Supporting Information for

A fluorescent light-up probe based on AIE and ESIPT process for β-galactosidase activity detection and visualization in living cells

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Table S1. Fluorescence spectroscopic data (maximum exication, emission and the quantum yield) of **SA-βGal (**100 μM) before and after addition of β-galactosidase (4.0 U/mL) in phosphate buffer solutionat pH 7.4 (10 mM, 37 ℃).

Sample	Fluorescence maximum		Apparent Quantum Yield (Φ)
	λ_{ex} [nm]	λ_{em}[nm]	
SA-βGal	387	545	<0.001
SA-βGal +βGal	387	545	0.246

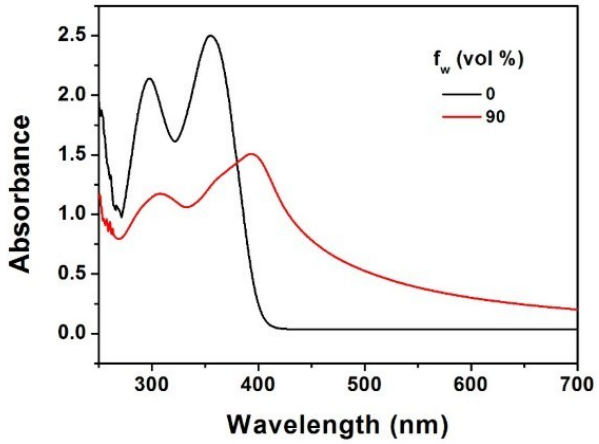

Fig. S1. Absorption spectra of **SA** (100 μM) in DMSO and co-solvent mixtures containing 90% phosphate buffer solution (10 mM, pH 7.4).

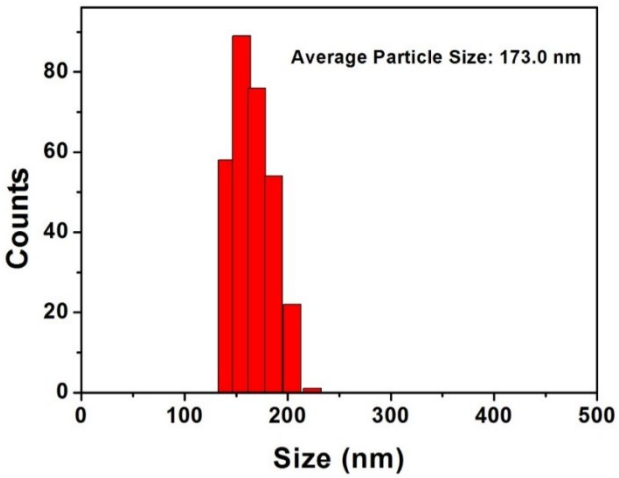

Fig. S2. Dynamic lighter scattering (DLS) analysis of **SA-βGal (**100 μM) in PBS buffer solution (10

mM, pH 7.4) containing 1% DMSO.

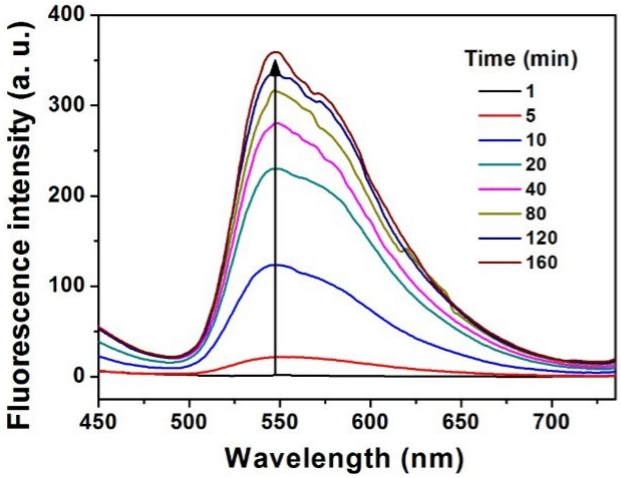

Fig.S3. (a) Time-dependent fluorescence spectra of **SA-βGal** (100 μM) upon addition of β-galactosidase (2.0 U/mL). Excitation at 387 nm.

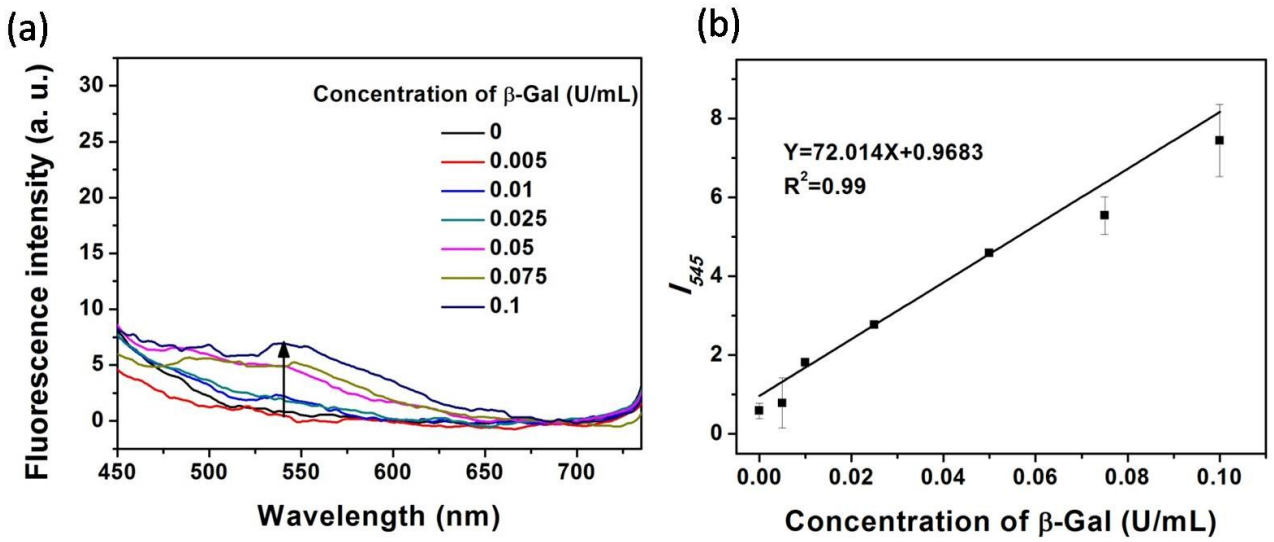

Fig. S4.(a) Fluorescence spectra of **SA-βGal (**100 μM) in the presence of different concentrations of β-galactosidase (0-0.1 U/mL) in phosphate buffer solution at pH 7.4 (10 mM, 37 ℃). (b) Calibration curve of the fluorescence intensities (I_{545}) versus β-galactosidase concentrations.

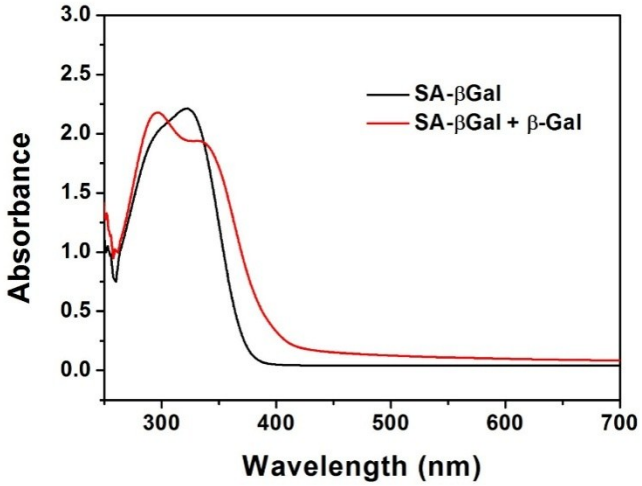

Fig. S5. Absorption spectra of **SA-βGal** (100 μM) before and after addition of β-galactosidase (4.0 U/mL) in phosphate buffer solution at pH 7.4 (10 mM, 37 ℃).

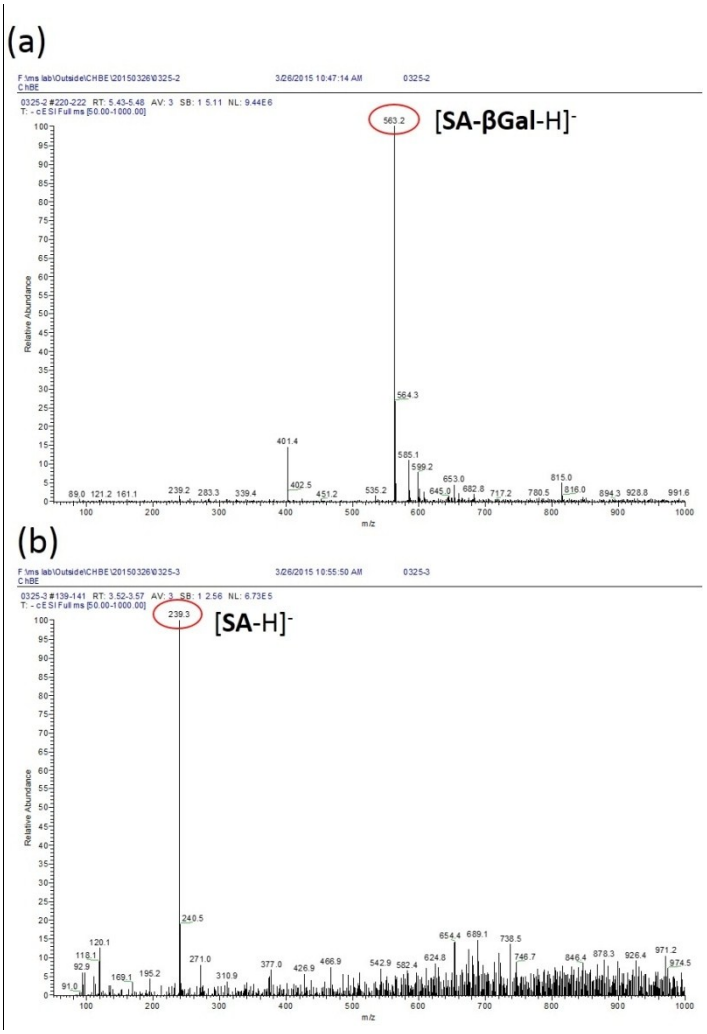

Fig.S6 (a) ESI mass spectrum of **SA-βGal** (1 mM) and (b) the isolated fluorescent product after **SA-βGal** (1 mM) was reacted with β-galactosidase (4.0 U/mL) for 30 min in PBS buffer solution (10

mM, pH 7.4, 37 ℃), suggesting the formation of **SA** from **SA-βGal.**

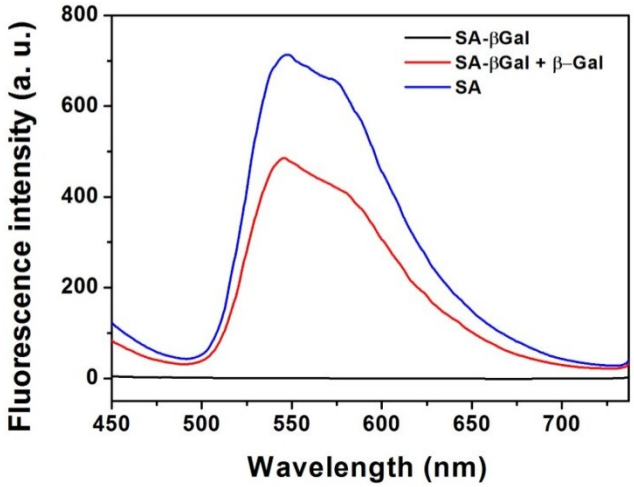

Fig. S7. Fluorescence spectra of **SA (**100 μM), **SA-βGal (**100 μM) before and after addition of β-galactosidase (4.0 U/mL) in phosphate buffer solutionat pH 7.4 (10 mM, 37 ℃).

Synthesis of compound **3**

In 20 mL of dichloromethane, 0.98 g of compound **1** (2.4 mmol), 244 mg of 2-hydroxybenzaldehyde (2.0 mmol), and 0.65 g of tetrabutylammonium bromide (2.0 mmol) were dissolved. Then 10.0 mL of sodium hydroxide solution (5%) was added, and the mixed solution was refluxed overnight. After the reaction was completed, the mixture was extracted three times using 30 mL of $CHCl_3$, washed with brine solution, and dried over anhydrous Na_2SO_4. Column chromatography was used to purify the coarse product to yield **3** as white powders (633 mg, 70%).

Synthesis of compound **4**

In 10 mL of ethanol, 452 mg of Compound **3** (1 mmol) and 25 mg of hydrazine hydrate (0.5 mmol) were dissolved. After refluxing for 4 hours, the reaction was completed. The resulting precipitates were filtrated and washed three times with 30 mL of ethanol to yield 4 as white powders (399 mg, 90% yield).

^{1}H NMR (100 MHz, $CDCl_3$): δ=8.92(s, 2H), 8.15 (d, 2H, J = 7.6 Hz,),7.43–7.39 (m, 2H), 7.17 (d, 4H, J = 8.0 Hz),5.59–5.45 (m, 4H),5.13–5.03 (m, 4H),4.26–4.04 (m, 6H),2.21(s, 6H),2.21(s, 6H),2.05(s, 6H),2.01(s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): 170.3, 170.2, 170.1, 169.4, 157.0, 156.5, 132.3, 127.5, 124.8, 124.0, 117.1, 100.5, 71.2, 70.8, 68.7, 66.9, 61.3, 20.7, 20.6, 20.5.

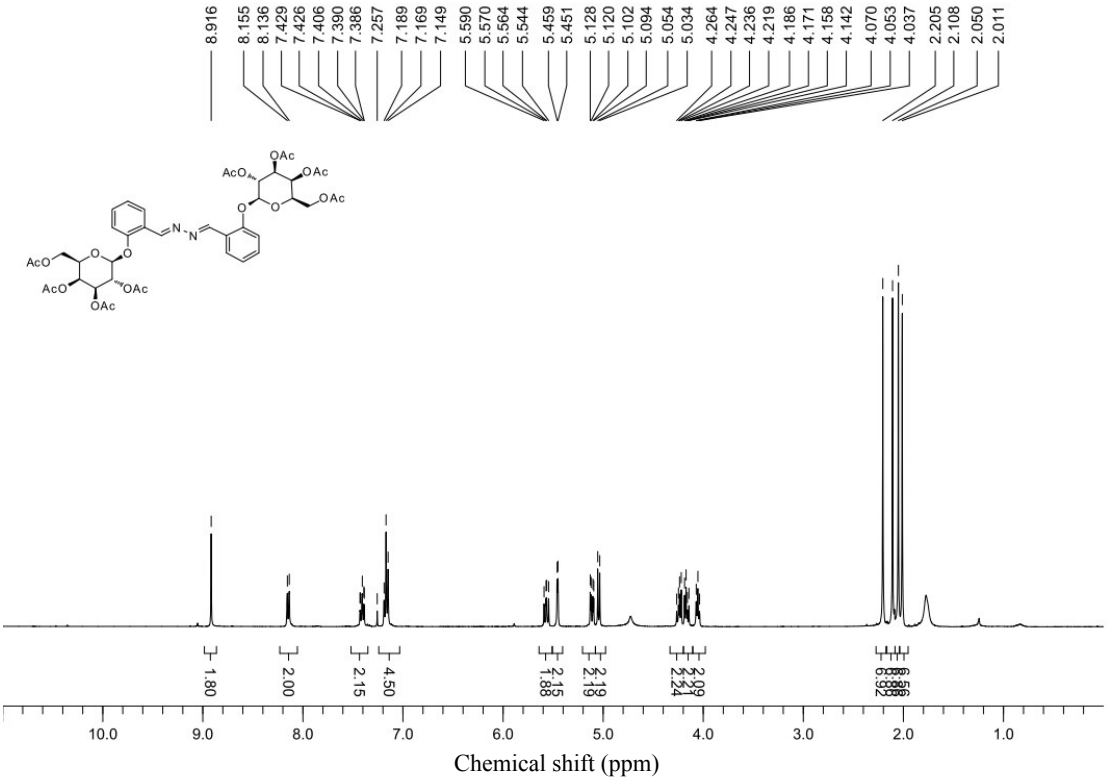

Fig. S8. ^{1}H NMR spectrum of compound **4**.

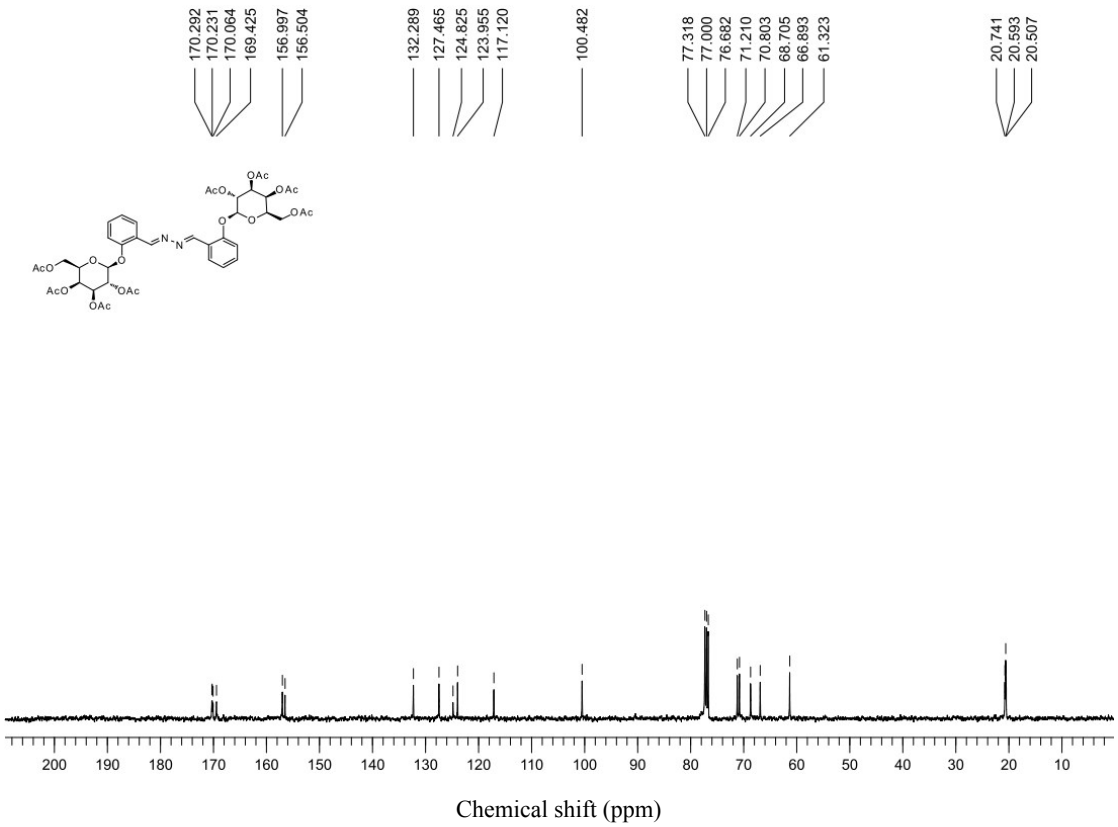

Fig. S9. ^{13}C NMR spectrum of compound **4**.

Synthesis of **SA-βGal**

In 5 mL of methanol, compound **4** (225mg, 0.25 mmol) and KOH (112 mg, 2 mmol) were added. After being stirred at r.t. for 2 hours, the mixed solution was neutralized by acetic acid. The product of **SA-βGal** was precipitated as white solid. The product was filtrated under vacuum, washed by cold methanol and dried under vacuum to afford pure product of **SA-βGal** (134 mg, 95% yield).

^{1}H NMR (400 MHz, d_6-DMSO): δ=9.07(s, 2H),8.03 (d, 2H, J = 6.4 Hz),7.48 (t, 2H, J = 6.8 Hz),7.26 (d,2H,J = 8.4 Hz), 7.11 (t, 2H, J = 7.2 Hz),4.91 (d, 2H, J = 7.6 Hz),4.64 (s, 8H), 3.73–3.42 (m, 12H); ^{13}C NMR (100 MHz, d_6-DMSO): 157.1, 156.7, 132.6, 126.4, 122.8, 122.0, 116.0, 101.5, 75.7, 73.2, 70.4, 68.0, 60.3. ESI-MS of $C_{26}H_{32}N_2O_{12}$ $[M-H]^+$ m/z 563.2(calc. 563.19)

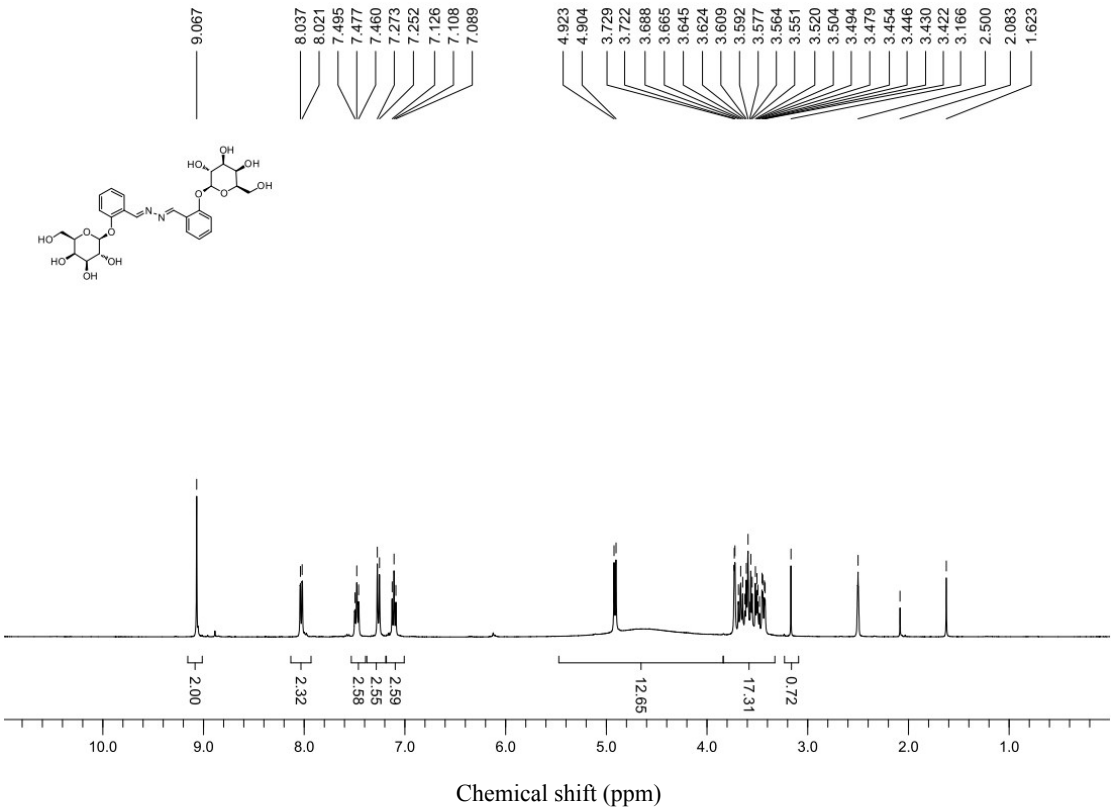

Fig. S10. ^{1}H NMR spectrum of **SA-βGal**.

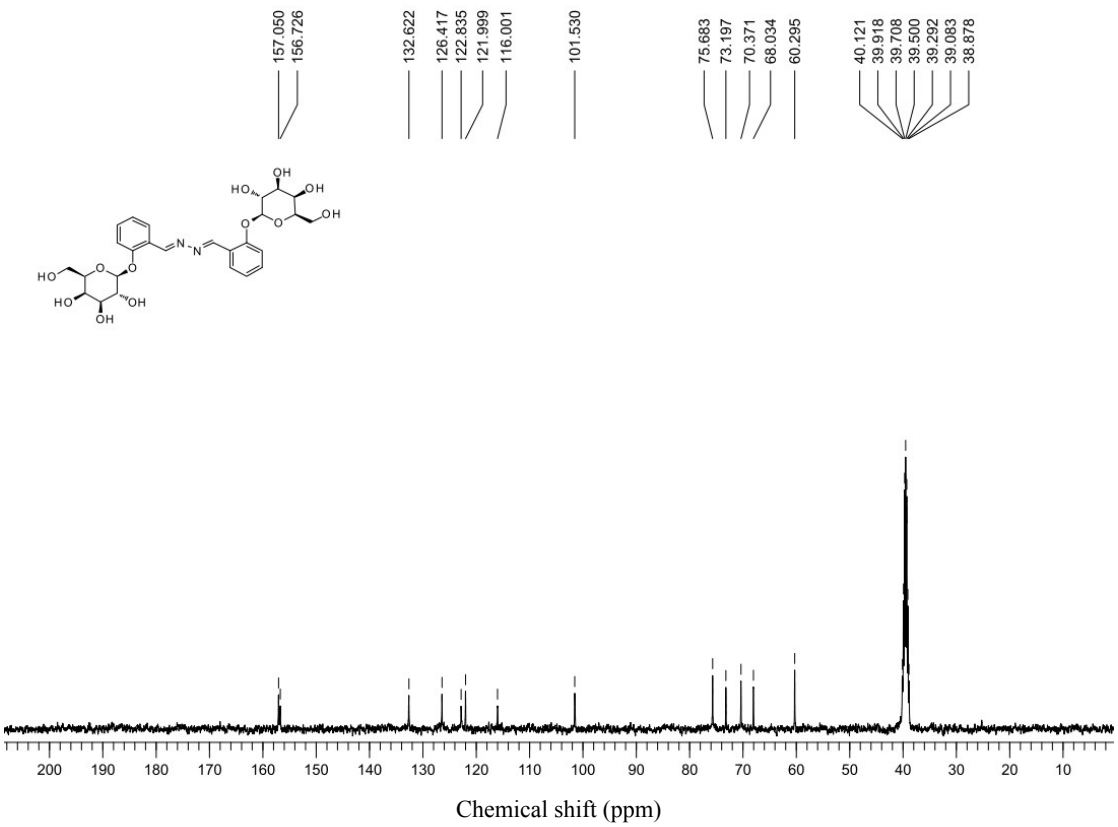

Fig. S11. ^{13}C NMR spectrum of **SA-βGal**.

Compound **SA** (salicylaldehyde azine) was prepared through our early reported procedure.[1, 2]

References

1. M. Gao, Q. Hu, G. Feng, B. Z. Tang and B. Liu, *Journal of Materials Chemistry B*, 2014, **2**, 3438-3442.
2. W. Tang, Y. Xiang and A. Tong, *The Journal of organic chemistry*, 2009, **74**, 2163-2166.