Electronic Supplementary Material (ESI) for Chemical Communications
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Supporting Information

Materials and general methods:

Chemicals:

Fmoc-amino acids were obtained from GL Biochem (Shanghai, China). All the other Starting materials were obtained from *Alfa*. Chemical reagents and solvents were used as received from commercial sources.

General methods:

^{1}H NMR spectra were obtained on Bruker ARX 400; HR-MS were received from VG ZAB-HS system (England). HPLC was conducted at LUMTECH HPLC (Germany) system using a C_{18} RP column with MeOH (0.1% of TFA) and water (0.1% of TFA) as the eluents; SEM images were obtained at QUANTA 200 (America). LC-MS was conducted at the Shimadzu LCMS-20AD (Japan) system, and rheology was performed on an AR 1500ex (TA instrument) system using a parallel plates (25 mm) at the gap of 400 μm.

Synthesis and characterizations:

Peptide systhesis: The peptide derivative was prepared by solid phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin and the corresponding N-Fmoc protected amino acids with side chains properly protected. The first amino acid was loaded on the resin at the C-terminal with the loading efficiency about 0.8 mmol/g. 20% piperidine in anhydrous N,N'-dimethylformamide (DMF) was used during deprotection of Fmoc group. Then the next Fmoc-protected amino acid was coupled to the free amino group using O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluroniumhexafluorophosphate (HBTU) as the coupling reagent. The growth of the peptide chain was according to the established Fmoc SPPS protocol. At the final step, 2-naphthylacetic acid was used to attach on the peptide.

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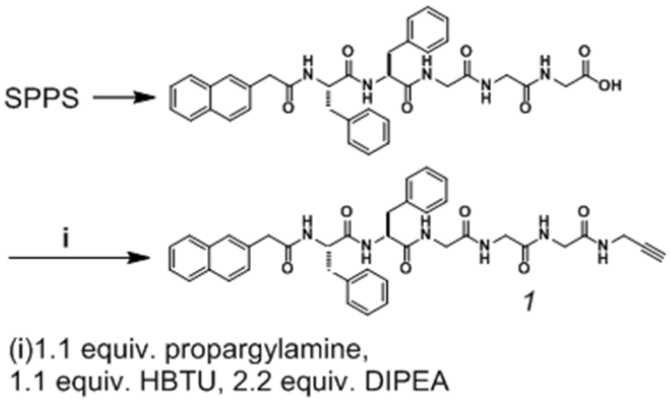
After the last coupling step, excessive reagents were removed by a single DMF wash for 5 minutes (5 mL per gram of resin), followed by five steps of washing using DCM for 1 min (5 mL per gram of resin). The peptide derivative was cleaved using 95% of trifluoroacetic acid with 2.5% of TMS and 2.5% of H_2O for 1 hours. 20 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 ^{0}C at 10,000 rpm. Then the supernatant was decanted and the resulting solid was directly used in the next synthesis steps without further purification.



Scheme S-1. Synthesis route of compound ***1.***

Preparation of Nap-FFGGG-propine (compound *1*):

Compound ***1*** was synthesized according to Scheme S-1. 1.0 mmol (651.7 mg) of peptide NapFFGGG, 1.1 mmol (416.9 mg) of HBTU and 2.2 mmol of DIPEA (284.4 mg) were dissolved in 2 mL of DMF, then 1.1 mmol of paopargylamine (60.5 mg) were added to the above solution. After stirring at room temperature (25 ^{o}C) over night, the reaction liquid were directly sent to HPLC purification, finally got about 75.8% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.07-8.25 (m, 6H), 7.91-7.70 (m, 3H), 7.59 (s, 1H), 7.47 (s, 2H), 7.16-7.23 (m, 11H), 4.55 (s, 2H), 3.70-3.86 (m, 6H), 2.97-3.09 (m, 5H), 2.70-2.87 (m, 2H). MS: calc. M^+ = 688.8032, obsvd. HR-MS: $(M+H)^+$= 689.3089.

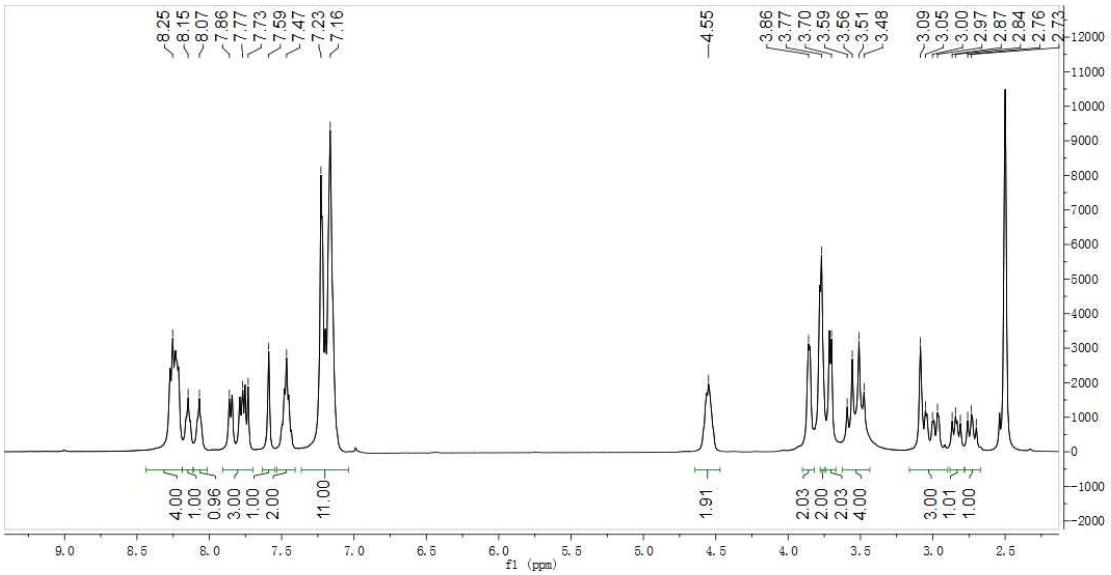


Fig. S-1. NMR spectrum of compound ***1***.

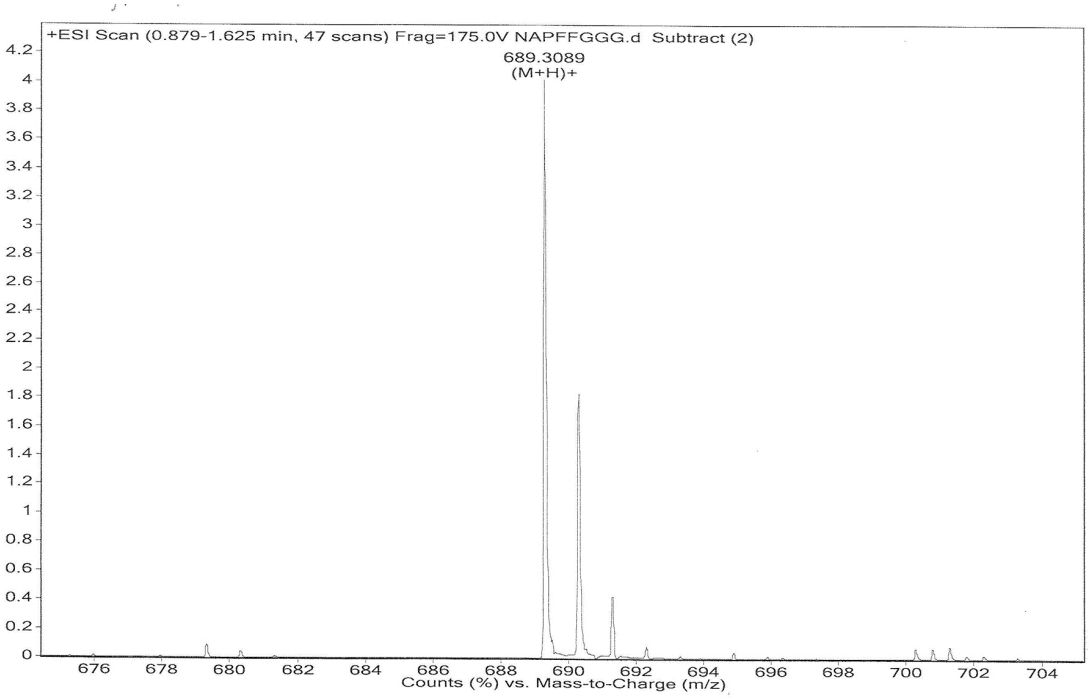
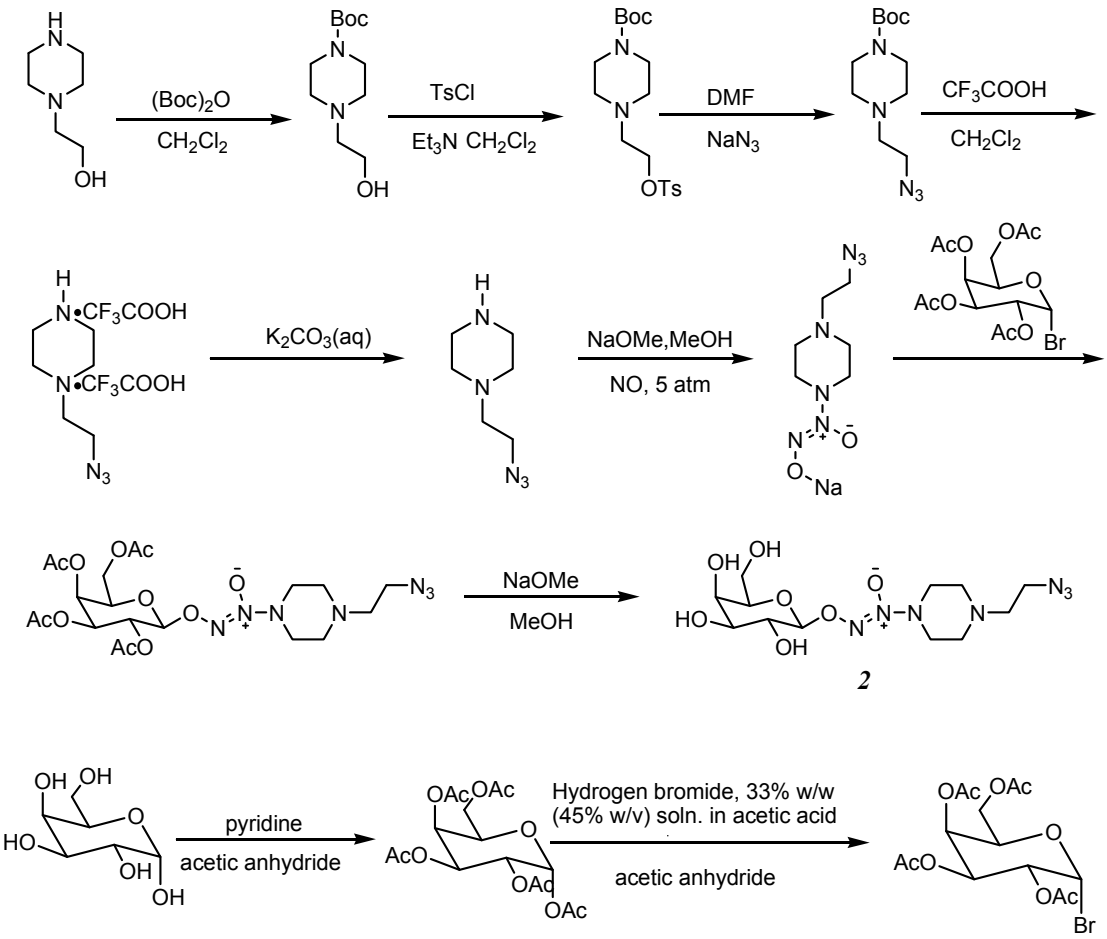


Fig. S-2. HR-MS of compound ***1***.

Preparation of caged NO donor (compound *2*):

Compound ***2*** was preparation according to the reported procedure.[1]



Sheme S-2. Synthetic pathway for compound ***2***

Preparation of compound *3*:

Compound ***3*** was synthesized according to Scheme 1, using a classical click chemistry method. Excess amount of compound ***1*** (0.2 mmol, 137.8 mg) were dissolved in10 mL of dd-H_2O and added with compound ***2*** (0.1 mmol, 37.7 mg) in 5 mL of dd-H_2O solution. The mixture was stirred to obtain a clear solution. Click reaction was initiated by subsequent addition of 1 mL of aqueous solution containing $CuSO_4$ (12.5 mg, 0.05 mmol) and sodium ascorbate (19.8 mg, 0.1 mmol). In nitrogen atmosphere, the reaction was allowed to proceed at room temperature (25 ^oC) and under stirring for 24 hours. The reaction liquid were frozen-dried and the resulting powder were dissolved in DMSO for HPLC purification, finally

yield ~36.3%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.07-8.25 (m, 6H), 7.91-7.70 (m, 3H), 7.59 (s, 1H), 7.47 (s, 2H), 7.16-7.23 (m, 11H), 4.87 (d, J = 8.1 Hz, 1H), 4.55 (s, 5H), 4.31 (d, J = 5.6 Hz, 2H), 3.80-3.64 (m, 7H), 3.48 (m, 16H), 3.11-2.62 (m, 9H). MS: calc. M^+ = 1065.4668, obsvd. HR-MS: $(M+H)^+$=1066.4743.

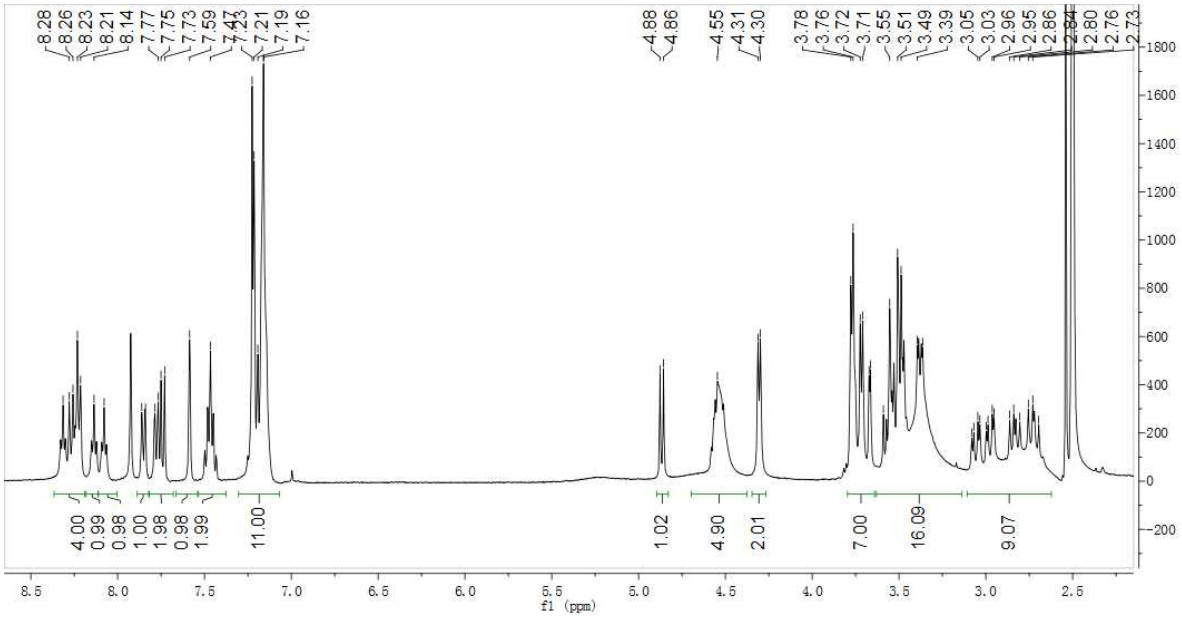


Fig. S-3. NMR spectrum of compound ***3***.

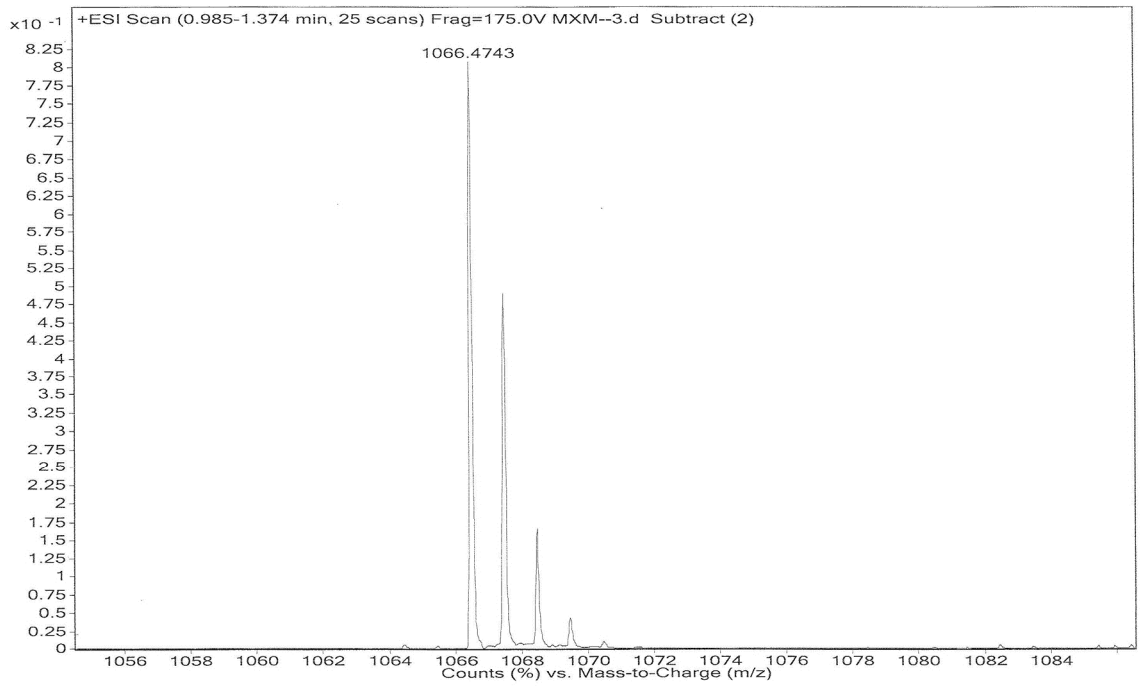


Fig. S-4. HR-MS of compound ***3***.

Preparation of NO-donor hydrogels:

NO-donor hydrogels: 1.0 mg of compound ***3*** and 1.5 equiv. of Na_2CO_3 were firstly suspended in 200 μL phosphate buffer saline (PBS, pH = 7.4), and then the suspensions were heated to form clear solutions. The gels would form after cooling back to room temperature within 5 minutes.

Rheology: Rheology test was done on an AR 1500ex (TA instrument) system, 25 mm parallel plates was used during the experiments at the gap of 400 μm. The dynamic time sweep was conducted at the frequency of 1 rad/s and the strain of 1%. Dynamic strain sweep was performed and the strain values within the linear range were chosen for the following dynamic frequency sweep. 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%.

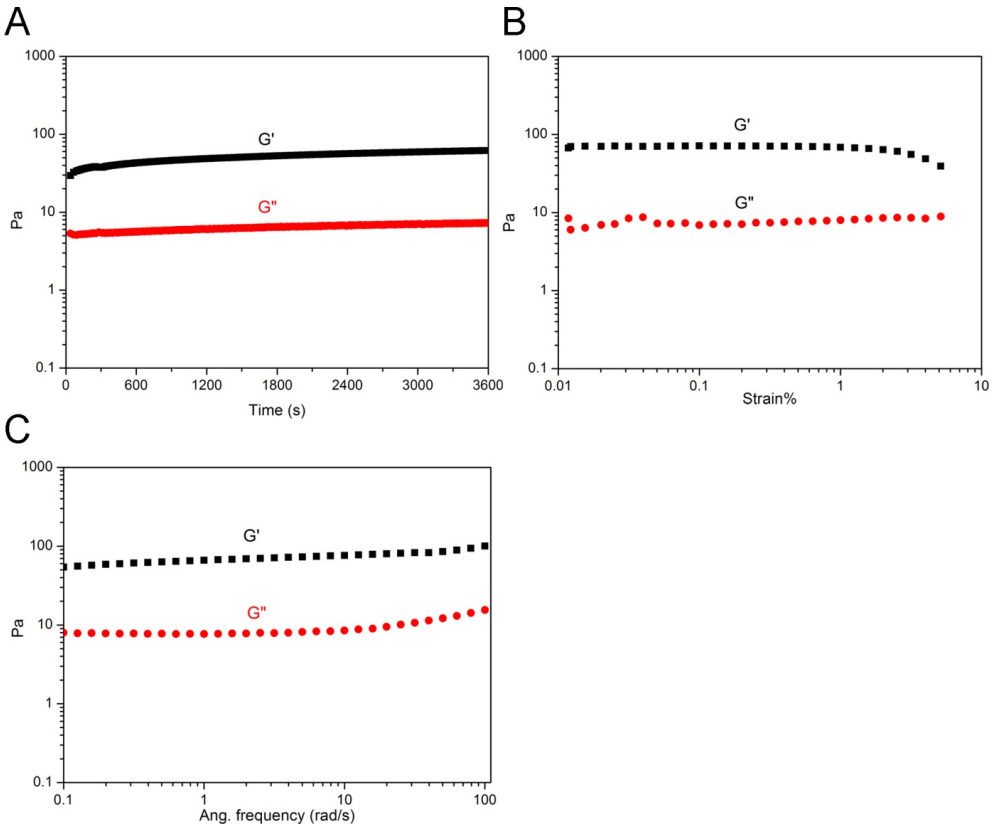
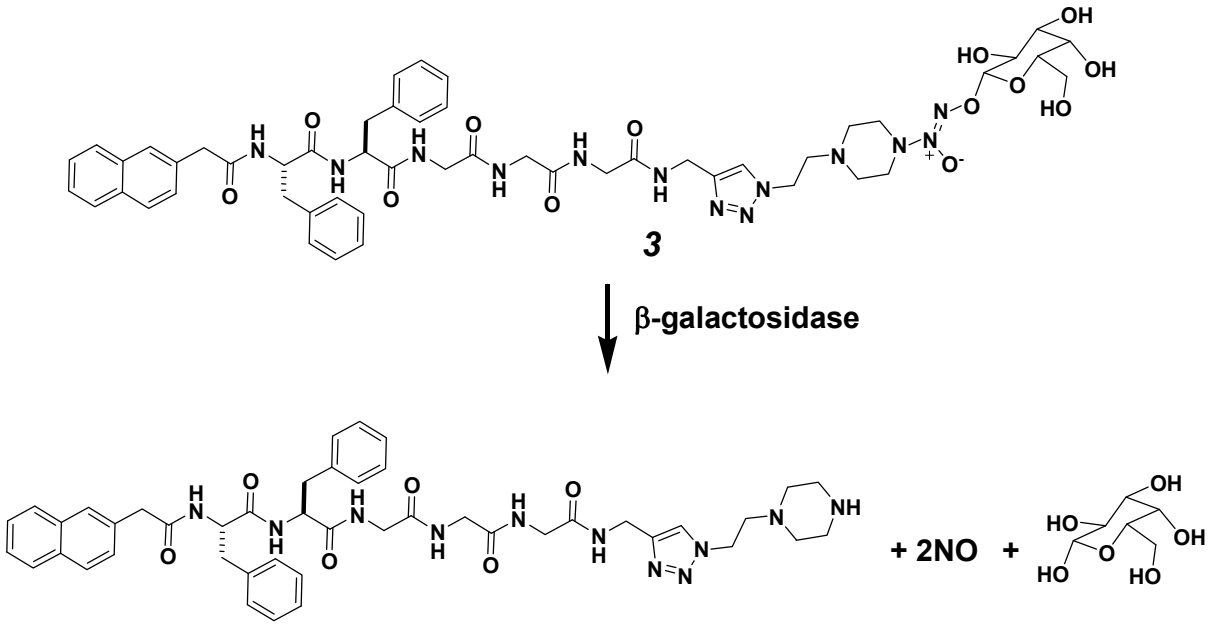


Fig. S-5. A) Rheological measurement in dynamic time sweep mode for hydrogel containing 0.5% compound ***3*** at the frequency of 1 rad/s and strain of 1%, B) dynamic frequency sweep at the strain of 1% and C) dynamic strain sweep at the frequency of 1 rad/s. (Black diamond: G' and red dots: G")

NO releasing measurement:

In this experiment, Griess method was adopted to detect NO, which is based on the chemical diazotization reaction that was originally described by Griess in 1879. Since NO was sensitive to oxygen and would gradually escape from the aqueous solution, we took out the entire upper solution for detection and replaced it with an equal volume of fresh-prepared enzyme solution. 50 μl NO-donor hydrogels were placed in each well of the 96 well plates, 50 μl PBS solutions of β-Galactosidase at different concentration were added on top of them. After releasing for a certain time period, the entire upper solution was take out and measured by Griess method according to the indication on the NO assay kit. 50 μl newly prepared PBS solutions of β-Galactosidase were added on top of the gel immediately for continuing release.



Scheme S-3. Schematic illustration of enzyme-triggered release of NO

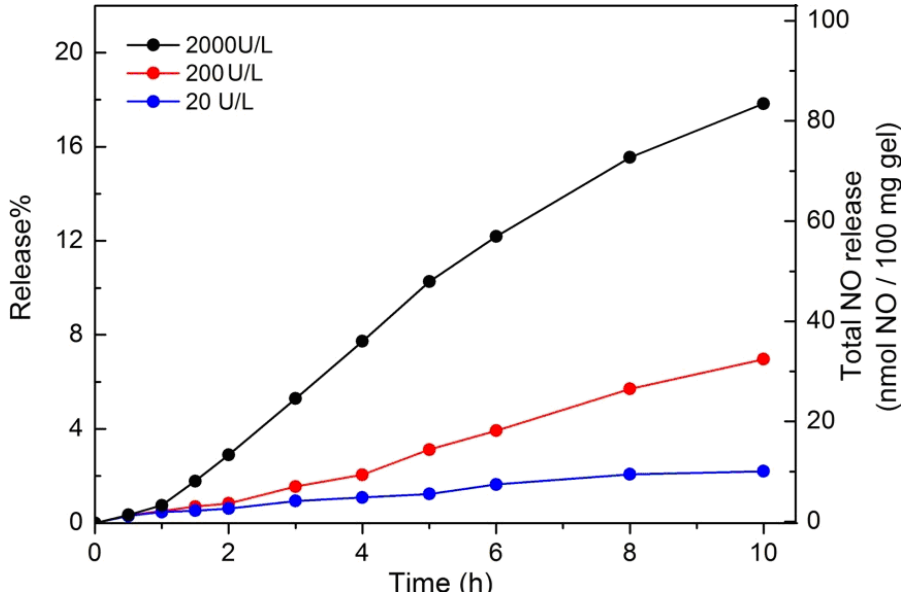


Fig. S-6. The releasing profile of NO from gel containing 0.5 wt% of compound ***3*** with the addition of 20 U/ L (blue dots), 200 U/ L (red dots) and 2000 U/ L (black dots) of β-Galactosidase.

MTT assay on NIH 3T3 cells:

The cytotoxicity of hydrogel formed by compound ***3*** was evaluated by MTT assay. NIH 3T3 mouse fibroblast cells were seeded into 96-well plates at a density of 10,000 cells per-well and incubated for 24 h. The powder resulting from freeze-dried hydrogel were dissolved in DMEM (Dulbecco's Modified Eagle Medium) solutions, and were added into the cells (final concentrations were 100, 200, 400, and 800 μM). After incubation for another 24 h, 20 μL of MTT solution (5 mg/ mL in PBS) was added to each well. Four hours later, the medium was removed and the samples in the wells were air dried. 150 μL of DMSO was added to dissolve the formed crystals. The optical density of the solution was measured at 492 nm using a microplate reader (Labsystem, Multiskan, Ascent, Finland). The 3T3 cells without any treatments were used as the control and its cell viability were set to 100%.

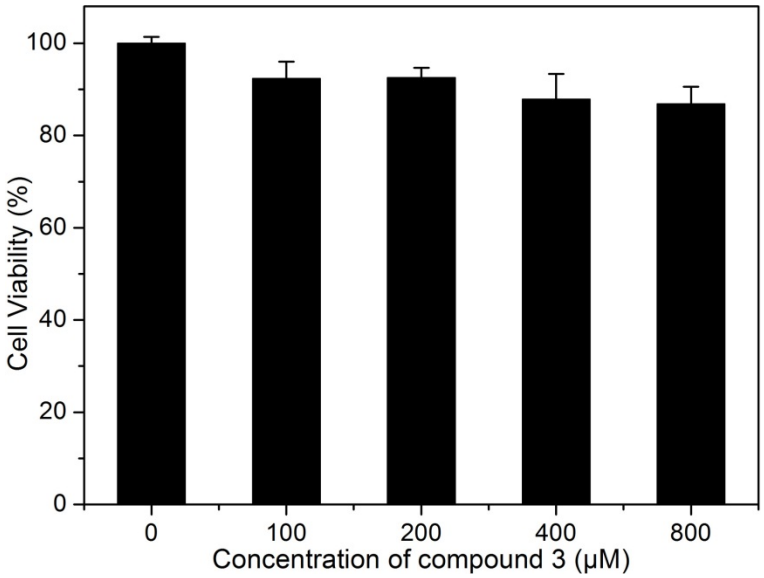


Fig. S-7. Cell viability of NIH 3T3 mouse fibroblast cells treated with different concentrations of compound ***3*** for 24 hours (determined by the MTT assay, N = 3).

In Vivo wound model and treatment with hydrogels:

All animal procedures were approved by the Animal Care Committee in Nankai University. Sixteen female C57 mice (20g; the Laboratory Animal Center of the Academy of Military Medical Sciences, Beijing, China) were divided into four treatment groups, comprising four animals each. We anesthetized mice by intraperitoneal injection of 4% chloral hydrate (mg/kg); then we shaved the dorsum and applied a depilatory (Nair; Church & Dwight Co, Inc.). After that, we removed full-thickness skin and generated a round wound with a single 8-mm punch biopsy specimen on the back of mice. For NO+GAL and NO groups, the wounds were covered with co-assembly hydrogel (20 μL) mentioned in hydrogel preparation section; for NapFF group and Control group, the wounds were covered with NapFF hydrogel (1.0 wt%, 20 μL) and PBS (20 μL), respectively. Finally, all wounds were covered with DuoDerm dressing. During the first 4 days, β-Galactosidase (1.5×10^{-4} U) in 5 μL was added to the wound bed daily in the NO+GAL group.

Preparation of two-component hydrogel for wound healing:

127.9 μg of compound ***3*** (120 nmol), 2.0 mg of NapFF and 1.5 equiv. of Na_2CO_3 were suspended in 200 μL phosphate buffer saline (PBS, pH = 7.4), and then the suspensions were heated to form clear solutions. The gels would form after cooling back to room temperature within 3 minutes.

Histological and immunofluorescence examinations:

Animals were sacrificed 7 days after wounding. Samples of lesion and normal tissue were excised from euthanized mice, fixed in 4% paraformaldehyde (PFA) for 24 hours, processed, and embedded in paraffin. Vertical sections (5μm thick) were fixed to glass slides and subjected to H&E, Masson Trichrome staining to examine tissue morphological characteristics and collagen deposition, respectively, followed by observation with a regular light microscope (Nikon Eclipse TE2000-U Kanagawa, Japan).

For immunofluorescence staining, the paraffin sections underwent an antigen recovery process. Briefly, the sections were put into sodium citrate solution and heated in a 600-W microwave, and then left to cool to room temperature. Rabbit anti-human vWF (1:100, DAKO Cytomation, Glostrup, Denmark), mouse anti-a-SMA (a-SMA, 1:100, Boster, China) were used as primary antibodies to examine vascularization and fibroblast infiltration. Alexa Fluor 488 IgG(H+L) (goat anti-mouse and goat anti-rabbit, 1:200, invitrogen) were used as the secondary antibody. The nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI) containing mounting solution (Dapi Fluoromount G, Southern Biotech, England). Slides were observed under a fluorescence microscope (Zeiss Axio Imager Z1, Germany), and the images were acquired with a digital camera (AxioCam MRm, Germany).

Statistical Analysis: All data were subjected to statistical analysis using GraphPad Prism version 5.0. All quantitative results were obtained from at least three samples for analysis. Data were expressed as the mean ± standard error of the mean (SEM). A two-tailed paired

Student's t-test was used to compare the differences. Difference with $p < 0.05$ was considered to be statistically significant.

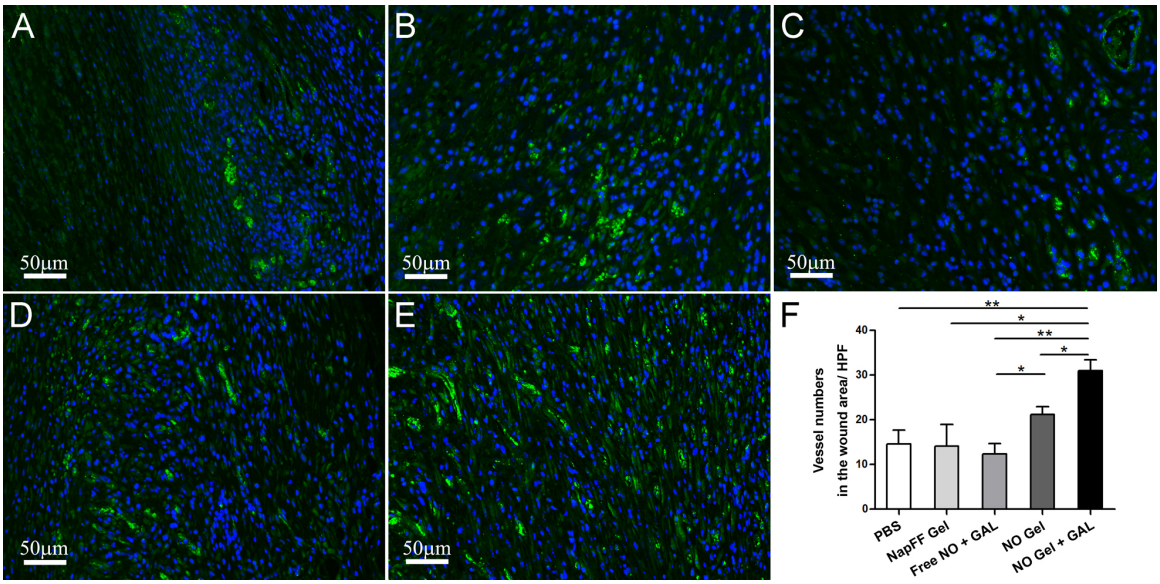


Fig. S-8. Angiogenic response at Day 7. Endothelial cells were stained with vWF antibody (green) and cell nucleus was stained with DAPI (blue) within wound sites. Fluorescence microscope images (200×) of wound area in groups of A) PBS, B) NapFF, C) Free NO+GAL, D) NO Gel and E) NO Gel+GAL. F) Number of stained micro-vessels per HPF (200×; 10 fields) of wound area in different groups. Significance levels were set at: *$p < 0.05$ and **$p < 0.01$

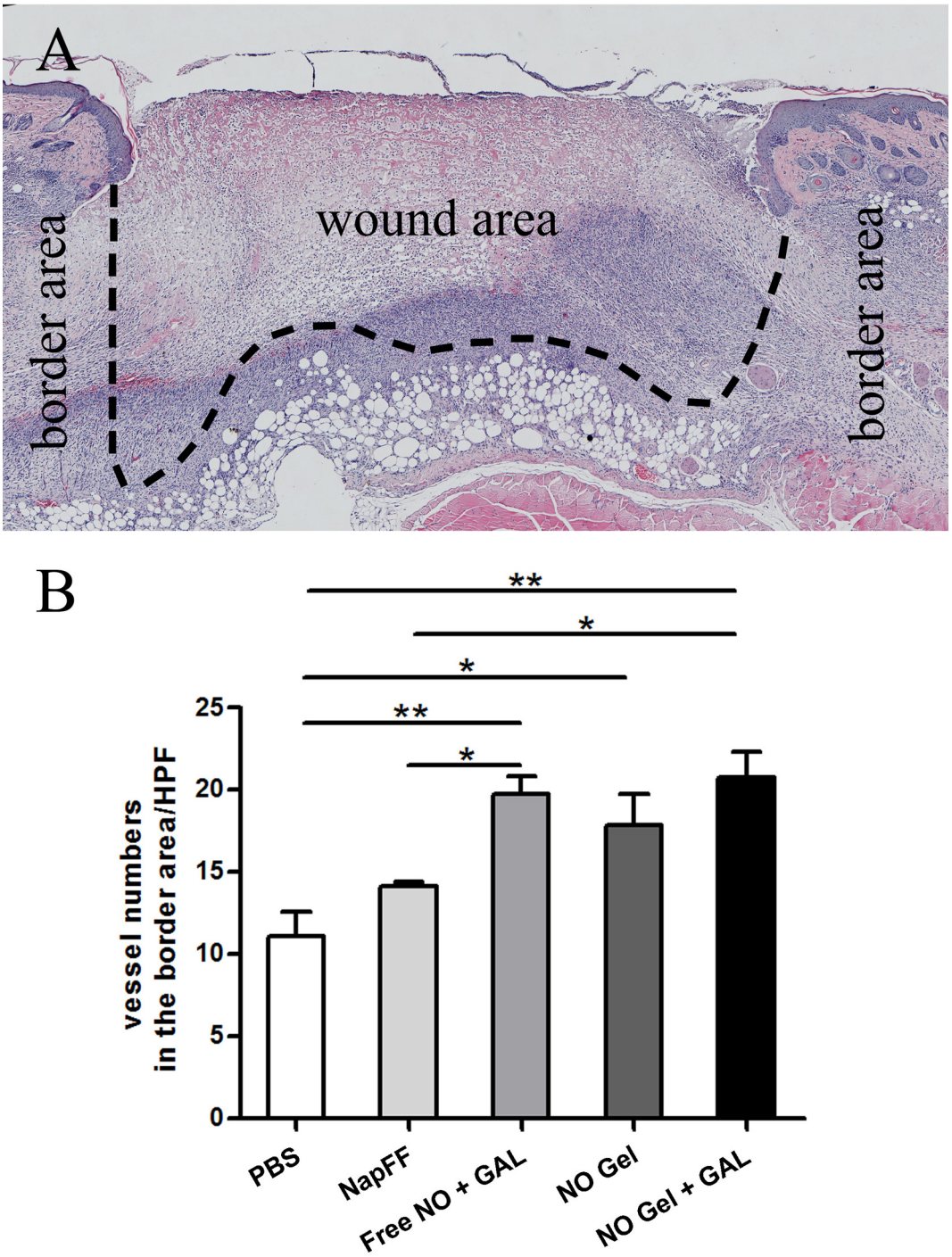


Fig. S-9. A) H&E stained image showing the bounds between wound and border area. B) Number of stained micro-vessels per HPF (200×; 10 fields) of border area in different groups. Significance levels were set at: *$p < 0.05$ and **$p < 0.01$.

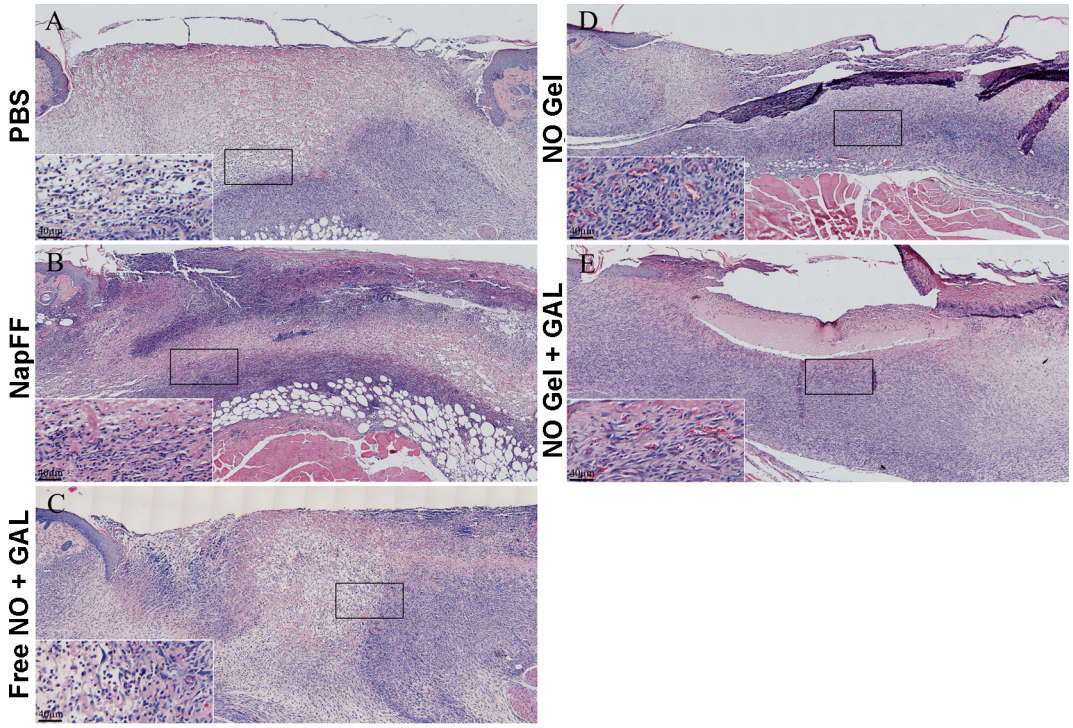


Fig. S-10. H&E-stained histologic sections of wound area in each group.

[1]. Zhao Q, Zhang JM, Song LJ, Ji Q, Yao Y, Cui Y, Shen J*, Wang PG, Kong DL*. Polysaccharide-based biomaterials with on-demand nitric oxide releasing property regulated by enzyme catalysis. **Biomaterials**, DOI: 10.1016/j.biomaterials.2013.07.045.