Supporting Information

Ratiometric sensing of β-galactosidase based on excited-state intramolecular proton transfer (ESIPT) and solid-state luminescence enhancement

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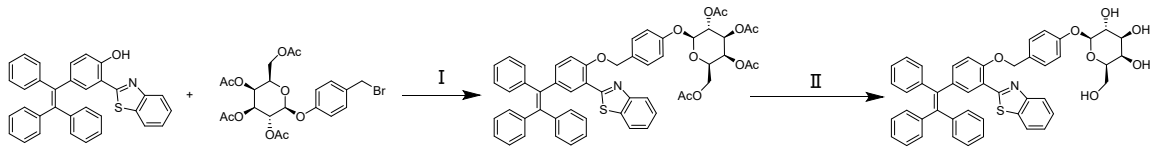
1 Experimental Section

General remarks. All purchased chemicals and reagents were of analytical grade. The β-galactosidase (β-Gal) used in this study was purchased from Sigma-Aldrich (origin: *Escherichia coli*). Absorption spectra were measured on a Varian Cary 500 UV−vis spectrophotometer. Fluorescence spectra were obtained on a Varian Cary Eclipse fluorescence spectrophotometer with a function of automatic wavelength correction. High-resolution transmission electron microscopy (HRTEM) was performed on JEOL 2100 equipped with a Gatan Orius charged-coupled device camera (Tridiem energy filter operating at 200 kV) and Talos F200X TEM. Dynamic light scattering was measured by a Malvern Zetasizer Nano ZS instrument.



TPE-BT **a** **b** **Gal-TPE-BT**

Scheme S1. Synthesis of **Gal-TPE-BT**. Reagents and conditions: (I) K_2CO_3/DMF; (II) CH_3ONa/CH_3OH.

Synthesis of b. To a solution of **TPE-BT**[1] (192 mg, 0.4 mmol) and K_2CO_3 (110 mg, 0.8 mmol) in CH_3CN (10 mL), **a**[2] (248 mg, 0.48 mmol) was added, and the resulting mixture was stirred at 40 °C temperature for 5 h under an argon atmosphere. Then, the mixture was diluted by CH_2Cl_2 and washed by brine. The combined organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuum to give a crude product, which was then purified by column chromatography (Petroleum ether (PE)/CH_2Cl_2 = 1:1, v/v) to obtain the acetyl intermediate **b** as a white solid (250 mg, 68% yield). TLC: R_f 0.3 (PE/CH_2Cl_2 = 1:2, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.23 (d, J = 2.1 Hz, 1H), 8.01 (d, J = 8.2 Hz, 1H), 7.85 (d, J = 7.9 Hz, 1H), 7.46 (t, J = 9.0 Hz, 3H), 7.34 (t, J = 7.5 Hz, 1H), 7.15-7.10 (m, 13H), 7.06 (dd, J = 7.9, 3.1 Hz, 5H), 6.86 (d, J = 8.6 Hz, 1H), 5.55 (dd, J = 10.4, 8.0 Hz, 1H), 5.50 (d, J = 3.3 Hz, 1H), 5.21 (s, 2H), 5.16 (dd, J = 10.0, 2.9 Hz, 1H), 5.12 (d, J = 8.0 Hz, 1H), 4.24 (dd, J = 11.3, 6.7 Hz, 2H), 4.11 (t, J = 6.7 Hz, 1H), 2.22 (s, 3H), 2.11 (s, 3H), 2.05 (s, 6H);^{13}C NMR (151 MHz, $CDCl_3$) δ 170.3, 170.2, 170.1, 169.4, 162.9, 156.8, 154.8, 151.9, 143.7, 143.6, 143.2, 141.2, 139.7, 137.1, 136.0, 134.8, 132.4, 131.4, 131.3, 131.2, 130.9, 129.6, 127.8, 127.7, 127.7, 126.6, 126.4, 125.8, 124.5, 122.8, 122.1, 121.2, 117.0, 116.9, 112.2, 99.5, 71.1, 70.8, 70.5, 68.6, 66.8, 61.3, 20.8, 20.7, 20.7, 20.6. HR-ESI-MS (m/z) $[M+H]^+$: calcd for $C_{54}H_{48}NO_{11}S^+$ 918.2943, found 918.2939.

Synthesis of Gal-TPE-BT. To a CH_3OH (20 mL) solution of **b** (200 mg, 0.22 mmol) was added CH_3ONa (1.4 mL, 5.9 mmol). The mixture was stirred over night at room temperature, and then concentrated in vacuum to give a crude product. This product was then purified by column chromatography (CH_2Cl_2/MeOH = 20:1, v/v) to obtain **Gal-TPE-BT** as a white solid (120 mg, 72.7% yield). TLC: R_f 0.4 (CH_2Cl_2/MeOH = 10:1, v/v). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.11 (d, J = 2.0 Hz, 1H), 8.04 (d, J = 8.0 Hz, 1H), 7.95 (d, J = 8.1 Hz, 1H), 7.48 (dd, J = 14.3, 8.0 Hz, 3H), 7.37 (t, J = 7.6 Hz, 1H), 7.19-7.07 (m, 12H), 7.07-7.02 (m, 5H), 7.02-6.97 (m, 2H), 5.28 (s, 2H), 5.22 (d, J = 5.0 Hz, 1H), 4.91 (d, J = 5.3 Hz, 1H), 4.88 (d, J = 7.7 Hz, 1H), 4.69 (t, J = 5.1 Hz, 1H), 4.55 (d, J = 4.0 Hz, 1H), 3.72 (s, 1H), 3.63-3.49 (m, 4H), 3.46-3.41 (m, 1H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 162.3, 157.9, 155.0, 151.7, 143.7, 143.6, 143.4, 141.3, 139.9, 136.6, 135.8, 135.2, 131.2, 131.2, 131.1, 131.1, 130.4, 129.4, 128.4, 128.3, 127.2, 127.1, 127.0, 126.7, 125.4, 122.8, 122.2, 121.4, 116.5, 113.6, 101.3, 76.0, 73.8, 70.7, 68.6, 60.9. HR-ESI-MS(m/z) $[M+Na]^+$: calcd for $C_{46}H_{39}NNaO_7S^+$ 772.2339, found 772.2338.

2 Additional Figures

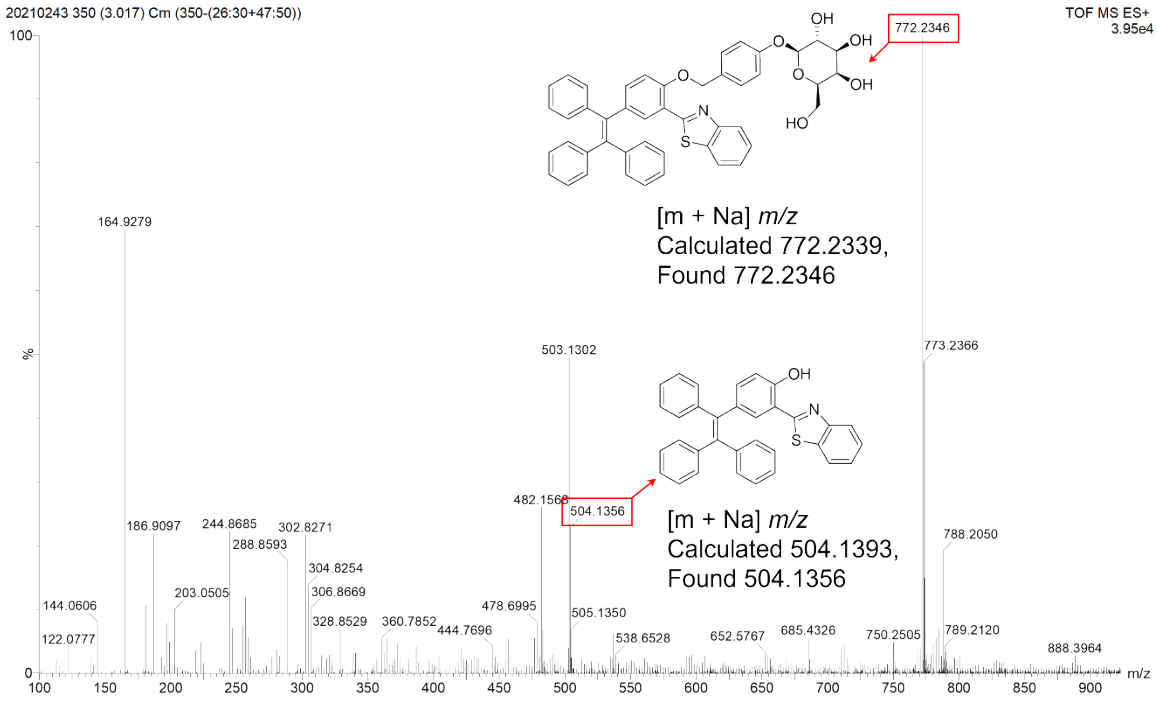


Figure S1. High-resolution mass spectrum of **Gal-TPE-BT** after treatment with β-Gal. The deglycosylation product **TPE-BT** is detected in the spectrum.

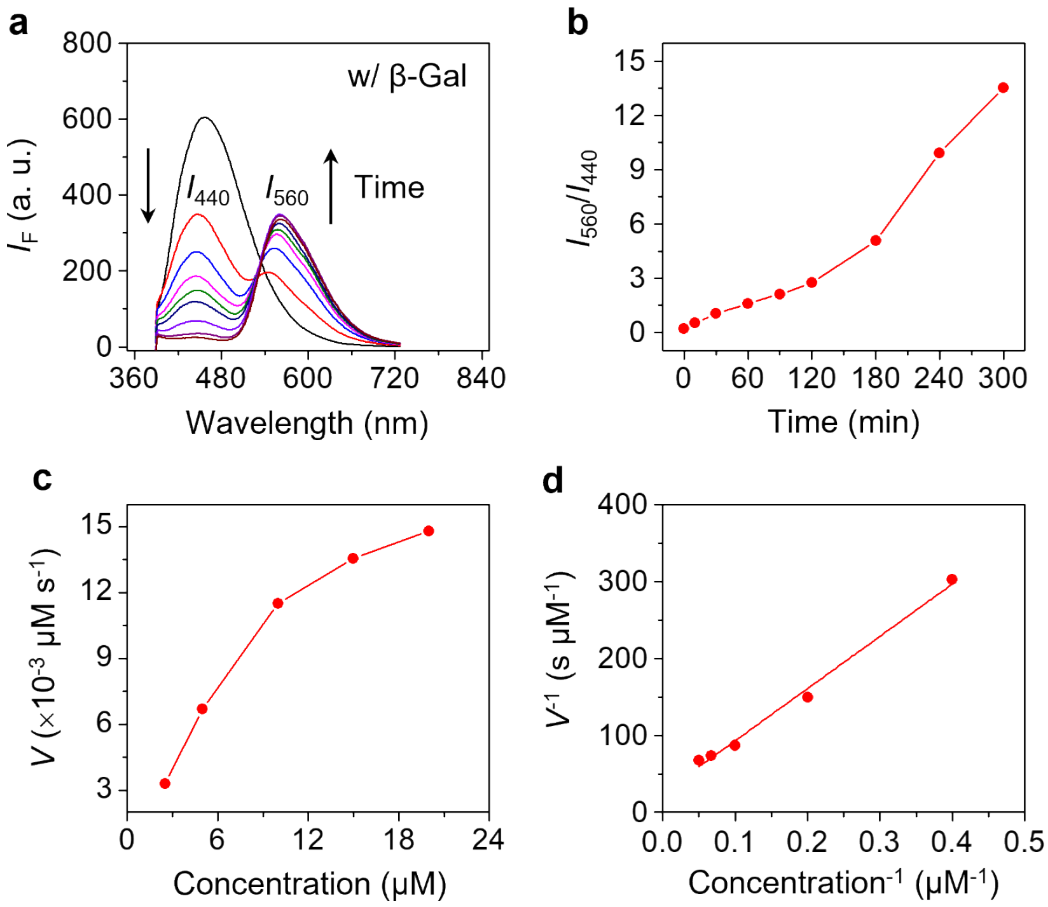


Figure S2. (a) Fluorescence emission spectra of **Gal-TPE-BT** (10 μM) in the presence of β-Gal (8 U mL^{-1}) with time (0-300 min). (b) Time-dependent changes in the fluorescence emission intensity ratio (I_{560}/I_{440}) of **Gal-TPE-BT** (10 μM) in the presence of β-Gal (8 U mL^{-1}). (c) Michaelis-Menten kinetics of increasing concentrations of **Gal-TPE-BT** (2.5, 5, 10, 15 and 20 μM) in the presence of β-Gal (8 U mL^{-1}) in a solvent mixture of phosphate buffered saline (PBS) (0.01 M, pH 7.4)/DMSO (7:3, v/v). (d) Double reciprocal plots of $1/V$ vs 1/[**Gal-TPE-BT**], where the Y-intercept of the equation is $1/V_{max}$, and the absolute value of the X-intercept is $1/K_m$.[3]

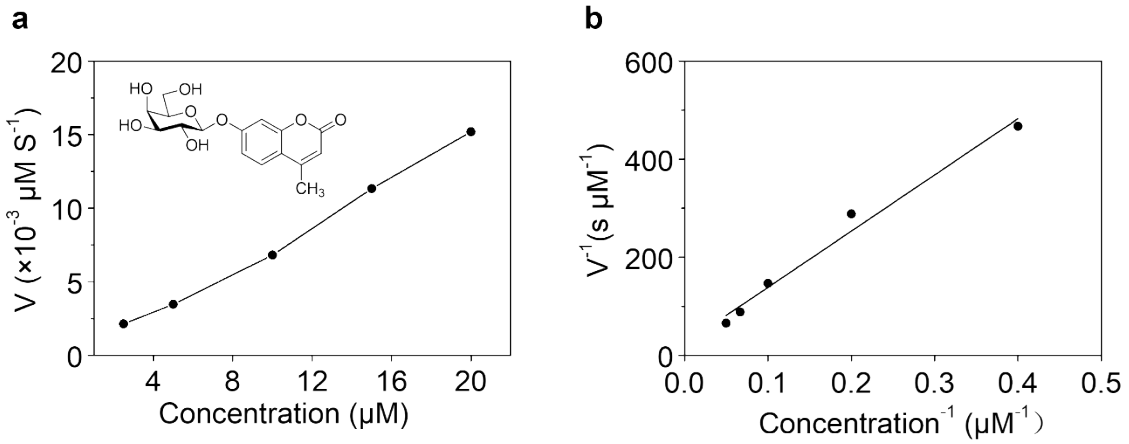


Fig. S3. (a) Michaelis-Menten kinetics of increasing concentrations of **4-MU-β-Gal** (2.5, 5, 10, 15 and 20 μM) in the presence of β-Gal (8 U mL^{-1}) in phosphate buffered saline (0.01 M, pH 7.4) (Inset: Chemical structure of **4-MU-β-Gal**). (b) Double reciprocal plots of $1/V$ vs $1/$[**4-MU-β-Gal**], where the Y-intercept of the equation is $1/V_{max}$, and the absolute value of the X-intercept is $1/K_m$.

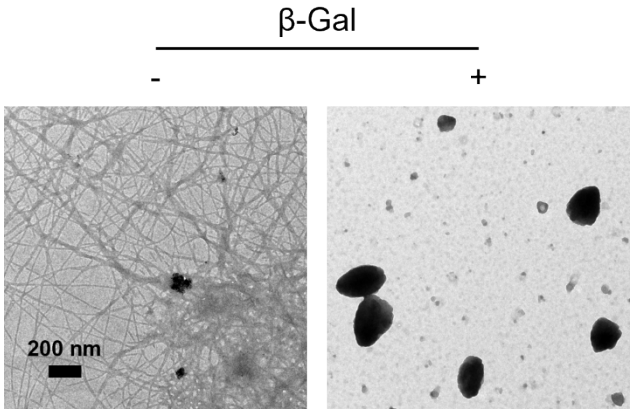


Fig. S4. Transmission-electronic microscopic images of **Gal-TPE-BT** (10 μM)) without and with treatment of β-Gal (8 U mL^{-1}) in a solvent mixture of phosphate buffered saline (PBS) (0.01 M, pH 7.4)/DMSO (7:3, v/v).

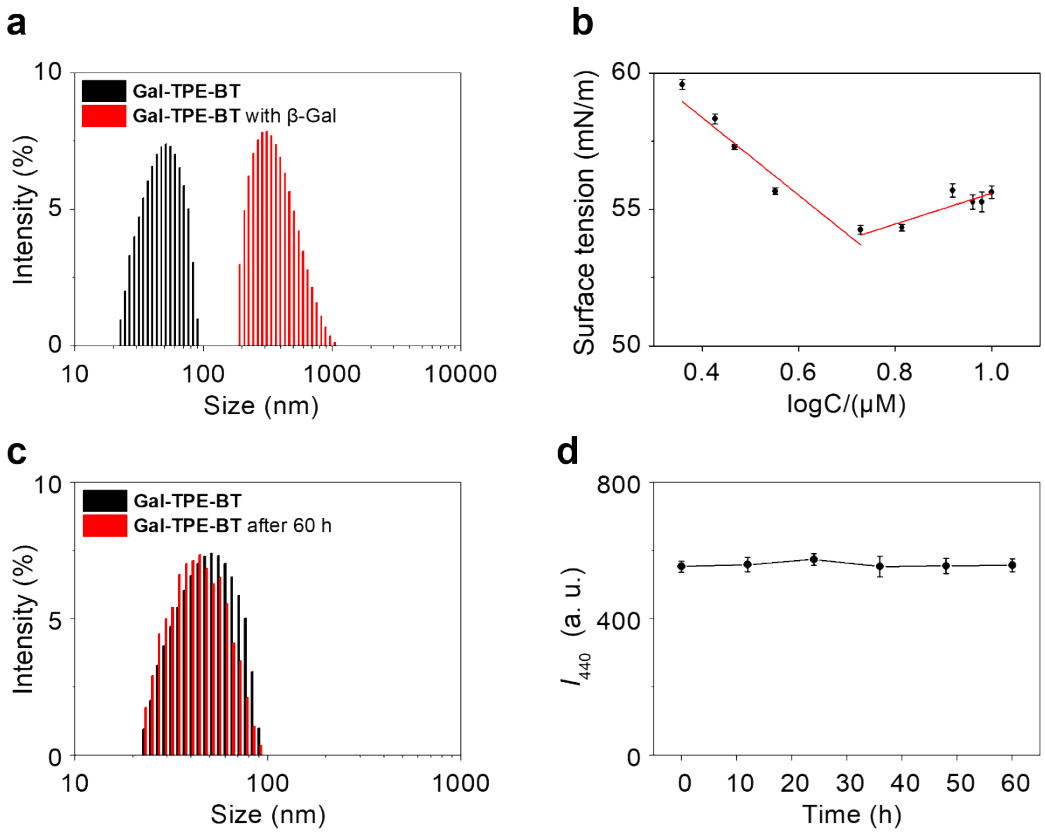
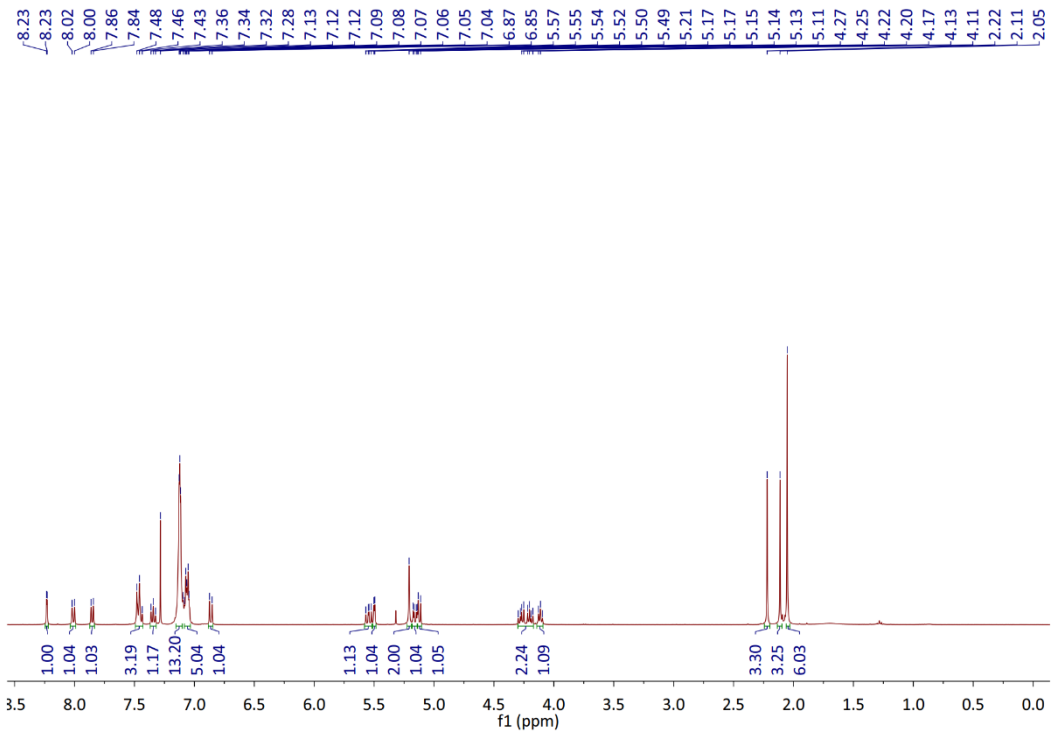
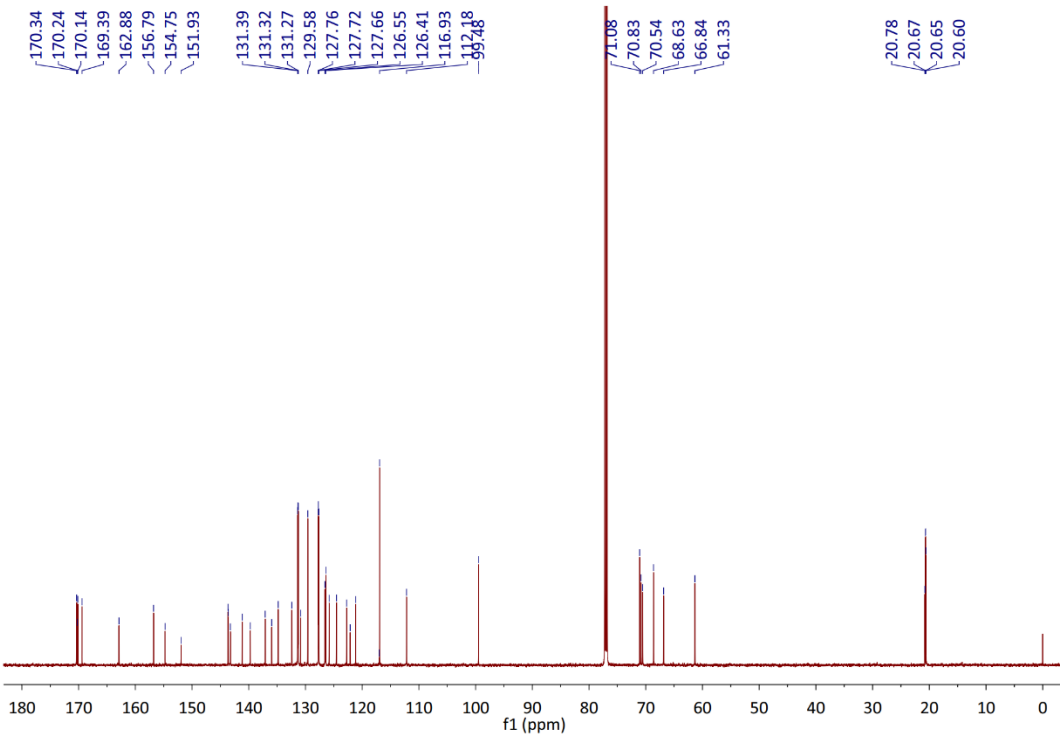


Fig. S5. (a) Dynamic light scattering (DLS) of **Gal-TPE-BT** (10 μM) without and with treatment of β-Gal (8 U mL^{-1}). (b) Surface tension-log C plot of **Gal-TPE-BT**. (c) DLS of **Gal-TPE-BT** (10 μM) before and after incubation for 60 h in PBS. (d) Time-dependent fluorescence emission intensity changes of **Gal-TPE-BT** (10 μM) at 440 nm in PBS. All measurements were performed in a solvent mixture of phosphate buffered saline (PBS) (0.01 M, pH 7.4)/DMSO (7:3, v/v).

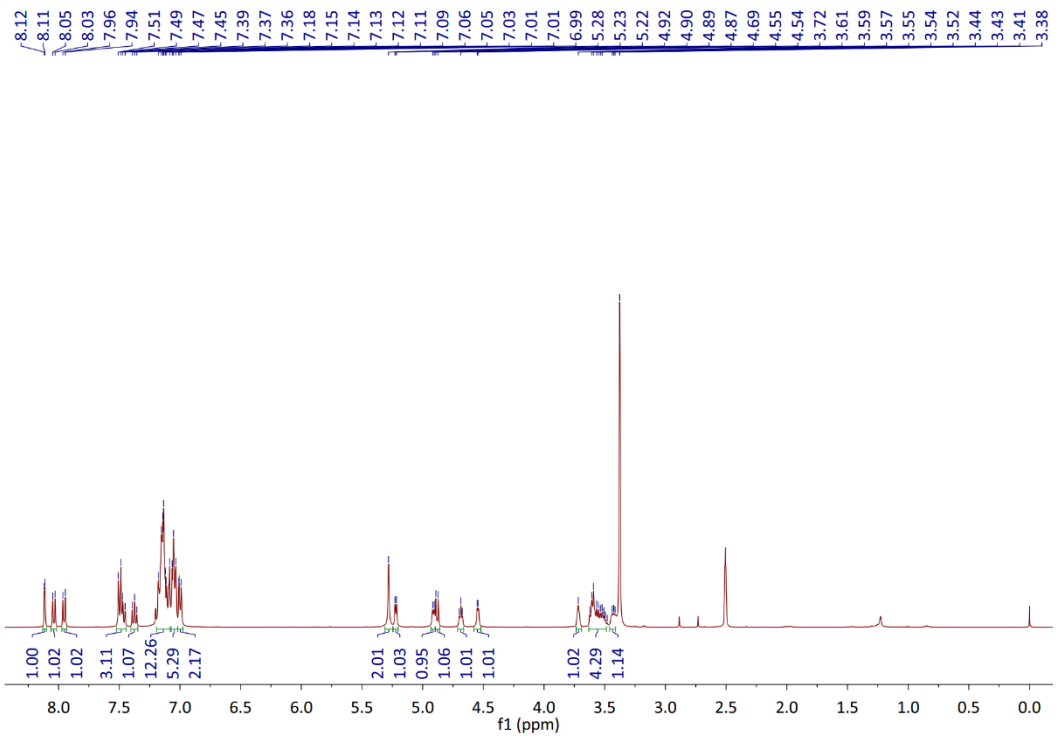
3 Original spectra of new compounds



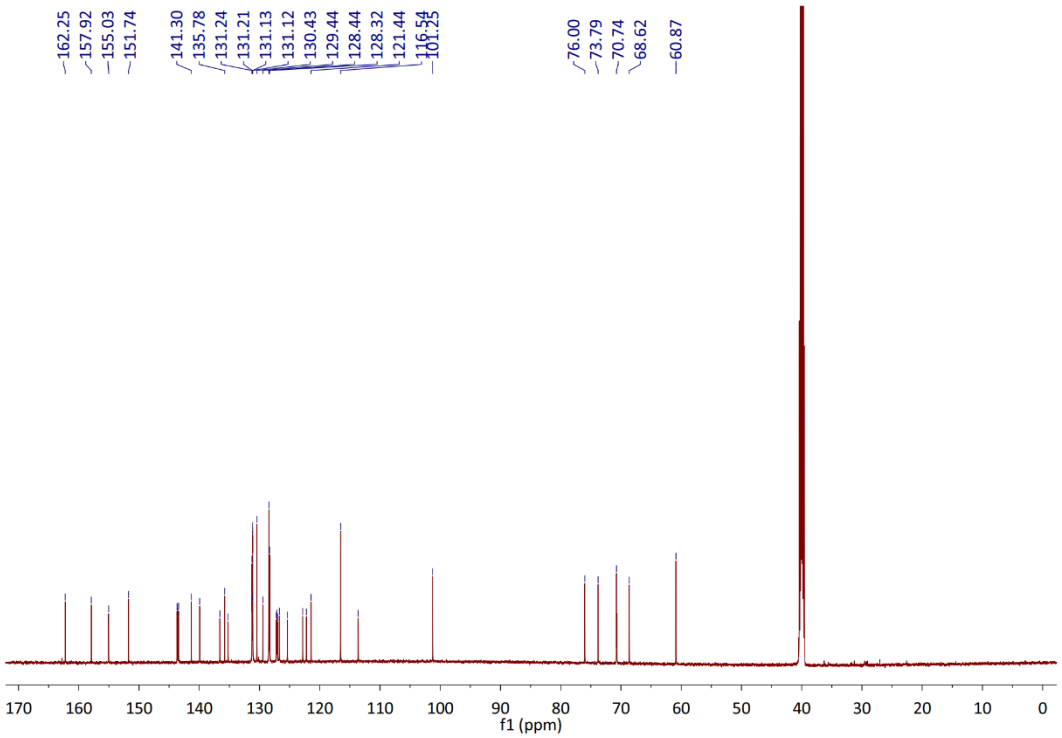
^{1}H NMR of **b**.



^{13}C NMR of **b**.



^{1}H NMR of **Gal-TPE-BT**.



^{13}C NMR of **Gal-TPE-BT**.

4 Additional References

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