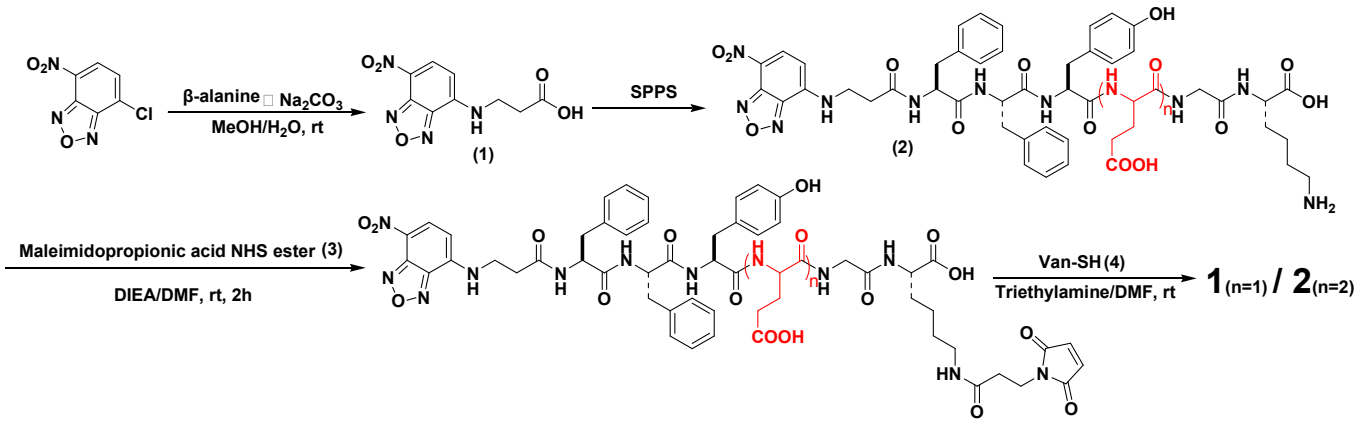
Supporting Information

Materials and methods:

Chemicals: Fmoc-amino acids were obtained from GL Biochem (Shanghai). 4-Chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl), vancomycin hydrochloride and tris(2-carboxyethyl)phosphine Hydrochloride (TCEP) were purchased from Aladdin Chemistry CO. Ltd. 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 and MALDI-TOF-MS spectrometric analyses were performed at the Thermo Finnigan LCQ AD System and AutoflexIII LRF200-CID System respectively. 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. 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). Fluorescence spectrum was recorded on a BioTek Synergy™ 4 Hybrid Microplate Reader. Confocal microscopy images were obtained on a Leica TCS SP5 system (Germany). TEM images were done on a Tecnai G2 F20 system, operating at 200 kV.

Syntheses and characterizations:



Scheme S-1. Synthesis of compounds ***1*** and ***2***

Synthesis of β-alanine conjugated NBD-Cl (1): To a solution of β-alanine (98mg, 1.1 mmol) and K_2CO_3 in MeOH and H_2O under N_2, 5 mL of 4-Chloro-7-nitrobenzo-2-oxa-1,3-diazole (200 mg, 1 mmol) solution was injected slowly. The reaction was completed 5 hours later at room temperature. The reaction mixture was concentrated by vacuum to remove the MeOH, then the pH of the resulted solution was

adjusted to 1~2 by HCl (2 mol/L). The aqueous mixture was extracted with diethyl ether (50 mL * 2) and dichloromethane (50 mL * 2), the organic layer was combined and concentrated to give crude 200 mg, which was directly reacted for next step without purification.

Peptide (2) systhesis: The peptide 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. The first amino acid was loaded on the resin at the C-terminal with the loading efficiency about 1.2 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, β-alanine conjugated NBD-Cl (1) was used to attach on the peptide. 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). The peptide derivative was cleaved using 95% of trifluoroacetic acid with 2.5% of TIS and 2.5% of H_2O for 30 minutes. 20 mL per gram of resin of ice-cold diethylether was then added to cleavage reagent. The resulting precipitate was filtrated and washed by ice-cold diethylether. The crude product was purified by HPLC and dried by lyophilizer.

Synthesis of maleimidopropionic acid NHS ester (3): The mixture of maleic anhydride (98 mg, 1 mmol) and β-alanine (89 mg, 1 mmol) in DMF was stirred at room temperature under N_2. After 2 hours, the reaction mixture was cooled to 0℃, NHS (140 mg, 1.2 mmol) and DCC (430mg, 2.1 mmol) were subsequently added to the above solution. The mixture was further stirred at room temperature overnight and then filtered to remove the solid. The filtrate was added to ice water and the precipitate was obtained by a further filter. The crude product was directly reacted for next step without purification.

Synthesis of Van-SH (4): The sulfydryl modifing vancomycin (Van-SH) was obtained from the reduction of dimeric vancomycin (V-S-S-V) by TCEP in water for 0.5 hour. The synthesis of bis(van) was according to a reported coupling protocol. Cystamine dihydrocholoride (8.3 mg, 0.03 mmol) was dissolved in 1 mL dry DMF firstly, then the resulted solution was added to the solution of vancomycin hydrocholoride (100 mg, 0.07 mmol) in 1 mL DMSO. The mixture was cooled to 0 °C, HBTU (34 mg, 0.09 mmol) and diisopropylethylamine (DIEA) (60 μL, 0.36 mmol) was added following. The solution was allowed to warm to room temperature and stirred overnight. Reaverse-phase preparative HPLC was used to purify the product and lyophilized to afford 80 mg bis(van).

Compound NBDFFYEGK: ^{1}H NMR (400 MHz, DMSO) δ 8.19 – 7.99 (m, 6H), 7.67 (s, 2H), 7.22 – 7.02 (m, 12H), 6.64 (d, *J* = 8.4 Hz, 2H), 6.32 (d, *J* = 11.8 Hz, 1H), 4.50 (t, *J* = 10.3 Hz, 3H), 4.31 – 4.25 (m, 1H), 4.20 (d, *J* = 11.5 Hz, 1H), 3.89 – 3.79 (m, 1H), 3.67 (dd, *J* = 16.8, 5.5 Hz, 1H), 2.95 (dd, *J* = 25.5, 14.1 Hz, 4H), 2.79 – 2.63 (m, 7H), 2.27 (t, *J* = 8.0 Hz, 2H), 2.02 – 1.87 (m, 2H), 1.75 (dd, *J* = 32.7, 25.7 Hz, 3H), 1.56 (dd, *J* = 29.4, 14.5 Hz, 4H), 1.38 (d, *J* = 20.3 Hz, 4H), 1.25 (d, *J* = 12.8 Hz, 2H). MS: calc. M = 1025.42, obsvd. $(M-H)^{-}$ =1024.4155.

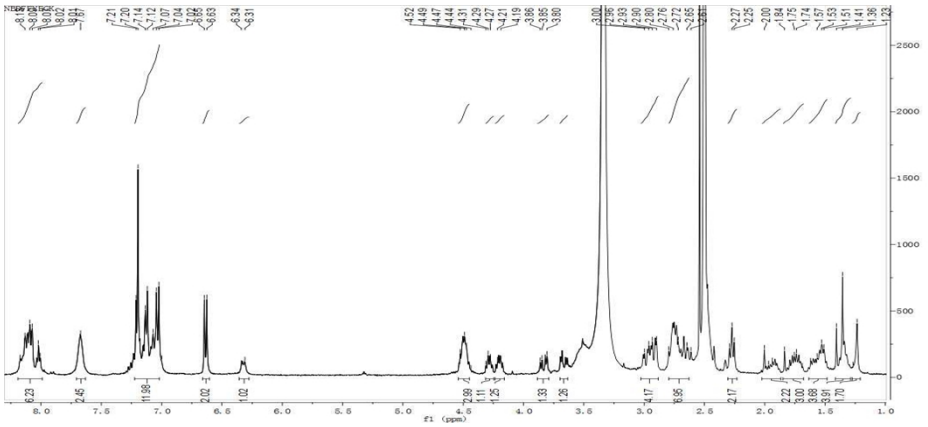


Fig. S-1. ^{1}H NMR of Compound ***NBDFFYEGK***

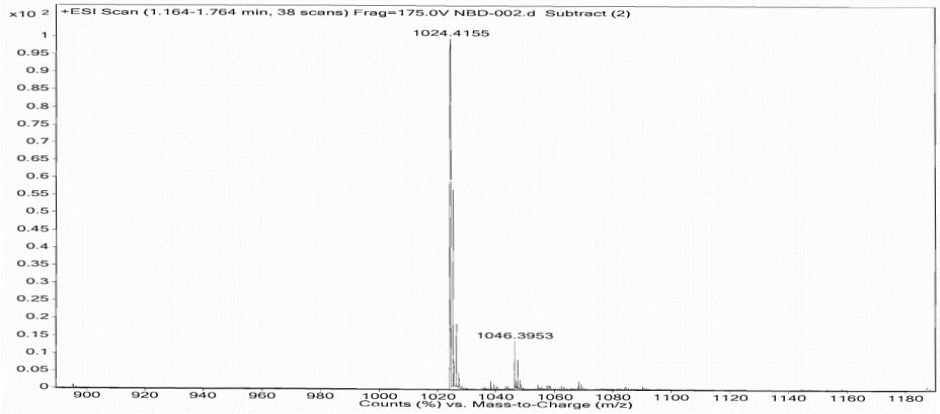


Fig. S-2. HR-MS of Compound ***NBDFFYEGK***

Compound NBDFFYEGK-Mal: ^{1}H NMR (400 MHz, DMSO) δ 8.16 – 7.87 (m, 7H), 7.14 (dd, *J* = 59.9, 28.4 Hz, 16H), 6.63 (d, *J* = 8.4 Hz, 2H), 6.36 – 6.28 (m, 1H), 4.51 (t, *J* = 12.0 Hz, 3H), 4.29 (dd, *J* = 25.4, 13.4 Hz, 2H), 4.16 (dd, *J* = 13.4, 8.1 Hz, 1H), 3.81 (dd, *J* = 14.4, 8.5 Hz, 1H), 3.69 – 3.63 (m, 1H), 3.59 (t, *J* = 9.3 Hz, 2H), 2.97 (dd, *J* = 34.3, 14.7 Hz, 5H), 2.68 (dd, *J* = 47.1, 17.7 Hz, 5H), 2.26 (s, 5H), 1.91 (d, *J* = 15.7 Hz, 2H), 1.84 – 1.62 (m, 4H), 1.57 (d, *J* = 16.3 Hz, 1H), 1.39 – 1.21 (m, 6H). MS: calc. M = 1176.45, obsvd. $(M-H)^{-}$ =1175.4426.

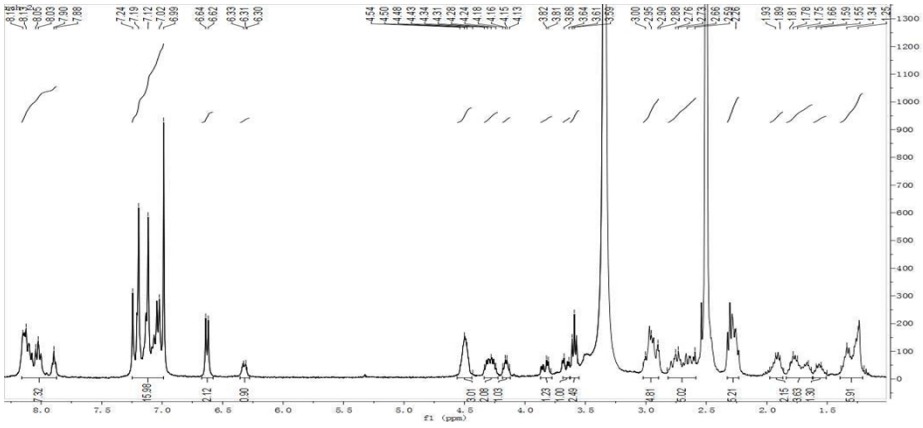


Fig. S-3. [1]H NMR of Compound ***NBDFFYEGK-Mal***

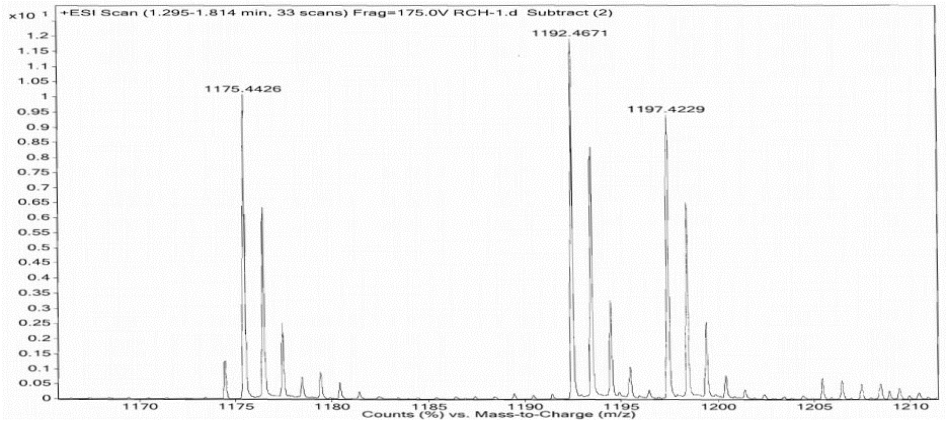


Fig. S-4. HR-MS of Compound ***NBDFFYEGK-Mal***

Compound NBDFFYEEGK: [1]H NMR (400 MHz, DMSO) δ 8.20 – 7.99 (m, 7H), 7.66 (s, 2H), 7.24 – 7.01 (m, 12H), 6.63 (d, *J* = 8.4 Hz, 2H), 6.35 – 6.27 (m, 1H), 4.54 – 4.44 (m, 3H), 4.32 – 4.18 (m, 3H), 3.89 – 3.81 (m, 2H), 3.65 (d, *J* = 11.6 Hz, 2H), 2.95 (dd, *J* = 28.6, 5.4 Hz, 5H), 2.74 (dd, *J* = 26.6, 17.8 Hz, 8H), 2.27 (t, *J* = 14.2 Hz, 4H), 1.90 (d, *J* = 2.5 Hz, 2H), 1.85 – 1.66 (m, 4H), 1.61 – 1.50 (m, 3H), 1.33 (dd, *J* = 36.0, 23.8 Hz, 4H). MS: calc. M = 1154.47, obsvd. $(M-H)^-$ = 1153.4582.

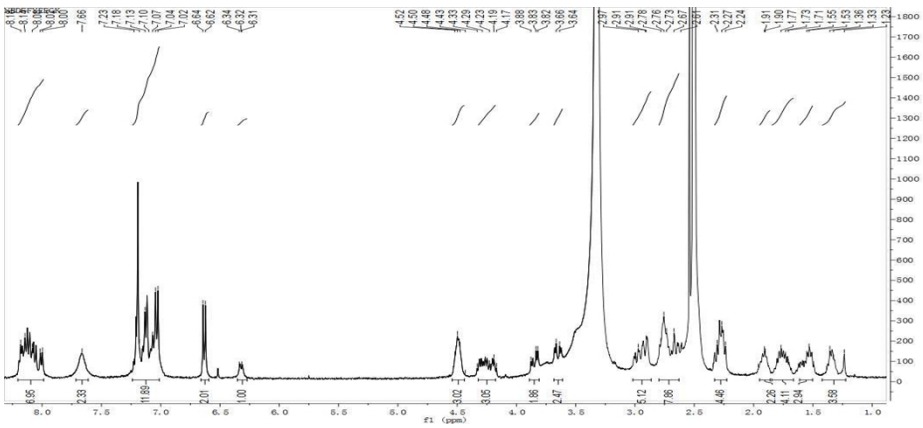


Fig. S-5 **.** [1]H NMR of Compound ***NBDFFYEEGK***

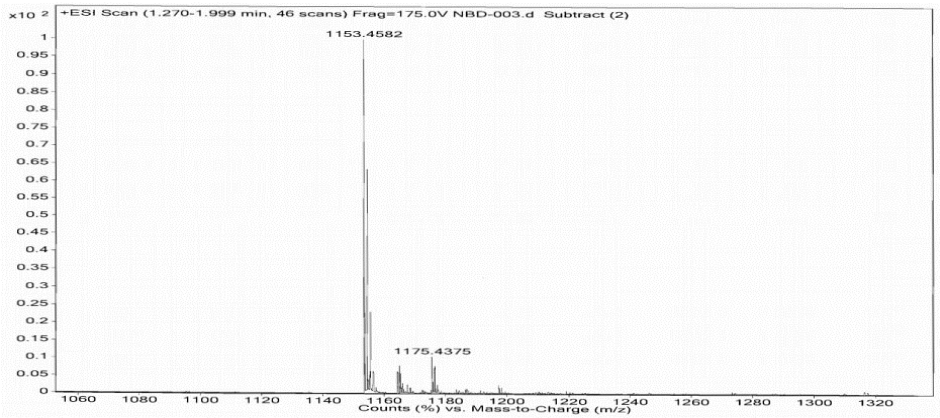


Fig. S-6. HR-MS of Compound ***NBDFFYEEGK***

Compound NBDFFYEEGK-Mal： ^{1}H NMR (400 MHz, DMSO) δ 8.17 – 7.89 (m, 8H), 7.25 – 7.00 (m, 16H), 6.63 (d, *J* = 8.4 Hz, 2H), 6.32 (d, *J* = 7.0 Hz, 1H), 4.55 – 4.45 (m, 3H), 4.27 (d, *J* = 29.2 Hz, 2H), 4.16 (t, *J* = 9.4 Hz, 1H), 3.84 (dd, *J* = 16.9, 6.0 Hz, 1H), 3.66 (d, *J* = 22.0 Hz, 1H), 3.59 (t, *J* = 7.2 Hz, 2H), 2.96 (dd, *J* = 30.0, 16.1 Hz, 6H), 2.73 (dd, *J* = 32.7, 21.0 Hz, 5H), 2.34 – 2.23 (m, 6H), 1.99 – 1.85 (m, 3H), 1.83 – 1.63 (m, 4H), 1.57 (d, *J* = 15.1 Hz, 1H), 1.39 – 1.22 (m, 6H). MS: calc. M = 1305.49, obsvd. $(M-H)^-$ = 1304.4823.

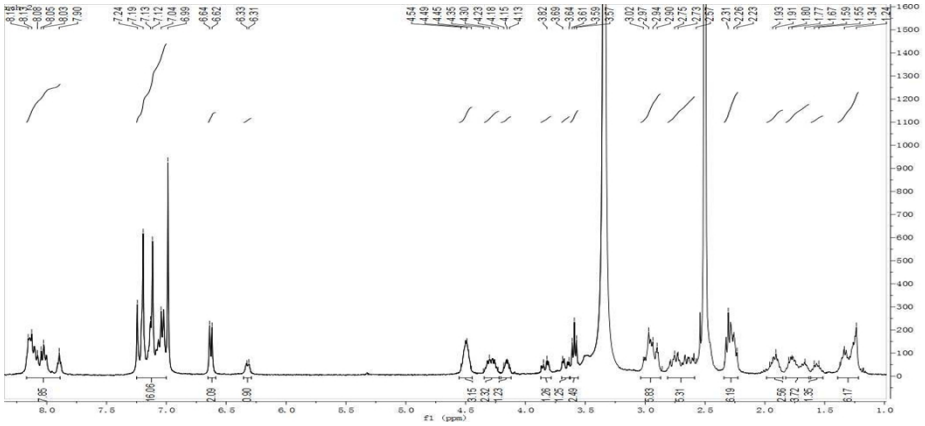


Fig. S-7. ^{1}H NMR of Compound ***NBDFFYEEGK-Mal***

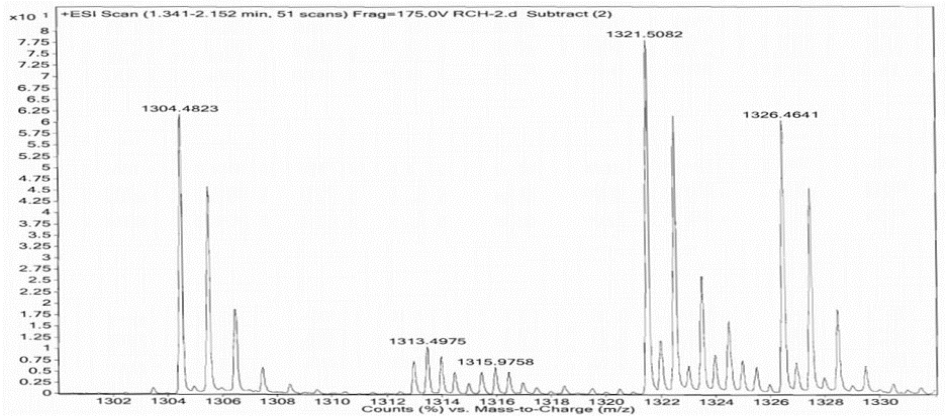


Fig. S-8. HR-MS of Compound ***NBDFFYEEGK-Mal***

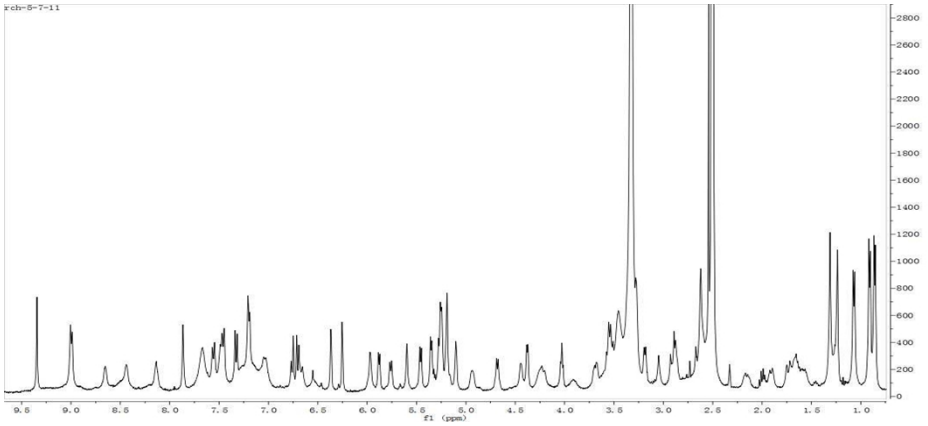


Fig. S-9. ^{1}H NMR of Compound ***Bis(van)***

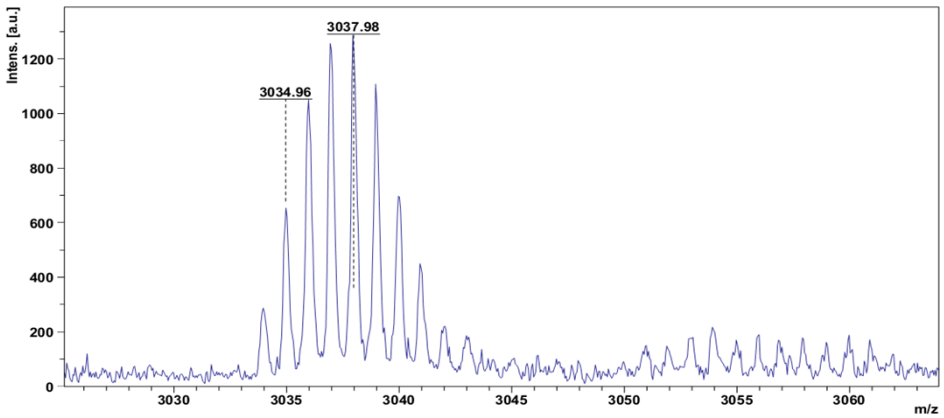


Fig. S-10. MALDI-TOF-MS of ***Bis(van)*** : calc. M = 3014.76, obsvd. $(M+Na)^+$ =3037.98

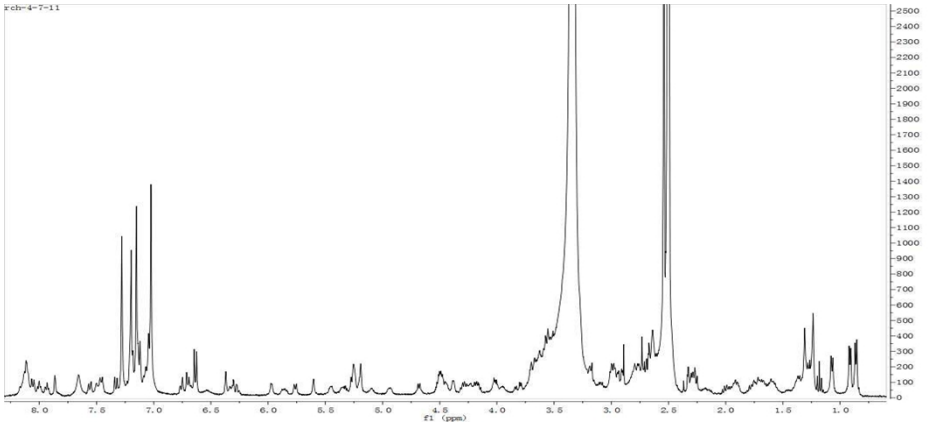


Fig. S-11. ^{1}H NMR of Compound ***1***

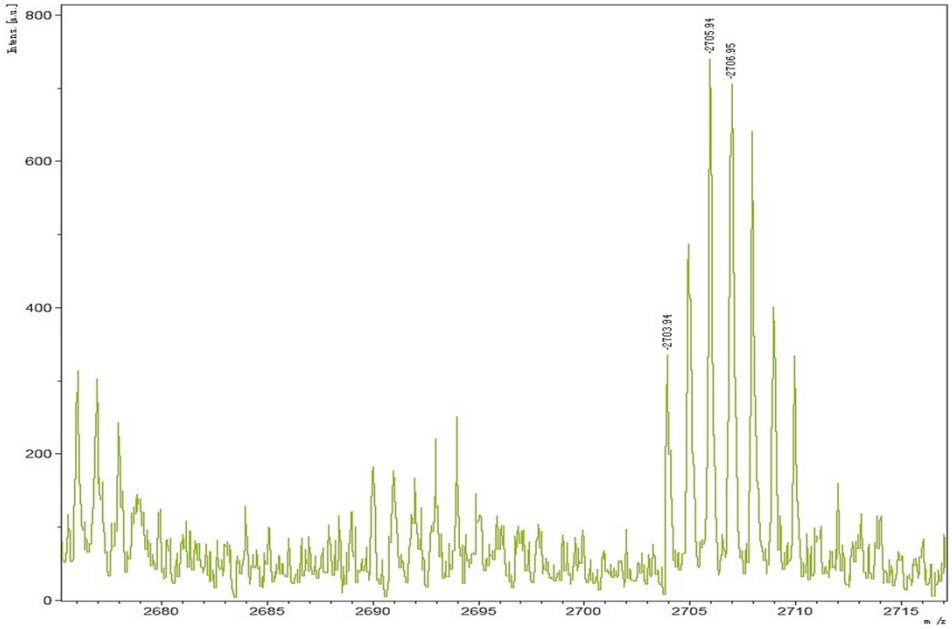


Fig. S-12. MALDI-TOF-MS of Compound ***1***: calc. M = 2682.84, obsvd. $(M+Na)^{+}$ = 2795.94.

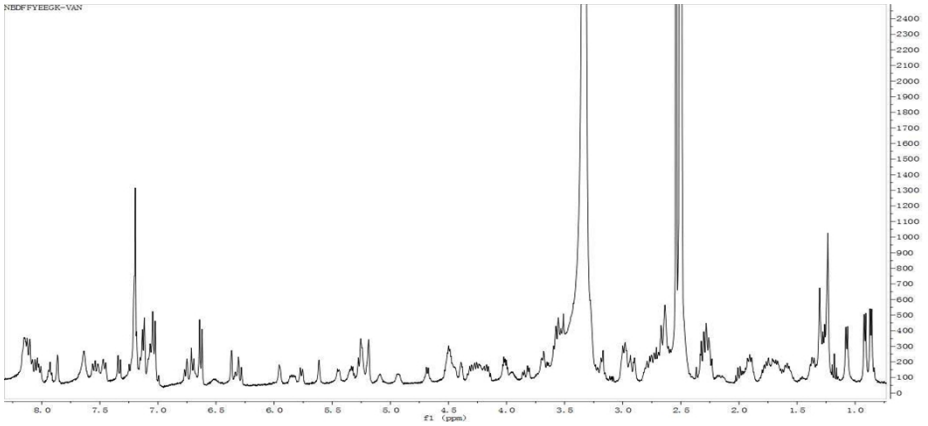


Fig. S-13. ^{1}H NMR of Compound ***2***

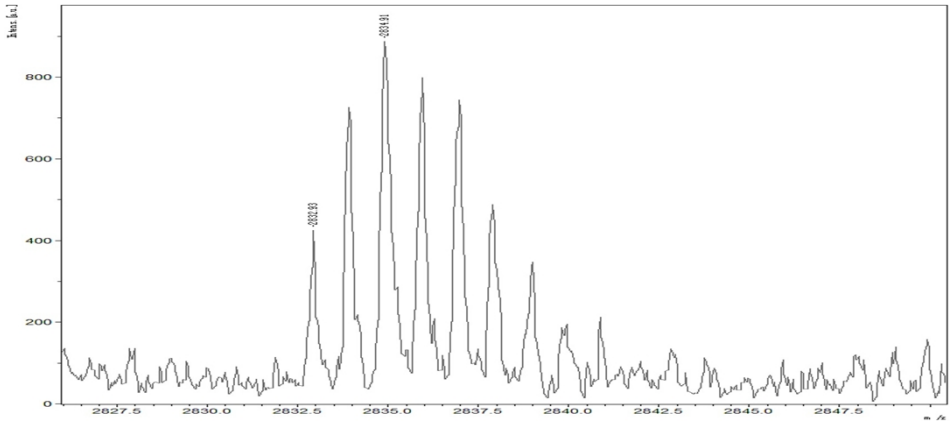


Fig. S-14. MALDI-TOF-MS of Compound ***2***: calc. M = 2812.42, obsvd. $(M+Na)^+$ = 2834.91.

Critical micelle concentration (CMC) of compound *1* and compound *2*: The CMC values of compound ***1*** and compound ***2*** in PBS buffer solutions were determined by dynamic light scattering (DLS). Solutions containing different concentrations of compound ***1*** and compound ***2*** were tested and the light scattering intensity was recorded for each concentration analyzed.

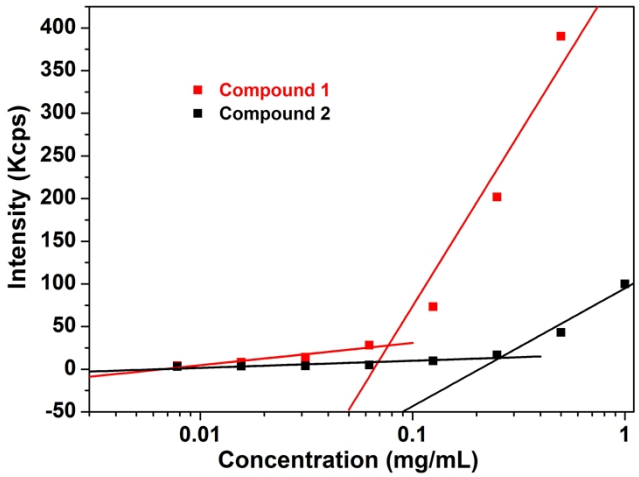


Fig. S-15. The curves used to determine the CMC value of compound ***1*** and compound ***2***

Preparation of TEM samples of compounds: compound ***1*** and ***2*** were dissolved in 1×PBS buffer and homogeneous solution were obtained by sonication. A carbon-coated copper grid was vertically dipped into the solution and then placed in a desicator overnight before the TEM measurement.

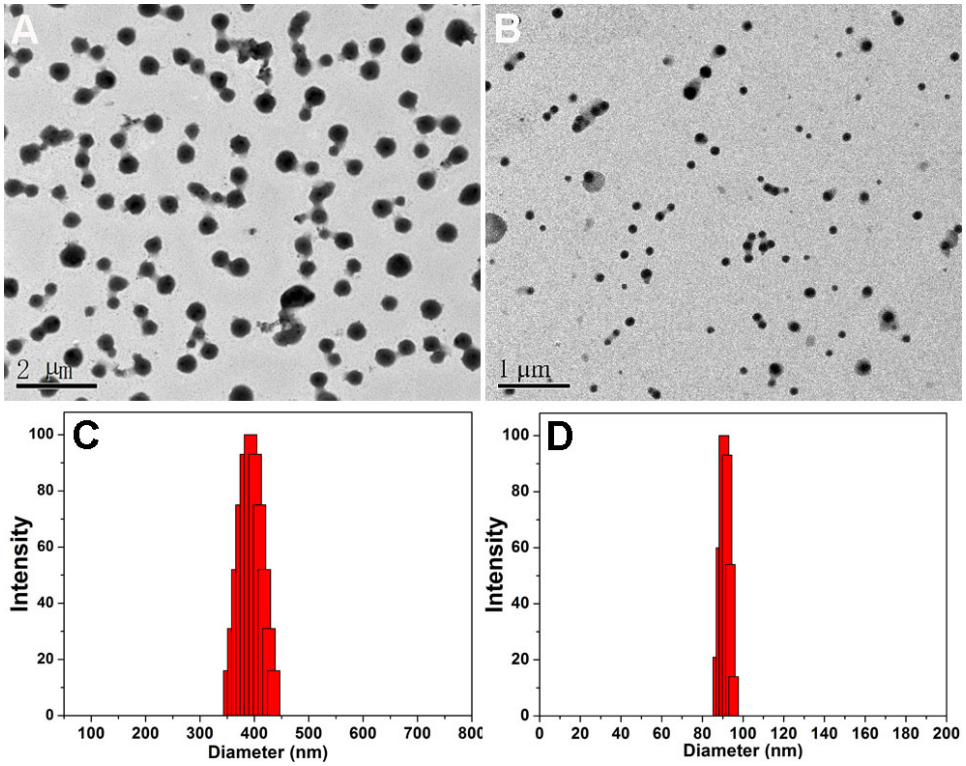


Fig. S-16. Transmission electron microscopy (TEM) images of A) PBS solution of ***1*** at 200 μg/mL and B) PBS solution of ***2*** at 200 μg/mL and size distribution of nanoparticles of C) ***1*** and D) ***2*** determined by dynamic light scattering (DLS) at the concentration of 200 μg/mL

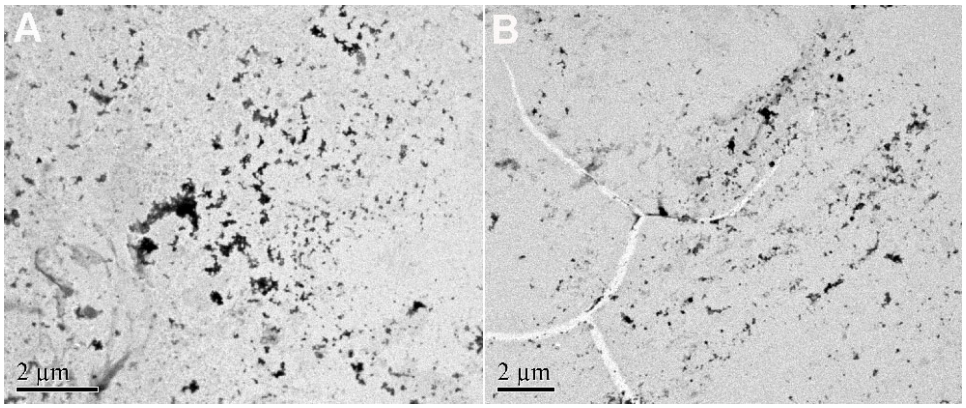


Figure S-17. The TEM images of A) PBS solution of ***1*** at 25μg/mL and B) PBS solution of ***2*** at 25μg/mL

MIC test: A standard broth dilution method was used to determine the MICs of bacterial. Compound 1, 2 and vancomycin hydrochloride were dissolved in DMSO to obtain 100 mg/mL stock solution. A total of 100 μl of LB solution was added to a series of holes on a sterile 96 well plates, with an additional 98 μL added to the first one. 2 μl of a 100 mg/mL compound stock solution was added to the first hole, and a series of 2-fold dilutions were prepared by transferring 100 μL to successive tubes. 5 μL bacterial solution at an OD_{600} value of 0.5 was added to each hole containing different concentration of compounds.

The compound-treated cultures were incubated at 37°C for 24 h, and the OD_{600} was measured. Each measurement was performed in triplicate.

Fluorescence detection: Glycerol strains of *B. subtilis* and *E. faecalis* were transferred to 5mL of liquid LB culture medium and were grown at 37 ˚ C. At an OD_{600} value of 0.5, bacteria were harvested by centrifuging (4000 rpm for 10 min) to remove the LB solution and were re-suspended in PBS. Different volumes of bacteria solution were added into the solution of compound ***1*** and ***2*** in a 96 wells plate. After incubation at 37℃ for 0.5 hour, fluorescence spectrum was recorded on a BioTek Synergy™ 4 Hybrid Microplate Reader.

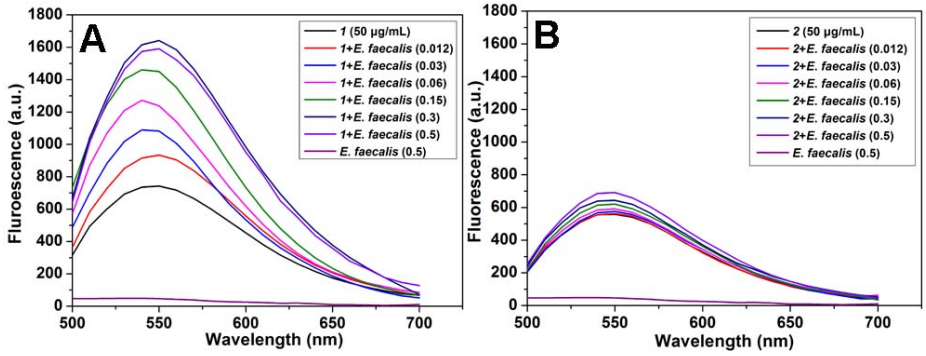


Figure S-18. Fluorescence spectra of PBS solutions (pH = 7.4) of ***1***A) and ***2*** B) in the presence of different OD values of *E. faecalis* (excitation wavelength = 480 nm)

Determination of compounds on bacterial surface: The standard fluorescence emission curves of compound ***1*** and ***2*** at different concentrations were established at the wavelength of 550 nm. Different amounts of compound ***1*** and ***2*** were added to each tube containing 500 μL bacterial solution with an OD_{600} value of 0.5. After incubation at 37℃ for 0.5 hour, redundant compound ***1*** and ***2*** were removed by centrifugation (10000 rpm for 10 min) and PBS washing for three times. The volume of the remaining bacteria was calculated to be about 0.6 μL by a pipette. 100 μL DMSO was added to the bacteria, the bacteria were then sonicated for 20 minutes to make the compounds dissolve in DMSO. The intensity of emission peak at 550 nm was detected and the concentration of compound ***1*** and ***2*** was calculated through the standard curves established prior.

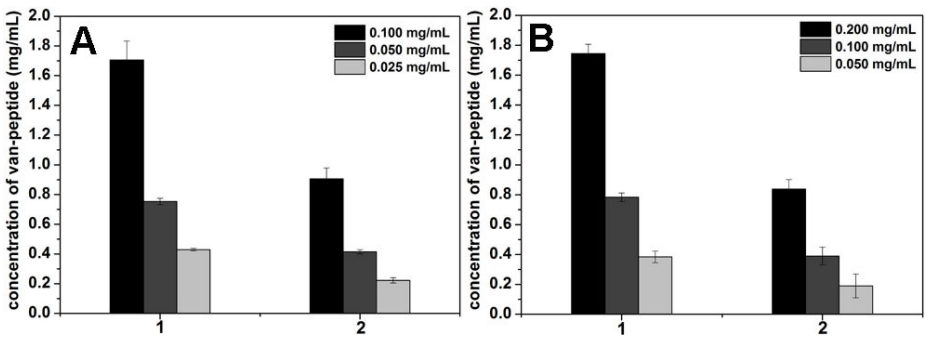


Figure S-19. Concentration of *1* and *2* on bacteria treated with different concentrations of compounds: A) *B.subtilis* and B) *E. faecalis*

Confocal fluorescence imaging test: Bacteria incubated with compound ***1*** and ***2*** at 37℃ in LB culture media for 0.5 hour were harvested by centrifuging (4000 rpm for 10 min). The supernatant was discarded and the remaining bacteria were re-suspended in PBS. After three times PBS washing, bacterial cells were spotted on glass slides and immobilized by the coverslips. Confocal microscopy images were obtained on a OLYMPUS FV1000S-IX81 system with 600×oil immersed optics.

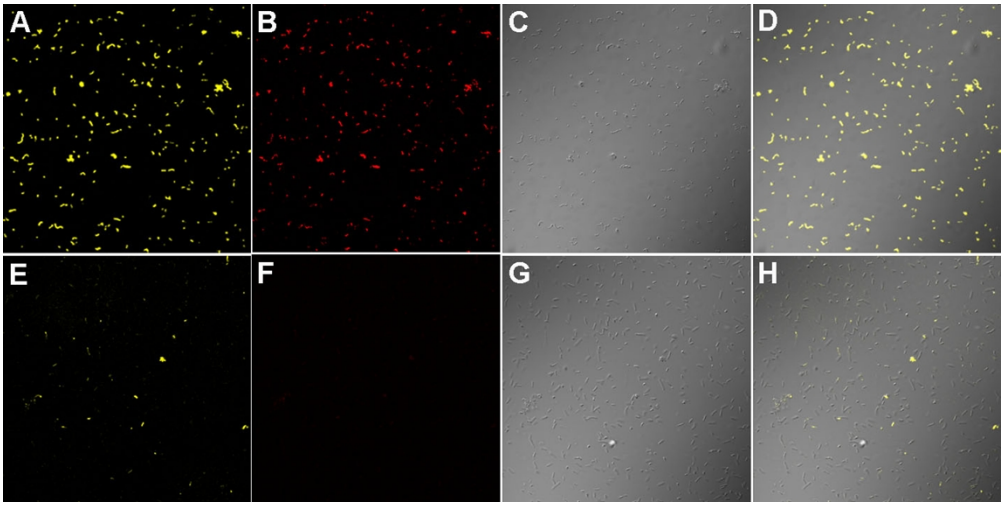


Figure S-20. Confocal fluorescence microscopy images of *B. subtilis* treated with compound ***1*** (A-D) and ***2*** (E-H) (the yellow emission was from NBD and red emission from nile blue)

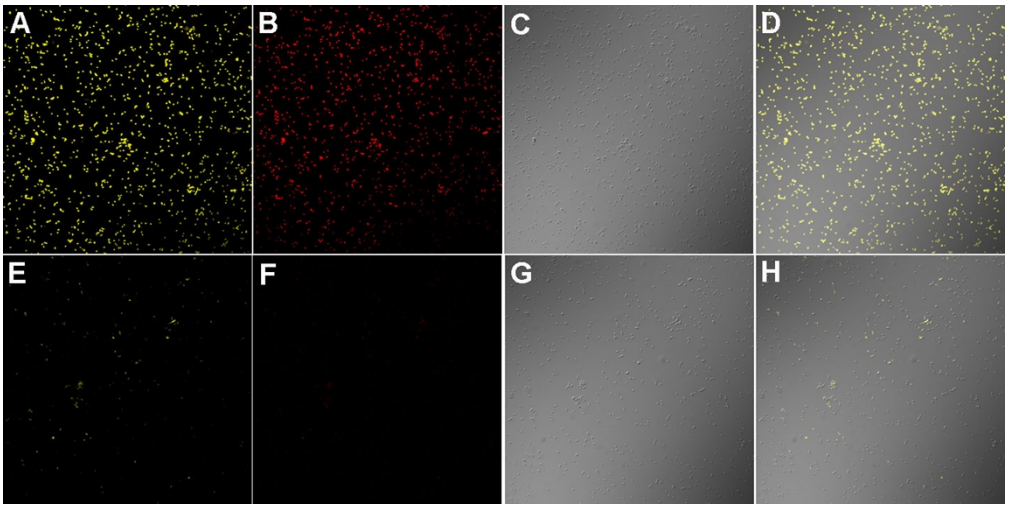


Figure S-21. Confocal fluorescence microscopy images of *E. faecalis* treated with compound ***1*** (A-D) and ***2*** (E-H) (the yellow emission was from NBD and red emission from nile blue)

Preparation of TEM samples of bacterial: *B. subtilis* and *E. faecalis* were incubated with compound ***1*** and ***2*** for 0.5 hour, then washed with 1×PBS buffer for three times and re-suspended in PBS. A carbon-coated copper grid was vertically dipped into the bacterial solution and was put into liquid nitrogen instantly to make the bacterial immobilized. The samples were vacuum-dried by an oil pump before TEM measurement.

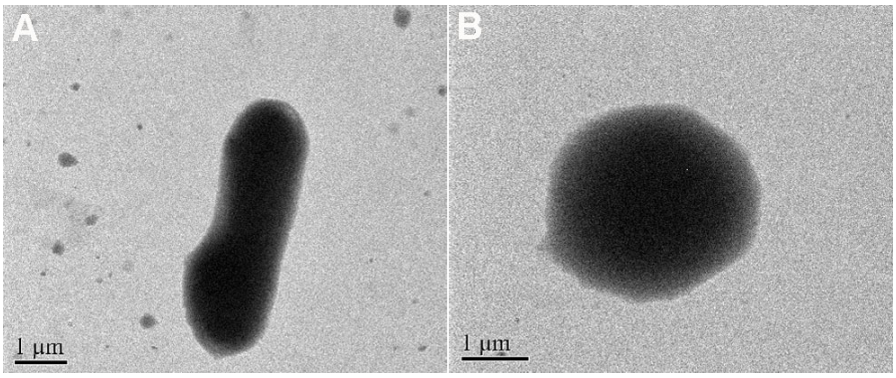


Figure S-22. The TEM images of strains without compounds: A) *B. subtilis* and B) *E. faecalis*

Fluorescence quantum yield of compound *1* and *2*: The fluorescence quantum yield of compound ***1*** and ***2*** were obtained according to a reported method. Rhodamine B was choosen as the standard substance [the quantum yield (Φ) was 0.97 in ethanol solution]. The absorption curves of dilute compound ***1***, ***2*** and Rhodamine B between 300-700 nm were both performed in ethanol solution, and the crossover point of that was observed at 506 nm (Figure S-23). Then the wavelength of 506 nm was set as excitation wavelength, the fluorescence spectrum of compound ***1***, ***2*** and Rhodamine B between 530-700 nm were obtained. The fluorescence quantum efficiency of compound ***1*** and ***2*** were calculated with a standard formula:

$$\Phi_f = \left(\frac{n_x}{n_{std}}\right)^2 \cdot \frac{F_x}{F_{std}} \cdot \Phi_{std}$$

x: unknown substance, *std*: standard substance;

n: refractive index of solvent;

F: the maximum peak weight of fluorescence spectrum.

Φ_1 (fluorescence quantum efficiency of compound ***1***) was calculated about 0.15 and Φ_2 (fluorescence quantum efficiency of compound ***2***) was calculated about 0.11.

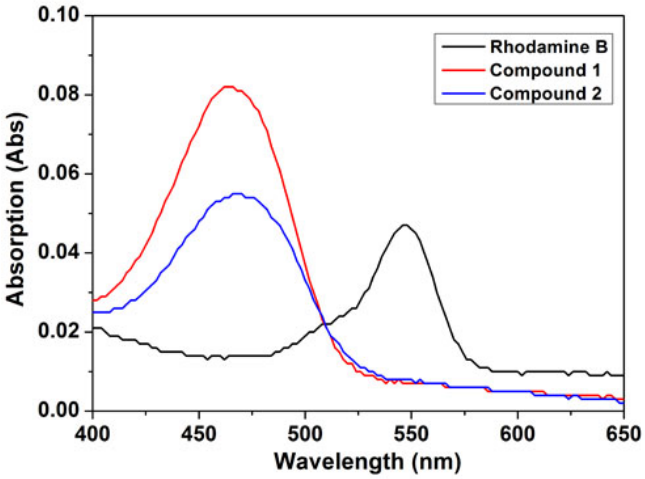


Figure S-23*:** The absorption curves of dilute compound ***1, ***2,*** and Rhodamine B between 300-700 nm

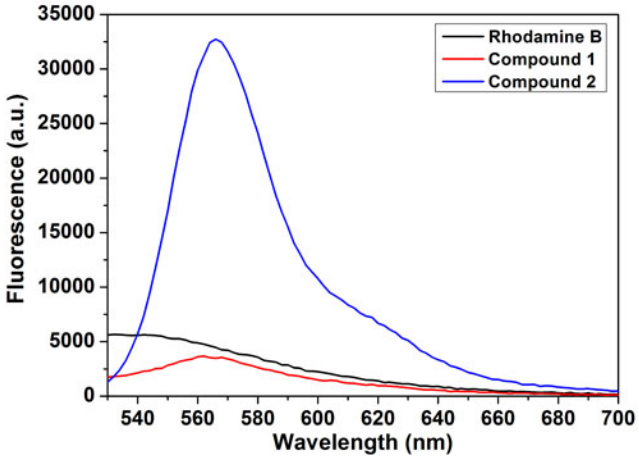


Figure S-24*:** Fluorescence spectrum of compound ***1, ***2,*** and Rhodamine B (excitation wavelength = 506 nm)