Electronic Supplementary Information

A selective and light-up fluorescent probe for β-galactosidase activity detection and imaging in living cells based on an AIE tetraphenylethylene derivative

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Experimental section

All solvents were purified and dried following standard procedures unless special statements. Compound **1** was prepared according to reported procedures.[S1] Compound **3** was synthesized according to reported methods.[S2] All other chemical reagents were purchased from Heowns, Sigma Aldrich or Aladdin, unless otherwise stated. ^{1}H NMR spectra were obtained on a Brucker DMX-400MHz spectrophotometer. High resolution mass spectra were obtained on Brucker APEX IV (7.0 T) FT_MS. Fluorescence emission spectra were recorded on a Hitachi F-4500 fluorescence spectrophotometer. Dynamic light scattering (DLS) experiments were carried out with Malvern Instrument (Nano Series). Fluorescent micrograms were performed on an Olympus Eclipse Fluorescence Spectrophotometer and the magnification employed was 10 × 10. Confocal fluorescence imaging experiments were performed with an Olympus FV-1000 laser scanning microscopy system, based on an IX81 (Olympus, Japan) inverted microscope. The microscope was equipped with 375 nm (CW) laser lines and UPLSAPO 60x/N.A 1.42 objective. Images were

collected and processed with Olympus FV10-ASW Ver.2.1b software.

Synthesis of compound 1

Compound **1** was prepared according to reported procedures.[S1] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (d, *J* = 4.6 Hz, 2H), 7.20 (m, 3H), 7.00 (m, 2H), 6.95 – 6.87 (m, 6H), 6.67 – 6.61 (m, 4H), 3.76 (s, 3H), 3.75 (s, 3H).

Synthesis of compound 2

A mixture of compound **1** (236 mg, 0.500 mmol), 4-pyridinylboronic acid (90 mg, 0.730 mmol), $Pd(dppf)Cl_2$ (80 mg, 0.100 mmol), CH_2Cl_2 (1 mL), Bu_4NI (25 mg, 0.068 mmol) and potassium carbonate aqueous solution (2 M, 10 mL) in degassed toluene (20 mL) was refluxed under nitrogen atmosphere for16 h. After cooling to room temperature, the mixture was washed with brine and extracted with ethyl acetate twice. The organic layer was combined and dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was subjected to column chromatography with ethyl acetate/petroleum ether (1/20~1/2, v/v) as eluent. Compound **2** was obtained as a yellow solid. Yield: 63 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (d, *J* = 5.9 Hz, 2H), 7.48 (d, *J* = 6.1 Hz, 2H), 7.41 (d, *J* = 8.3 Hz, 2H), 7.14 – 7.12 (m, 5H), 7.06 – 7.04 (m, 2H), 7.00 – 6.94 (m, 4H), 6.67 – 6.63 (m, 4H), 3.75 (s, 6H).

Synthesis of compound 4

To a solution of compound **2** (20.0 mg, 0.043 mmol) in toluene, compound **3** (24.2 mg, 0.047 mmol) was added. The mixture was stirred at 110 ℃ overnight at a nitrogen atmosphere. After cooling to room temperature, the mixtures was concentrated and subjected to column chromatography, using dichloromethane/methanol (100/1~10/1) as eluent. Compound **4** was obtained as an orange yellow solid. Yield: 87 %. ^{1}H NMR (400 MHz, MeOD) δ 8.92 (br, 2H), 8.29 (br, 2H), 7.75 (br, 2H), 7.52 (br, 2H), 7.38 – 6.86 (m, 14H), 6.66 (br, 3H), 5.74 (br, 2H), 5.46 – 5.25 (m, 4H), 4.33 (br, 1H), 4.16 (br, 2H), 3.71 (s, 6H), 2.16 (s, 3H), 2.02 (s, 6H), 1.98 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 170.60, 170.51, 170.04, 169.85, 158.85, 158.70, 157.89, 156.16, 149.48, 143.97, 143.53, 142.66, 137.73, 135.60, 135.50, 132.57, 132.38, 132.27, 131.08, 130.83, 130.56, 129.91, 128.31, 128.25, 128.19, 127.95, 127.91, 127.79, 127.68, 127.21, 126.31, 124.37, 124.27, 117.25, 113.01, 112.78, 98.24, 70.82, 70.77, 68.70, 67.25, 62.73, 61.13, 54.27, 19.57, 19.30, 19.18, 13.13. HRMS (MALDI-TOF) m/z: $[M]^+$ calcd for $C_{54}H_{52}NO_{12}^+$, 906.3484, found: 906.34871.

Synthesis of TPE-Gal

To a solution of compound **4** (15 mg, 0.015 mmol) in MeOH (3 mL), K_2CO_3 (8.4 mg, 0.061 mmol) was added. The mixture was stirred at room temperature for 1 h. After that, chloroform was added, and the mixture was washed with brine. The organic layer was collected and dried over anhydrous Na_2SO_4. TPE-Gal was obtained by recrystallization from a mixed solvent of ethyl acetate, petroleum ether and dichloromethane. Yield: 92 %. ^{1}H NMR (400 MHz, DMSO) δ 9.14 (d, *J* = 6.3 Hz, 2H), 8.43 (d, *J* = 5.5 Hz, 2H), 7.87 (d, *J* = 7.8 Hz, 2H), 7.51 (d, *J* = 7.2 Hz, 3H), 7.28 – 6.81 (m, 13H), 6.70 (t, *J* = 8.6 Hz, 3H), 5.72 (s, 2H), 5.31 (s, 1H), 4.83 – 4.81 (m, 3H), 3.67 (s, 6H), 3.55 – 3.52 (m, 3H). ^{13}C NMR (101 MHz, DMSO) δ 158.61, 158.46, 154.64, 150.64, 148.70, 144.86, 143.70, 142.15, 138.02, 135.67, 135.65, 132.58, 132.49, 132.46, 132.42, 132.04, 131.25, 130.76, 129.21, 128.74, 128.64, 128.53, 128.41, 128.30, 128.14, 127.93, 127.05, 126.55, 124.76, 121.21, 117.20, 116.90, 113.90, 113.68, 101.21, 100.85, 76.02, 73.82, 70.71, 68.42, 62.35, 60.73, 55.42. HRMS (MALDI-TOF) m/z: $[M]^+$ calcd for $C_{46}H_{44}NO_8^+$, 738.3061, found: 738.30674.

Determination of the detection limit of TPE-Gal toward addition of β-galactosidase

Based on the linear fitting in Fig. 1b, the detection limit (*C*) is estimated as follows:

$$C = 3\sigma/B$$

Where σ is the standard deviation obtained from three individual fluorescence measurements (I_{512} nm) of TPE-Gal (10 μM) without any β-galactosidase and B is the slope obtained after linear fitting the titration curves within certain ranges.

Determination of *n*-octanol/water partition coefficeints (log *P*)

The *n*-octanol/water partition coefficients were measured at room temperature following a reported method.[S3] Typically, solutions of TPE-Gal (100 mM) in equal volumes of 5 mM PBS, pH 7.4 (2 mL) and *n*-octanol (2 mL) were mixed and sonicated for 30 min. After separation by centrifugation, the amounts of TPE-Gal in each phase were determined after dilution with DMSO using the calibration curve based on the absorption spectra obtained on a Shimadzu UV-1601PC spectrophotometer and the results were the average of three independent measurements.

Cell culture and cell imaging

HeLa cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10 % fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin. OVCAR-3 cells were grown in RPMI 1640 containing 10 % fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin. TPE-Gal was dissolved in DMSO at a storage concentration of 1 mM. Cells were incubated with 1 mL probe solution (10 μM) for 40 min at 37 ℃, then washed 3 times with PBS and underwent imaging measurement.

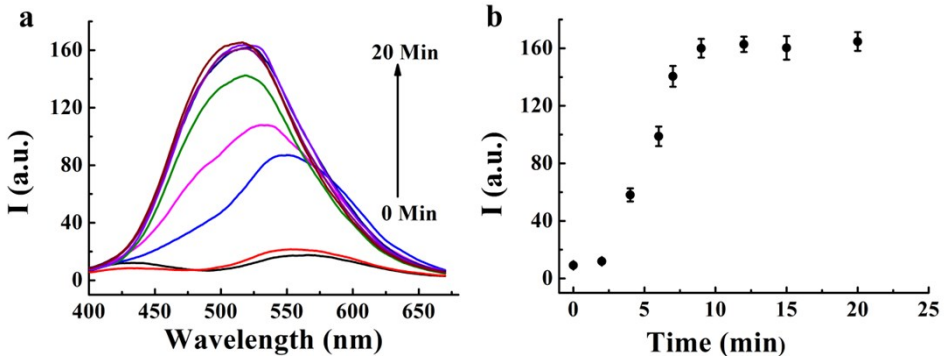

Fig. S1. (*a*) Fluorescent responses of TPE-Gal (10 μM) toward β-galactosidase (8.0 U/mL) at different time points. (*b*) The fluorescence intensity at 512 nm of TPE-Gal (10 μM) incubated with β-galactosidase (8.0 U/mL) as a function of time. (E_{ex} = 344 nm).

ESI(P),W20160316-1,20160316

Analysis Info

Analysis Name D:\Data\ESI\2016\2016-03\0316\W20160316-1_000001.d

Acquisition Date 3/16/2016 11:19:49 AM

Sample Name W20160316-1

Instrument solariX

Acquisition Parameter

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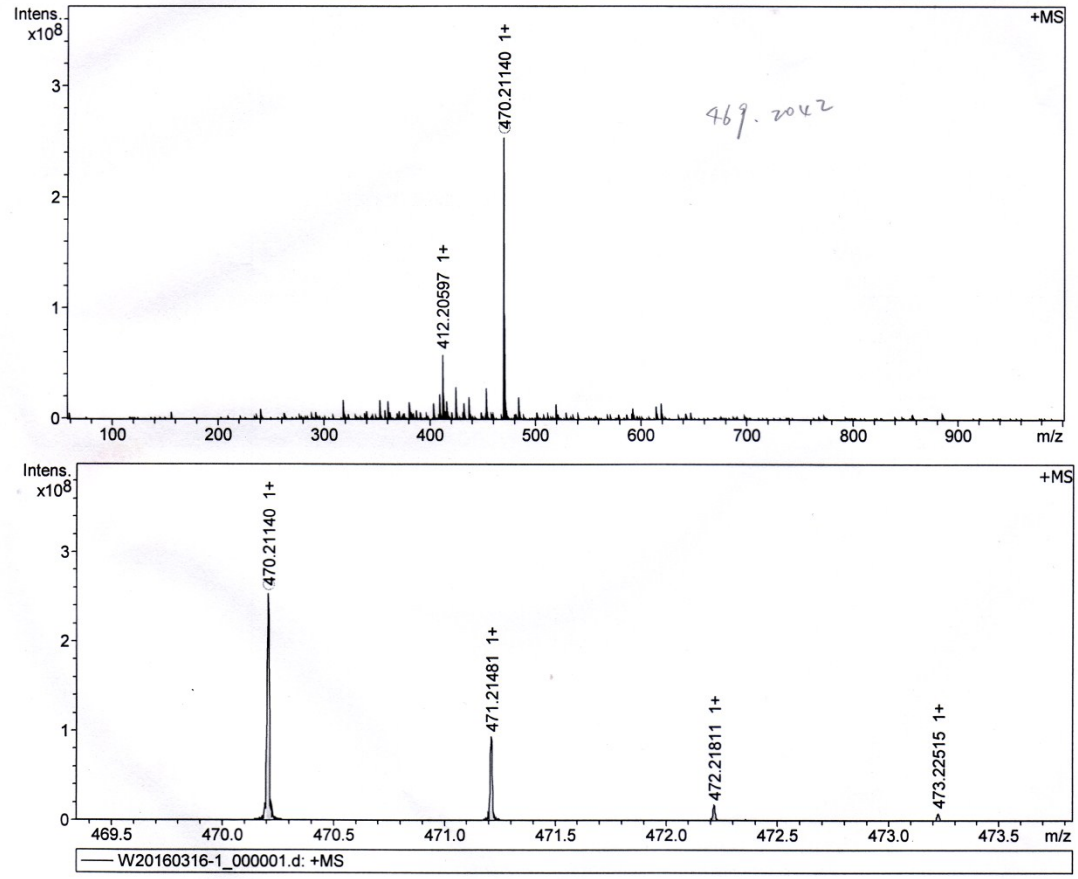

Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e¯ Conf	N-Rule
470.211402	1	C33H28NO2	100.00	470.211456	0.1	-0.2	12.0	20.5	even	ok

Fig. S2. HRMS spectrum of TPE-Gal after 10 min incubation with β-galactosidase at 37℃.

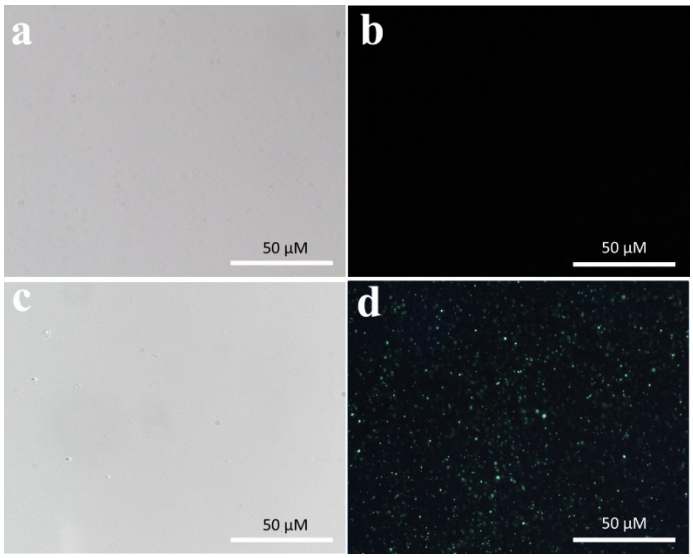

Fig. S3. Fluorescence microscope images of TPE-Gal (10.0 μM) in PBS before (*a* and *b*) and after (*c* and *d*) incubation with β-galactosidase (12.8 U/mL). Scale bar represents 50 μm.

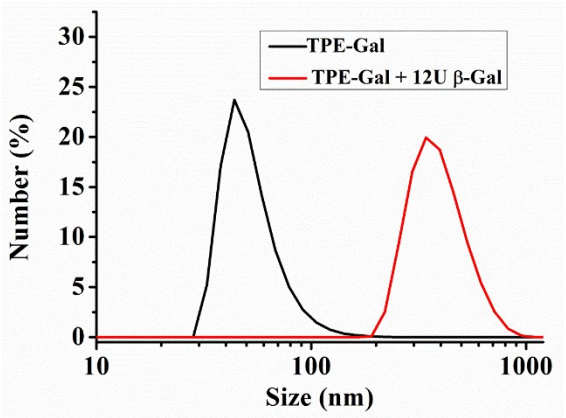

Fig. S4. DLS data of TPE-Gal (10 μM) before and after 10 min incubation with 12.8 U/mL of β-galactosidase in PBS.

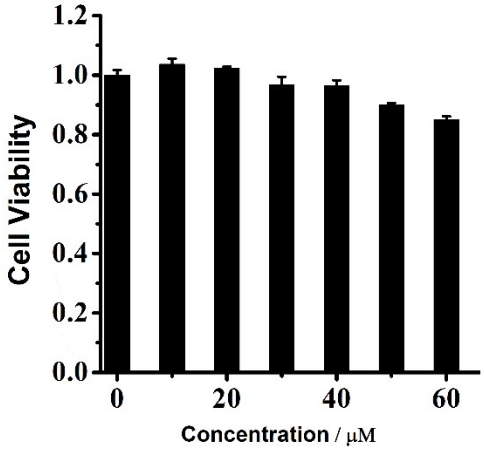

Fig. S5. Cell viability of HeLa cells at varied concentrations of TPE-Gal using MTT assay.

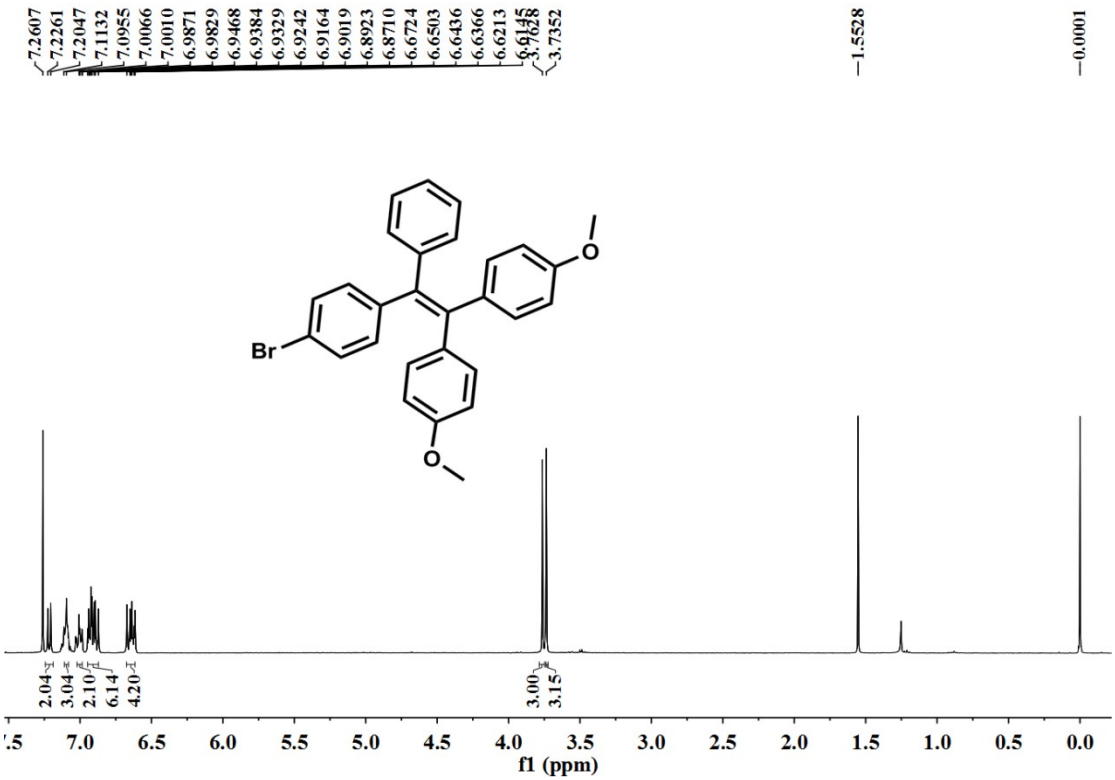

Fig. S6. ^{1}H NMR spectrum of compound **1** in $CDCl_3$.

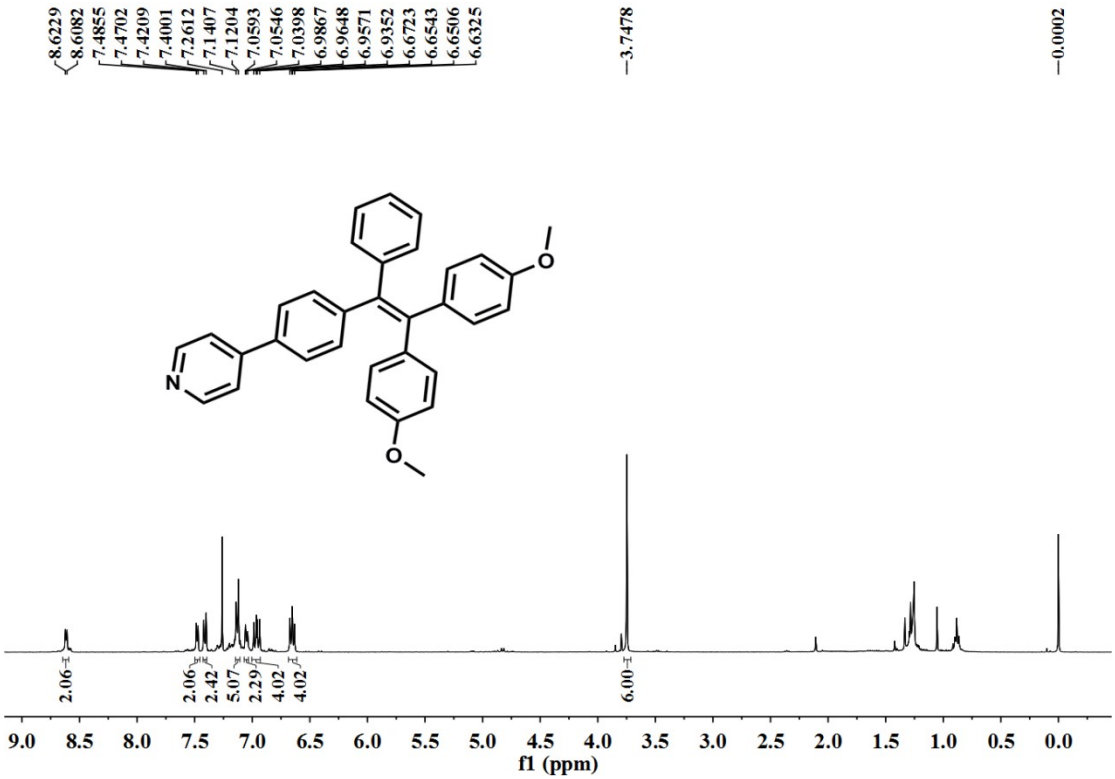

Fig. S7. ^{1}H NMR spectrum of compound **2** in $CDCl_3$.

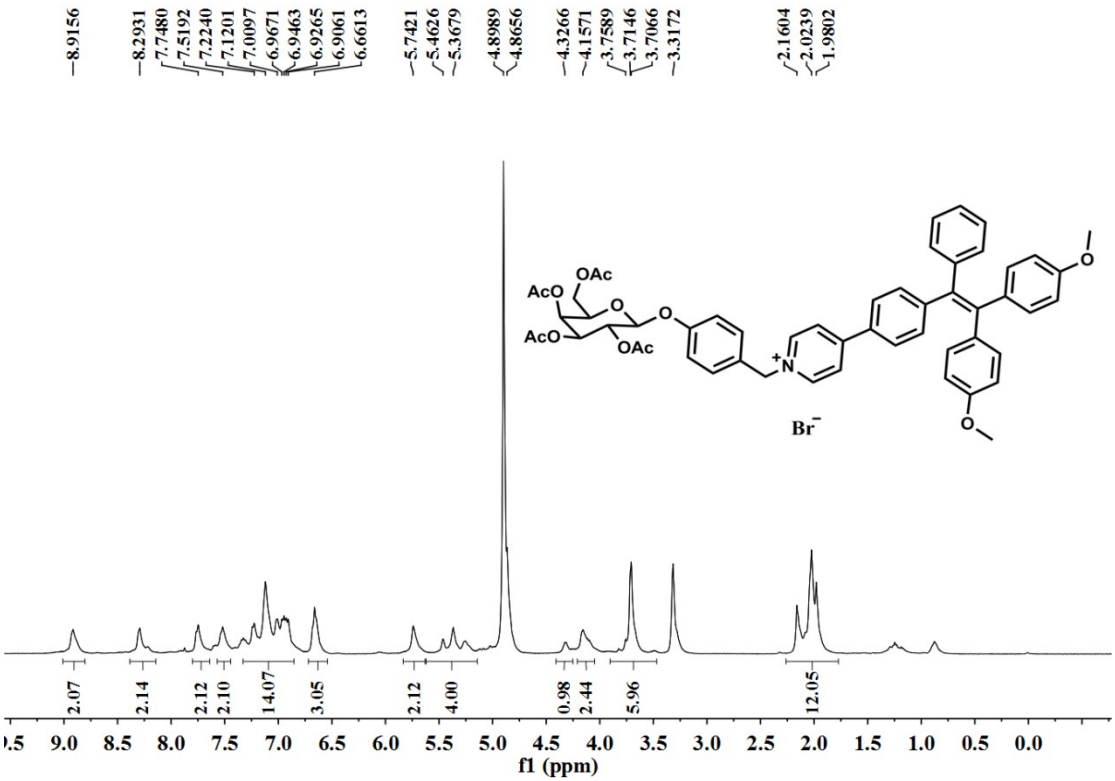

Fig. S8. ^{1}H NMR spectrum of compound **4** in CD_3OD.

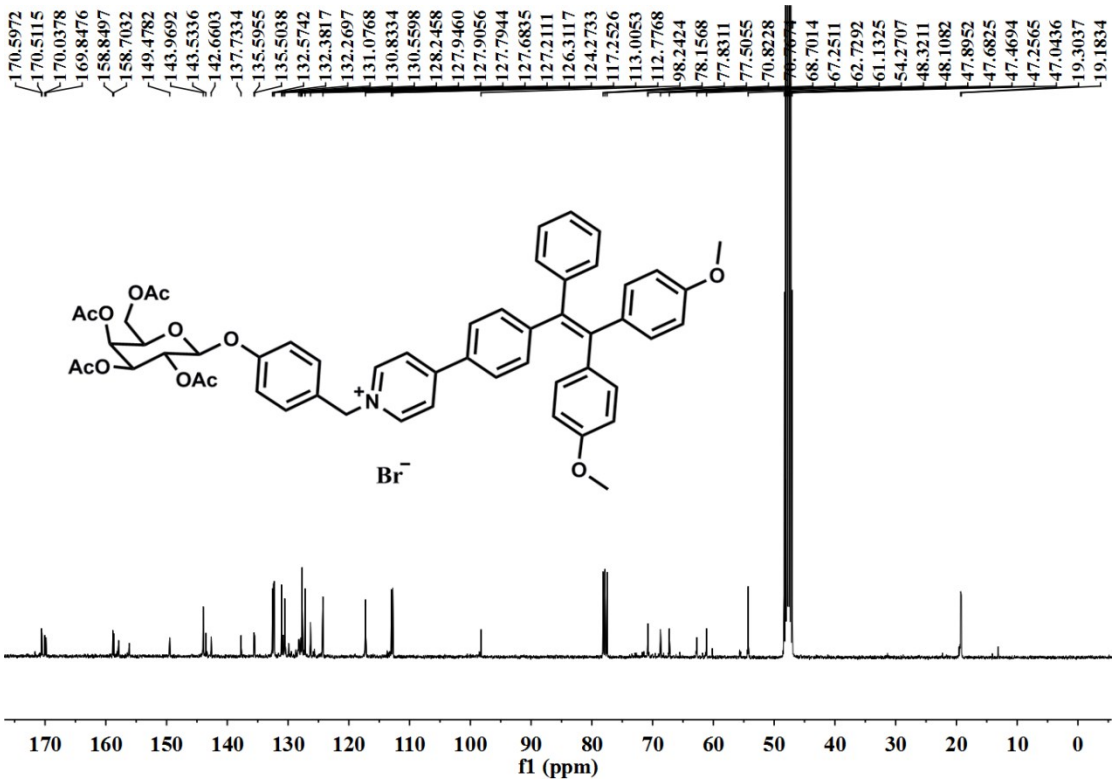

Fig. S9.^{13}C NMR spectrum of compound **4** in CD_3OD.

ESI(P),W20160316-3,20160316

Analysis Info

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Sample Name	W20160316-3	Instrument	solariX

Acquisition Parameter

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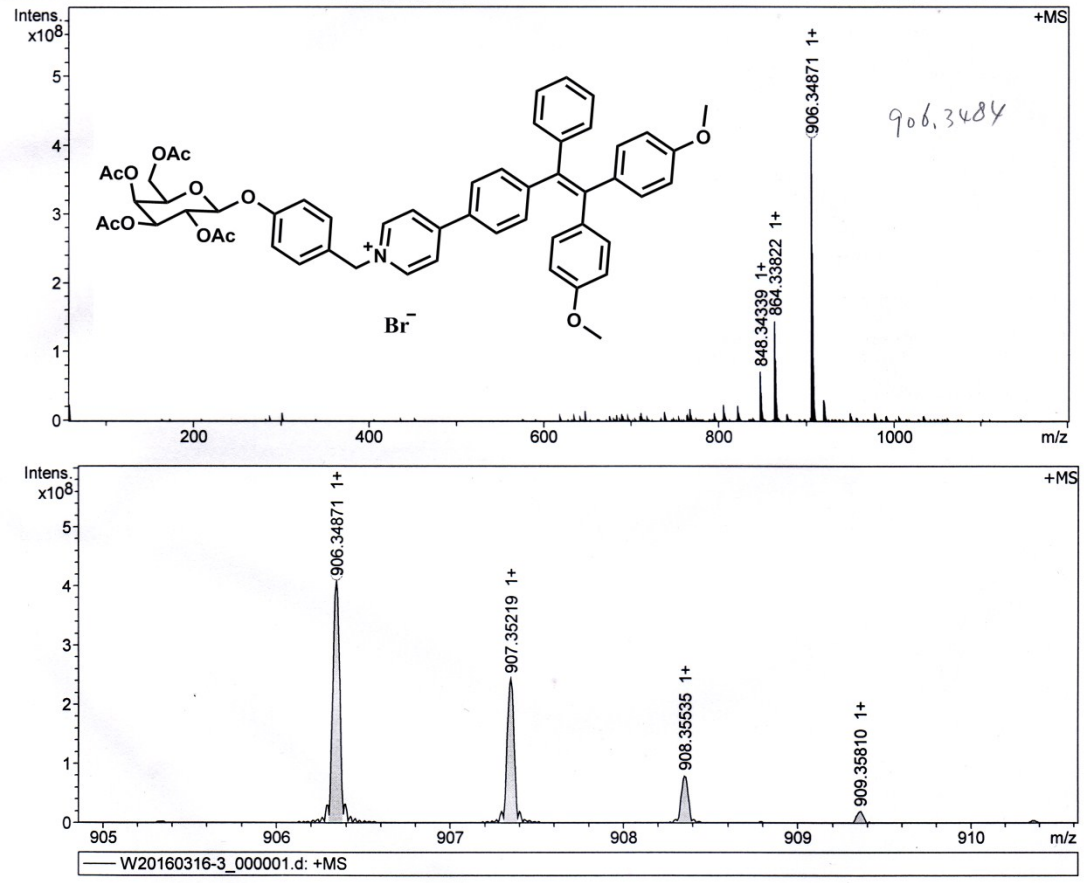
Broadband High Mass	1200.0 m/z	Ion Accumulation Time	0.100 sec	Apodization	Sine-Bell Multiplication



Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
906.348714	1	C54H52NO12	100.00	906.348403	0.3	-0.4	2.2	29.5	even	ok

Fig. S10. HRMS spectrum of compound **4**.

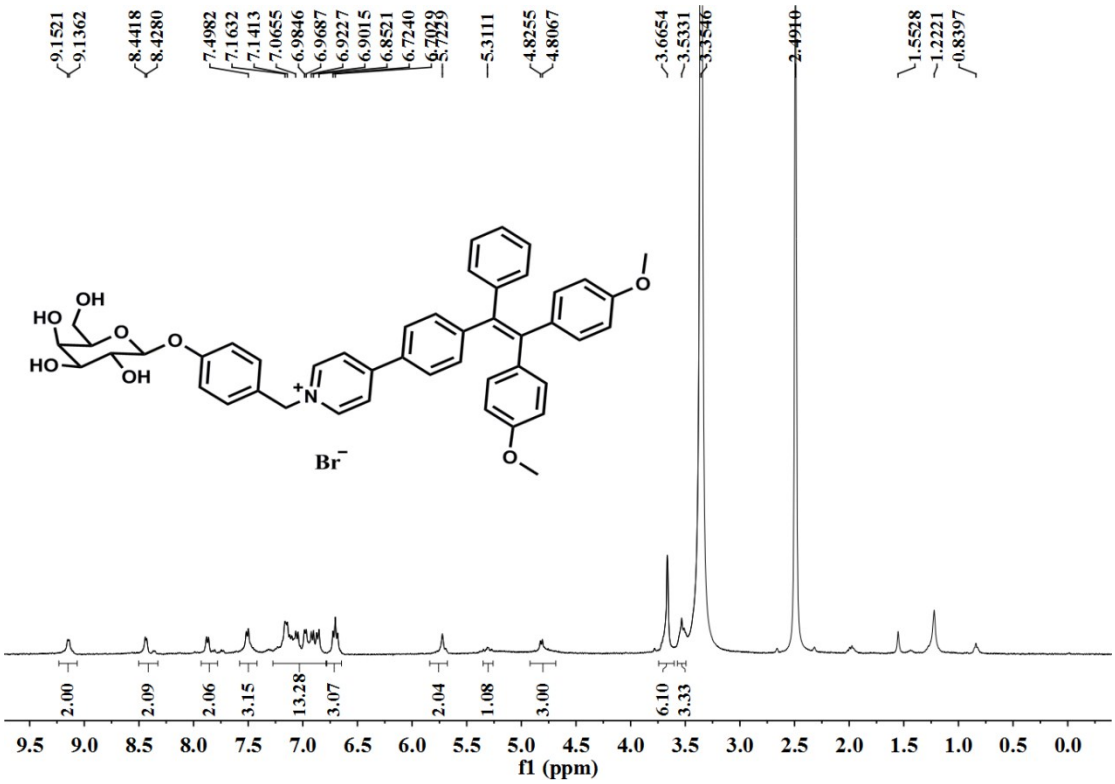


Fig. S11. ^{1}H NMR spectrum of **TPE-Gal** in d_6-DMSO.

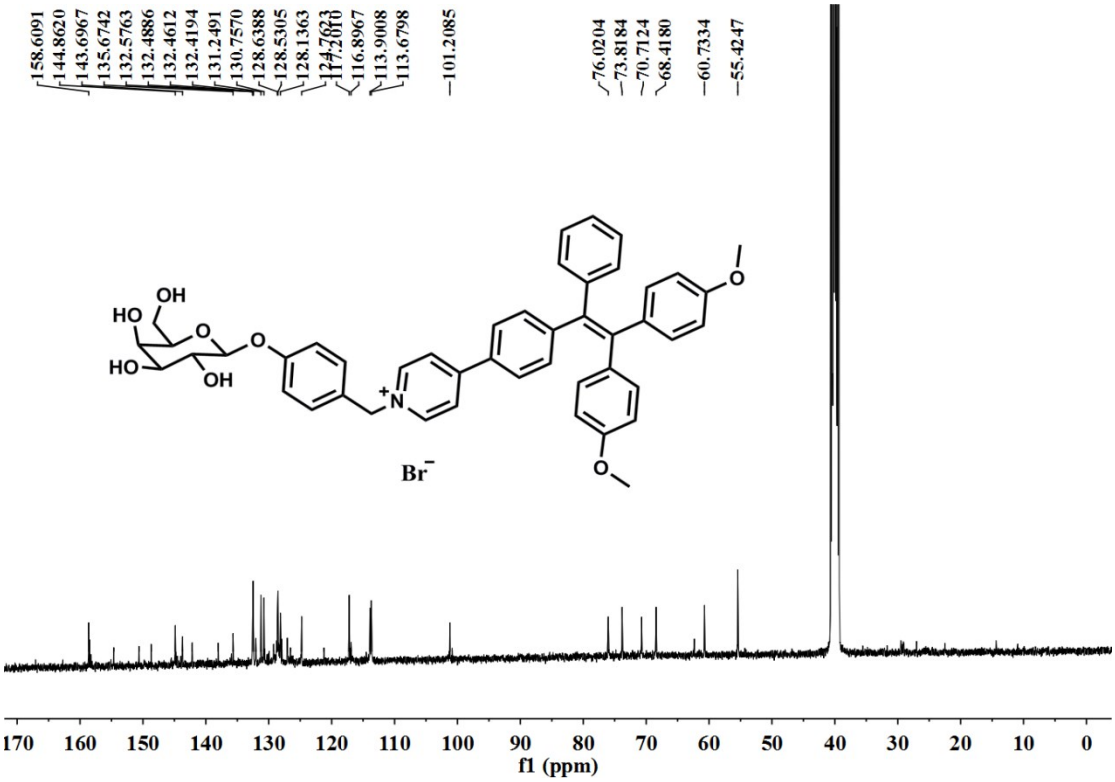


Fig. S12. ^{13}C NMR spectrum of **TPE-Gal** in d_6-DMSO.

ESI(P),W20160316-2,20160316

Analysis Info

Analysis Name D:\Data\ESI\2016\2016-03\0316\W20160316-2_000002.d

Acquisition Date 3/16/2016 12:24:46 PM

Sample Name W20160316-2

Instrument solariX

Acquisition Parameter

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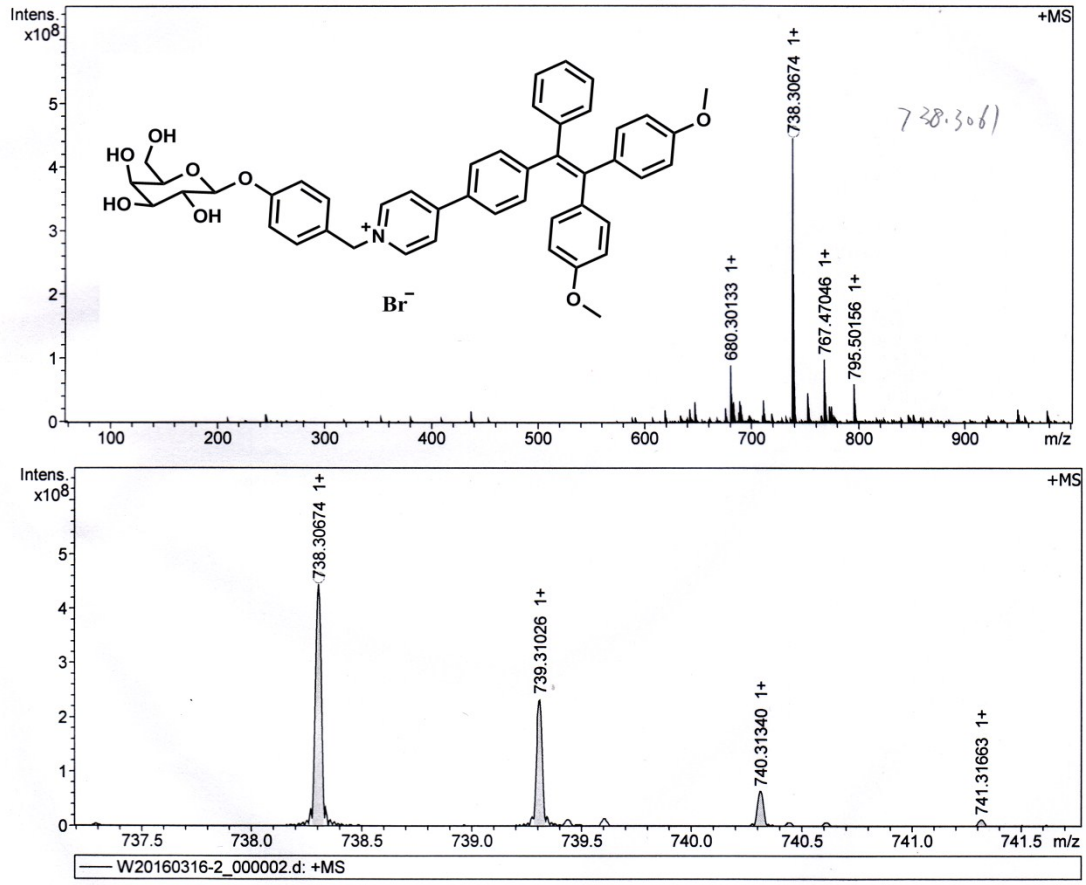
Broadband High Mass	1000.0 m/z	Ion Accumulation Time	0.200 sec	Apodization	Sine-Bell Multiplication



Meas. m/z	#	Ion Formula	Score	m/z	err [ppm]	Mean err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
738.306741	1	C46H44NO8	100.00	738.306144	0.8	-0.9	7.4	25.5	even	ok

Fig. S13. HRMS spectrum of **TPE-Gal**.

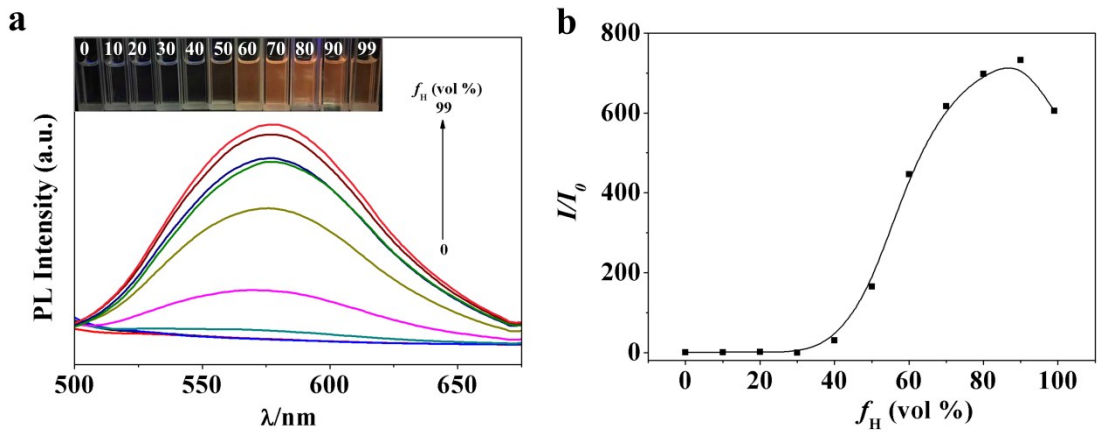


Fig. S14. (a) PL spectra of **TPE-Gal** in hexane/THF mixtures with different hexane fractions (f_H). Excitation wavelength: 344 nm. [TPE-Gal] = 10 μM. Inset: fluorescent photographs of TPE-Gal in hexane/THF mixtures with different f_H taken under 365 nm UV irradiation. (b) Plot of relative PL intensity (I/I_0) versus the composition of the hexane/THF mixtures of TPE-Gal. I_0 = PL intensity in pure THF.

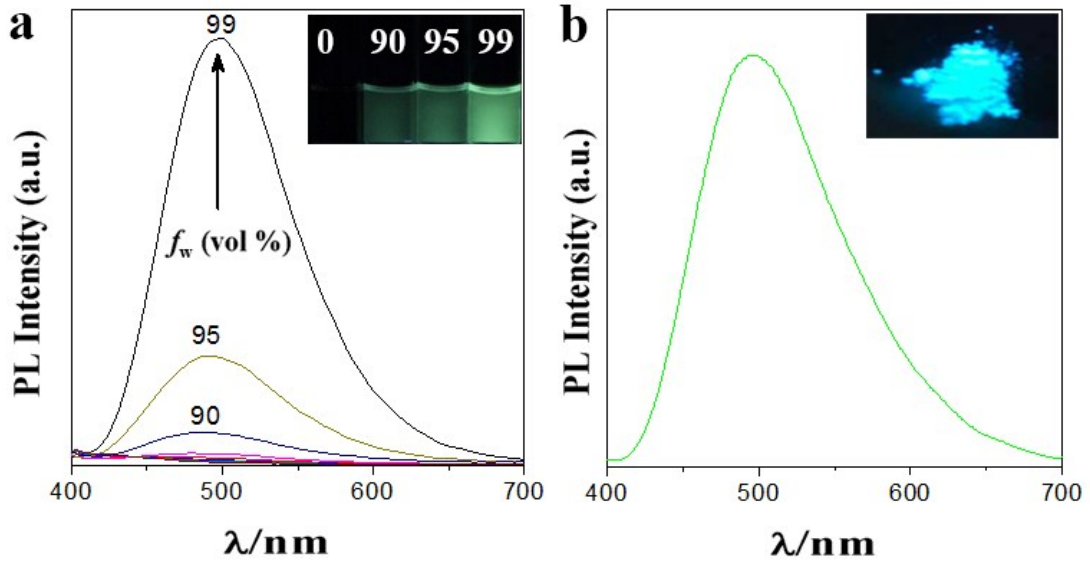


Fig. S15. (a) PL spectra of compound **2** in water/THF mixtures with different water fractions (f_w). Excitation wavelength: 344 nm. [**2**] = 10 μM. Inset: fluorescent photographs of **2** in water/THF mixtures with different f_w taken under 365 nm UV irradiation. (b) PL spectra of **2** in solid state. Inset: fluorescent photographs of **2** in solid state taken under 365 nm UV irradiation.

Table. S1. Comparison of TPE-Gal with other small molecular fluorescent probes for β-galactosidase

probe	ACQ or AIE	Detection limit	Linear range	Wavelength (E_x/E_m)(nm)	Literature
7-hydroxy-9*H*-(1,3-dichloro-9,9-dimethylacridin-2-one) derivative	ACQ	No mentioned	No mentioned	646/659	Tung et al, *Cancer Res.*, 2004, **64**, 1579
Squarylium dyes	ACQ	No mentioned	No mentioned	626/644	Oushiki et al, *Anal. Chem.*, 2012, **84**, 4404
6-(benzo[*d*]thiazol-2′-yl)-2-(methylamino)-Naphthalene derivative	ACQ	0.25 nM	0-2.0 nM	378/540	Lee et al, *Anal. Chem.*, 2014, **86**, 10001
TokyoMagenta derivative	ACQ	No mentioned	No mentioned	582/598	Egawa et al, *Chem. Commun.*, 2011, **47**, 4162
Cyanine dyes	ACQ	No mentioned	No mentioned	570/680	Han et al, *Mol. Biosyst.*, 2013, **9**, 3001
Rhodol derivative	ACQ	No mentioned	No mentioned	498/505–600	Asanuma et al, *Nat. Commun.*, 2015, **6**, 6463
4-hydroxyl-N-butyl-1,8-naphthalimide derivative	ACQ	No mentioned	No mentioned	445/545	Zhang et al, *Chem. Commun.*, 2016, **52**, 8283
9-di-3-sulfonyl-propylaminobenzo[a]phenoxazonium perchloride derivative	ACQ	No mentioned	No mentioned	630/670	Ho et al, *ChemBioChem*, 2007, **8**, 560
Fluorescein derivative	ACQ	No mentioned	No mentioned	492/509	Urano et al, *J. Am. Chem. Soc.*, 2005, **127**, 4888
Fluorescein derivative	ACQ	No mentioned	No mentioned	491-494/510-516	Kamiya et al, *J. Am. Chem. Soc.*, 2007, **129**, 3918
Rhodamine derivative	ACQ	No mentioned	No mentioned	501/524	Sakabe, et al, *J. Am. Chem. Soc.*, 2013, **135**, 409
Rhodol derivative	ACQ	No mentioned	No mentioned	525/~550	Kamiya et al, *J. Am. Chem. Soc.*, 2011, **133**, 12960
dicyanomethylene-4*H*pyran derivative	ACQ	0.17 mU/mL	0-12 U	535/685	Gu et al, *J. Am. Chem. Soc.*, 2016, **138**, 5334
salicylaldehyde azine derivatives	AIE	0.014 U/mL	0−0.1 U/mL	387/545	Peng et al, *J. Mater. Chem. B*, 2015, **3**, 9168
tetraphenylethylene derivative	AIE	0.33 U/mL	0.8-4.8 U/mL	344/512	This work

References:

S1. (*a*) X. Gu, J. Yao, G. Zhang, C. Zhang, Y. Yan, Y. Zhao and D. Zhang, *Chem-Asian J.*, 2013, **8**, 2362; (*b*) F. Hu, G. Zhang, C. Zhan, W. Zhang, Y. Yan, Y. Zhao, H. Fu and D. Zhang, *Small*, 2015, **11**, 1335.

S2. (*a*) O. Redy-Keisar, E. Kisin-Finfer, S. Ferber, R. Satchi-Fainaro and D. Shabat, *Nat. Protoc.*, 2014, **9**, 27; (*b*) R. Orth, M. Pitscheider and S. A. Sieber, *Synthesis*, 2010, **13**, 2201.

S3. M. Kepczyński, R. P. Pandian, K. M. Smith and B. Ehrenberg, *Photochem. Photobiol.*, 2002, **76**, 127.