Electronic Supplementary Material (ESI) for RSC Advances

Self-immolative trigger-initiated polydiacetylene probe for β-glucuronidase activity

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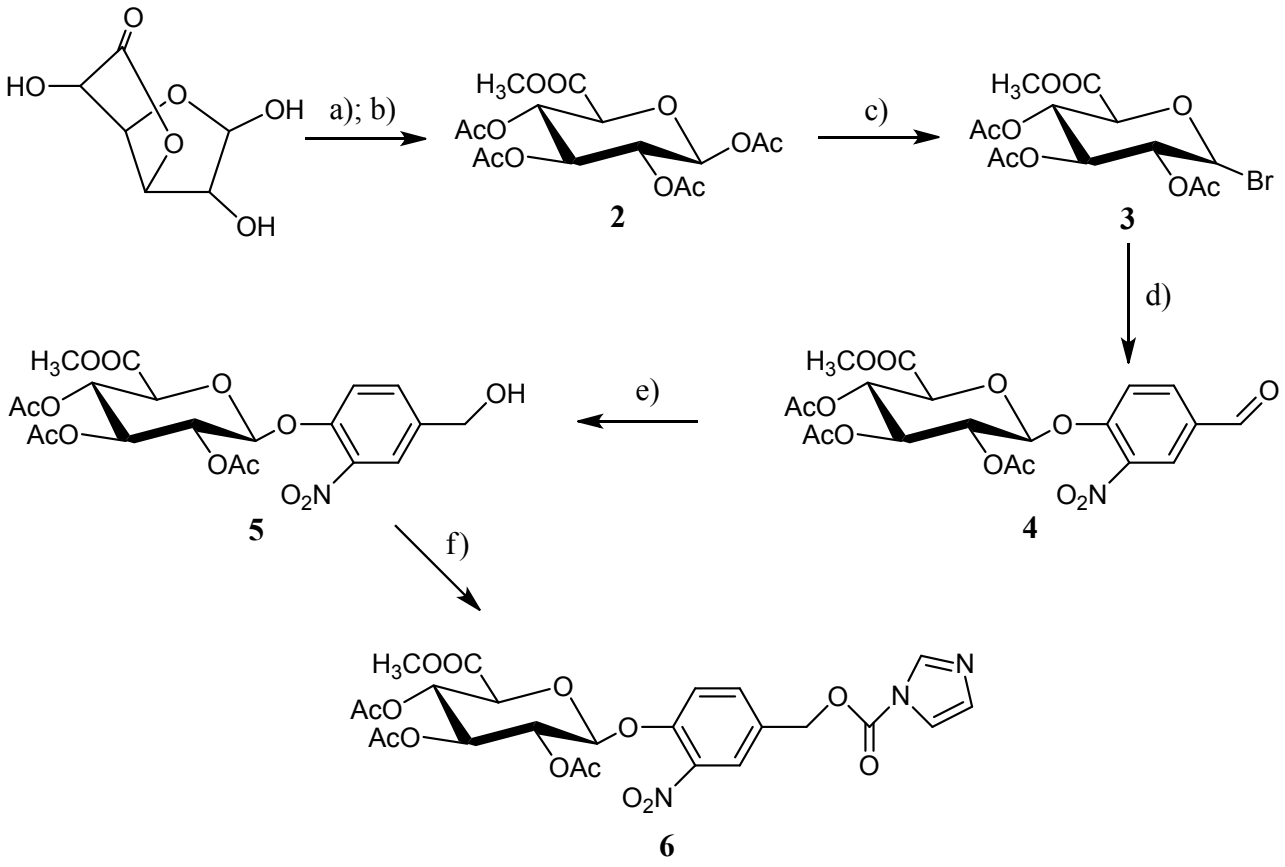
1. Materials and general methods

10,12-Pentacosadiynoic acid (PCDA) and 2,2'-(ethylenedioxy)-bis(ethylamine) (EDEA) were purchased from Alfa Aesar (Lancaster, England). The PCDA powder was dissolved in chloroform and filtered through a nylon 0.45 μm filter to remove oligomers prior to use. *β*-Glucuronidase (from *E. Coli*, 1,000,000-5,000,000 U/g of protein) and D-glucaric acid-1,4-lactone were purchased from Sigma–Aldrich (St. Louis, MO, USA). D-glucurono-3,6-lactone and methyl trifluoromethanesulphonate (MeOTf) were obtained from Aladdin (Shanghai, China). 1,1'-Carbonyl-diimidazolen (CDI) and 4-hydroxy-3-nitrobenzaldehyde were purchased from Energy Chemical (Shanghai, China). Dry solvents used in the synthesis were purified using standard procedures. All other reagents and solvents were of analytical grade and supplied by local commercial suppliers. Ultrapurified water was supplied by a Milli-Q system (Millipore).

Reactions were monitored by thin-layer chromatography (TLC) using silica gel 60 GF254 (Qingdao Haiyang Chem. Co., Ltd., Shangdong, China). Sugar-containing compounds were visualized on silica TLC plates with 8% H_2SO_4 followed by heating; PCDA-containing compounds were directly visualized under UV light (254 nm) (ZF-6 type-III ultraviolet analyzer; Shanghai Jiapeng Technology Co., Ltd., Shanghai, China). Column chromatography was conducted using silica gel 60 (Qingdao Haiyang Chem. Co., Ltd.). ^{1}H-nuclear magnetic resonance (NMR) and ^{13}C-NMR spectra were recorded using a BrukerAvance DMX 500 MHz/125 MHz spectrometer or BrukerAvance DMX 400 MHz/100 MHz spectrometer (Bruker, Billerica, MA, USA). Peaks were based on a tetramethylsilane (TMS) internal standard. Electrospray ionization mass spectroscopy (ESI-MS) data were obtained using a Thermo Scientific LCQ FLEET mass spectrometer equipped with an electrospray ion source and controlled by Xcalibur software (Thermo Fisher Scientific, Waltham, MA, USA).

2. Synthesis of compounds

Compound **6** was synthesized according to **Scheme S1**.[S1, S2]



Scheme S1. Reagents and conditions: (a) Sodium methylate, MeOH, RT, 1 h; (b) $HClO_4$, acetic anhydride, RT, 24 h; (c) 30% HBr/acetic acid, 4 °C, 24 h; (d) 4-hydroxy-3-nitrobenzaldehyde, Ag_2O, CH_3CN, RT, 4 h; (e) $NaBH_4$, IPA, CH_3Cl, 0 °C, 45 min; (f) CDI, DMAP, CH_2Cl_2, 2 h.

Synthesis of 2: D-glucurono-3,6-lactone (8.8 g, 50 mmol) was dissolved in methanol (50 mL) containing sodium methylate (75 mg, 1.4 mmol). The mixture was stirred at room temperature for 1 h. Subsequently, the solvent was removed under reduced pressure to obtain a yellow–orange oily residue. The residue was dissolved in acetic anhydride (34 mL), after which a mixture of perchloric acid (0.15 mL) in acetic anhydride (10 mL) was added (dropwise) to the

resultant solution at 0 °C. This solution was allowed to stir at room temperature for at least 6 h and then stored at 4 °C overnight. The resulting precipitate was filtered and washed with cooled ether to yield **2** as a white solid (4.7 g, 25.3%). ^{1}H NMR (400 MHz, $CDCl_3$)δ: 2.05 (s, 9H), 2.14 (s, 3H), 3.76 (s, 3H), 4.18–4.21 (d, *J* = 9.4 Hz, 1H), 5.14–5.18 (t, *J* = 8.3 Hz, 1H), 5.23–5.28 (t,*J* = 9.4 Hz, 1H), 5.31–5.35 (t, *J* = 9.2 Hz, 1H), 5.77–5.79 (d, *J* = 7.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.50, 20.57, 20.59, 20.81, 53.06, 68.90, 70.11, 71.80, 72.97, 91.33, 166.81, 168.87, 169.21, 169.45, 169.94. ESI-MS *m/z*: calculated for $C_{15}H_{20}O_{11}$, 376.1, found $[M+H]^+$, 377.2.

Synthesis of 3: Compound **2** (3 g, 8 mmol) was dissolved in 30% hydrobromic acid in acetic acid (15 mL). This mixture was stored at 4 °C overnight and then concentrated on a rotary evaporator to remove most of the solvent. The residue was dissolved in chloroform (20 mL) and washed first with cold saturated $NaHCO_3$ solution (6×40 mL) and then with water. The solution was dried over Na_2SO_4 and concentrated under vacuum to produce syrup. The residual syrup was dissolved in absolute ethyl alcohol (10 mL) and stored at 4 °C to obtain a white precipitate. The solid was filtered and washed with cooled ether to yield **3** as a white solid (1.8 g, 56.8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.06 (d, *J* = 2.1 Hz, 6H), 2.11 (s, 3H), 3.77 (s, 3H), 4.57–4.59 (d, *J* = 10.3 Hz, 1H), 4.85–4.88 (dd, *J* = 10.0, 4.1 Hz, 1H), 5.22–5.27 (t, *J* = 9.4 Hz, 1H), 5.59–5.64 (t, *J* = 9.8 Hz, 1H), 6.65–6.66 (d, *J* = 4.0 Hz, 1H).^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.45, 20.60, 20.61, 53.14, 68.45, 69.25, 70.28, 72.01, 85.39, 166.68, 169.48, 169.66, 169.68. ESI-MS *m/z*: calculated for $C_{13}H_{17}BrO_9$, 396.0, found $[M+H]^+$, 397.2.

Synthesis of 4: Compound **3** (1.53 g, 3.84 mmol) was dissolved in anhydrous acetonitrile (50 mL), after which 4-hydroxy-3-nitrobenzaldehyde (1.29 g, 7.70 mmol) and Ag_2O (1.8 g, 7.76 mmol) were added to the resultant solution under nitrogen. The resulting slurry was stirred in the dark for 4 h at room temperature. The solution was filtered and concentrated under vacuum. The

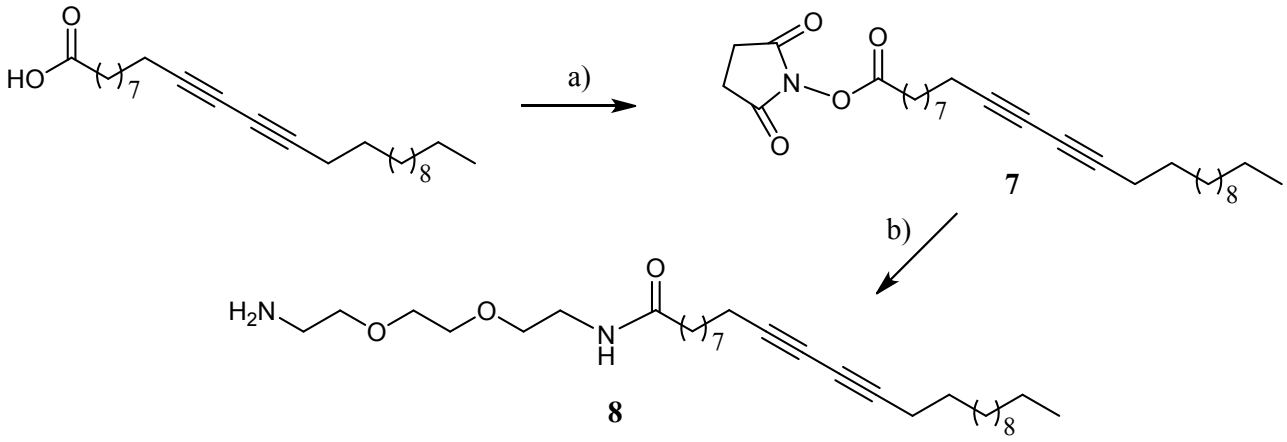
residue was dissolved in ethyl acetate (50 mL) and washed with saturated $NaHCO_3$ (6×20 mL), brine, and water. After drying over Mg_2SO_4, the solvent was removed under vacuum to yield **4** as a beige solid (1.17 g, 63.2%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.01 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 3.64 (s, 3H), 4.79–4.81 (d, J = 10.3 Hz, 1H), 5.11–5.19 (m, 2H), 5.45–5.50 (m, 1H), 5.93–5.95 (d, J = 8.0 Hz, 1H), 7.63–7.65 (d, J = 9.0 Hz, 1H), 8.21–8.24 (dd, J = 9.0 Hz, 1H), 8.44–8.45 (d, J = 1.5 Hz, 1H), 9.98 (s, 1H).^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.14, 20.20, 20.24, 52.61, 68.48, 69.66, 70.43, 71.18, 97.25, 117.58, 126.18, 131.06, 134.69, 140.25, 152.07, 166.81, 168.68, 169.29, 169.45, 190.44. ESI-MS *m/z*: calculated for $C_{20}H_{21}NO_{13}$, 483.1, found $[M+Na]^+$, 506.2.

Synthesis of 5: Compound **4** (1.12 g, 2.33 mmol) was dissolved in isopropanol (10 mL) and chloroform (50 mL) containing 5 g of silica gel. Sodium borohydride (120 mg, 3.17 mmol) was added to the solution at 0 °C, and the resulting slurry was reacted at this temperature for 45 min. Afterward, the mixture was poured into 100 mL of ice water and then filtered to remove the silica gel. The organic layer was then separated and washed with brine and water, dried over Mg_2SO_4, concentrated under vacuum to afford **5** as a white solid (0.94 g, 84.2%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.99 (s, 3H), 2.02 (s, 6H), 3.65 (s, 3H), 4.51 (s, 2H), 4.72–4.75 (d, J = 10.3 Hz, 1H), 5.06–5.13 (m, 2H), 5.43–5.48 (m, 1H), 5.70–5.72 (d, J = 8.0 Hz, 1H), 7.37–7.39 (d, J = 9.0 Hz, 1H), 7.60–7.62 (d, J = 8.0 Hz, 1H), 7.80 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.16, 20.19, 20.25, 52.60, 61.31, 68.71, 69.94, 70.76, 71.02, 98.06, 117.71, 122.27, 131.96, 138.53, 140.18, 146.90, 166.90, 168.71, 169.27, 169.47. ESI-MS *m/z*: calculated for $C_{20}H_{23}NO_{13}$, 485.1, found $[M+Na]^+$, 508.2.

Synthesis of 6: 1,1'-Carbonyl-diimidazolen (336 mg, 2.07 mmol) was added to a mixture of **5** (500 mg, 1.03 mmol) and 4-dimethylamiopyridine (DMAP) (25 mg, 0.21 mmol) in anhydrous

methylene chloride (10 mL) under nitrogen. The solution was stirred at room temperature, and the reaction was monitored by TLC (silica, methylene chloride:methanol, 19:1, v/v) until the reaction was complete (3 h). The solution was then washed with water, saturated $NaHCO_3$, and brine. After drying over $MgSO_4$, the solvent was removed under vacuum to yield **6** as a white solid (510 mg, 91.3%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.06 (s, 6H), 2.11 (s, 3H), 3.73 (s, 3H), 4.25–4.27 (d, J = 8.3 Hz, 1H), 5.28–5.37 (m, 4H), 5.41 (s, 2H), 7.07 (s, 1H), 7.42–7.44 (d, J = 8.5 Hz, 2H), 7.65–7.67 (d, J = 8.6 Hz, 1H), 7.92 (s, 1H), 8.14 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$)δ: 20.52, 20.54, 20.58, 53.10, 53.49, 67.87, 68.55, 70.01, 70.82, 72.51, 99.42, 117.11, 119.99, 125.73, 130.01, 130.95, 134.35, 137.12, 148.41, 149.59, 166.67, 169.21, 169.33, 169.98. ESI-MS *m/z*: calculated for $C_{24}H_{25}N_3O_{14}$, 579.1, found $[M+Na]^+$, 602.3.

Compound **8** was prepared according to **Scheme S2**.[S3]



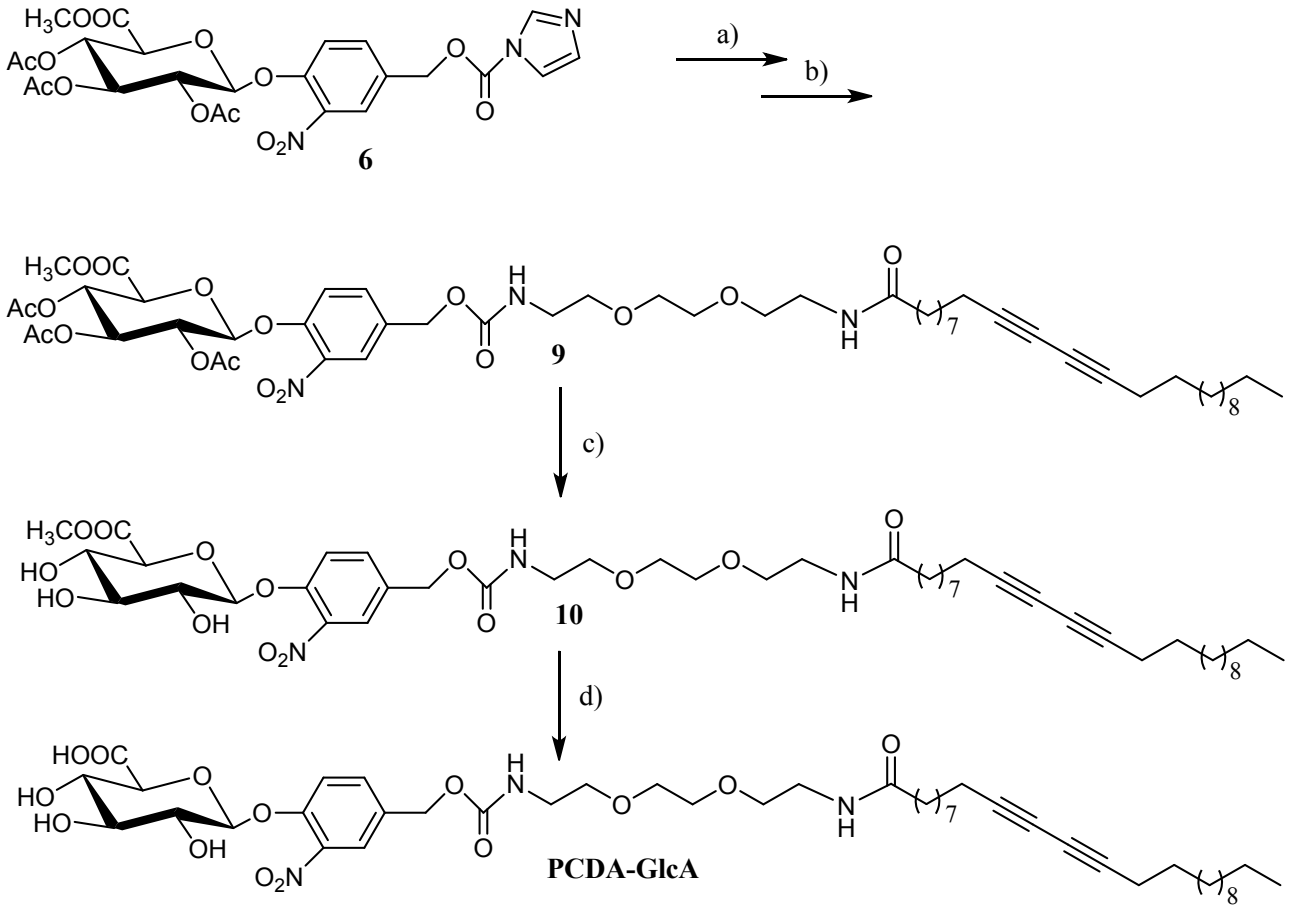
Scheme S2. Reagents and conditions: (a) NHS, EDC, CH_2Cl_2, RT, 2 h; (b) EDEA, CH_2Cl_2, RT, 2 h.

Synthesis of 7: *N*-hydroxysuccinimide (173 mg, 1.50 mmol) and *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (300 mg, 1.56 mmol) were addedto a solution of 10,12-pentacosadiynoic acid (500 mg, 1.33 mmol) in anhydrous methylene chloride (20 mL). The

solution was stirred at room temperature for 2 h and then concentrated under vacuum. The residue was extracted using ethyl acetate (20 mL) and washed with water. The organic layer was dried over $MgSO_4$ and concentrated in vacuo to obtain **7** as a white solid (580 mg, 92.4%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.88–0.91 (t, *J* = 6.7 Hz, 3H), 1.27–1.55 (m, 30H), 1.72–1.79 (m, 2H), 2.24–2.28 (t, *J* = 6.9 Hz, 4H), 2.60–2.64 (t, *J* = 7.5 Hz, 2H), 2.85–2.86 (d, *J* = 4.1 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.16, 19.20, 19.22, 22.72, 24.55, 25.61, 28.28, 28.36, 28.72, 28.81, 28.88, 28.92, 29.12, 29.37, 29.50, 29.63, 29.65, 29.67, 30.93, 31.94, 65.19, 65.28, 77.46, 77.64, 168.69, 169.23. ESI-MS *m/z*: calculated for $C_{23}H_{45}NO_4$, 471.3, found $[M+H]^+$, 472.4.

Synthesis of 8: A mixture of **7** (500 mg, 1.06 mol) in methylene chloride (10 mL) was slowly added to a solution of 2,2'-(ethylenedioxy)-bis(ethylamine) in methylene chloride. The resultant solution was stirred at room temperature for 3 h, and the solvent was removed under vacuum. The residue was dissolved in methylene chloride (30 mL), washed with water, and dried over $MgSO_4$. The solution was then concentrated and purified by column chromatography (silica, methylene chloride: methanol, 19:1, followed by 9:1, v/v). The desired compound **8** was obtained as a white solid (300 mg, 56.1%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86–0.90 (t, *J* = 6.8 Hz, 3H), 1.26–1.40 (m, 26H), 1.47–1.55 (m, 4H), 1.58–1.66 (m, 2H), 2.17–2.20 (t, *J* = 7.5 Hz, 2H), 2.22–2.26 (t, *J* = 7.0 Hz, 4H), 2.91–2.93 (t, *J* = 4.7 Hz, 2H), 3.44–3.48 (m, 2H) 3.55–3.59 (m, 4H), 3.63 (s, 4H), 6.38 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.13, 19.18, 19.19, 22.68, 23.15, 25.73, 28.31, 28.34, 28.79, 28.85, 28.95, 29.09, 29.23, 29.28, 29.34, 29.47, 29.60, 29.62, 29.63, 31.91, 36.60, 39.15, 39.34, 65.20, 65.27, 70.07, 70.11, 70.18, 70.21, 77.28, 77.59, 173.49. ESI-MS *m/z*: calculated for $C_{31}H_{56}N_2O_3$, 504.4, found $[M+H]^+$, 505.3.

PCDA-GlcA was prepared according to **Scheme S3**.



Scheme S3. Reagents and conditions: (a) MeOTf, CH_2Cl_2, 0 °C, 30 min; (b) **8**, CH_2Cl_2, 0 °C, 6 h; (c) NaOMe, MeOH, 0 °C, 2 h; (d) NaOH, acetone, 0 °C, 20 min.

Synthesis of 9: Compound **6** (500 mg, 0.86 mmol) was dissolved in anhydrous methylene chloride (7 mL). Methyl trifluoromethanesulphonate (90 µL, 0.80 mmol) was then added to this solution over 5 min under nitrogen at 0 °C. The resulting solution was allowed to stir at 0 °C for 30 min. The reaction was then diluted with 4 mL of ether and cooled to –20 °C to allow the methylated product to precipitate. The solid was collected by filtration, washed with ether, and dried. The activated compound was suspended in anhydrous methylene (5 mL) under nitrogen. Afterward, compound **8** (400 mg, 0.79 mmol) was added. This solution was cooled to 0 °C and

then mixed with 110 μL of triethylamine (0.79 mmol). The reaction was carried out at 0 °C under nitrogen for 4 h and then warmed to room temperature. After another 2 h of stirring, the solution was washed with water and brine, dried over $MgSO_4$, concentrated under vacuum, and purified by column chromatography (silica, methylene chloride: methanol, 9:1, v/v) to yield **9** as a pale yellow solid (640 mg, 79.8%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.90–0.93 (t, J = 6.9 Hz, 3H), 1.26–1.43 (m, 26H), 1.50–1.57 (m, 4H), 1.61–1.67 (m, 2H), 2.09–2.10 (d, *J* = 3.7 Hz, 6H), 2.15 (s, 3H), 2.18–2.21 (t, *J* = 7.5 Hz 2H), 2.25–2.28 (t, *J* = 6.9 Hz, 4H), 3.39–3.51 (m, 4H), 3.55–3.69 (m, 8H), 3.78 (s, 3H), 4.26–4.27 (d, *J* = 8.7 Hz, 1H), 5.12 (s, 2H), 5.23–5.26 (m, 1H), 5.31–5.41 (m, 4H), 7.39–7.41 (d, *J* = 8.5 Hz, 1H), 7.55–7.57 (d, *J* = 7.7 Hz, 1H), 7.84 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.12, 19.19, 19.22, 20.51, 20.55, 20.59, 22.69, 25.68, 28.33, 28.38, 28.80, 28.88, 28.95, 29.11, 29.20, 29.25, 29.35, 29.49, 29.62, 29.63, 29.65, 31.93, 36.71, 39.13, 40.96, 53.06, 64.75, 65.26, 65.34, 68.76, 69.92, 70.02, 70.23, 70.30, 71.14, 72.59, 77.26, 77.47, 77.65, 99.77, 120.11, 124.51, 133.19, 133.29, 141.27, 148.70, 155.97, 166.72, 169.25, 169.32, 170.01, 173.25. ESI-MS *m/z*: calculated for $C_{52}H_{77}N_3O_{17}$, 1015.5, found $[M+H]^+$, 1016.3 and $[M+Na]^+$, 1038.5.

Synthesis of 10: 28 μL of 30% sodium methylate in methanol was added to a solution of **9** (200 mg, 0.20 mmol) in anhydrous methanol (5 mL) at 0 °C. This solution was stirred at room temperature for 2 h. The reaction system was then quenched by adding 5 μL of acetic acid and concentrated under vacuum. The crude residue was purified by column chromatography (silica, methylene chloride:methanol, 9:1, v/v) to yield **10** as a colorless wax (115 mg, 64.6%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.88–0.91 (t, *J* = 6.8 Hz, 3H), 1.22–1.43 (m, 26H), 1.47–1.56 (m, 4H), 1.57–1.66 (m, 2H), 2.16–2.19 (t, *J* = 7.6 Hz, 2H), 2.23–2.27 (t, *J* = 6.9 Hz, 4H), 3.39–3.46 (m, 4H), 3.53–3.67 (m, 8H), 3.72–3.76 (t, *J* = 6.3 Hz, 2H), 3.83 (s, 3H), 3.86–3.90 (t, *J* = 8.5 Hz, 1H),

4.07–4.09 (d, J =9.5 Hz, 1H), 5.00–5.02 (d, J = 6.7 Hz, 1H), 5.08 (s, 2H), 5.72–5.75 (t, J = 5.6 Hz, 1H), 6.16–6.19 (t, J = 5.1 Hz, 1H), 7.29–7.31 (d, J = 9.0 Hz, 1H), 7.54 (d, J = 8.7 Hz, 1H), 7.83 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 14.16, 19.20, 19.22, 22.71, 25.69, 28.33, 28.38, 28.82, 28.89, 28.97, 29.12, 29.23, 29.26, 29.37, 29.51, 29.64, 29.65, 29.67, 31.94, 36.66, 39.10, 40.89, 53.01, 64.81, 65.22, 65.30, 69.95, 70.13, 70.96, 72.74, 74.69, 75.06, 77.26, 77.50, 77.67, 102.03, 118.44, 125.03, 132.11, 134.14, 140.10, 149.76, 156.15, 169.01, 173.64. ESI-MS *m/z*: calculated for $C_{46}H_{71}N_3O_{14}$, 889.5, found $[M+H]^+$, 890.2 and $[M+Na]^+$, 912.5.

Synthesis of PCDA-GlcA: Compound **10** (140 mg, 0.16 mmol) was dissolved in acetone (7 mL). The resulting solution was cooled to 0 °C, after which a solution of 1 mLNaOH (1 M) was added to it. After 10 min of stirring, the mixture was neutralized with 1 M HCl, concentrated under vacuum, and purified by column chromatography (silica, methylene chloride:methanol:water, 60:35:5, v/v/v) to obtain the desired diacetylene monomer PCDA-GlcA as a light yellow solid (100 mg, 72.5%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.84–0.88 (t,J = 6.8 Hz, 3H), 1.16–1.35 (m, 26H), 1.41–1.47 (m, 4H), 1.70 (s, 2H), 2.03–2.07 (t, J = 7.4 Hz, 2H), 2.25–2.29 (t, J = 6.8 Hz, 4H), 3.13–3.18 (m, 4H), 3.20–3.22 (m, 1H), 3.24–3.32 (m, 3H), 3.37–3.43 (m, 5H), 3.49 (s, 4H), 3.52 (s, 1H), 3.55 (s, 1H), 5.02 (s, 2H), 5.09–5.10 (d, J = 7.4 Hz, 1H), 7.34–7.38 (m, 1H), 7.42–7.44 (d, J = 8.8 Hz, 1H), 7.59–7.61 (d, J = 7.0 Hz, 1H), 7.84–7.88 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.32, 22.54, 24.14, 25.68, 28.08, 28.14, 28.54, 28.61, 28.79, 28.98, 29.07, 29.14, 29.27, 29.34, 29.43, 31.74, 35.76, 38.90, 40.58, 64.33, 65.75, 65.79, 69.44, 69.89, 69.90, 72.11, 73.33, 74.47, 76.83, 78.28, 78.32, 100.62, 117.67, 124.30, 131.29, 134.07, 140.02, 149.64, 156.57, 172.71, 173.36. ESI-MS *m/z*: calculated for $C_{45}H_{69}N_3O_{14}$, 875.5, found $[M-H]^+$, 874.3 and $[M+Na]^+$, 898.3.

3. Preparation of PDA liposomes

The PDA liposomes used in the current study were prepared according to a probe sonication method described in previous studies.[S4] In Brief, a mixture of PCDA and various mole amounts (0%–40%) of PCDA-GlcA was dissolved in 1 mL chloroform. The organic solution was then evaporated in vacuo and an appropriate amount of ultrapurified water was subsequently added to yield a total lipid concentration of 1 mM. The resulting solution was sonicated for 20 min at approximately 80 °C to afford a clear or translucent solution. The formed liposome solution was stored at 4 °C overnight and then warmed to ambient temperatures. The solution was polymerized for 15 min under a 254 nm lamp to obtain a deep-blue solution. The obtained solution can be stored at 4 °C for at least two weeks without the formation of precipitate.

4. Absorption and emission spectra of the liposome solution for enzymatic assay

UV-vis absorption spectra were recorded on a Shimadzu UV1800 spectrometer (Shimadzu, Kyoto, Japan). Fluorescence emission spectra were obtained on a Shimadzu RF-5301PC fluorescence spectrometer (Shimadzu). *β*-GUS was dissolved in phosphate-buffered saline (PBS, 50 mM, pH 7.4) containing 0.1% (w/v) bovine serum albumin (BSA) to obtain 1 mg/mL *β*-GUS stock solutions. For enzymatic assays, an appropriate volume of enzyme stock solution was mixed with 600 μL of the liposomes (1 mM). Afterward, the mixture solution was diluted to 2.4 mL with 50 mM PBS (pH 7.4). The molecular weight of *β*-GUS is 68259 Da for monomer in our experiments.The enzymatic reaction was monitored through UV-vis and fluorescent spectraobtainedat different incubating time intervals. The fluorescence emission spectra were obtained under excitation at 520 nm. Both excitation and emission slit widths were 5 nm.

5. Color responses (CR, %) and fluorescence intensity for monitoring enzyme hydrolysis

To quantify the color change induced by the enzyme and to monitor the process of the enzymatic hydrolysis, the colorimetric response (CR, %) was employed to determine the extent of color transition at different time points measured. The formula was defined as follows:

$$CR = [PB_0 - PB_1]/PB_0 \times 100\%$$

where $PB = A_{645}/[A_{645} + A_{545}]$. PB_0 is the ratio of the absorbance at 645 nm to that at 545 nm in the absence of the enzyme, while PB_1 is the ratio of the absorbance at 645 nm to that at 545 nm when incubated with β-GUS.

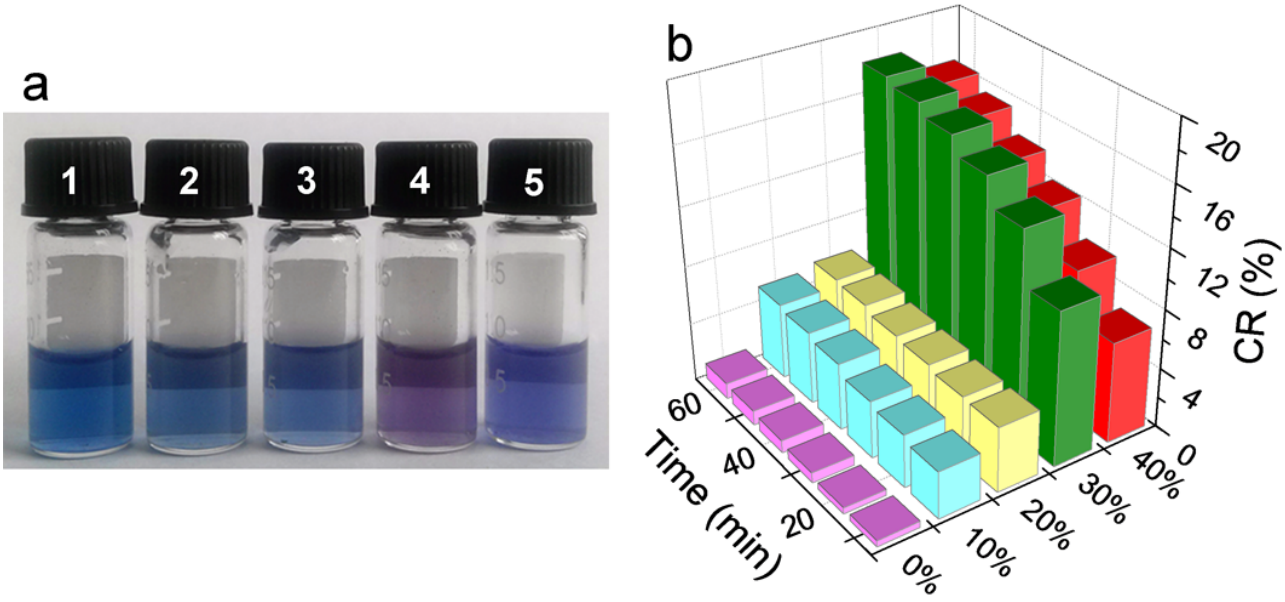


Figure S1. (a) Color changes of PDA liposomes (250 μM) containing different molar percents of PCDA-GlcA (1: 0%, 2: 10%, 3: 20%, 4: 30%, 5: 40% of PCDA-GlcA) and PCDA after incubation with *β*-GUS (1.2 μM) for 60 min in PBS (50 mM, pH 7.4) at 35 °C. (b) Corresponding colorimetric responses (CR, %) as a function of incubation time. Liposomes obtained from 30% PCDA-GlcA and 70% PCDA show the most rapid and obvious color changes after incubation with *β*-GUS. Increasing the PCDA-GlcA to 40% in liposomes slowed the color change. In addition, a clear color change with a CR value of ~19% and a large fluorescence enhancement was observed only after prolonged incubation. This finding may be ascribed to the increasing PCDA-GlcA concentration, which causes the bulky conjugation of enzyme substrates on the liposome surface and thus decreases the enzymatic reaction rate on this surface.[S5] Therefore, the molar percentof 30% PCDA-GlcA and 70% PCDA were used to prepare the PDA liposomes for enzymatic assays.

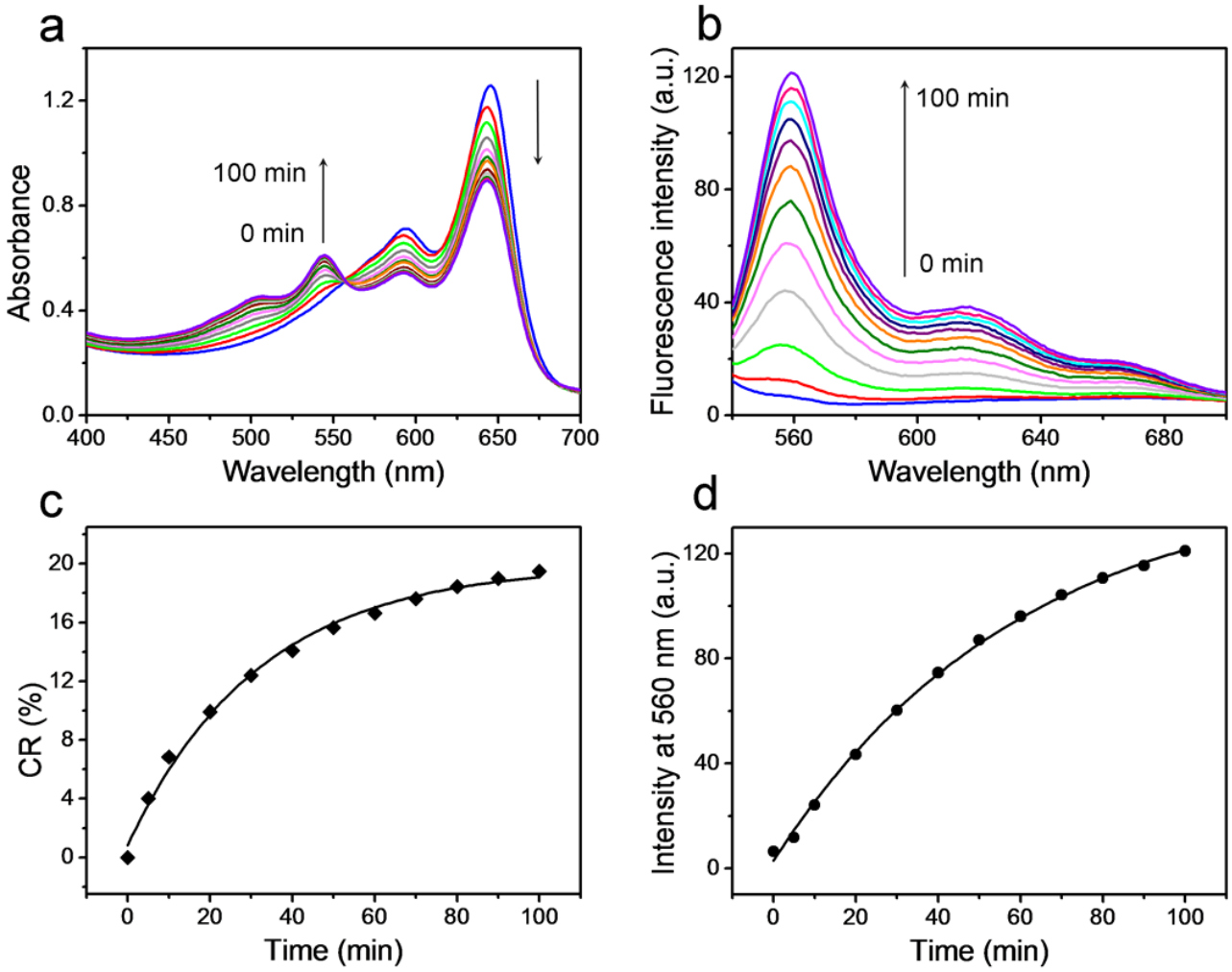


Figure S2. (a) UV-vis absorbance and (b) fluorescence emission spectra of PDA liposomes (250 μM) prepared from 40% PCDA-GlcA and 60% PCDA (molar percent) as a function of reaction time upon incubation with *β*-GUS (1.2 μM) for 100 min in PBS (50 mM, pH 7.4) at 35 °C. (c) Colorimetric responses (CR, %) and (d) fluorescence intensity (at 560 nm) of the abovementioned PDA liposomes versus reaction time upon incubation with *β*-GUS.

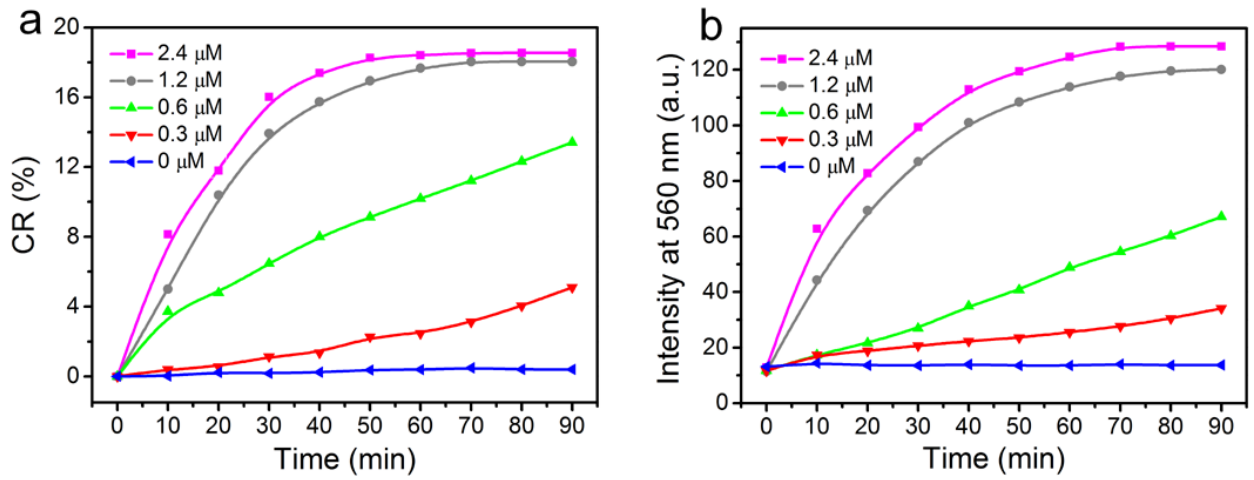


Figure S3. (a) Plots of colorimetric responses (CR, %) and (b) fluorescence intensity (at 560 nm) of PDA liposomes prepared from 30% PCDA-GlcA and 70% PCDA versus reaction time in various *β*-GUS concentrations (0, 0.3, 0.6, 1.2, and 2.4 μM).

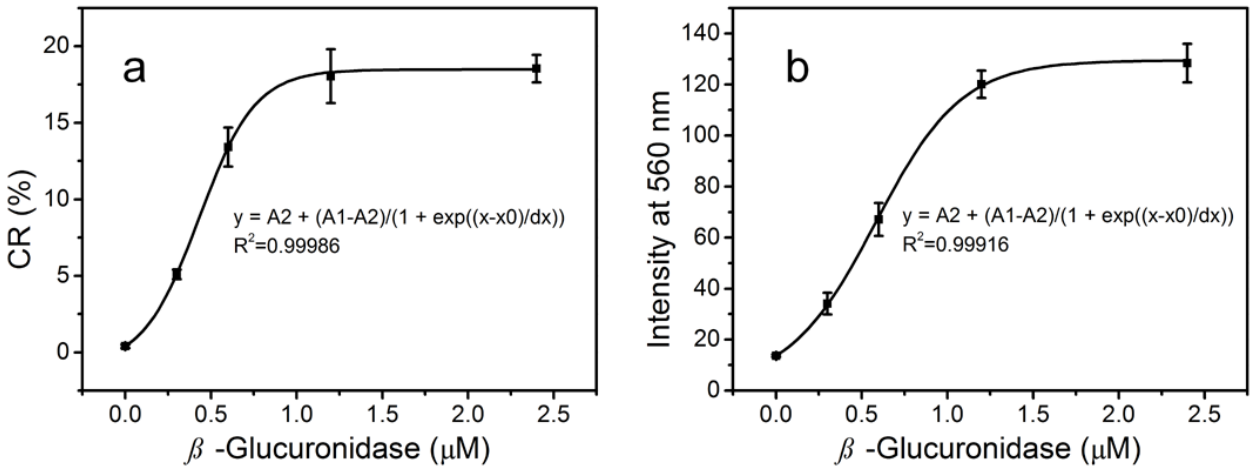


Figure S4. (a) Colorimetric response (CR, %) and (b) fluorescence intensity (at 560 nm) change in the presence of different concentrations of *β*-GUS (0, 0.3, 0.6, 1.2 and 2.4 μM) after 90 min incubation.

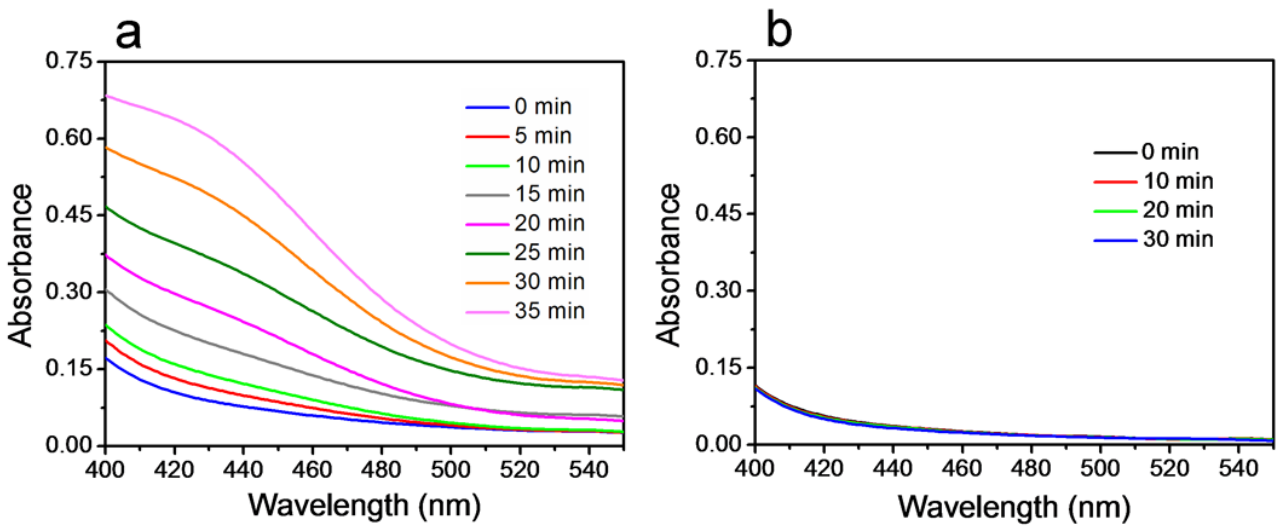


Figure S5. UV-vis absorbance spectra of PCDA-GlcA solution (250 μM) versus reaction time (a) in the presence and (b) in the absence of *β*-GUS (1.2 μM) in PBS (50 mM, pH 7.4) at 35 °C.

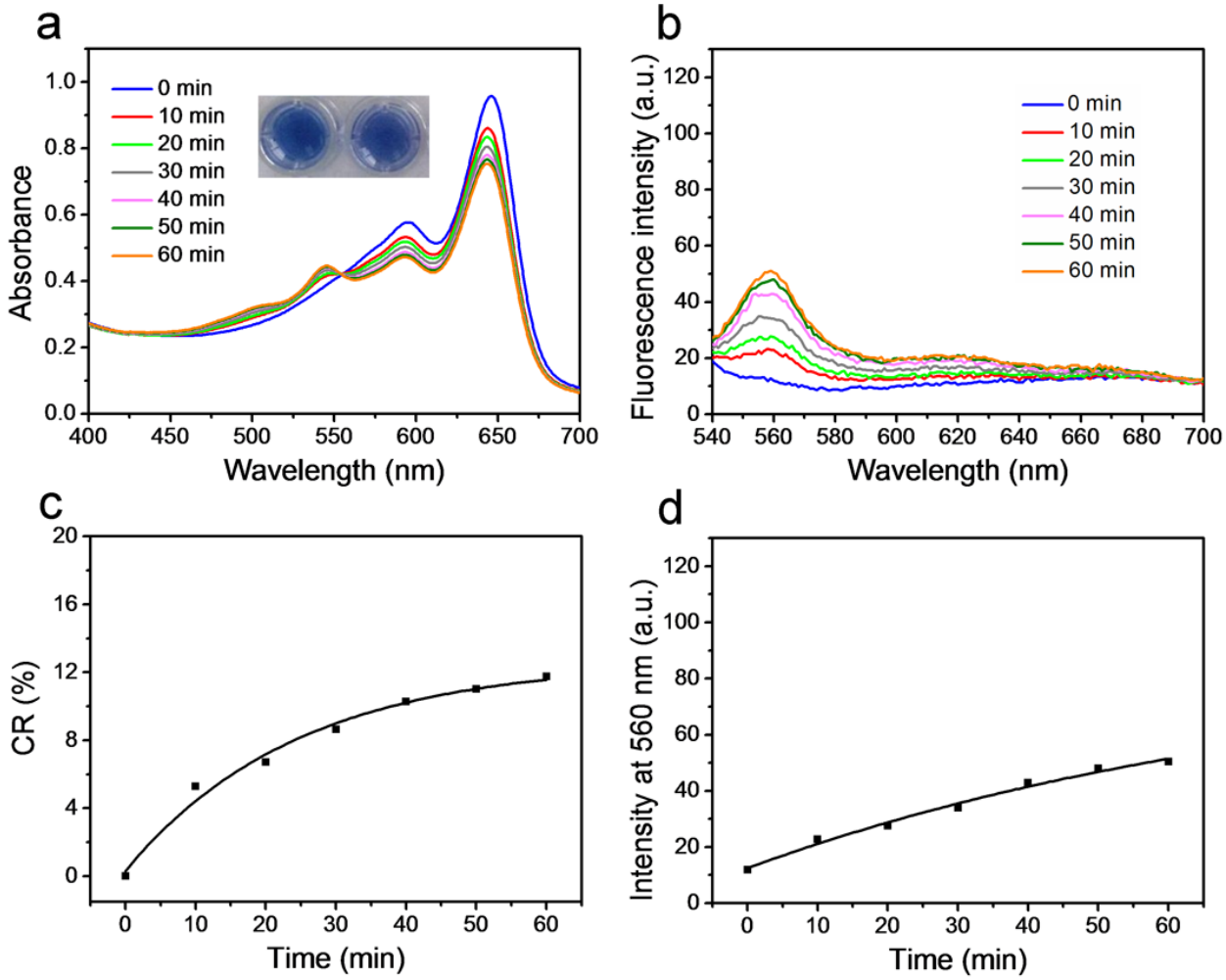


Figure S6. (a) UV-vis absorbance and (b) fluorescence emission spectra of PDA liposomes (250 μM) prepared from 30% PCDA-GlcA and 70% compound **7**(molar percent) versus reaction time upon incubation with *β*-GUS (1.2 μM) in PBS (50 mM, pH 7.4) at 35 °C. Inset: (Left) PDA liposomes in the absence of the enzyme, (right) PDA liposomes in the presence of the enzyme. (c) Colorimetric responses (CR, %) and (d) fluorescence intensity (at 560 nm) of the abovementioned PDA liposomes versus reaction time upon incubation with *β*-GUS.

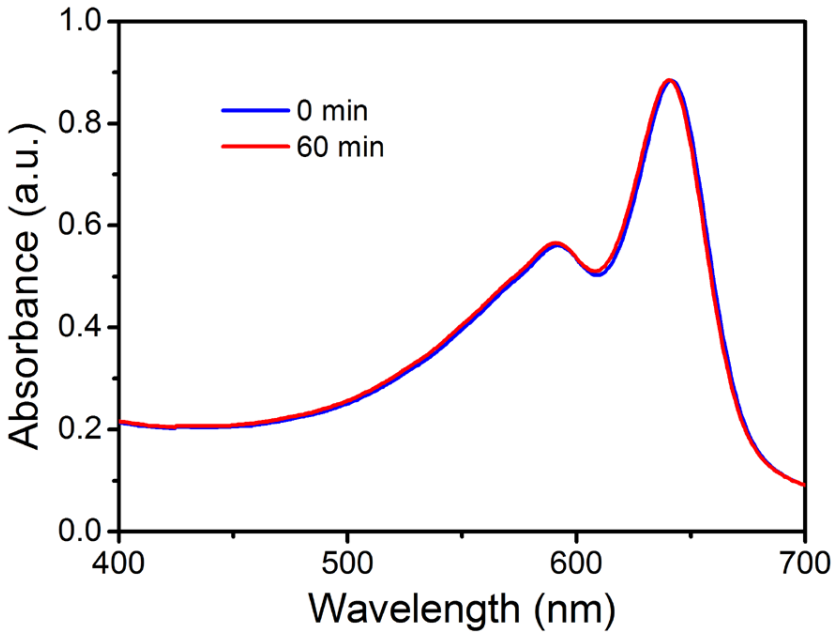


Figure S7. UV-vis absorbance spectra of liposome solution prepared with pure compound **7** (250 μM) in the presence of *β*-GUS (1.2 μM) in PBS (50 mM, pH 7.4) at 35 °C.

6. Limit of Detection

The limit of detection (LOD) of this method using the fluorescence enhancement is estimated using Equation (1) at S/N=3, in which S_0 is the standard deviation of the background and S is the sensitivity.[S6, S7]

$$\text{LOD} = 3 \times \frac{S_0}{S} \qquad (1)$$

7. Electrospray ionization mass spectroscopic (ESI-MS) analysis

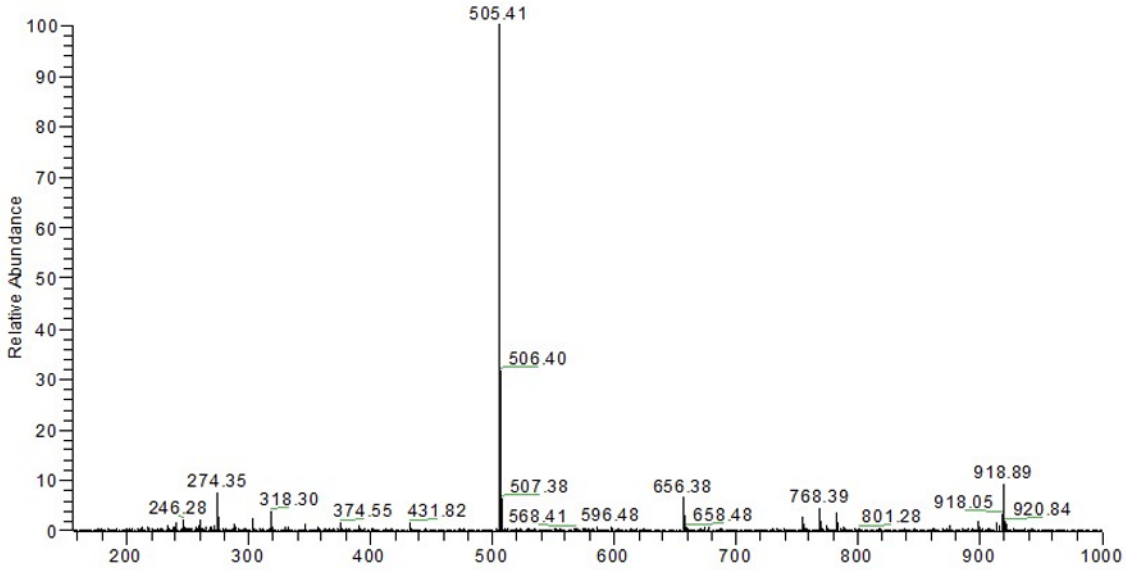


Figure S8. ESI mass spectrum of the solution extracted with ethyl acetate from the PCDA-GlcA solution (250 μM) after incubation with *β*-GUS (1.2 μM) in PBS (50 mM, pH 7.4) at 35 °C. Calculated mass values for $[M+H]^+$ and $[M+2H]^{2+}$ of PCDA-GlcA residues are 505.43 and 506.44, respectively.

8. Dynamic light scattering (DLS) and zeta potential (ζ) measurements for liposome size distribution determination

The average size and size distribution of the PDA liposomes as well as the zeta potentials (ζ) before and after *β*-GUS treatment were determined using a Zetasizer Nano ZS (Malvern Instruments, Malvern, U.K.) instrument. Samples were prepared in test tubes, and measurements were performed at room temperature.

9. Scanning electron microscopy (SEM) for liposome morphology determination

SEM images of liposome samples with and without enzyme treatment were obtained using a field emission scanning electron microscope (FE-SEM; S-4800, Hitachi, Tokyo, Japan). Samples

were freshly prepared and dropped on small silicon wafers. The silicon wafers were allowed to dry at room temperature overnight. Samples were then coated with gold nanoparticles for 10 min before FE-SEM examination at an accelerating voltage of 10 kV.

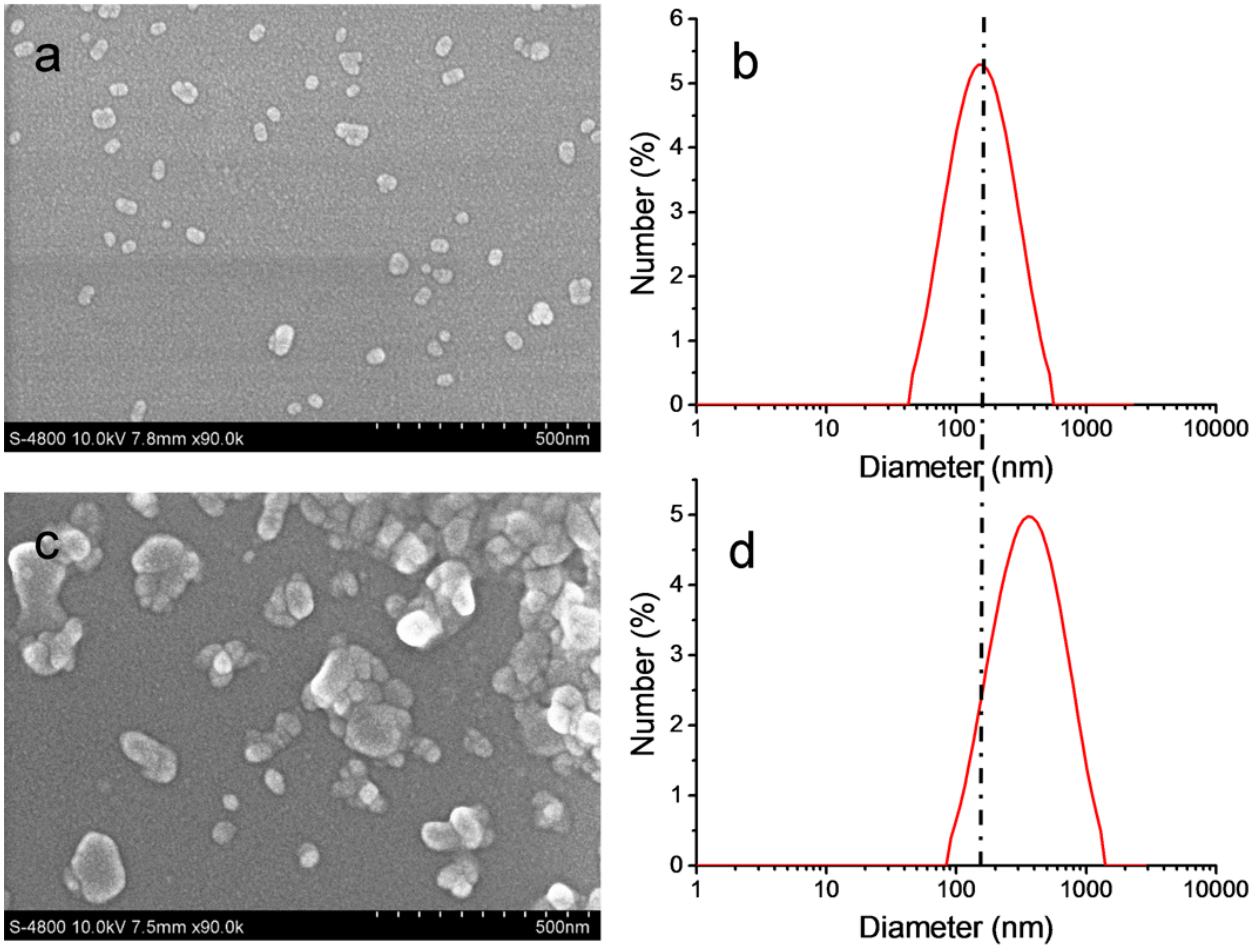


Figure S9. SEM images (a, c) and DLS size distribution (b, d) of PDA liposomes prepared from 30% PCDA-GlcA and 70% PCDA (a, b) before and (c, d) after *β*-GUS treatment.

10. Inhibition assays of *β*-GUS activity

The inhibition assay for the enzyme was performed in a standard 96-well microtiter plate. About 50 μL of liposome solution (1 mM), 20 μL of enzyme stock solution and 30 μL of inhibitor were added to 100 μL of PBS solution (50 mM, pH 7.4). Mixed solutions containing

1.2 μM β-GUS, different inhibitor concentrations (1, 5, 10, 30, 80, and 200 μM D-glucaric acid-1,4-lactone), and 250 μM liposome solution were incubated for 60 min at 35 °C. The absorbance of each sample at 645 nm and 545 nm was directly obtained using a microplate reader (Model 680, BIO-RAD, Hercules, CA, USA). Fluorescence images were acquired using a fluorescence microscope (Olympus, CKX41) equipped with a CCD camera (Olympus, DP72). The fluorescence intensity of each sample was analyzed using Software Image-Pro Plus 6.0 (Media Cyternetics).

11. Inhibition efficiency[S7, S8]

The inhibition efficiency (IE) of the inhibitor was estimated using two methods. The first approach utilized Equation (2), in which R_0 is the A_{545}/A_{645} ratio of the liposome solution in the absence of β-GUS and its inhibitor. $R_{(inhibitor)}$ and $R_{(no\ inhibitor)}$ are the A_{545}/A_{645} ratios of the assay solutions in the presence and absence of inhibitor, respectively, and A is the absorbance at either 645 nm (blue phase) or 545 nm (red phase) in the UV-vis spectrum.

$$\mathrm{IE} = \frac{R_{(inhibitor)} - R_{(no\ inhibitor)}}{R_0 - R_{(no\ inhibitor)}} \times 100\% \qquad (2)$$

Another method for IE estimation utilized Equation (3), in which F_0 is the fluorescence intensity of the liposome solution in the absence of β-GUS and its inhibitor. $F_{(inhibitor)}$ and $F_{(no\ inhibitor)}$ are the fluorescence intensity of the assay solutions in the presence and absence of inhibitor, respectively.

$$\mathrm{IE} = \frac{F_{(inhibitor)} - F_{(no\ inhibitor)}}{F_0 - F_{(no\ inhibitor)}} \times 100\% \qquad (3)$$

The half maximal inhibitory concentration (IC_{50}), which indicates the inhibitor concentration necessary to inhibit enzyme activity by half, was employed to determine the inhibition ability of an inhibitor. IC_{50} values were obtained from an inhibition efficiency curve.

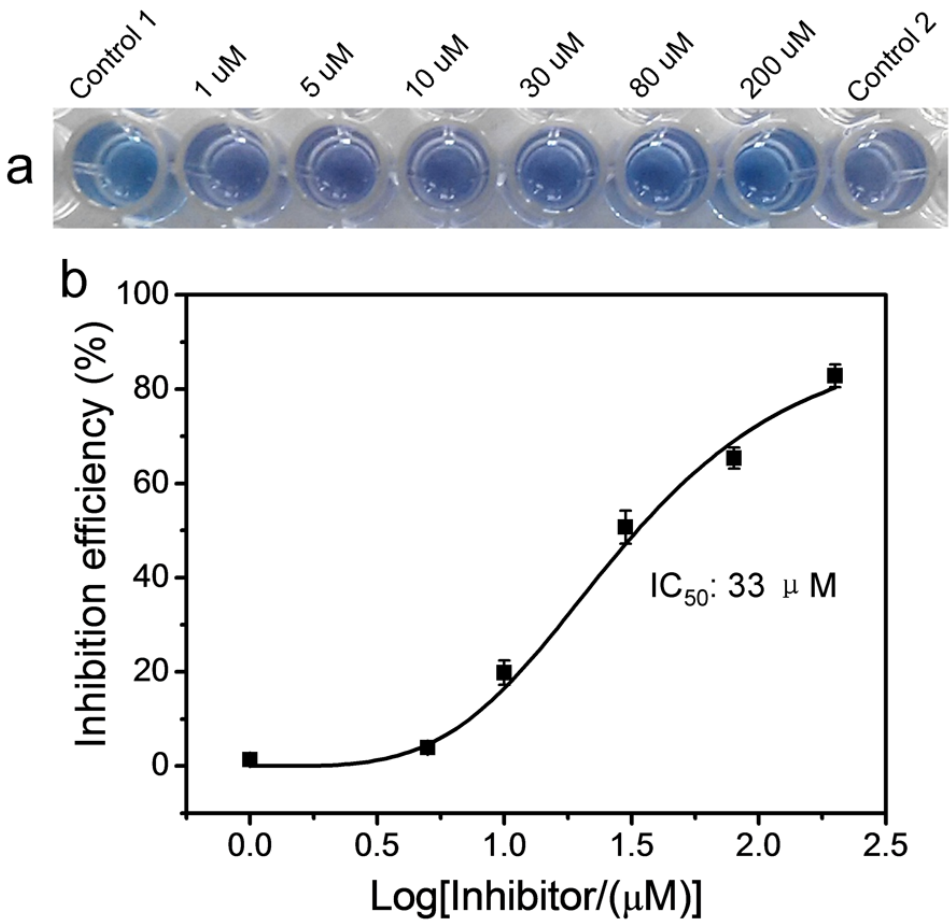


Figure S10. (a) Color changes in PDA liposomes prepared from 30% PCDA-GlcA and 70% PCDA after incubation with *β*-GUS (1.2 μM) in different concentrations of the inhibitor (concentrations: 1, 5, 10, 30, 80, and 100 μM) for 1 h in PBS (50 mM, pH 7.4) at 35 °C. Control groups are set as **control 1** (in the absence of the enzyme and the inhibitor) and **control 2** (in the absence of the inhibitor). (b) Plot of D-glucaric acid-1,4-lactone inhibition efficiency for *β*-GUS versus the inhibitor concentration determined by absorbance measurements.

12. ^{1}H and ^{13}C NMR spectra of Compounds 9, 10, and PCDA-GlcA

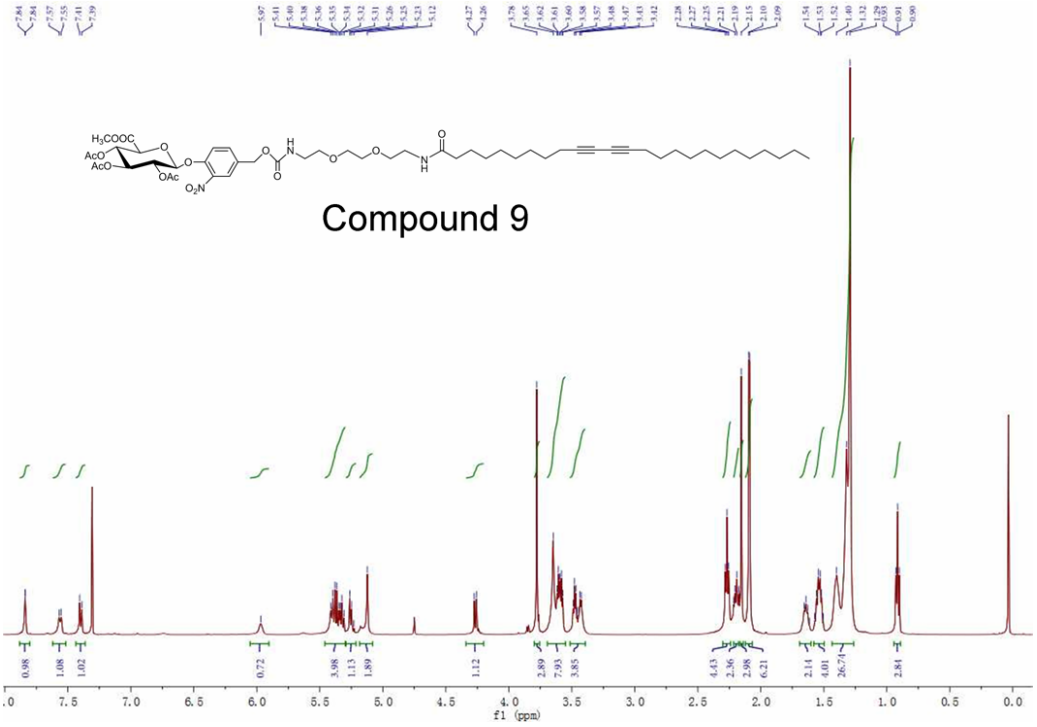


Figure S11. ^{1}H NMR spectrum of Compound **9**

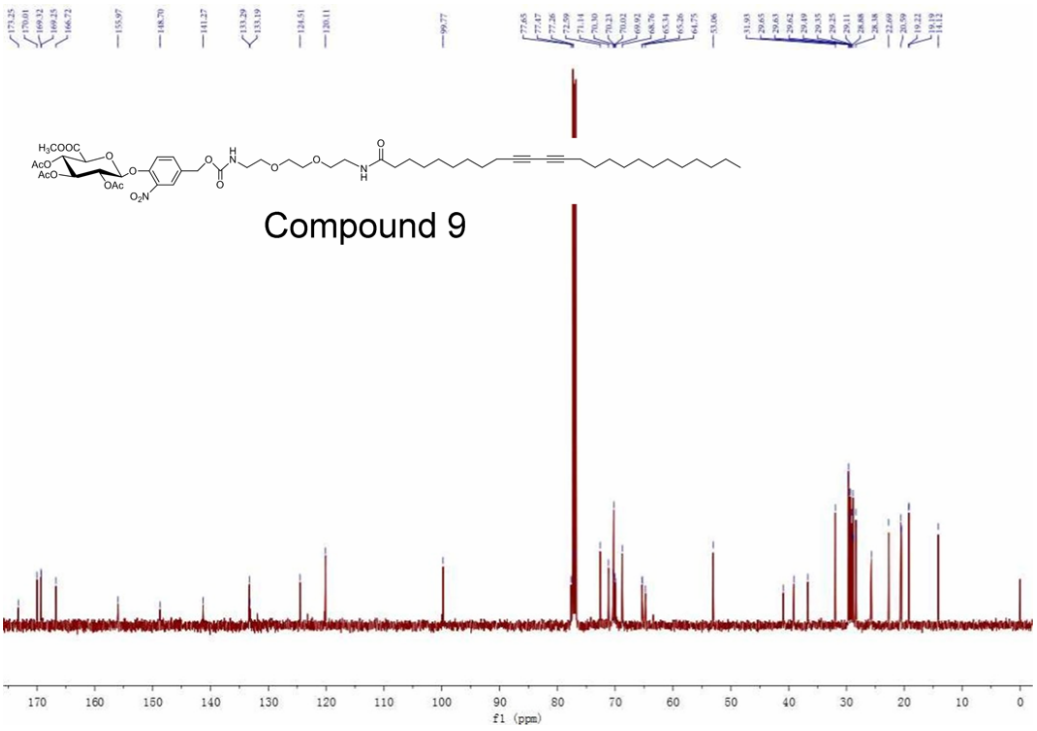


Figure S12. ^{13}C NMR spectrum of Compound **9**

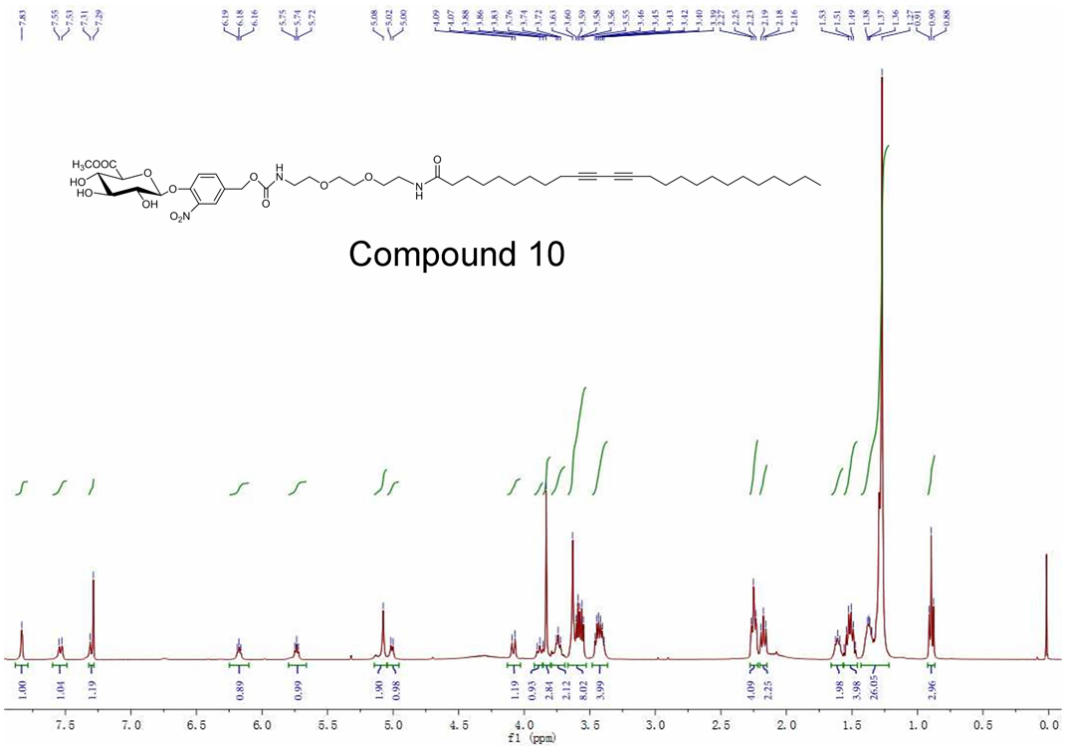


Figure S13. ^{1}H NMR spectrum of Compound **10**

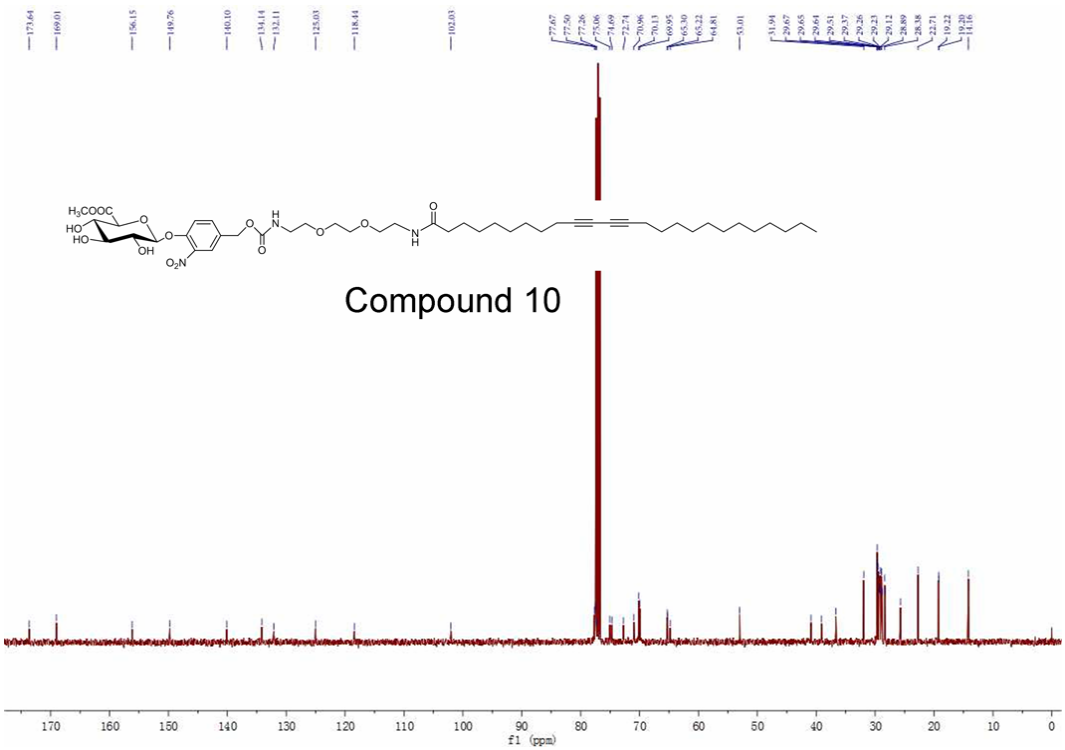


Figure S14. ^{13}C NMR spectrum of Compound **10**

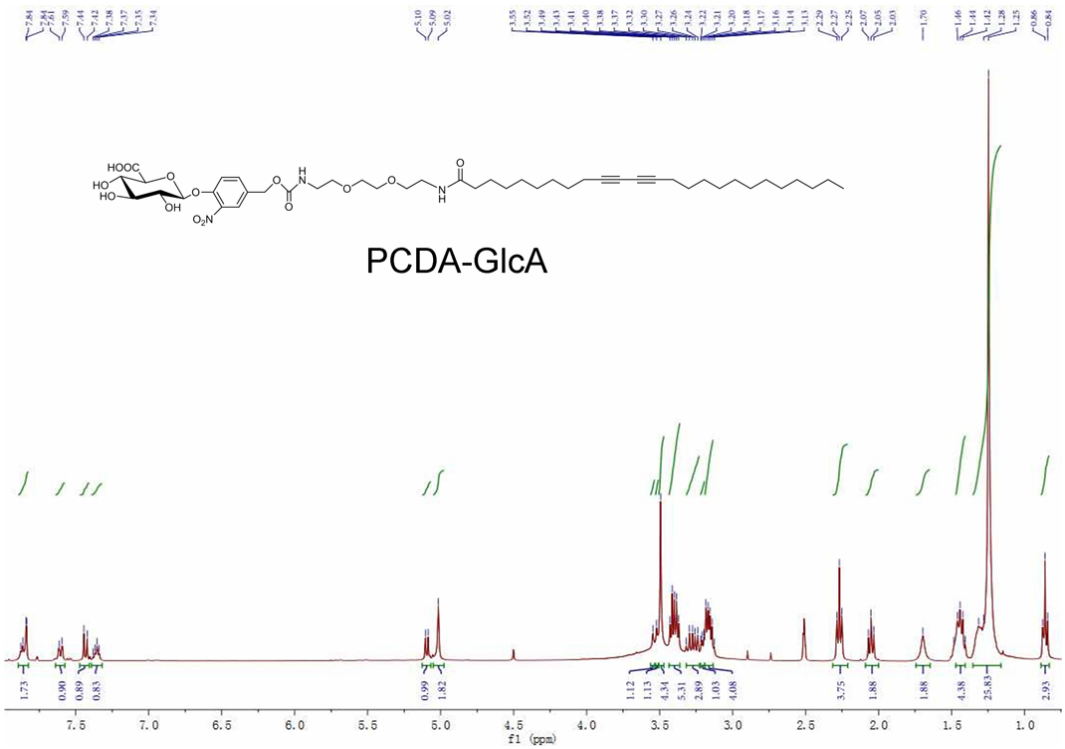


Figure S15. ^{1}H NMR spectrum of PCDA-GlcA

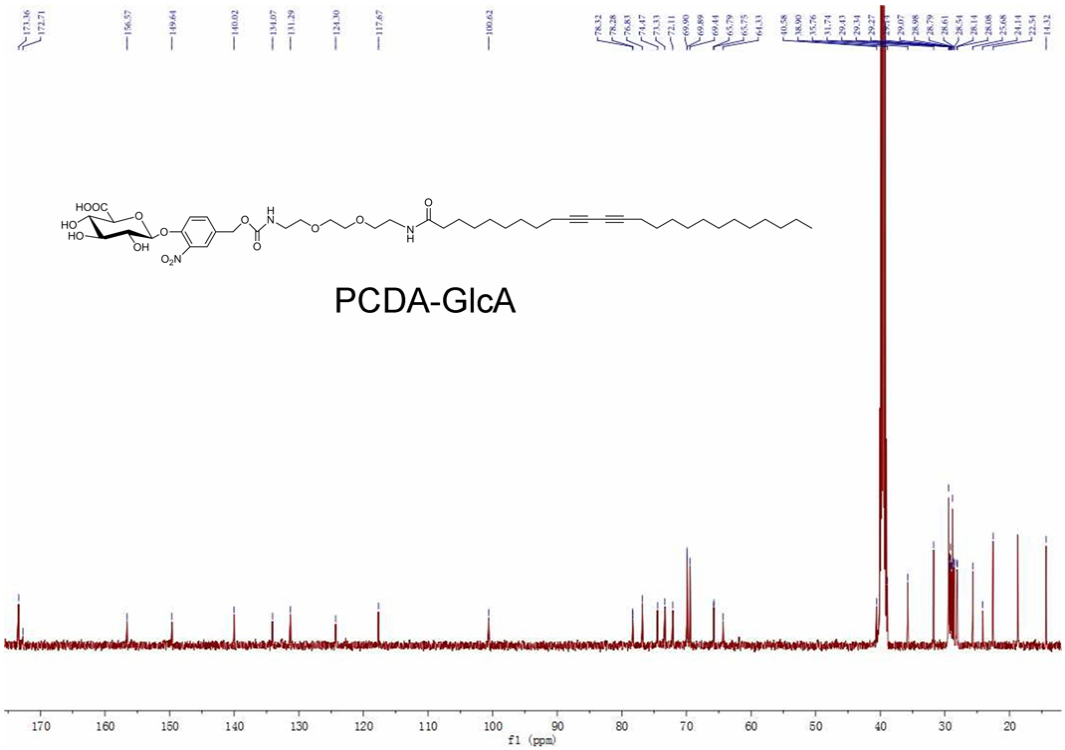


Figure S16. ^{13}C NMR spectrum of PCDA-GlcA

13. References for supporting information

(S1) G. N. Bollenback, J. W. Long, D. G. Benjiamin, J. A. Lindquist, *J. Am. Chem. Soc.*, 1955,**77**, 3310–3315.

(S2) J. A. Duimstra, F. J. Femia, T. J. Meade, *J. Am. Chem. Soc.*, 2005, **127***,* 12847–12855.

(S3) J. Lee, H. J. Kim, J. Kim, *J. Am. Chem. Soc.,*2008, **130**, 5010–5011.

(S4) A. Reichert, J. O. Nagy, W. Spevak, D. Charych, *J. Am. Chem. Soc.*, 1995, **117***,* 829–830.

(S5) Z. Zeng, S. Mizukami, K. Kikuchi, *Anal. Chem.,*2012, **84***,* 9089–9095.

(S6) G. Zhou, F. Wang, H. Wang, S. Kambam, X. Chen, *Macromol. Rapid Commun.,* 2013, **34**, 944–948.

(S7) F. Feng, Y. Tang, S.Wang, Y. Li, D. Zhu, *Angew. Chem. Int. Ed.,* 2007, **46***,* 7882–7886.

(S8) G. Zhou, F. Wang, H. Wang, S. Kambam, X. Chen, J. Yoon, *ACS Appl. Mater. Interfaces,* 2013, **5**, 3275–3280.