Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

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.

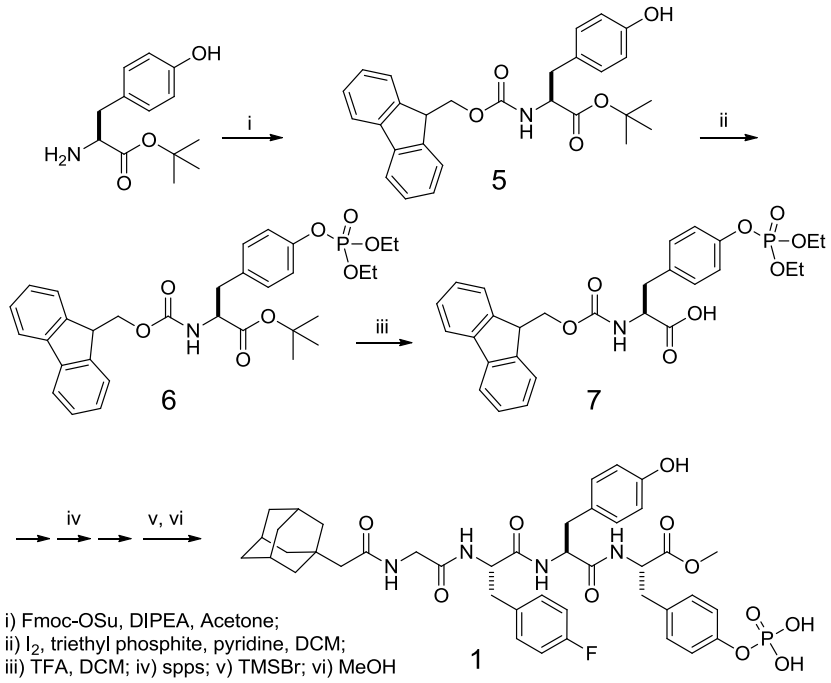
Alkaline Phosphatase (30 U/μL): 1 U corresponds to the amount of enzyme which hydrolyzes *p*-nitrophenyl phosphate to form 1 μmol of *p*-nitrophenol at pH 8.0 and 37 ^{o}C.

Tyrosinase (25 U/μL): 1 U will cause an increase in A280 nm of 0.001 per minute at pH 6.5 at 25°C in a 3 mL reaction mixture containing L-tyrosine.

General methods: ^{1}H and ^{19}F 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 C18 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.

Synthesis and Characterization

Compound ***1*** was synthesized according to Scheme S-1. Other compounds were synthesized using the same procedure.



Scheme S-1. Synthetic route of Compound ***1***.

The compounds of Fmoc-L-Tyr-OtBu (5), Fmoc-L-Tyr(PO(OEt)2)-OtBu (6) and Fmoc-L-Tyr(PO(OEt)$_2$-OH (7) were synthesized using the same procedures we reported before.[1]

Preparation of Fmoc-L-Tyr-O^{t}Bu (5): L-Tyrosine methyl ester hydrochloride (1.17 g, 5 mmol) and $NaHCO_3$ (840 mg, 10 mmol) were dissolved in 50 mL of water under stirring, the solution of Fmoc-OSu (1.65 g, 4.9 mmol) in 100 mL of acetone was added, and the resulting reaction mixture was stirred at room temperature overnight. After drying the reaction mixture with air, the solid obtained was washed with acid water (pH of 3) (20 ml * 3) and water (20 ml * 2), successively. 1.95 g of ***2*** was collected (93.4%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.03-8.05 (d, 2H), 7.99-8.01 (d, 1H), 7.79-7.82 (t, 1H), 7.55-7.59 (t, 2H), 7.44-7.50 (m, 2H), 7.18-7.20 (d, 2H), 6.80-6.83 (d, 2H), 4.30-4.40 (m, 4H), 3.76 (s, 3H), 3.05-3.10 (m, 1H), 2.90-2.96 (m, 1H). MS: calc. M^+ = 417.2, obsvd. $(M+H)^+$ = 418.2.

Preparation of Fmoc-L-Tyr(PO(OEt)$_2$)-O^{t}Bu (6): I_2 (5.0 mmol, 1.25 equivalents)

was added to a solution of the Triethyl phosphate (5.2 mmol, 1.3 equivalents) in 20 mL of dichloromethane (DCM) at 0 °C. 5 minutes after the addition, the clear, colorless solution was allowed to warm to room temperature (25°C). Then the solution was added dropwise, over a period of 30 minutes, to a flask containing Fmoc-Tyr-O^{t}Bu (4.0 mmol, 1.0 equivalent) and pyridine (16.0 mmol, 4.0 equivalents) in 80 mL of DCM at 0°C. After an additional 30 minutes, the reaction mixture was diluted with Et_2O (150 mL). The organic layer was washed with $KHSO_4$ (5 wt%) (30 ml*2), saturaed brine (30 ml), then dried with anhydrous $MgSO_4$. The residue was purified by flash chromatography on silica gel (eluent: Hexane/Ethyl acetate = 3/2) to afford 1.93 g of title compound (87.2%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.03-8.07 (m, 3H), 7.79-7.82 (m, 2H), 7.55-7.59 (m, 2H), 7.42-7.48 (m, 3H), 7.25-7.27 (d, 2H), 4.35-4.40 (m, 3H), 4.24-4.28 (q, 4H), 3.78 (s, 3H), 3.18-3.22 (m, 1H), 3.00-3.04 (m, 1H), 1.37-1.40 (t, 6H). ^{31}P NMR (δ -6.36 ppm). MS: calc. M^+ = 553.2, obsvd. $(M+H)^+$ = 554.2.

Preparation of Fmoc-L-Tyr(PO(OEt)$_2$-OH (7): To a solution of **6** (600 mg, 1.0 mmol) in 5 mL CH_2Cl_2, TFA (10 ml) was added at 0 ^{o}C. After stirred at room temperature for 4 hours, the solvent was removed by rotary evaporator. The solid obtained was co-evaporated with toluene twice. 530 mg of title compound was obtained after removing the solvent in vacuum (yield of 98.3%) and it was used directly for solid phase peptide synthesis. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.23-8.25 (d, 2H), 8.01-8.05 (t, 2H), 7.76-7.79 (t, 2H), 7.59-7.62 (m, 4H), 7.47-7.52 (d, 2H), 4.30-4.60 (m, 8H), 3.45-3.48 (m, 1H), 3.21-3.27 (m, 1H), 1.58-1.61 (t, 6H). ^{31}P NMR (δ -6.348 ppm). MS: calc. M^+ = 539.2, obsvd. $(M+H)^+$ = 540.1.

Peptide Synthesis: 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 by a tert-butyl group. The first amino acid was loaded on the resin at the C-terminal with the loading efficiency about 0.6 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, the N-terminus of the peptides was coupled with 1-Adamantaneacetic acid to attach the adamantane group on the 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 1 min (5 ml per gram of resin). The peptide derivative was cleaved using 1% of trifluoroacetic acid in DCM for ten times (one minute for each time, 5 ml per gram). All the solutions were combined and concentrated, and then 20 mL of ice-cold diethylether was added. The resulting precipitate was centrifuged for 10 min at 2 ^{0}C at 10,000 rpm. Afterward the supernatant was decanted and the resulting solid was dissolved in DMSO for HPLC separation.

Ada-GfFYpY-OMe. The compound obtained by general solid phase peptide synthesis (spps) was treated with 20 equiv. of TMSBr in 10 ml of dry DCM for 24 hours. The solvent was removed and then 10 mL of methanol was added, the mixture was stirred at room temperature for another 2 hours. The solid obtained after evaporating the solvents was purified by HPLC to afford title compound **1** in a yield of about 43%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.18 (d, J=7.6 Hz, 4H), 7.11-6.95 (m, 6H), 6.64 (d, J = 8.3 Hz, 2H), 4.43-4.47 (m, 4H), 3.61-3.85 (m, 3H), 3.56 (s, 3H), 2.86-3.01 (m, 4H), 2.62-2.71 (m, 2H), 1.85-1.86 (m, 5H), 1.51-1.62 (m, 13H). HR-MS: calc. M^{+} = 836.3270, obsvd. $(M+H)^{+}$ = 837.3273.

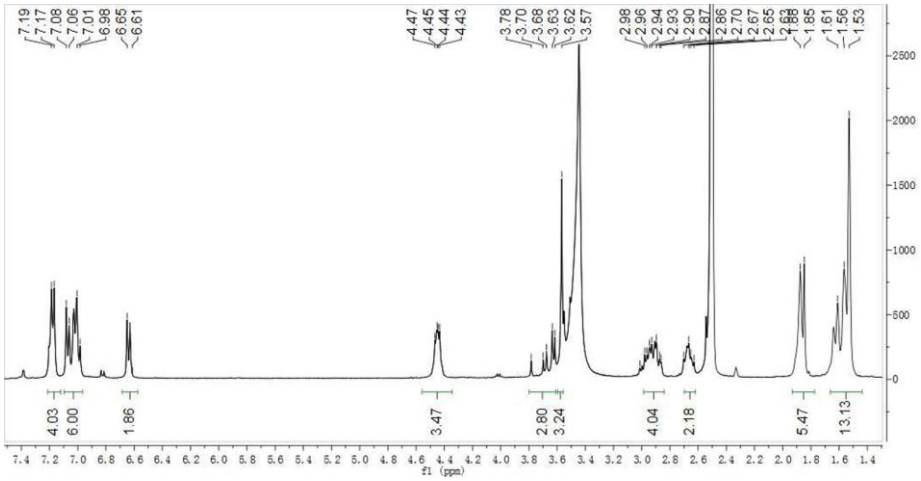


Fig. S-1. ^{1}H NMR of Ada-GfFYpY-OMe

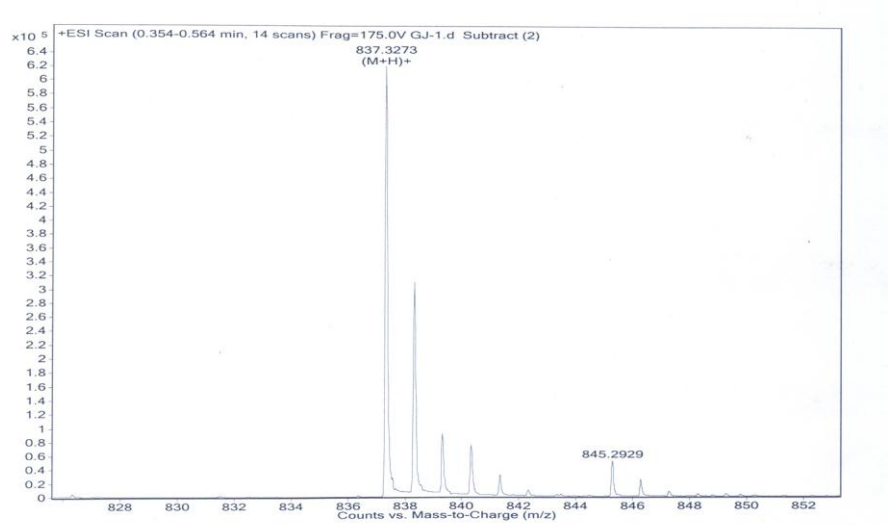


Fig. S-2. HR-MS of Ada-GfFYpY-OMe

Ada-GfFYpY-OH: The same procedure was used to synthesize Ada-fFYpY-OH with a little change, which was to add dd H_2O to the peptide after treated with TMSBr and stirring. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.19 (t, J=8.3 Hz, 4H), 6.97-7.09 (m, 6H), 6.63 (d, J = 8.1 Hz, 2H), 4.45 (s, 3H), 2.86-3.01 (m, 4H), 2.66 (s, 2H), 1.85-1.88 (m, 5H), 1.53-1.64 (m, 13H). HR-MS: calc. M^+ = 822.3106, obsvd. $(M+H)^+$ = 823.3114.

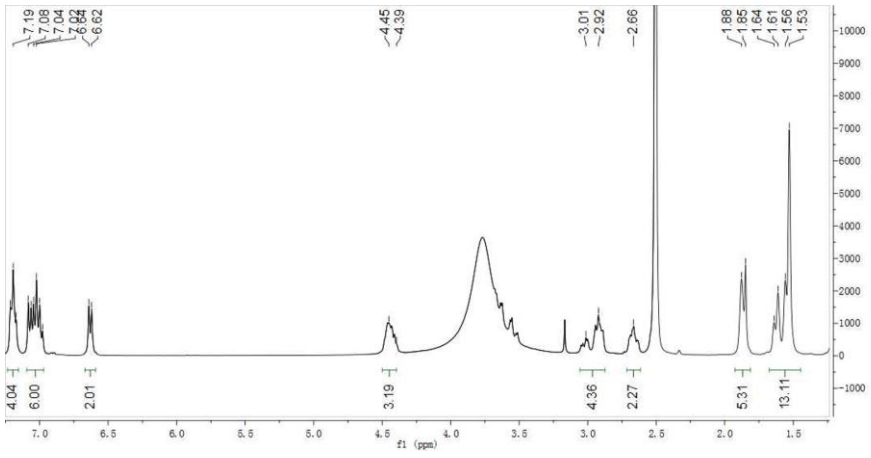


Fig. S-3. ^{1}H NMR of Ada-GfFYpY-OMe

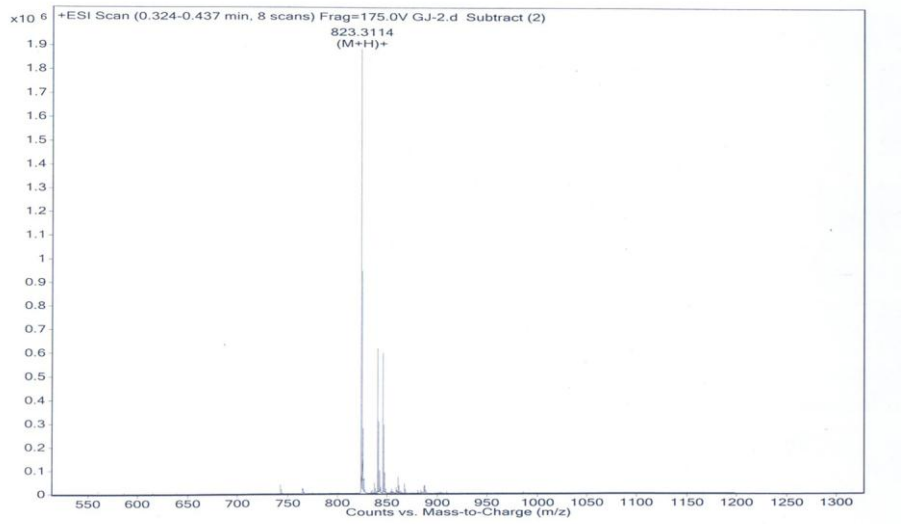


Fig. S-4. HR-MS of Ada-GfFYpY-OMe

Ada-fFYpY-OH: The same procedure with the synthesis of Ada-GfFYpY-OH. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.13-7.30 (m, 4H), 6.90-7.12 (m, 6H), 6.61 (d, J=7.9 Hz, 2H), 4.35-4.60 (m, 4H), 2.89-3.04 (m 4H), 2.61-2.70 (m, 2H), 1.70-1.76 (m, 5H), 1.13-1.57 (m, 13H). HR-MS: calc. M+ = 765.2827, obsvd. (M+H)+ = 766.2899.

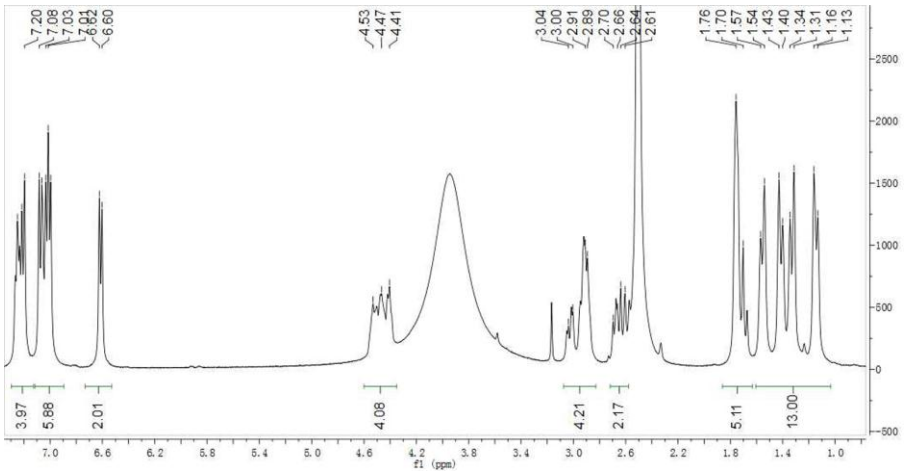


Fig. S-5. ^{1}H NMR of Ada-fFYpY-OMe

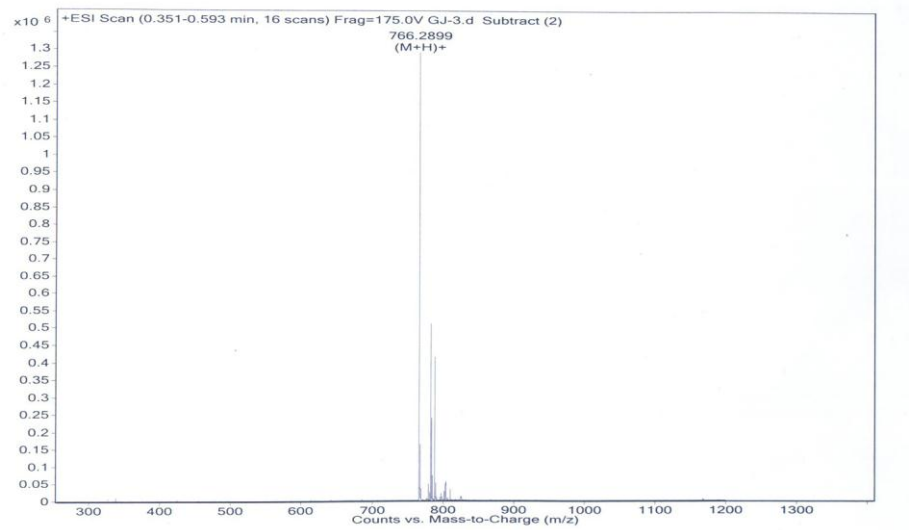


Fig. S-6. HR-MS of Ada-fFYpY-OMe

Formation of nanospheres

Compound 1 was dissolved in ddH_2O and the pH of the solution is carefully adjusted to 7.4 by Na_2CO_3. To above solution, phosphatase was added and nanospheres would form in less than 5 mins. The suspension containing nanospheres were directly used in the following experiments.

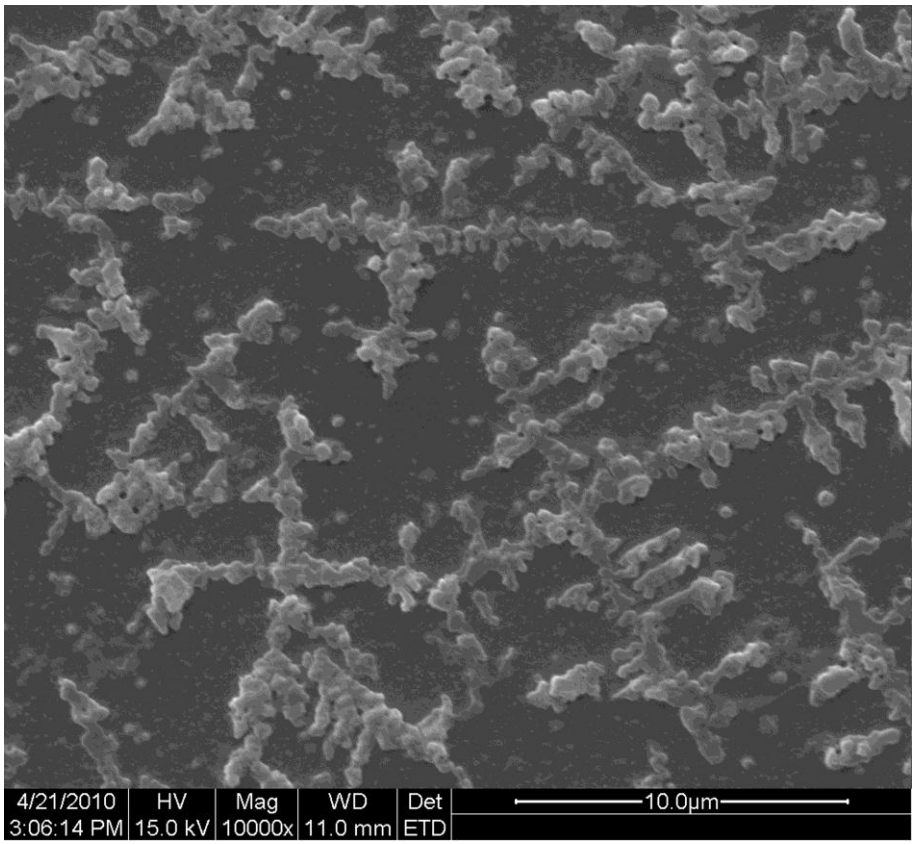


Fig. S-7. SEM images of nanospheres treated with 800 U/L tyrosinase after 1 hour, clearly showing the disassembly of the nanosphere.

Measurements of dynamic light scattering

The solutions containing nanosphers for the light scattering measurements were prepared in a clean vials. Dynamic light scattering (DLS) measurements were performed on a laser-light-scattering spectrometer (BI-200SM) that was equipped with a digital correlator (BI-10000AT) and a laser source of 532 nm at atmosphere temperature (22-25 °C).

Table S-1 DLS measurements of spheres formed at different AP concentrations

Time (min)	AP[a] Conc. (U ml^{-1})	DLS Intensity (kcps)	Radius (nm)
25	7.5	767.8±5.7	635.0
25	3.0	668.9±4.6	685.9
25	1.5	560.2±3.4	666.2
25	0.7	480.5±8.9	669.0
600	7.5	782.7±5.1	697.7
600	3.0	792.9±0.8	678.3
600	1.5	795.3±4.7	611.3
600	0.7	799.8±9.6	646.7

a AP is short for Alkaline Phosphatase

Table S-2 Diameter difference between different concentrations of compound **1**

Compound 1 Conc. (wt%)	Radius (nm)
0.005	684.4
0.05	482.2
0.1	377.5
0.2	246.9

^{19}F NMR spectrum of nanospheres with tyrosinase

Compound 1 (1.0 eqiv.) was dissolved in ddH_2O (10% D_2O(v/v), pH 7.4, 0.33 eqiv. of TFA) and certain amount of phosphatase was added to form nanospheres. Then tyrosinase (200 U) in ddH_2O (5 μL) was added to above solution and the sample were directly analysed by ^{19}F NMR spectroscopy with TFA as an internal standard (-75.6 ppm) at 37 ^{o}C.

^{1}H NMR spectrum of nanospheres with tyrosinase

Compound 1 (1.0 eqiv.) was dissolved in D_2O, and certain amount of phosphatase (D_2O solution) was added to form nanospheres. Then tyrosinase (200 U) in D_2O (5 μL) was added to above solution and the sample were directly analysed by ^{1}H NMR spectroscopy every 15 minutes.

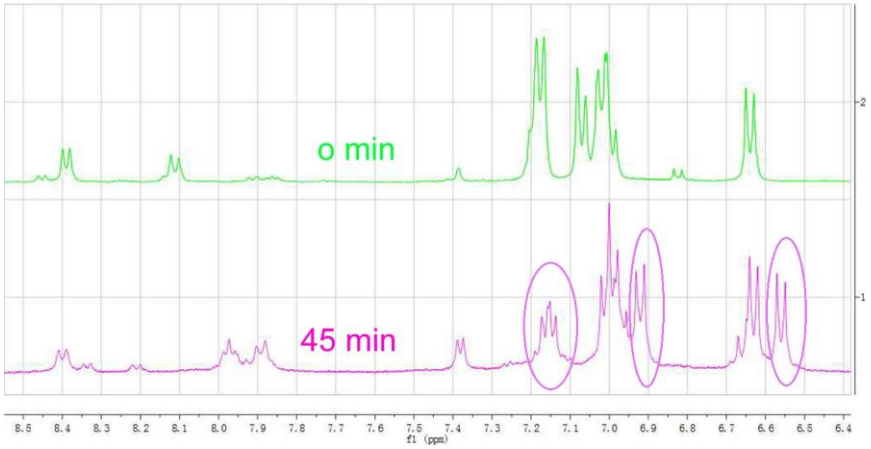


Fig. S-8. ^{1}H NMR spectroscopy on nanospheres before and 45 minutes after the addition of tyrosinase (Frozen-dried powder dissolved in DMSO-d_6). 45 minutes after the oxidation, some of the peaks moved from low field to higher field, indicating the formation of more electron-donating group of -OH on benzene ring.

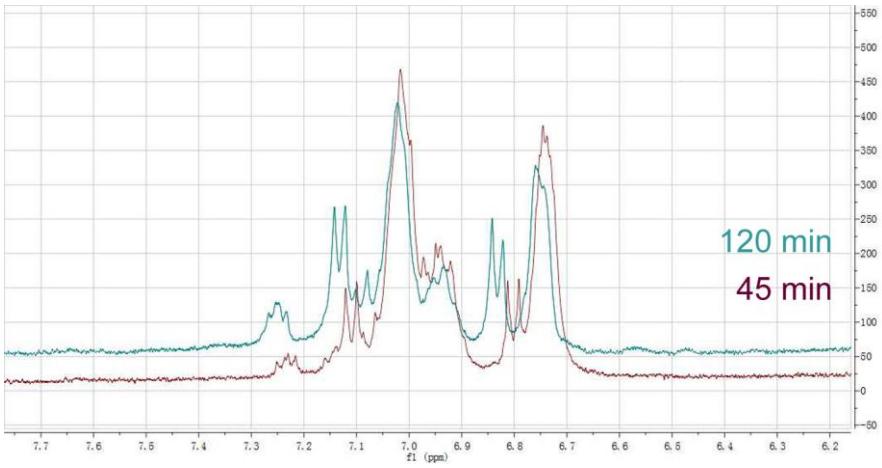


Fig. S-9. Time sweep of ^{1}H NMR spectroscopy on nanospheres (D_2O solutions). Compared with the spectrum acquired at 45 minutes, almost all the peaks moved slightly from high field to lower field 120 minutes after the oxidation, indicating the formation of electron withdrawing group of quinone on benzene ring.

MTT assay on NIH 3T3 cells

The cytotoxicity of the compounds were evaluated by MTT assay.

The MTT assay is a colorimetric assay for determing cell viability. Under defined conditions, NAD(P)H-dependent cellular oxidoreductase enzymes may reflect the number of viable cells present. These enzymes are capable of reducing the tetrazolium

dye MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to its insoluble formazan, which has a purple color. [2]

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 nanospheres and the solution formed after the addition of tyrosinase were dissolved in DMEM (Dulbecco's Modified Eagle Medium) solutions, and were added into the cells (final concentrations were 0.3, 0.6 and 1.2 mM). 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%.

1. J. Gao, H. Wang, L. Wang, J. Wang, D. Kong and Z. Yang, *J. Am. Chem. Soc.*, 2009, **131**, 11286-+; H. Wang, Z. Wang, D. Song, J. Wang, J. Gao, L. Wang, D. Kong and Z. Yang, *Nanotechnology*, 2010, **21**.
2. M. V. Berridge, P. M. Herst and A. S. Tan, Biotechnology annual review, 2005, **11**, 127-152.