-MS spectrum of NBD-FFY(p)G

Hydrogel Formation: Peptides were prepared at a final concentration of 5 mg mL^{-1} in PBS buffer (pH=7.4), the sodium carbonate was used to neutralize the terminal carboxylic acid of peptides. 10 U mL^{-1} of β-Galactosidase was then added to the solution to initiate hydrogelation. A transparent gel would form within 12 h.

Preparation of TEM samples of compound: The compuond were dissolved in 1×PBS buffer and homogeneous solution were obtained by sonication. A micropipet was used to load 5 μL of sample solution to a carbon-coated copper grid. The excess solution was removed by a piece of filter paper. The samples were dried overnight in a desiccator and then conducted on a Tecnai G2 F20 system, operating at 200 kV.

Circular dichroism (CD) spectrum: CD spectrum was measured by a BioLogic (MOS-450) system. All samples were placed in 0.1 cm quartz spectrophotometer cell (20-C/Q/0.1). The wavelength range was from 185 to 250 nm. The acquisition period was 0.5s and the step was 0.5 nm. The resultant CD spectra was acquired after subtracting the solvent background.

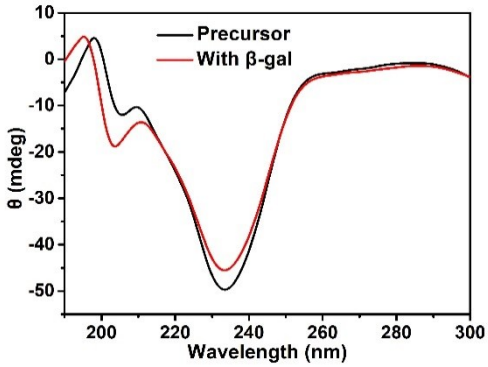


Figure S9. Circular dichroism (CD) spectra of ***Comp. 1*** before and after subjection of β-galactosidase.

Rheology: Rheology test was carried out on an AR 2000ex (TA instrument) system, 40 mm parallel plates were used during the experiment at the gap of 500 μm. For the dynamic time sweep, the solution of compounds were directly transferred to the rheometer and it was conducted at the frequency of 1 rad/s and the strain of 1% immediately. The gels were also characterized by the mode of dynamic frequency sweep in the region of 0.1-100 rad/s at the strain of 1%. A dynamic strain sweep at the frequency of 1 rad $s^{-1}$was conducted finally.

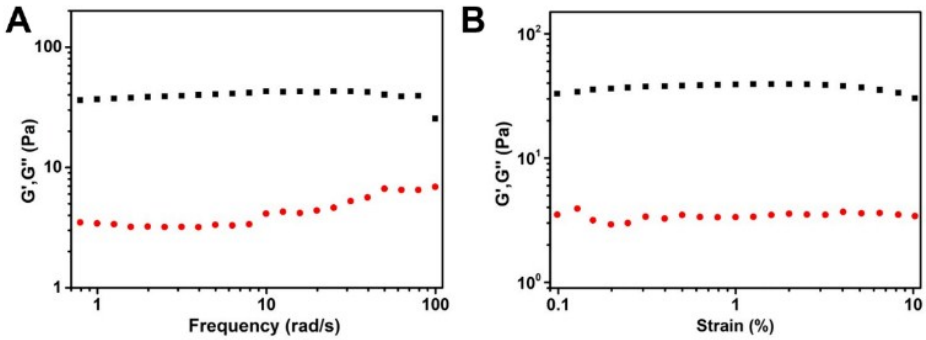


Figure S10. A) Dynamic frequency sweep of the resulting gel at the strain value of 1%. B) Dynamic strain sweep at frequency of 1 rad/s.

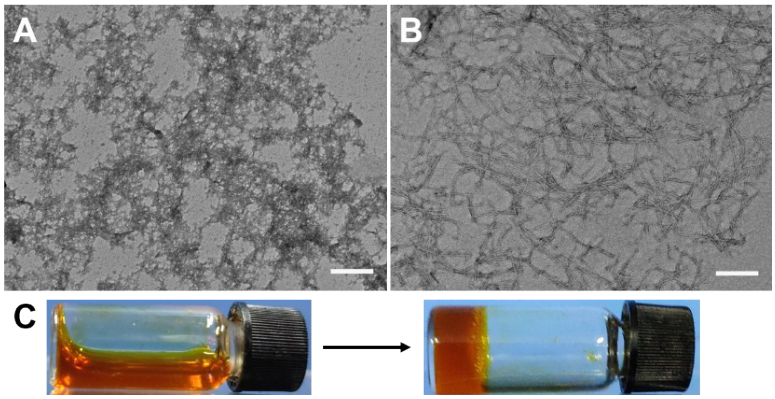


Figure S11. TEM images of A) solutions of 0.5 wt. % of ***Comp. 2*** and B) hydrogels of ***Comp. 2*** after the treatment of heating-cooling. C) Sol–gel transitions of ***Comp. 2*** triggered by heating-cooling.

Critical micelle concentration (CMC) of compounds: The CMC values of compound in PBS buffer solutions were determined by dynamic light scattering (DLS). Solutions containing different concentrations of the compound was tested and the light scattering intensity was recorded for each concentration analyzed

LC-MS analysis to determine the conversion percentage from NBD-FFY(Gal)G to NBD-FFYG: The samples were used for the analysis at different time intervals and the total number of samples for each measurement was 3. 2 mL of the samples (100 μM) were prepared for this experiment. After adding β-Galactosidase, 100 μL of each sample was taken out at each time point and 100 μL DMSO was added to terminate the reaction. The areas of peaks in LC-MS spectra were used to determine the conversion percentage from NBD-FFY(Gal)G to NBD-FFYG.

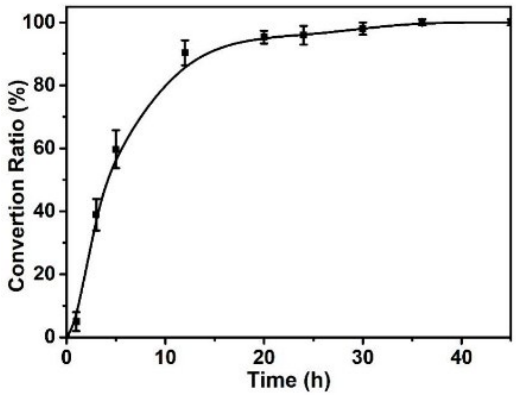


Figure S12. The Conversion of NBD-FFY(Gal)G to NBD-FFYG in the presence of β-Gal in PBS buffer solution (Mean±SD, N=3, enzyme concentration =10 U mL^{-1})

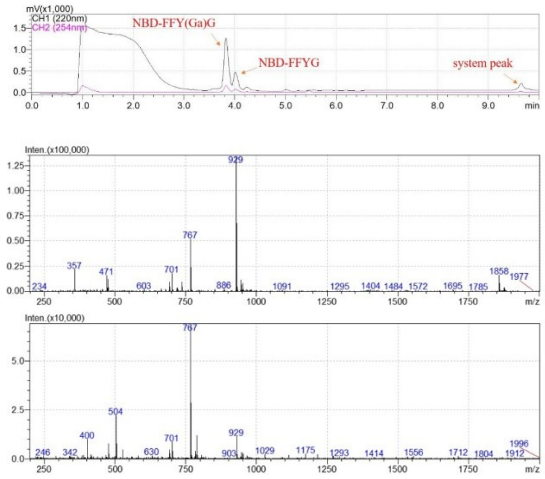


Figure S13. Representative HPLC spectra to indicate the conversion from NBD-FFY(Gal)G to NBD-FFYG by β-Gal at 3 hours time point.

Fluorescence detection in PBS solutions: 100 μL solution of compound 100 μM were added in a 96 wells plate. 3μL PBS buffer with β-Galactosidase (final enzyme concentration was 10 U/mL) was added. After incubation at room temperature for 12 h. Fluorescence spectrum was acquired on a BioTek SynergyTM 4 Hybrid Microplate Reader, the excitation wavelength was 488 nm.

Induction of senescent HeLa cells: HeLa cells were grown until 80% confluent (CTRL) and treated with 1 μg/mL of cisplatin for 24 hours. After treatment, the cells were rinsed twice with PBS. Continue to culture for 6-7 days with fresh complete

medium, and change every 2-3 days. The senescent HeLa cells showed abnormal morphology, increased SA-βGal staining.

Induction of senescent HUVECs: Lipopolysaccharide (LPS), a glycan secreted by bacteria that promotes inflammatory responses was used to construct senescence model of human umbilical vein endothelial cells (HUVECs) in vitro. The procedure was as follows: 3-5×10^4 cells were planted in a 35mm culture dish, after the cells were attached, they were starved for 12 hours without serum. Afterwards, the cells were cultured in medium containing 5% FBS and 0.5 μg/ml LPS with changing medium every two days. Senescent HUVECs could be induced successfully after continuous intervention for 6 days.

SA–β-galactosidase staining: SA–β-gal staining was done using a SA–β-gal staining kit (catalog no.C0602; Beyotime Biotechnology, Shanghai, China). After the induction of senescent cells (SCs) was completed, the cells were washed 3 times with PBS, fixed with a staining fixative for 15 minutes at room temperature, and then washed with PBS × 3 times at least for more than 5 minutes. The prepared staining working solution containing 930 μl of staining solution C + 50 μl of X-gal + 10 μl of staining solution A + 10 μl of staining solution B - was added to the culture dish. The culture dish was sealed with parafilm, incubated at 37 °C for 16-18 hours, and the staining solution was changed to PBS for observing under a microscope and taking photographs. SCs were identified as blue-stained cells under light microscopy. A total of 1,000 cells were counted in 20 random fields on a slide to determine the percentage of SA–β-gal^+ cells.

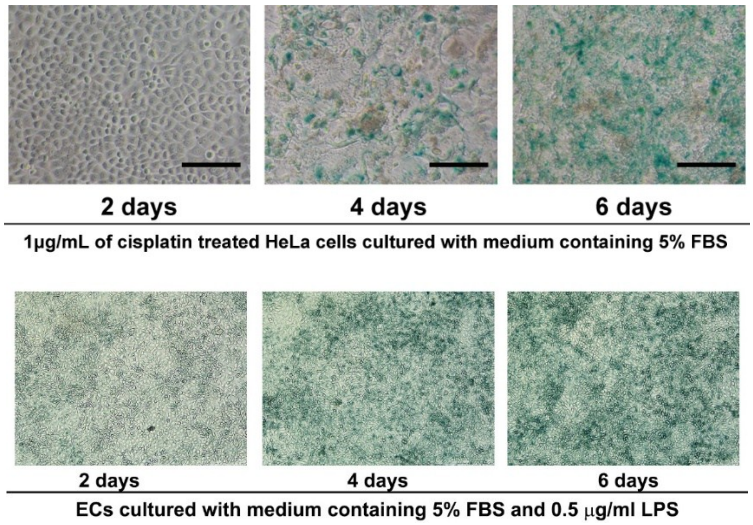


Figure S14. SA-β-Gal staining of senescent HeLa cells and senescent endothelial cells

Laser Scanning Confocal Microscopy for Imaging in Cells: The HeLa cells were incubated in class bottom cell culture dish at a density of $1x10^5$ cells per dish. After incubating with 1μg/mL of cisplatin for 24 hours, the cells were rinsed twice with PBS. Continue to culture for 6-7 days with fresh complete medium. the Dulbecco' s modified Eagle' s medium (DMEM) solution containing 100 μM of compound was then added to the cells. The DMEM solution was removed at 4 h and 12 h time point, respectively, and cells were washed for three times with PBS. Cells were then stained with 1.0 μg/mL of DAPI for 3 min at 37 ℃ in dark. After that, the cells were rinsed three times by PBS buffer, and kept in the live cell imaging solution for imaging. We recorded the images by a laser scanning confocal microscopy (λexc. = 488 nm for yellow channel; λexc. = 405 nm for blue channel). All images were taken by a laser scanning confocal microscopy (Leica TSC SP5) at the same voltage.

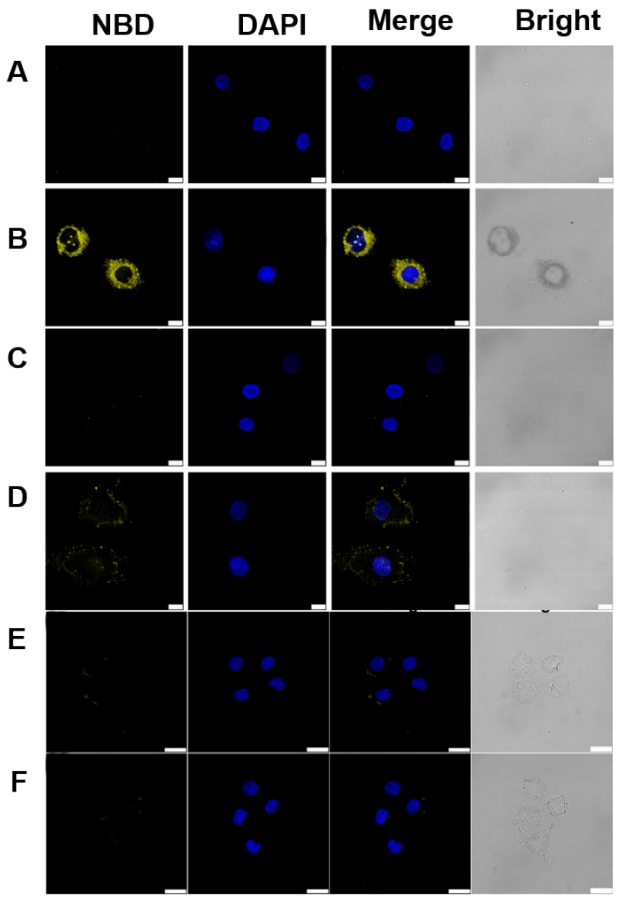


Figure S15. CLSM images of (A) Senescent HeLa cells treated with 200 μM of ***Comp. 1*** for 4 h. (B) senescent HeLa cells treated with 200 μM of ***Comp. 1*** for 12 h. (C) Senescent HeLa cells treated with 200 μM of ***Comp. 2*** for 4 h. (D) Senescent HeLa cells treated with 200 μM of ***Comp. 2*** for 12 h. (E) Normal HeLa cells treated with 200 μM of ***Comp. 1*** for 12 h. (F) Normal HeLa cells treated with 200 μM of ***Comp. 2*** for 12 h.

Cellular Uptake: Cells were incubated in 6-well plates for 24 h at a density of 3 × 10^6 cells. After incubating with 1 μg/mL of cisplatin for another 24 hours, the cells were rinsed twice with PBS. Continue to culture for 6-7 days with fresh complete medium. the Dulbecco' s modified Eagle' s medium (DMEM) solution containing 100 μM of ***comp. 1*** and ***comp. 2*** was then added to the cells. The DMEM solution was removed at 12 h time points, respectively, and cells were washed for three times with PBS. After being treated with Cell Lysis solution (1 mL per well contained 200 μL

DMSO) for 15 min, the solutions were centrifuged at 1570 g for 10 min. The amount of compound in the upper solution was determined by a microplate reader (Bio-RAD iMarkTM, America).

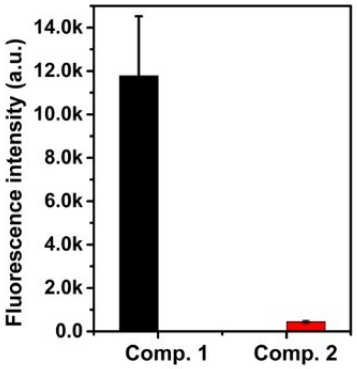


Figure S16. Cellular uptake of ***Comp. 1*** or ***Comp. 2*** (100 μM) in senescent HeLa cells at 12 h time points (SEM ± mean, n = 3).

Gene expression analysis by RT‑qPCR: Total RNA was extracted from the transfected endometrial cancer cells using TRIzol reagent (Takara Bio, Japan) according to the manufacturer's protocol. The miRNA analysis was performed using Taqman MicroRNAReverse Transcription kit (Takara Bio, Japan). qPCR was performed as follows: 95˚C for 10 min, followed by 40 cycles of 95˚C for 15 sec and 60˚C for 60 sec. The primer sequences used were as follows:P53, forward 5'-GCTCGACGCTAGGATCTGAC-3' and reverse 5'- CAGGTAGCTGCTGGGCTC-3'; P21, forward 5'-GTGGACCTGTCACTGTCTT -3' and reverse 5'-GCGTTTGGAGTGGTAGAAATC-3'; and P16, forward 5'-GACCTGGCTGAGGAGCTG-3' and reverse 5'-TTCAATCGGGGATGTCTGA-3'. was used as an endogenous control to calculate expression of p53/p21/p16 in endothelial cells. miRNA expression levels were measured based on the threshold

cycle (Cq) and relative expression levels were calculated using the 2-ΔΔCq method.

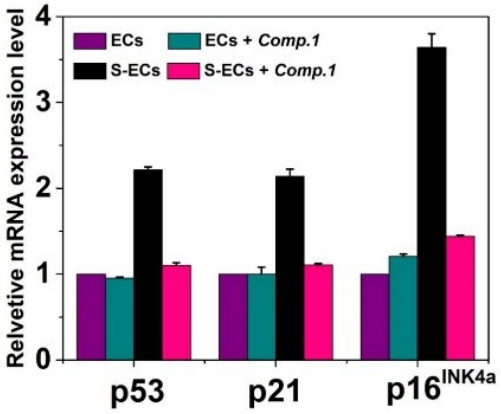


Figure S17. Detection of mRNA expression of p53, p21 and $p16^{INK4a}$ in four groups of cells via RT-qPCR.

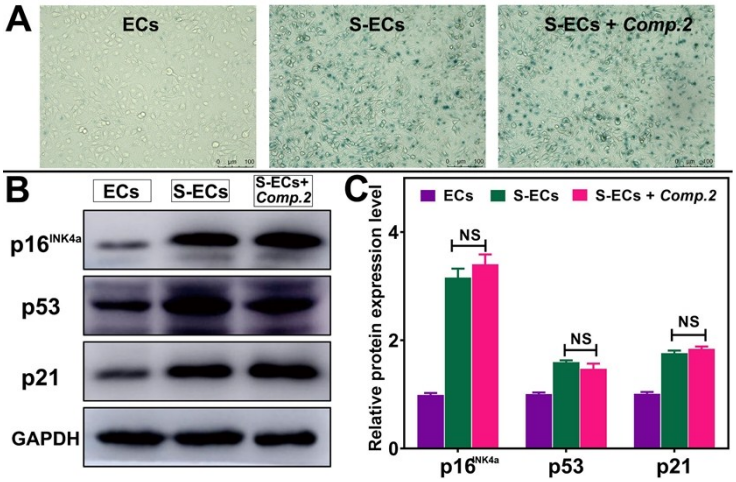


Figure S18. A) SA-β-Gal staining of ECs and S-ECs with or without treatment with 200 μM of ***Comp. 2*** for 24 h, respectively. B) The levels of p53, p21 and $p16^{INK4a}$ proteins in ECs and ones in S-ECs with or without treatment with 200 μM of ***Comp. 2*** for 24 h via western blotting. C) Relative statistical protein expression level data by density analysis (SEM ± mean, n = 3).

Cell viability assays: The cells (Control and SCs) were plated in 96-well plates (typically 3000-4000 cells per well). Viability was assessed 24 h later with CellTiter-

Lumi^{TM} luminescence assay as follows: (1) The control and treatment group cells were removed from the incubator and equilibrated at room temperature for 10 minutes (usually not more than 30 minutes); (2) Add 100 μl of CellTiter-Lumi^{TM} luminescence assay reagent to each well of a 96-well plate (25 μl per well); (3) Shake for 2 minutes at room temperature to promote cell lysis; (4) Incubate for 10 minutes at room temperature (about 25 °C) to stabilize the luminescent signal. (5) Chemiluminescence detection was performed at a GloMax 96 well plate reader (Promega).

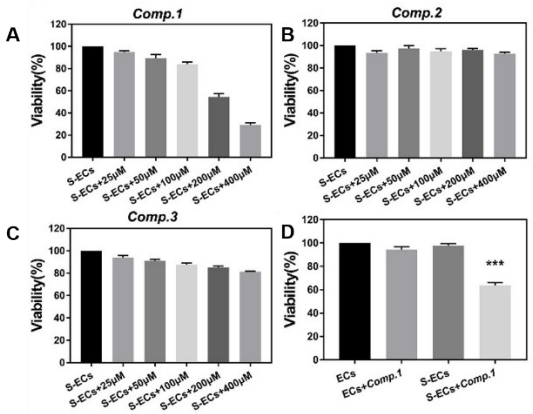


Figure S19. A-C) Relative cell viability of S-ECs treated with different concentration of compound for 24h, D) Relative cell viability of S-ECs or normal ECs with different compound for 24h.

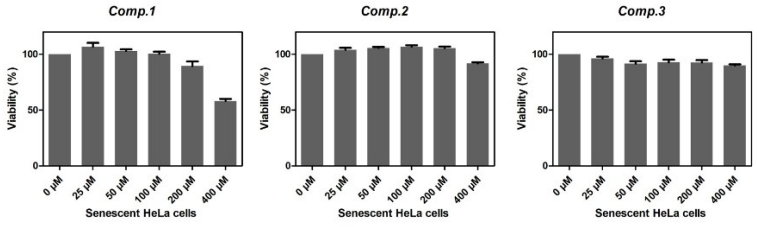


Figure S20. Relative cell viability of senescent HeLa cells treated with different concentration of compound for 24h

Western Blotting: To extract total protein from the cells, ice-cold radio-immunoprecipitation assay lysis and extraction buffer (Beyotime Biotechnology,

Guangzhou, China) was used for 1 h to suspend the cells, followed by extraction of protein supernatant through centrifugation at 12,000 x g, 4˚C for 30 min. Bicinchoninic acid (BCA) protein assay kit was applied to measure the protein concentration, and then an appropriate amount of loading buffer (both from Invitrogen; Thermo Fisher Scientifc, Inc.) was added for denaturation at 100˚C for 5 min. Every sample at an equal loading of 40 μg, underwent electrophoresis. Later, the proteins were transferred to the polyvinylidene fluoride (PVDF) membrane. Then the membrane was blocked in 5% skim milk at room temperature for 1 h, followed by incubation of rabbit anti-human P53, P21, P16, Bax, Bcl-2, and GAPDH monoclonal/polyclonal antibodies (1:1,000; cat nos. ab131442, ab109199, ab51243, ab32503, ab32124 and ab181602; all from Abcam, Cambridge, UK), rabbit anti-human Caspase3 polyclonal antibody （1:1000 ; cat nos . 9662, from Cell Signaling Technology, Inc）. The membrane was washed with Tris-buffered saline and Tween-20 (TBST), and incubated with corresponding horseradish peroxidase-conjugated goat anti-rabbit secondary polyclonal antibody (1:8,000; cat no. BA1039; Boster Biological Technology, Wuhan, China). Later, an enhanced chemiluminescence (ECL) detection system (Yeasen Biotech Co, Shanghai, China) was utilized to visualize the membrane, and a gel analyzer (GraphPad Prism 5; GraphPad Software, Inc., La Jolla, CA, USA) was applied for gray analysis. The relative content of the target protein was the ratio of the target protein to gray value of the corresponding internal control band.

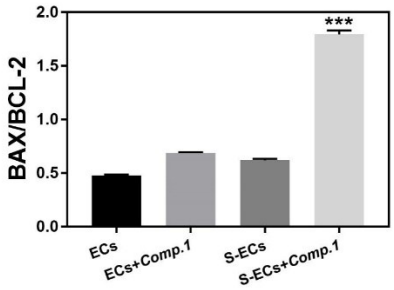


Figure S21. The value of BAX/BCL-2 reflected the apoptosis of cells directly in four groups via western blotting, Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma-2, ***P<0.001.

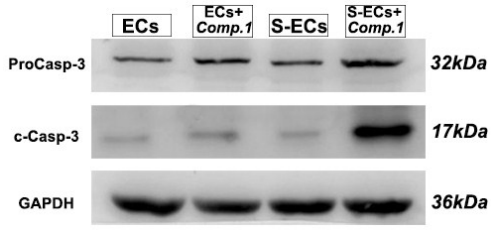


Figure S22. Detection of protein status of Caspase-3 in four groups of cells via western blotting.

Detection of the cell apoptosis level *via* flow cytometer: The apoptosis level of cells in each group was measured using an apoptosis kit (Yeasen Biotech Co, Shanghai, China). After the cells were treated with different water extracts for 48 h, the cell culture fluids were absorbed and reserved, which were centrifuged at 850 x g for 5 min together with the digested cells, followed by washing with phosphate-buffered saline (PBS) and centrifugation twice. After that, the samples were resuspended in 100 µl 1X binding buffer, to which 5 µl propidium iodide (PI) and 5 µl Alexa Fluor 647 were added, followed by incubation in the dark at room temperature for 15 min. Then the samples were sent to the scientific research center of the hospital within 1 h for detection by a flow cytometer (FACSCalibur; BD Biosciences, Detroit, MI, USA).

Cell apoptosis rate = early apoptosis rate + late apoptosis rate.