Electronic Supplementary Information (ESI)

An enzyme-activatable probe liberating AIEgen: on-site sensing and long-term tracking of β-galactosidase in ovarian cancer cells

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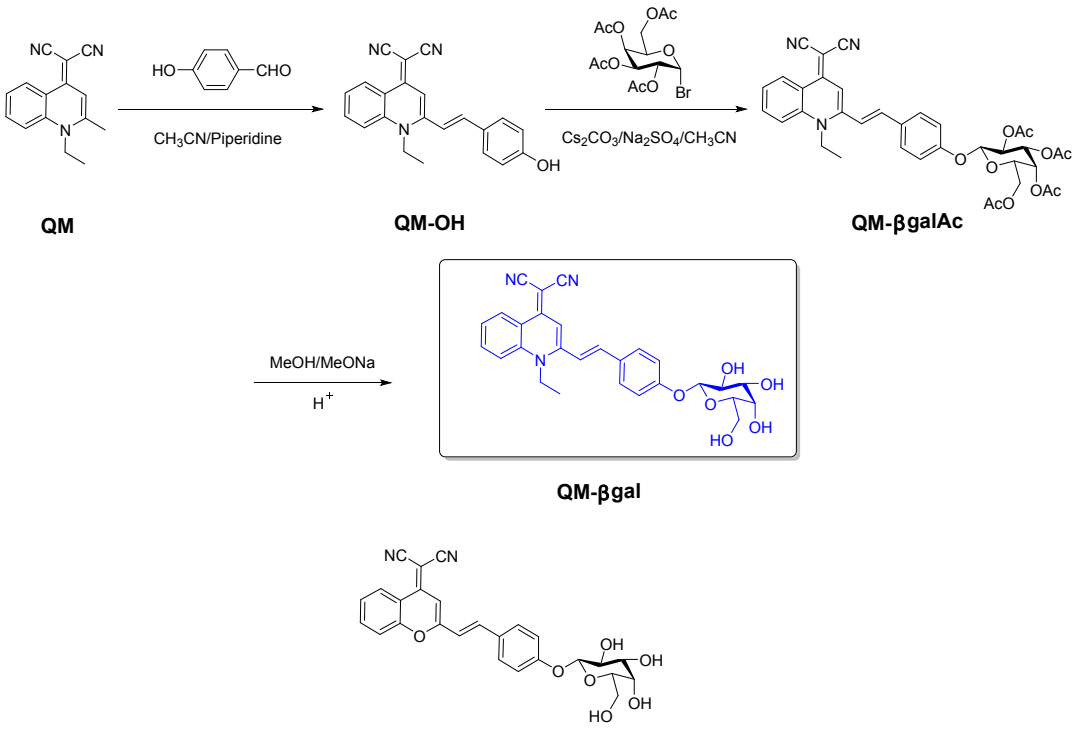
1. Experimental section

Materials and instruments

All solvents and chemicals, unless special stated, were purchased commercially in analytical grade and used without further purification. β-Galactosidase (β-gal) was supplied by *J&K* Scientific Ltd (Beijing, China). ^{1}H and ^{13}C NMR spectra in DMSO-d_6 were obtained with a Bruker AvanceIII 400 MHz NMR spectrometer using TMS as an internal standard. High resolution mass spectrometry (HRMS) spectra were measured with a Waters LCT Premier XE spectrometer. UV-Vis absorption and fluorescence spectra were recorded on an Agilent Cary 60 spectrophotometer and Varian Cary Eclipse fluorescence spectrophotometer, respectively (10 × 10 mm quartz cuvette). Dynamic light scatting (DLS) experiments were conducted with Zetasizer Nano-ZS (Malvern Instruments, Worcestershire, UK), and scanning electron microscope (SEM) images were operated on a JEOL JSM-6360 scanning electron microscope. HPLC chromatograms were carried out using an Agilent 1100 series. Confocal fluorescence images were performed on confocal laser scanning microscope (CLSM, Nikon A1R).

Synthesis of QM-*β*gal



Scheme S1. Synthetic route of QM-βgal

Synthesis of QM-OH

QM (1.0 g, 4.25 mmol) and 4-hydroxybenzaldehyde (623 mg, 5.11 mmol) were dissolved in acetonitrile (30 mL) with piperidine (1.0 mL) under argon protection at room temperature. The mixture then was refluxed for 10 h. The solvent was removed by filtration, and the crude product was purified by recrystallization to afford the desired product QM-OH (827 mg, 2.44 mmol): yield 57%. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ = 1.40 (t, J = 8.0 Hz, 3 H), 4.56 (q, J = 6.8 Hz, 2 H), 6.84 (d, J = 8.4 Hz, 2 H), 7.02 (s, 1 H), 7.29 (d, J = 16.0 Hz, 1 H), 7.36 (d, J = 15.6 Hz, 1 H), 7.61 (t, J = 7.6 Hz, 1 H), 7.67 (d, J = 8.4 Hz, 2 H), 7.92 (t, J = 7.6 Hz, 1 H), 8.08 (d, J = 8.8 Hz, 1 H), 8.92 (d, J = 8.4 Hz, 1 H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ = 13.63, 24.63, 26.28, 43.71, 46.24, 46.53, 106.42, 115.82, 116.58, 118.07, 120.60, 124.85, 125.09, 126.04, 130.04, 133.60, 137.84, 140.06, 149.68, 152.05, 159.80. High-resolution mass spectrometry (ESI negative ion mode for $[M - H]^-$): Calcd. for $C_{22}H_{16}N_3O$: 338.1293; found: 338.1299.

Synthesis of QM-*β*galAc

QM-OH (100 mg, 0.29 mmol) and tetra-O-acetyl-α-D-galactopyranosyl-1-bromide (200 mg, 0.48 mmol) were dissolved in acetonitrile (15 mL) with Cs_2CO_3 (479 mg, 1.47 mmol) and Na_2SO_4 (171.8 mg, 1.21 mmol) under argon protection at room temperature. The mixture then was stirred at room temperature for 4 h. After filtration, the solvent was removed under reduced pressure. The residue was taken up in sat.NH_4Cl, and extracted with CH_2Cl_2. Next, the solution was dried over anhydrous Na_2SO_4 and the solvent was removed by evaporation again. Finally, the crude product was purified by silica gel chromatography with dichloromethane/methanol (100:1) to afford the desired product QM-βgalAc (93 mg, 0.14 mmol) as yellow solid: yield = 48%. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ = 1.46 (t, J = 6.8 Hz, 3 H), 2.02 (s, 3 H), 2.09 (s, 3 H), 2.12 (s, 3 H), 2.22 (s, 3 H), 4.17 (d, J = 6.0 Hz, 2 H), 4.54 (t, J = 6.2 Hz, 1 H), 4.64 (d, J = 6.8 Hz, 2 H), 5.28-5.32 (m, 1 H), 5.35-5.36 (m, 1 H), 5.39-5.42 (m, 1 H), 5.65(d, J =7.6 Hz, 1 H), 7.08 (s, 1 H), 7.13 (d, J = 8.8 Hz, 2 H), 7.48 (d, J = 16 Hz, 1 H), 7.53 (d, J = 15.6 Hz, 1 H), 7.69 (t, J = 7.6 Hz, 1 H), 7.90 (d, J = 8.8 Hz, 2 H), 8.00 (t, J = 7.6 Hz, 1 H), 8.16 (d, J = 8.8 Hz, 1 H), 9.00 (d, J = 8.4 Hz, 1 H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ = 13.63, 20.32, 20.37, 20.43, 20.49, 43.83, 46.86, 61.29, 63.34, 67.19, 68.26, 70.10, 70.44, 97.25, 106.74, 116.51, 118.08, 119.41, 120.58, 124.93, 125.10, 129.76, 129.98, 133.68, 137.79, 138.93, 149.24, 152.23, 157.52, 169.22, 169.54, 169.84, 169.95. High-resolution mass spectrometry (ESI positive ion mode for $[M + Na]^+$): Calcd. for $C_{36}H_{35}N_3O_{10}Na$: 692.2220; found: 692.2217.

Synthesis of QM-*β*gal

QM-βgalAc (88 mg, 0.13 mmol) was added MeONa (70 mg, 1.3 mmol) in methanol (5 mL) and the mixture was stirred at room temperature for 3 h. Then the reaction mixture was neutralized with Amberlite IR-120 plus (H^+). After the Amberlite IR-120 plus (H^+) was filtered off, the solvent was removed by evaporation. Finally, the crude product was purified by silica gel chromatography with dichloromethane/methanol (40:1) to afford the desired product QM-βgal (41 mg, 0.08 mmol) as yellow solid: yield = 62%. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ = 1.41 (t, J = 6.4 Hz, 3 H), 3.44-3.63 (m, 4 H), 3.72 (s, 2 H), 4.57 (t, J = 6.8 Hz, 2 H), 4.68 (s, 2 H), 4.93 (d, J = 8.4 Hz, 2 H), 5.22 (d, J =4.4 Hz, 1 H), 7.03 (s, 1 H), 7.11 (d, J = 8.4 Hz, 2 H), 7.42 (s, 2 H), 7.62 (t, J = 8.0 Hz, 1 H), 7.79 (d, J = 8.4 Hz, 2 H), 7.93 (t, J = 8.0 Hz, 1 H), 8.10 (d, J = 8.8 Hz, 1 H), 8.94 (d, J = 8.4 Hz, 1 H). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ = 13.63, 13.92, 28.97, 31.11, 31.25, 43.82, 46.63, 60.32, 68.08, 70.21, 73.24, 75.55, 100.56, 106.67, 116.44, 118.09, 118.56, 120.59, 124.92, 125.09, 128.61, 129.62, 133.69, 137.81, 139.33, 149.41, 152.20, 158.76. High-resolution mass spectrometry (ESI positive ion mode for $[M + H]^+$): Calcd. for $C_{28}H_{28}N_3O_6$: 502.1978; found: 502.1976.

Cell experiment

Cell lines

Human embryonic kidney 293T cells was supplied by the Institute of Cell Biology (Shanghai, China). 293T cells were cultured at 37 ºC under a humidified 5% CO_2 atmosphere in DMEM (GIBCO/Invitrogen, Camarillo, CA, USA), which were supplemented with 10% fetal bovine serum (FBS, Biological Industry, Kibbutz Beit Haemek, Israel) and 1% penicillin-streptomycin (10,000 U mL^{-1} penicillin and 10 mg/ml streptomycin, Solarbio life science, Beijing, China).

Human ovarian adenocarcinoma cells (SKOV-3 cells) was supplied by the Institute of Cell Biology (Shanghai, China). SKOV-3 cells were cultured at 37 ºC under a humidified 5% CO_2 atmosphere in McCoy's 5A (GIBCO/Invitrogen, Camarillo, CA, USA), which were supplemented with 10% fetal bovine serum (FBS, Biological Industry, Kibbutz Beit Haemek, Israel) and 1% penicillin-streptomycin (10,000 U mL^{-1} penicillin and 10 mg/ml streptomycin, Solarbio life science, Beijing, China).

In vitro cytotoxicity assay

The cytotoxicity of QM-βgal or QM-OH in both cancer and normal cell lines was evaluated by a MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Briefly, cells were seeded into 96-

well plates at a density of 1×10^4 cells/well and were cultured at 37 ºC under a humidified 5% CO_2 atmosphere for 12 h. Then, the cells were exposed to the various concentrations (1, 2, 5, 10 μM) of QM-*β*gal or QM-OH, and for negative control group, 100.0 μL of culture medium were added. After incubation at 37 ºC under a humidified 5% CO_2 atmosphere for 24 h, MTT solution (5 mg/mL in PBS, 10 μL) was added to the media and incubated for another 4 h, and the absorbance at 490 nm was measured with a Multimode Plate Reader (BioTek, USA). The relative cell viability (%) was calculated by the following formula: viability (%) = mean absorbance value of the treatment group-blank/mean absorbance value of the control-blank × 100.

Cells imaging

Cells were seeded onto glass-bottom Petri dishes in culture medium (1.5 mL) and allowed to adhere for 12 h before imaging. Probe QM-βgal at a final concentration of 10 μM (containing 0.1% DMSO) were added into culture medium and incubated for different time at 37 °C under a humidified 5% CO2 atmosphere. Cells imaging was captured by using a confocal laser scanning microscope (CLSM, Nikon A1R system, Japan) with a 60× oil immersion objective lens. The fluorescence signals of cells incubated with probes were collected at 500−650 nm under excitation wavelength at 404 nm.

2. Hydrodynamic diameter of QM-OH aggregates

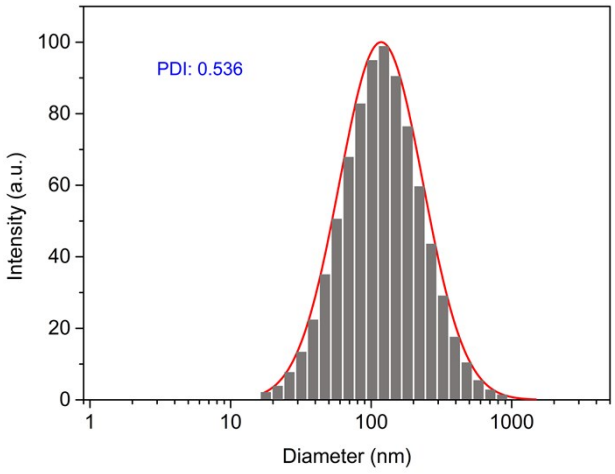


Fig. S1 Hydrodynamic diameter of QM-OH (10 μM) in a mixture of water/DMSO (v/v = 95/5) obtained from dynamic light scattering (DLS).

3. SEM image of QM-OH aggregates

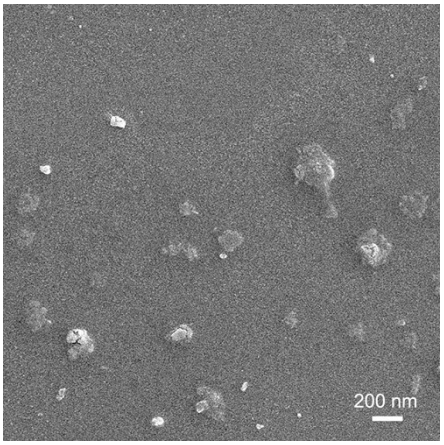


Fig. S2 SEM image of aggregates formed by QM-OH (10 μM) in a mixture of water/DMSO (v/v = 95/5).

4. The detection limit of QM-βgal

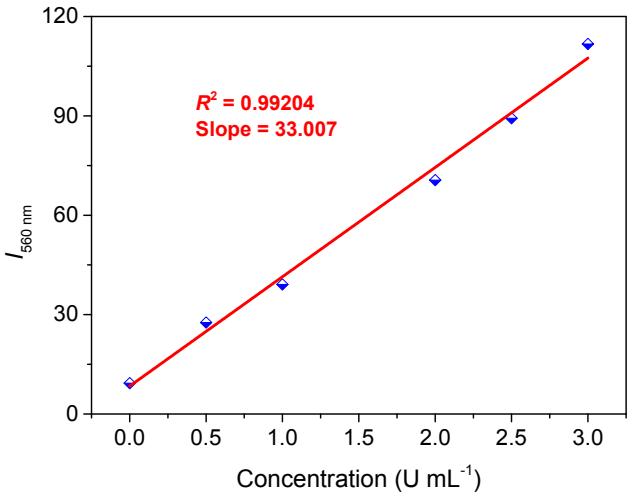


Fig. S3 A linear correlation between fluorescence intensity at 560 nm ($I_{560\ nm}$) and concentration of β-gal. Note: The detection limit was calculated to be 1.0×10^{-3} U mL^{-1} (3σ/slope).

5. Effects of ionic strength and culture medium

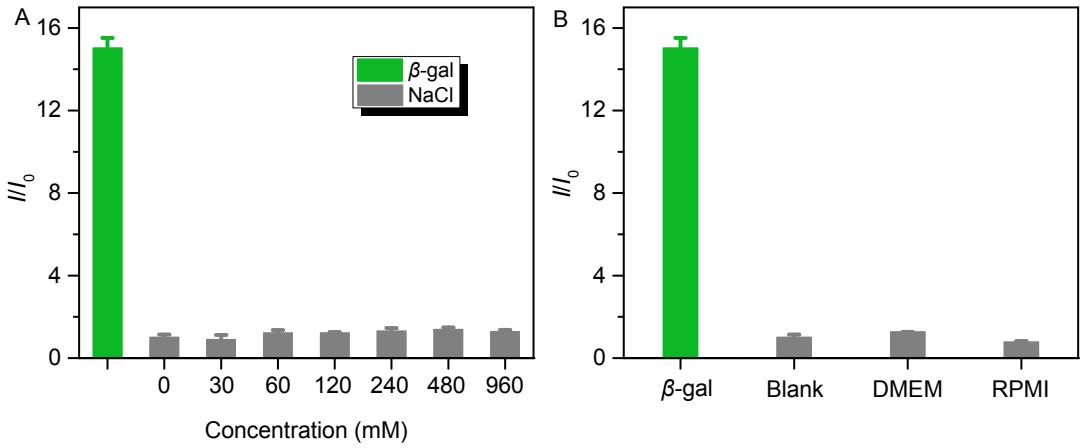


Fig. S4 Fluorescence response of QM-βgal (10 μM) to (A) varied concentrations of NaCl (0-960 mM) and (B) cell culture medium (DMEM and RPMI) for 30 min, λ_{ex} = 434 nm. I/I_0 represents the fluorescence intensity ratio at 560 nm, and I_0 is the fluorescence intensity of free QM-βgal.

6. The photostability of QM-βgal QM-OH

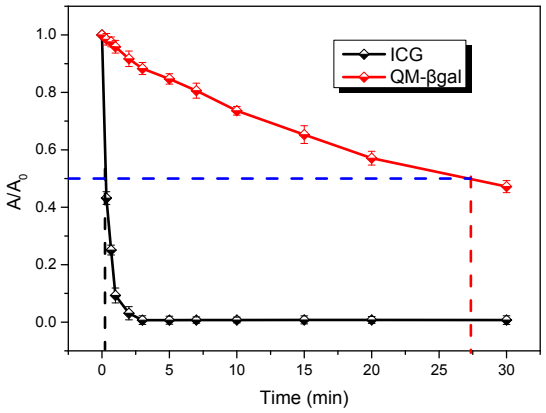


Fig. S5 Time-dependent absorption of ICG (10 μM, monitored at 780 nm), QM-βgal (10 μM, monitored at 434 nm) in a mixture of water/DMSO (v/v = 95/5) under continuous illumination.

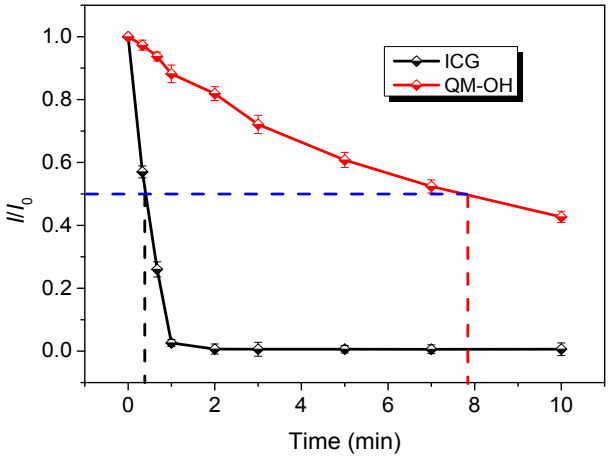


Fig. S6 Time-dependent fluorescence intensity of ICG (10 μM, monitored at 812 nm, and λ_{ex} = 780 nm), QM-OH (10 μM, monitored at 560 nm, and λ_{ex} = 434 nm) in a mixture of water/DMSO (v/v = 95/5) under continuous illumination.

7. *In vitro* cytotoxicity of QM-βgal or QM-OH

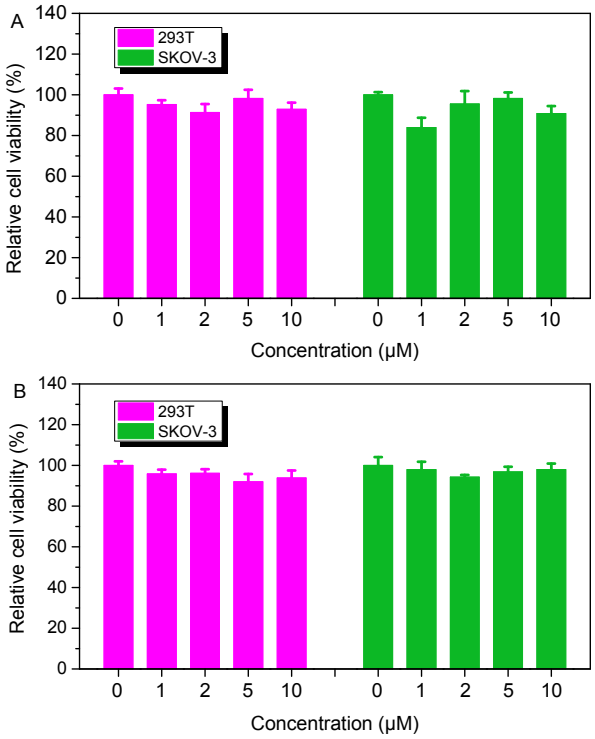


Fig. S7 Relative cell viability of 293T or SKOV-3 cells in vitro after incubation with (A) QM-βgal and (B) QM-OH at various concentrations for 24 h.

8. Pearson's correlation coefficient in colocalization experiments

Table S1 Pearson's correlation coefficient of various organelles in colocalization experiments

Organelle	Golgi body	Lysosome	Endoplasmic reticulum	Mitochondria
Pearson's correlation coefficient	0.629	0.335	0.835	0.933

9. Characterization of compounds

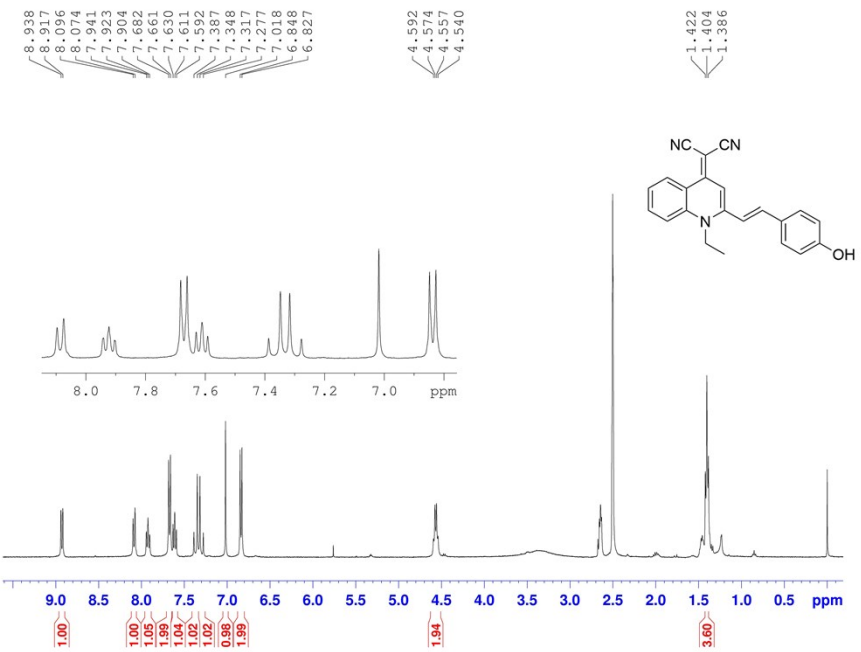


Fig. S8 ^{1}H NMR spectrum of QM-OH in DMSO-d_6.

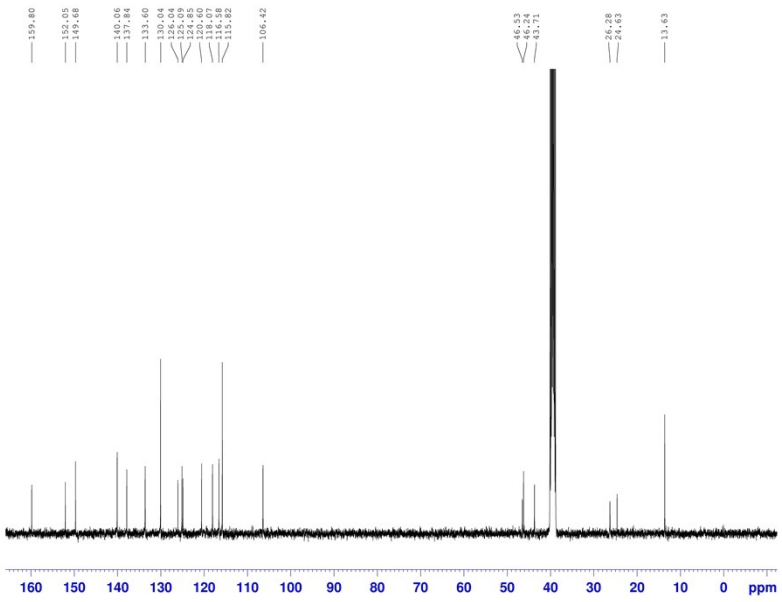


Fig. S9 ^{13}C NMR spectrum of QM-OH in DMSO-d_6.

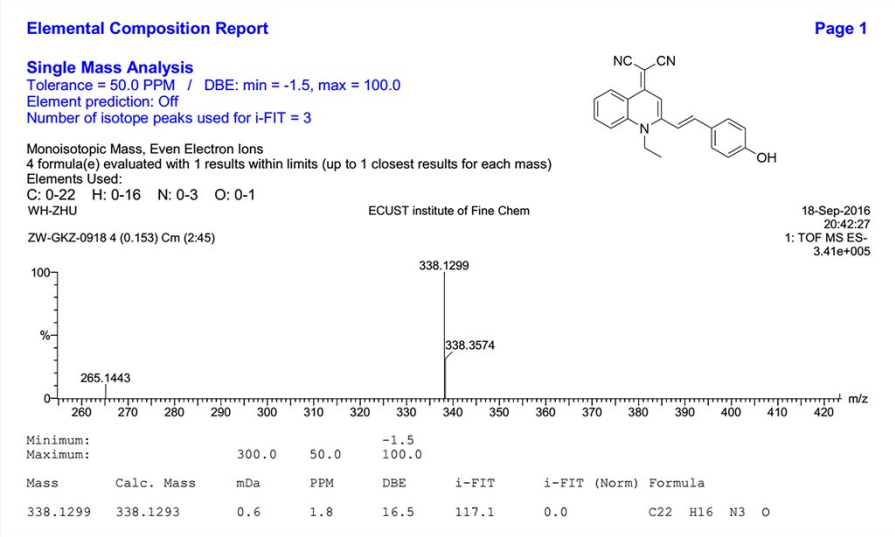


Fig. S10 HRMS spectrum of QM-OH.

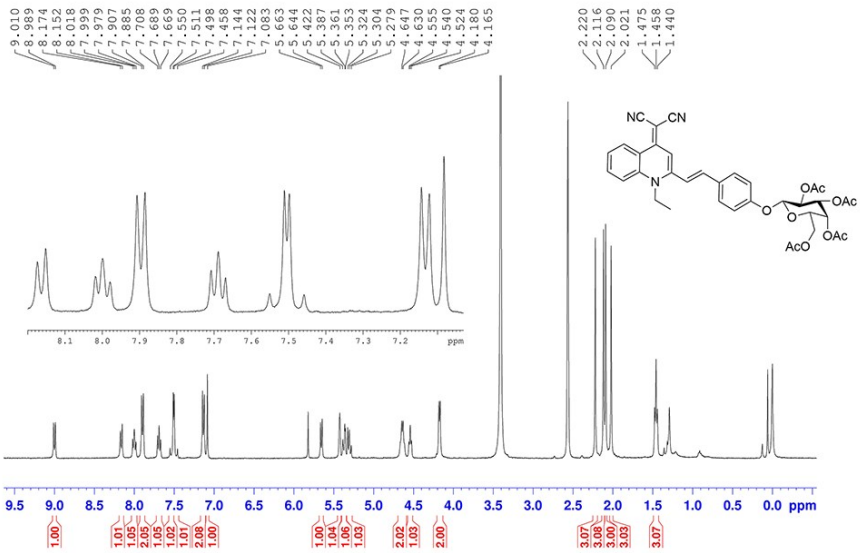


Fig. S11 ^{1}H NMR spectrum of QM-βgalAc in DMSO-d_6.

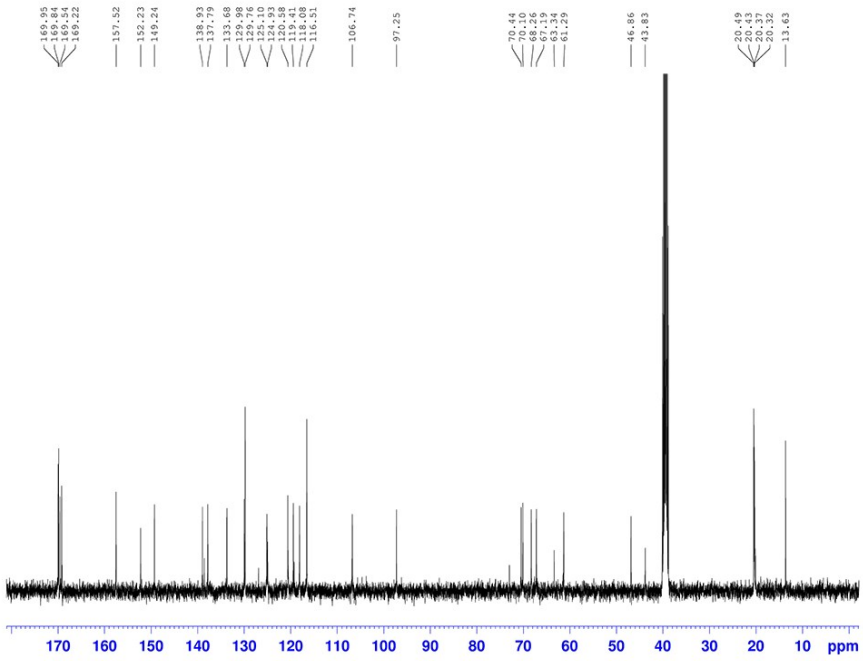


Fig. S12 ^{13}C NMR spectrum of QM-βgalAc in DMSO-d_6.

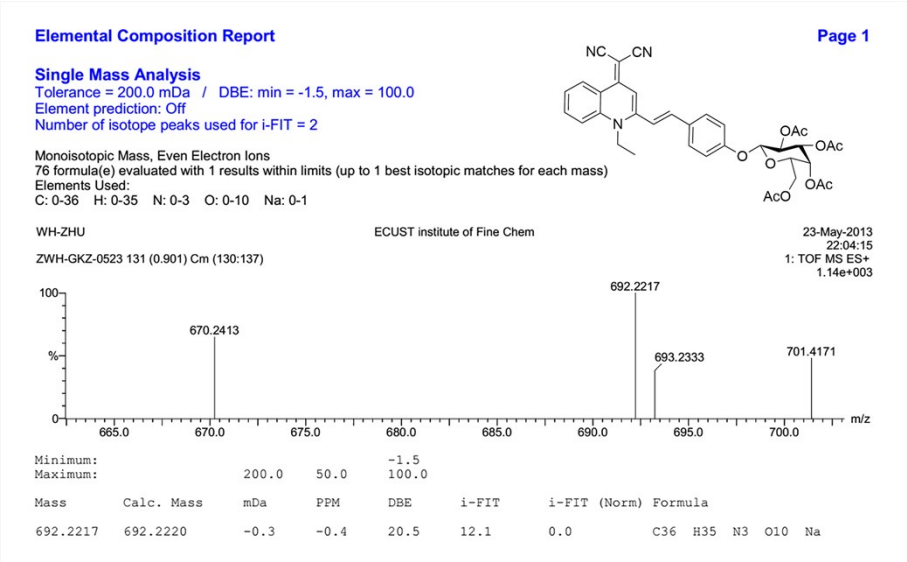


Fig. S13 HRMS spectrum of QM-βgalAc.

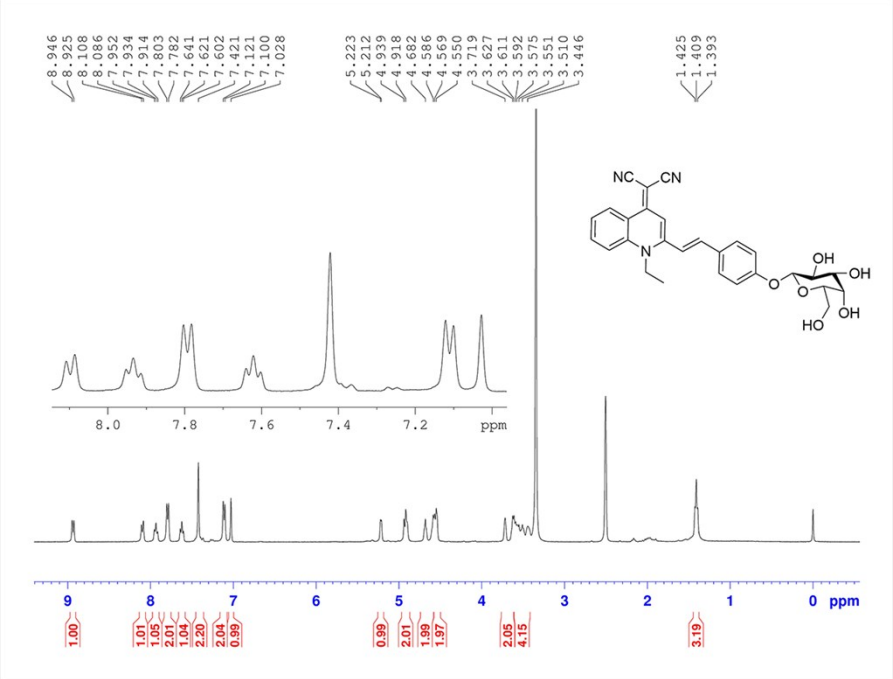


Fig. S14 ^{1}H NMR spectrum of QM-βgal in DMSO-d_6.

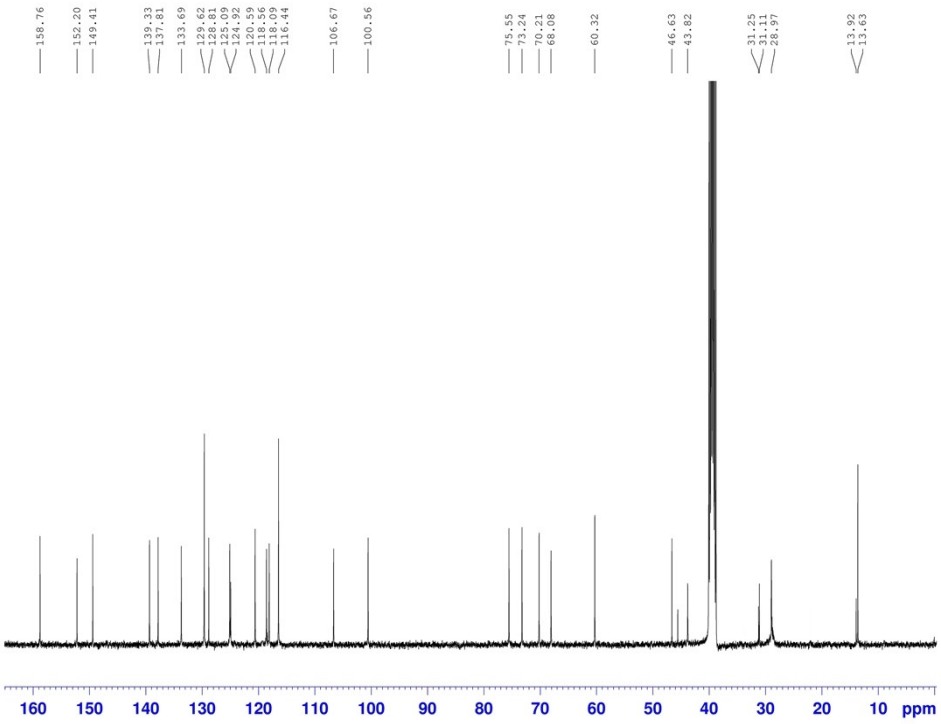


Fig. S15 ^{13}C NMR spectrum of QM-βgal in DMSO-d_6.

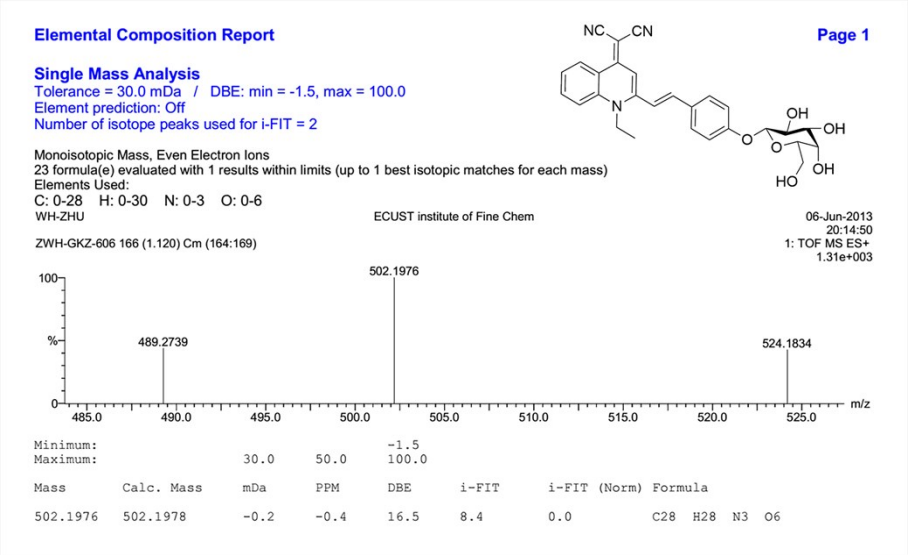


Fig. S16 HRMS spectrum of QM-*β*gal.