Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Informations for

Aminonaphthalimide-based pyridinium probes for selectively fluorescent sensing of maltose in aqueous media and living cells

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1. Experimental

1.1 Reagents and instruments

All reagents and solvents were of AR grade and used without further purification unless otherwise noted. 6-bromobenzo[de]isochromene-1,3-dione, 3-aminopyridine were purchased from Sigma-Aldrich Chemical Company**.** All solvents were dried using standard procedure prior to use.

^{1}H NMR and ^{13}C NMR spectra were recorded on a VARIAN INOVA-400 spectrometer with chemical shifts reported as ppm (in DMSO-d_6 or $CDCl_3$, TMS as internal standard). Mass spectrometric data were obtained on HP1100LC/MSD and LCQ-Tof mass spectrometers. Fluorescence emission spectra were obtained using EDINBURGH FS920 luminescence spectrometer. For all fluorescent measurements, both excitation and emission slit widths were 1 nm. Optical absorption spectra were measured on a TU-1900 Uv/Vis spectrophotometer at room temperature. Cell imaging were measured on Nikon eclipase TE2000-5 inverted fluorescence microscopy.

1.2 General procedures of spectra detection

Stock solutions (5×10^{-2} M) of the saccharides (*D*-Galactose, Erythrose, Mannose, Fructose, Xylose, Glucose, Lactose, Sucrose, Maltose) were prepared in DMF solution. The solution of **TPA1** and **TPA2** were prepared in CH_3CN:H_2O=9:1 (v:v) solution. Test solutions were prepared by placing 40 μL of host stock solution (1×10^{-3} mol/L) into a quartz cell of 1 cm optical path length including 2 mL distilled CH_3CN/H_2O=9:1 (v:v) solution, and then adding an appropriate aliquot of each saccharides stock by using a micro-syringe. All the spectroscopic measurements were performed at least in triplicate and averaged.

1.3 NMR titration method

All NMR spectra were measured on a VARIAN INOVA-400 spectrometer at 298 K. A solution (1 mM) of host **TPA1** in DMSO-d_6 was titrated with maltose (3 mM) in DMSO-d_6 by using a micro-syringe. The chemical shift changes of the proton of maltose units were monitored.

1.4 Cell incubation and imaging

HeLa cells were cultured in 1640 supplemented with 10% FCS (Invitrogen). Cells were seeded in 24-well flat-bottomed plates for Nikon eclipase TE2000-5 inverted fluorescence microscopy. After 12 h, HeLa cells were incubated with 10 μM compound **TPA1** (in the culture medium containing 0.5% DMSO) for 30 min at 37^{o}C under 5% CO_2 and then washed with phosphate-buffered saline (PBS) three times before incubating with 100 eq maltose for another 30 min, and cells were rinsed with PBS three times again. The fluorescence imaging of intracellular maltose in HeLa cells was observed under Nikon eclipase TE2000-5 inverted fluorescence microscopy with a 20×objective lens (excited with blue light). For all images, the microscope settings, such as brightness, contrast, and exposure time were held constant to compare the relative intensity of intracellular maltose fluorescence.

1.5 Synthesis of TPAs

1.5.1 Synthesis of 1

A mixture of 6-bromobenzo[de]isochromene-1,3-dione (2.77 g, 10 mmol) and 3-aminopyridine (0.94 g, 10 mmol) was heated to reflux in EtOH (20 mL) under nitrogen atmosphere for 10 h. After cool to room temperature, pale solid appered, filter cake was washed by EtOH to afford compound **1**. The yield was 3.06 g (82.5%), white powder. Compound **1** was used in the next reaction without any further characterization.

1.5.2 Synthesis of 2

A mixture of **1** (2.61 g, 7.4 mmol) and piperidine (1.2 g, 14 mmol) was heated to reflux in methoxylethanol (50 mL) under nitrogen atmosphere for 10 h. After cool to room temperature,

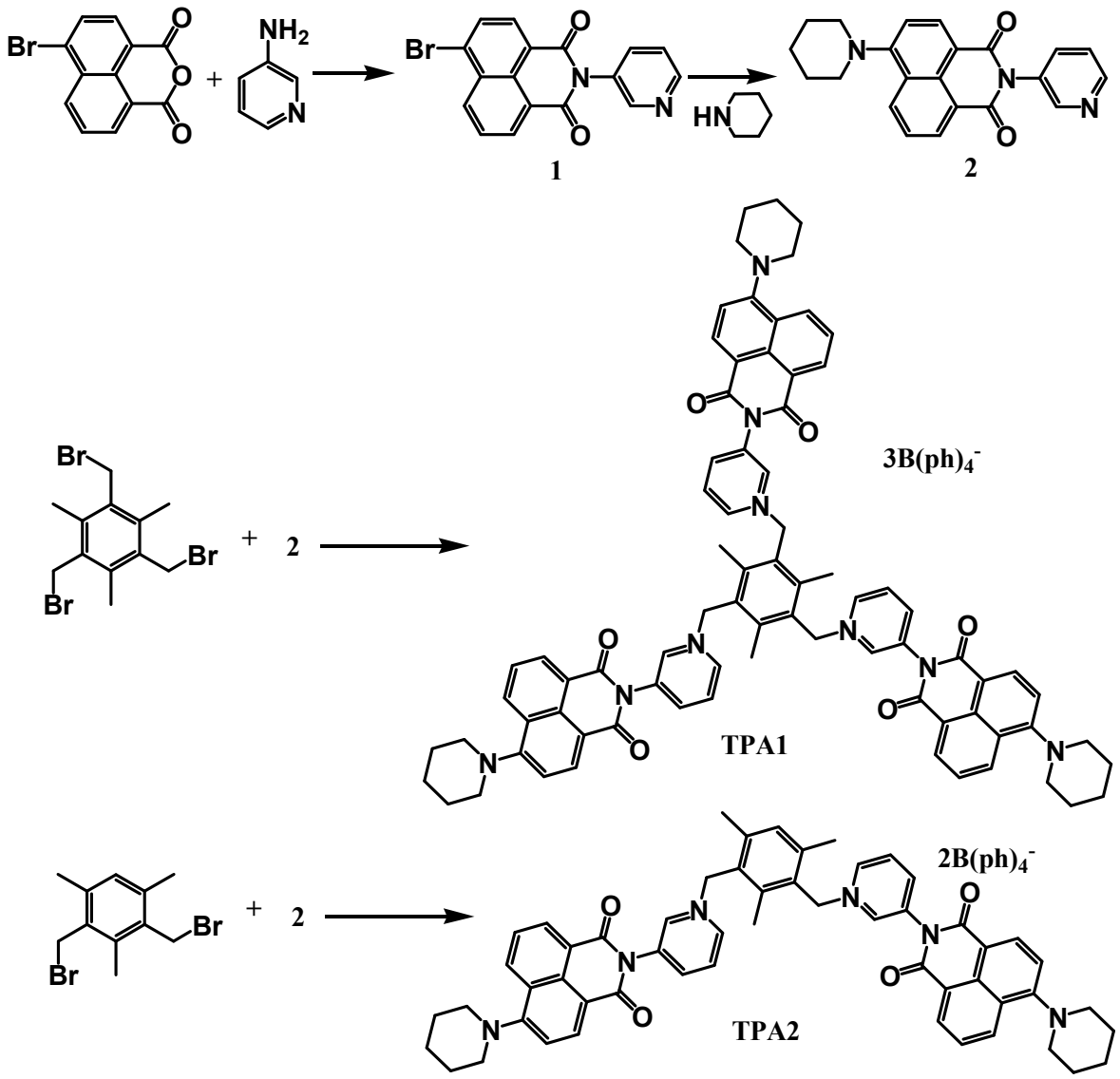
yellow solid appered, filter cake was washed by EtOH to afford compound **2**. The yield was 2.56 g (80%), yellow powder. Compound **2** was used in the next reaction without any further characterization.

1.5.3 Synthesis of TPA1

1,3,5-Tris(bromomethyl)-2,4,6-trimethylbenzene (0.4 g, 1 mmol) and 4-(piperidin-1-yl)-N-(pyridin -3-yl)-1,8-naphthalimide (1.13 g, 3.15 mmol) were dissolved in $CHCl_3$ (30 mL) and stirred at reflux for 15 h. During this time, a yellow precipitate formed. The product was filtered off and washed with $CHCl_3$ to give the desired tribromo anions product as a yellow powder. ^{1}H NMR (DMSO-d_6, ppm) δ: 9.16 (d, $3H_{Ar}$, J = 3), 8.86 (s, $3H_{Ar}$), 8.73 (d, $3H_{Ar}$, J = 4), 8.31 (d, $3H_{Ar}$, J = 8), 8.27 (d, $3H_{Ar}$, J = 8), 8.21 (d, $6H_{Ar}$, J = 8), 7.71 (t, $3H_{Ar}$, J = 8), 7.21 (d, $3H_{Ar}$, J = 8), 6.31 (s, $6H_{CH2}$), 3.15 (m, $12H_{piperidine}$), 2.39 (s, $9H_{CH3}$), 1.82 (m, $12H_{piperdine}$), 1.68 (m, $6H_{piperdine}$). LCQ-Tof MS: 410.20 $[M]^{3+}$, 410.22 $[M]^{2+}$, 655.77 $[M]^{+}$. A solution of the mixture of $3Br^-$ product (0.295 g, 0.2 mmol) and $NaB(C_6H_5)_4$ (0.246 g, 0.72 mmol) was stirred at room temperature in CH_3OH (30 mL) for 1 h. The yellow precipitated **TPA1** formed was filtered, washed with methanol and diethyl ether, and dried in vacuo. The yield was 0.26 g (76%). Anal calc. for $C_{150}H_{132}B_3N_9O_6$: C 82.30, H 6.08, B 1.48, N 5.76, O 4.38%. Found: C 82.33, H 6.05, B 1.51, N 5.74, O 4.37%. ^{1}H NMR (DMSO-d_6, ppm) δ: 9.04 (d, $3H_{Ar}$, J = 6), 8.79 (s, $3H_{Ar}$), 8.71 (d, $3H_{Ar}$, J = 8), 8.30 (d, $3H_{Ar}$, J = 8), 8.24 (d, $3H_{Ar}$, J = 8), 8.19 (d, $6H_{Ar}$, J = 8), 7.68 (t, $3H_{Ar}$, J = 8), 7.174 (d, $3H_{Ar}$, J = 8), 7.171 (m, $24H_{B(ph)4}$), 6.91 (t, $24H_{B(ph)4}$, J = 4), 6.78 (t, $12H_{B(ph)4}$, J = 4), 6.24 (s, $6H_{CH2}$), 3.13 (m, $12H_{piperidine}$), 2.36 (s, $9H_{CH3}$), 1.80 (m, $12H_{piperdine}$), 1.67 (m, $6H_{piperdine}$). ^{13}C NMR (DMSO-d_6, ppm) δ: 164.54, 164.05, 163.56, 163.52, 163.07, 162.78, 157.89, 147.50, 144.25, 144.26, 143.99, 135.99, 133.35, 131.85, 131.64, 129.80, 128.55, 125.81, 125.73, 125.39, 122.00, 115.12, 113.91, 54.34, 26.07, 24.25, 17.36. LCQ-Tof MS: 410.22 $[M]^{3+}$, 655.81 $[M]^{2+}$.

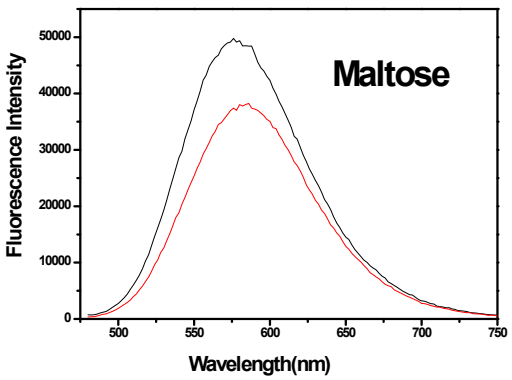
1.5.4 Synthesis of TPA2

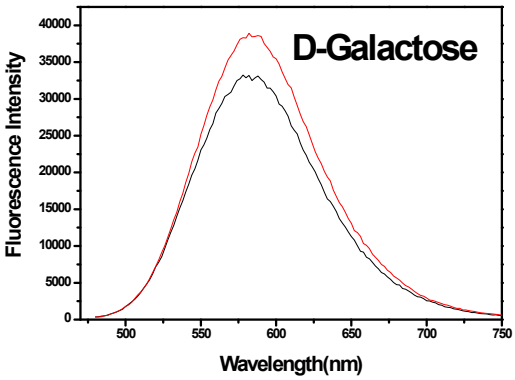
TPA2 was synthesized using the same method as **TPA1** (>60% yield). Anal calc. for $C_{103}H_{92}B_2N_6O_4$: C 82.50, H 6.18, B 1.44, N 5.60, O 4.27%. Found: C 82.48, H 6.20, B 1.42, N 5.62, O 4.27%. ^{1}H NMR (DMSO-d_6, ppm) δ: 9.10 (d, $2H_{Ar}$, J = 4), 8.74 (d, $2H_{Ar}$, J = 8), 8.71 (s, $3H_{Ar}$), 8.34 (d, $2H_{Ar}$, J = 4), 8.33 (d, $2H_{Ar}$, J = 4), 8.30 (d, $2H_{Ar}$, J = 8), 8.23 (d, $2H_{Ar}$, J = 8), 7.72 (t, $2H_{Ar}$, J = 16), 7.29 (s, $1H_{Ar}$), 7.22 (d, $2H_{Ar}$, J = 4), 7.21 (m, $16H_{B(ph)4}$), 6.92 (t, $16H_{B(ph)4}$, J = 16), 6.78 (t, $8H_{B(ph)4}$, J = 16), 6.12 (s, $4H_{CH2}$), 3.13 (m, $8H_{piperidine}$), 2.39 (s, $6H_{CH3}$), 2.10 (s, $3H_{CH3}$), 1.80 (m, $8H_{piperdine}$), 1.67 (m, $4H_{piperdine}$). ^{13}C NMR (DMSO-d_6, ppm) δ: 164.15, 163.59, 163.17, 163.09, 162.61, 162.45, 157.36, 146.88, 144.26, 143.64, 141.53, 140.61, 135.74, 135.52, 132.81, 131.44, 131.10, 129.50, 128.09, 127.79, 125.71, 125.29, 125.09, 121.86, 121.49, 113.80, 53.87, 25.59, 23.77, 19.91.LCQ-Tof MS: 430.33 $[M]^{2+}$, 582.35 $[M]^{+}$.

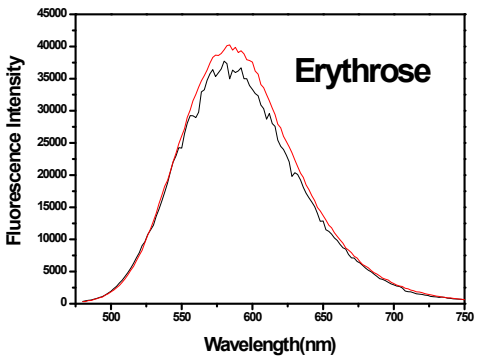


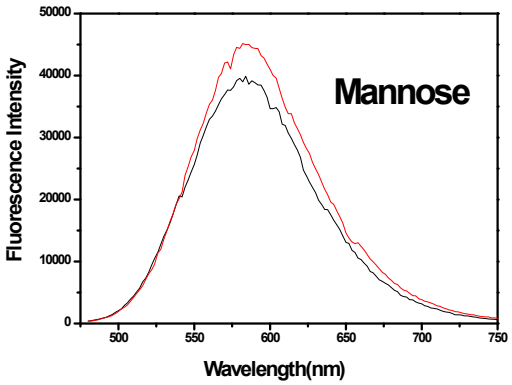
2. Scheme. S1 Synthetic procedure of **TPA1** and **TPA2**.

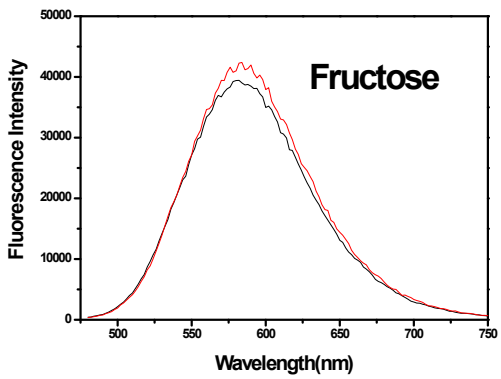
3. Figure S1 Family of fluorescence spectra of **TPA1** (red line, 20 μM) upon the addition of saccharides (black line, 1.6 mM).

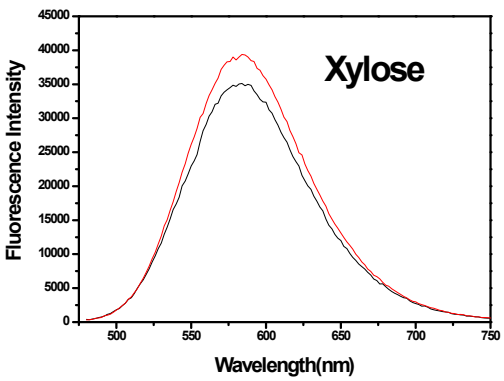


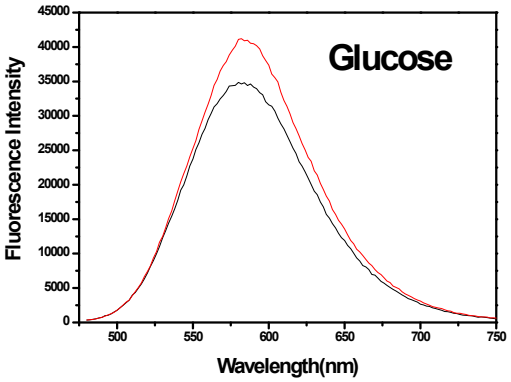








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Fluorescence Intensity
Wavelength(nm)
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30000
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20000
15000
10000
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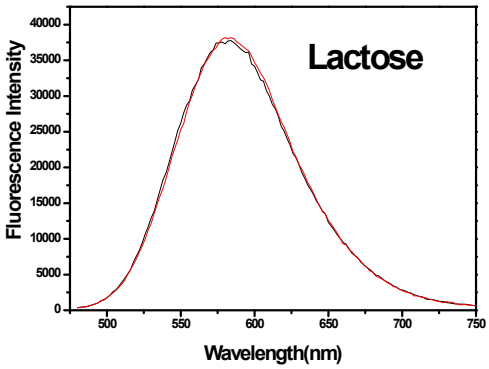
0
500
550
600
650
700
750


Lactose
Fluorescence Intensity
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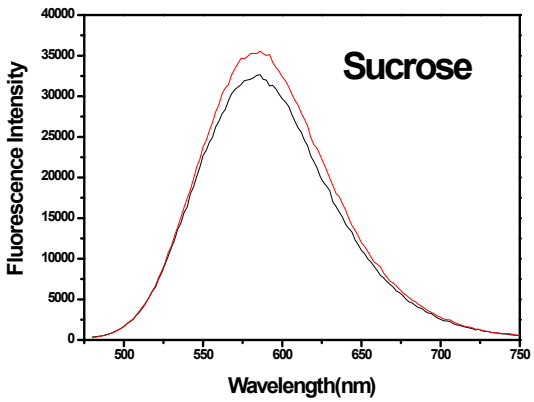
Sucrose
Fluorescence Intensity
Wavelength(nm)
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30000
25000
20000
15000
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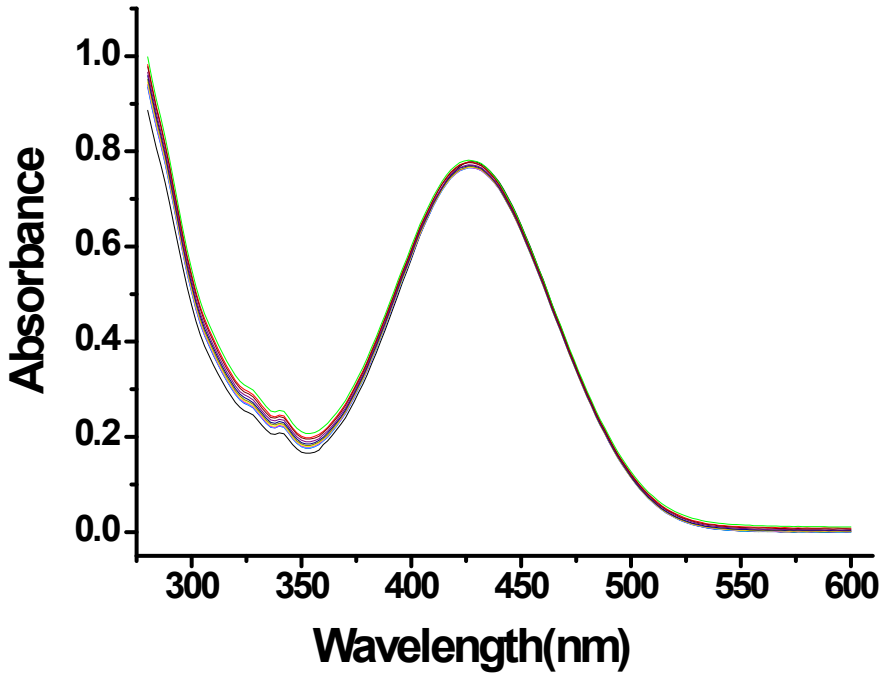
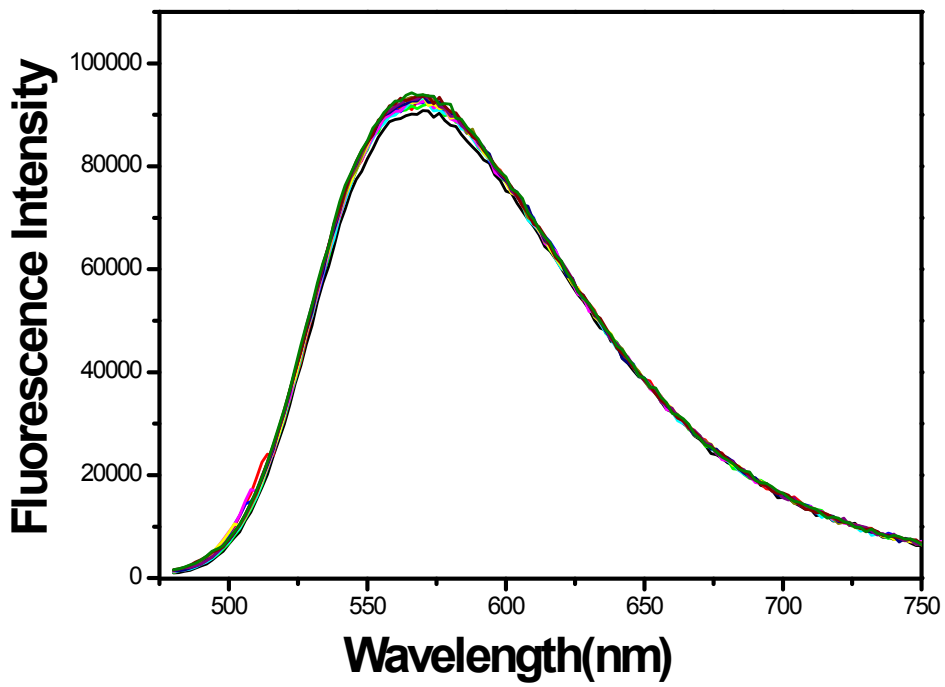
500
550
600
650
700
750

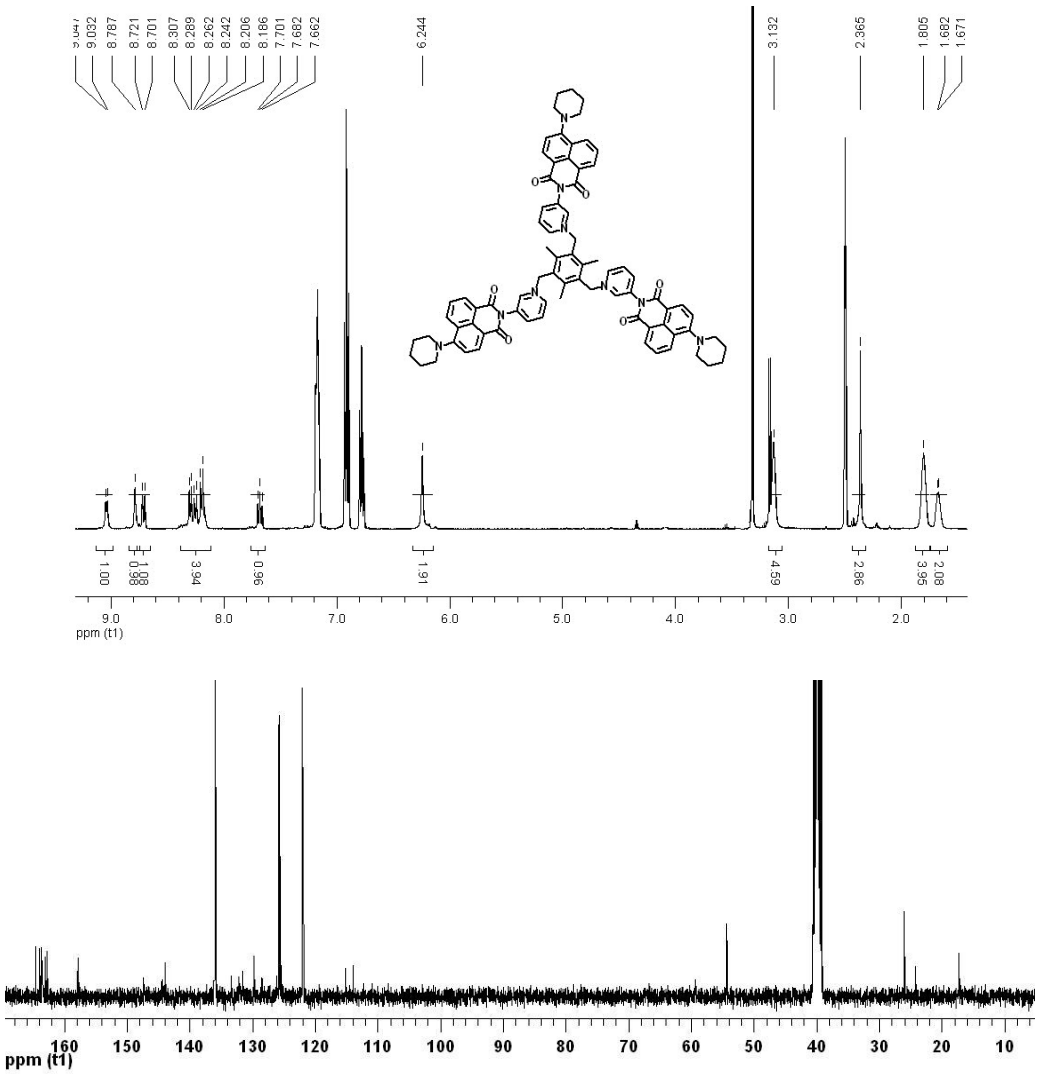
4. Figure S2 Absorption spectra of **TPA1** upon addition of increasing amounts of maltose

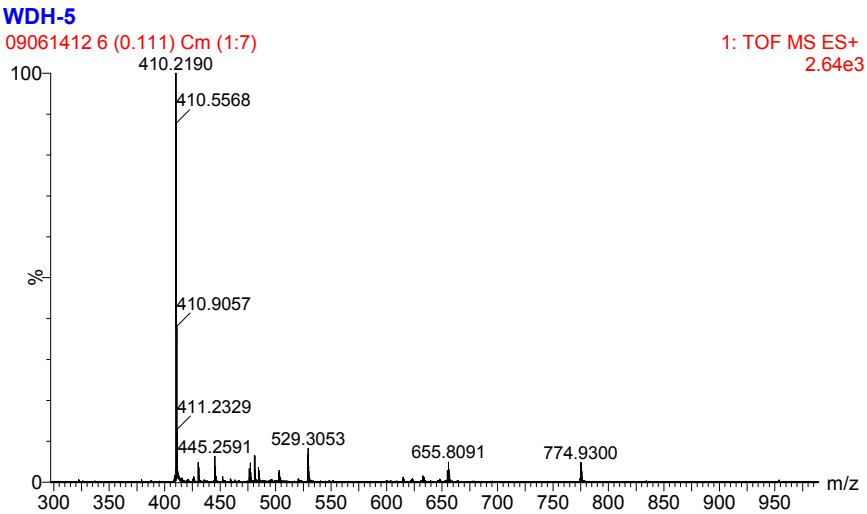


5. Figure S3 Fluorescence spectra of **TPA2** upon addition of increasing amounts of maltose.

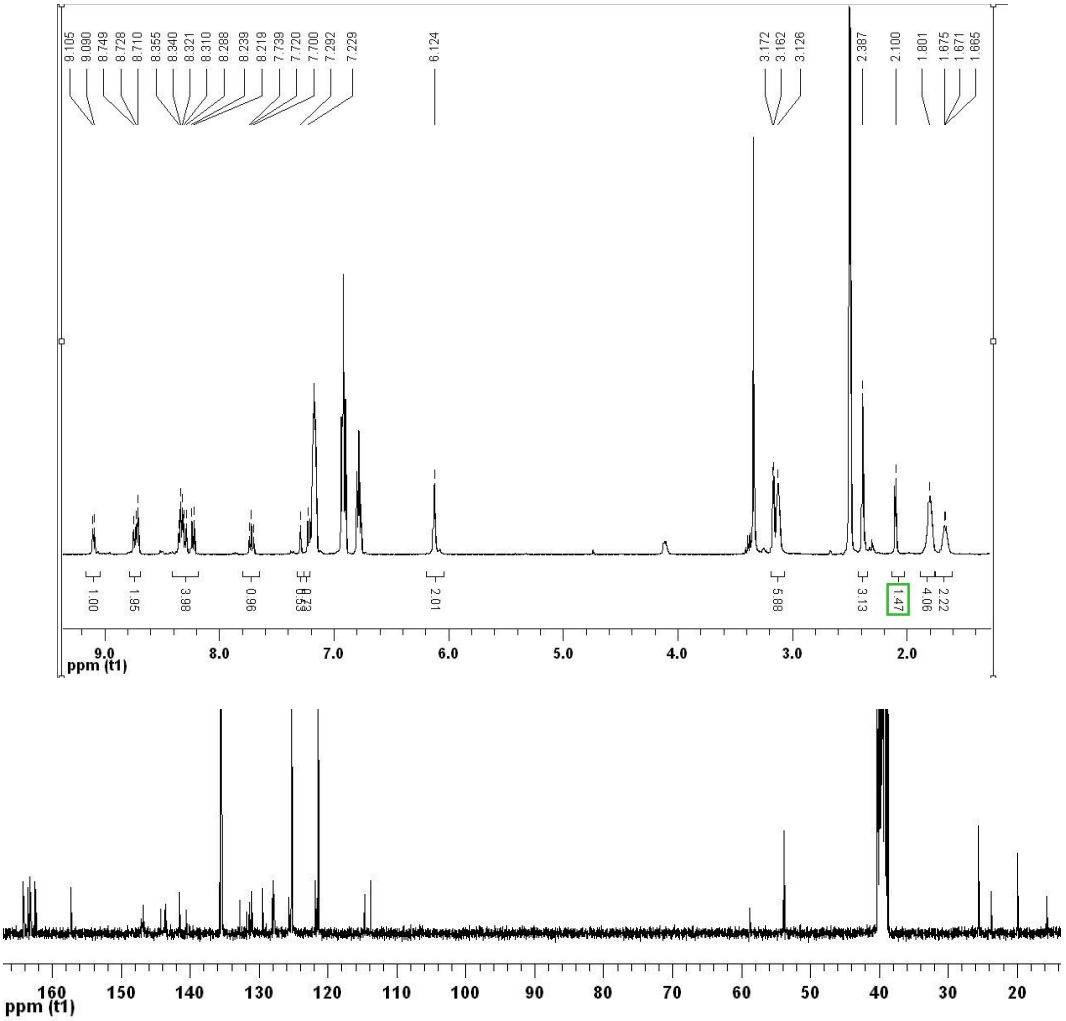


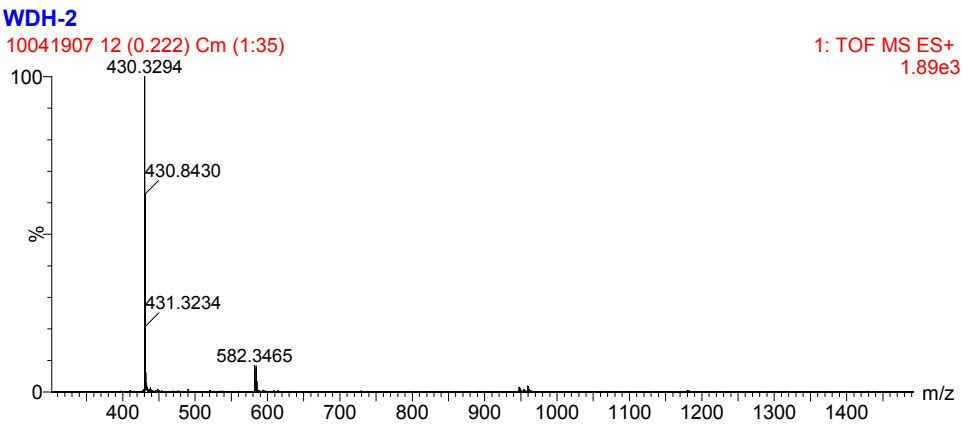
6. Figure S4 ^{1}H-NMR, ^{13}C-NMR and Mass spectra of **TPA1**.



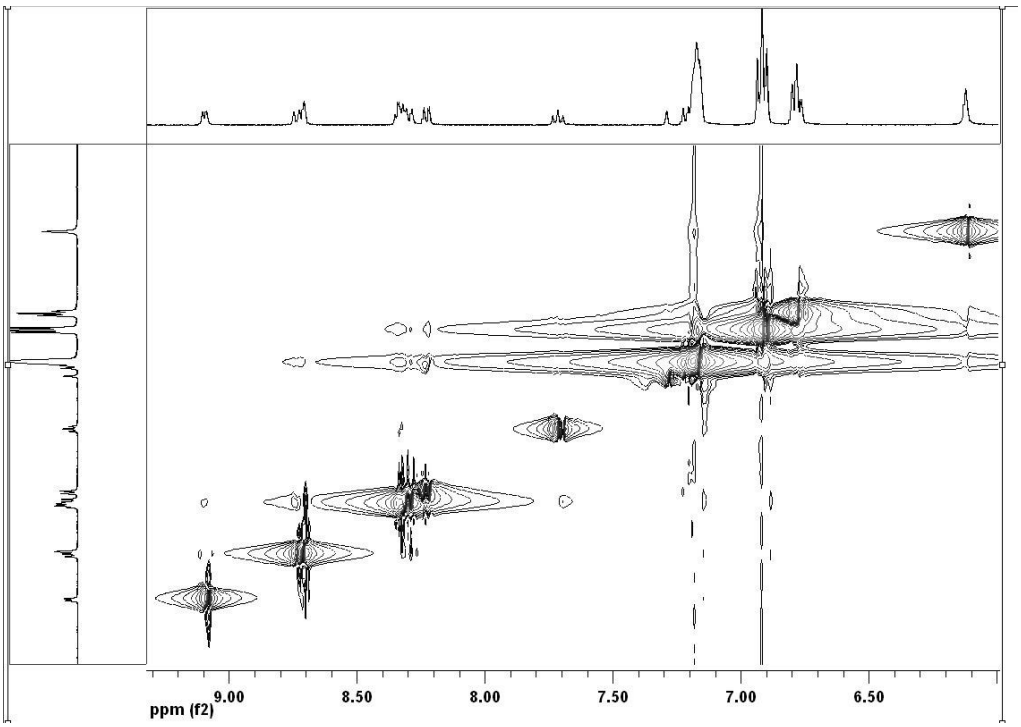


7. Figure S5 ^{1}H-NMR, ^{13}C-NMR and Mass spectra of **TPA2**.

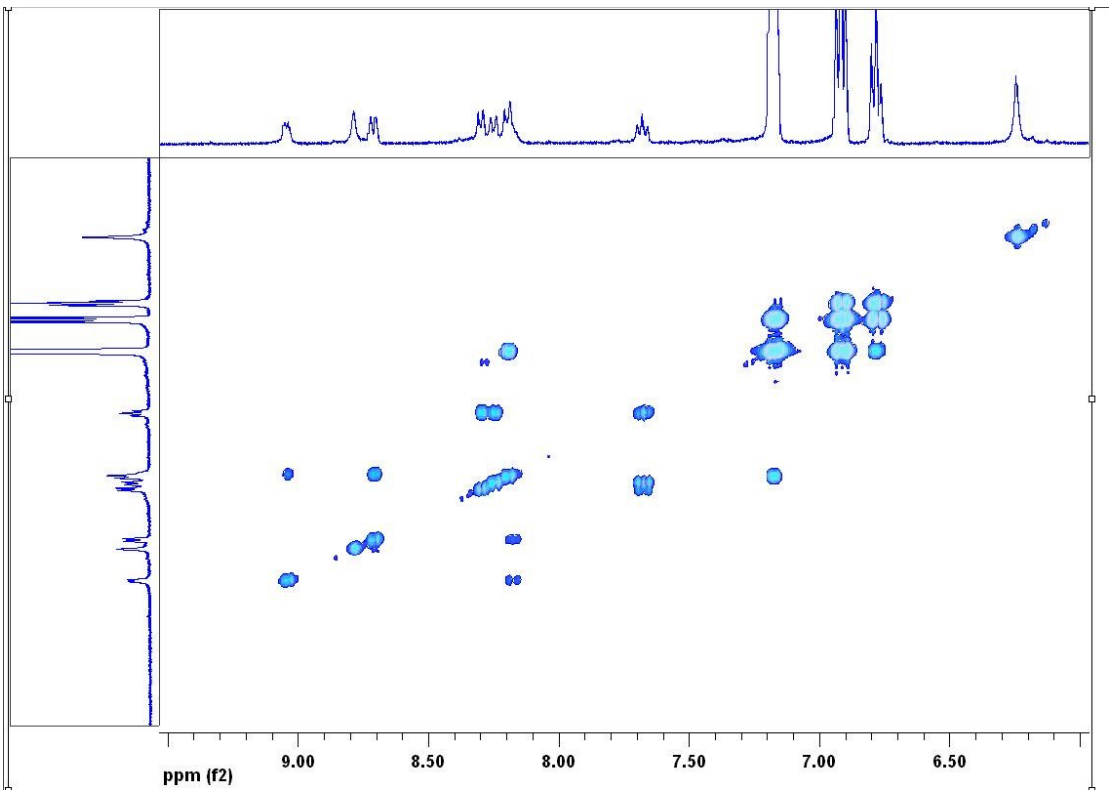




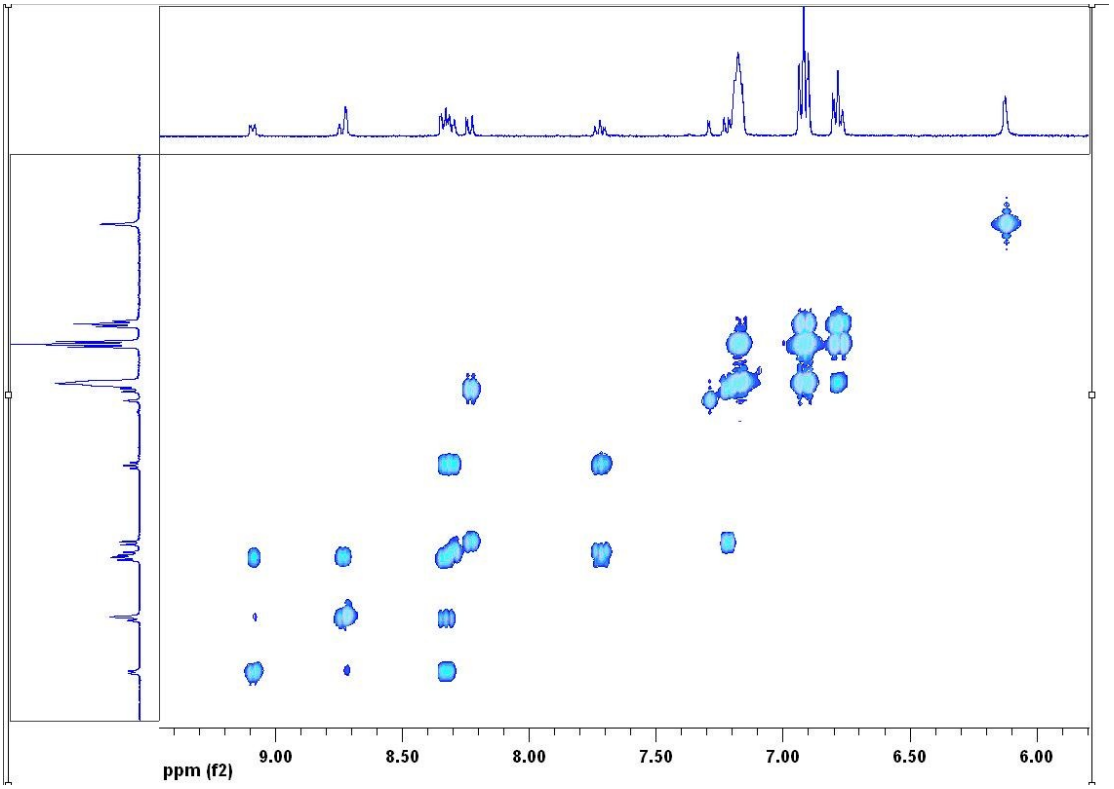
8. Figure S6 2D Noesy of **TPA2**



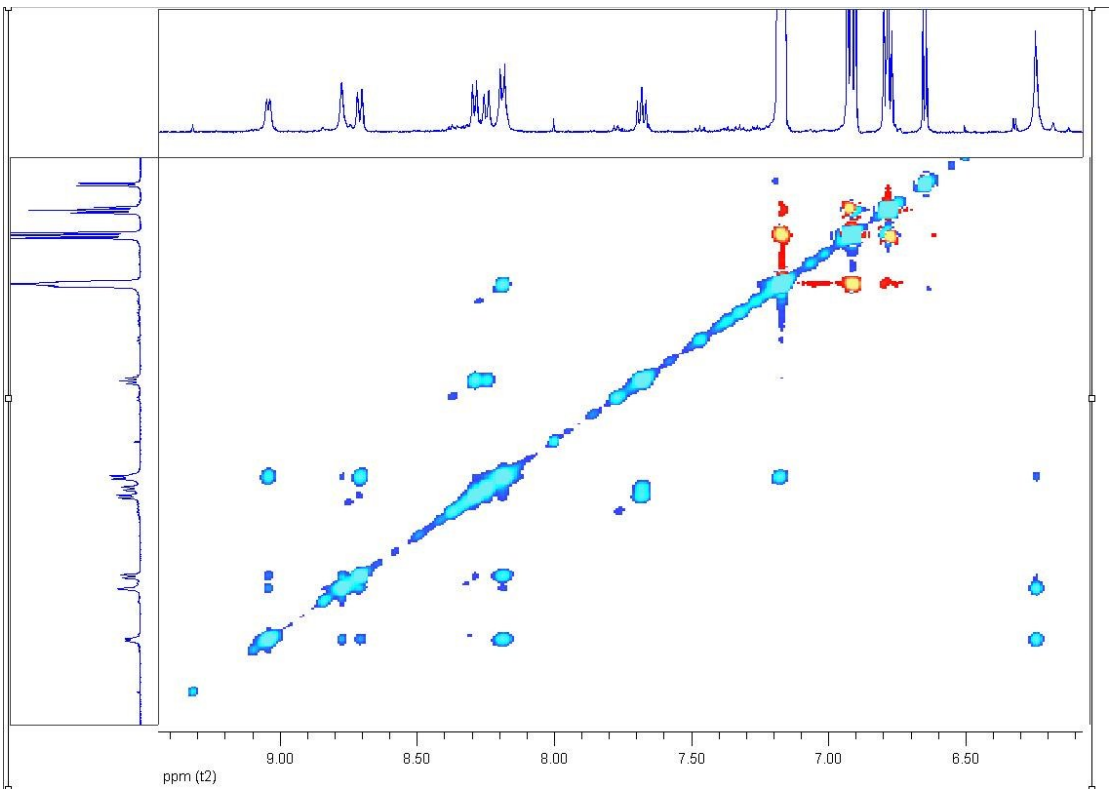
9. Figure S7 2D Cosy of **TPA1**



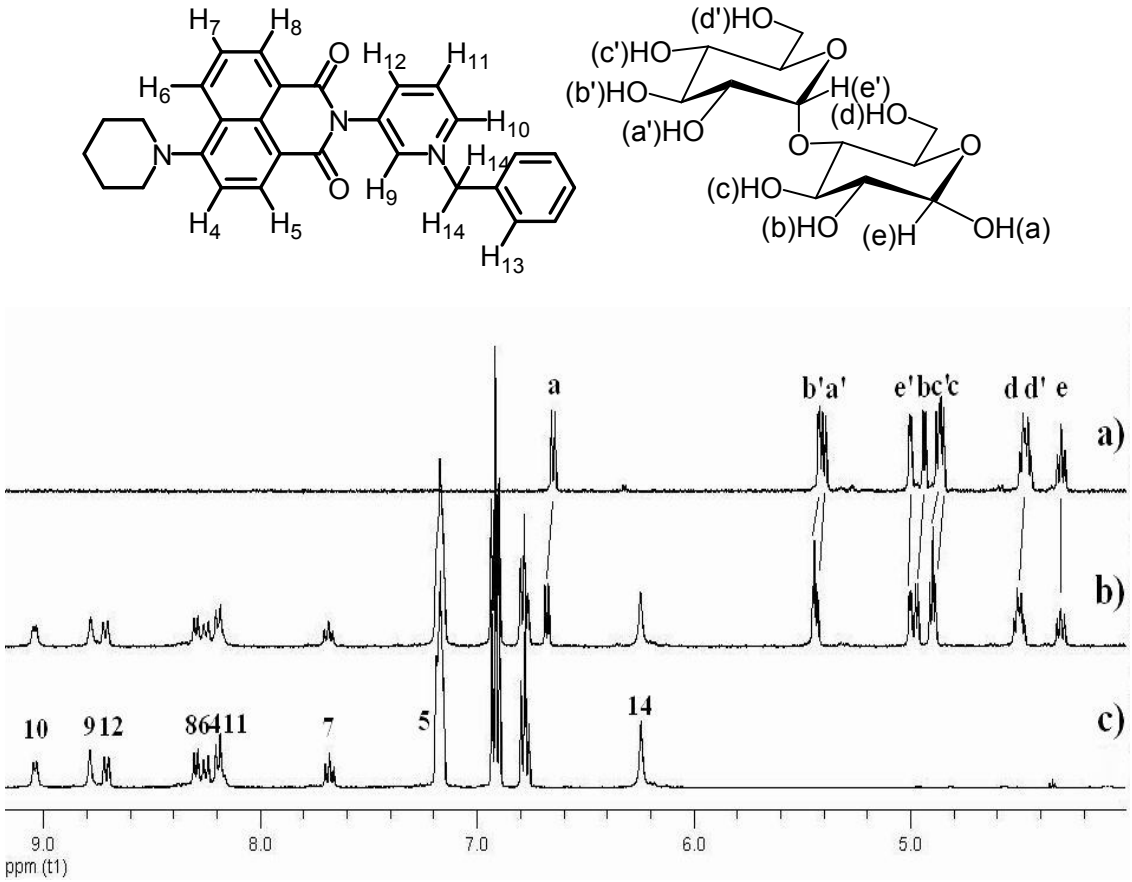
10. Figure S8 2D Cosy of **TPA2**



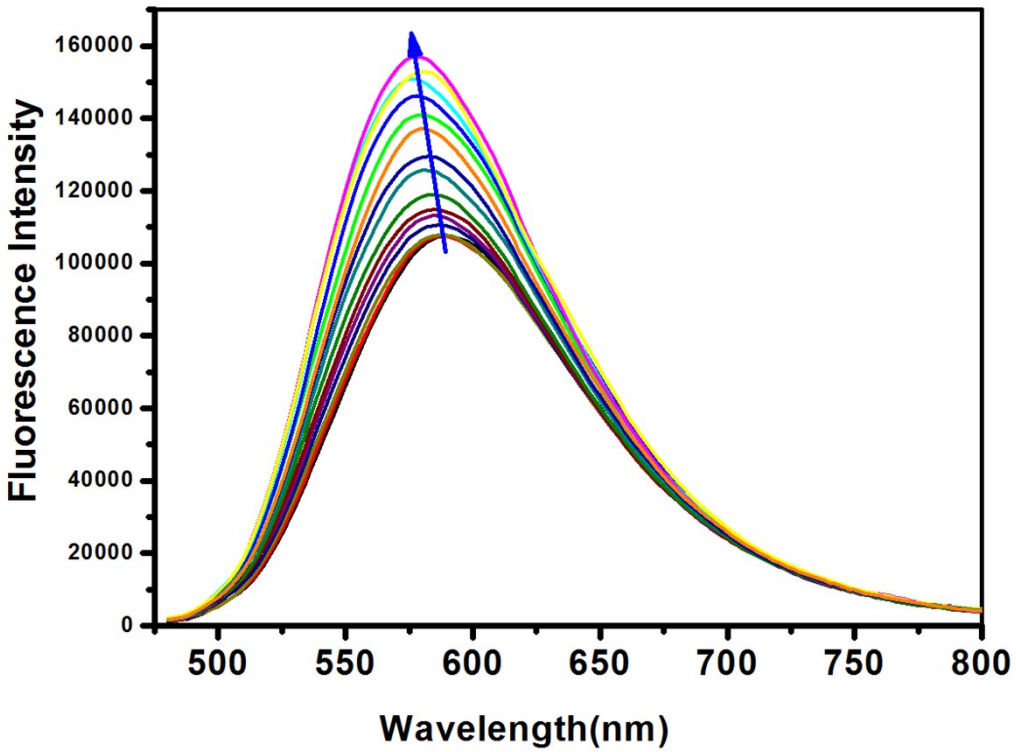
11. Figure S9 2D Noesy of **TPA1+Maltose.**

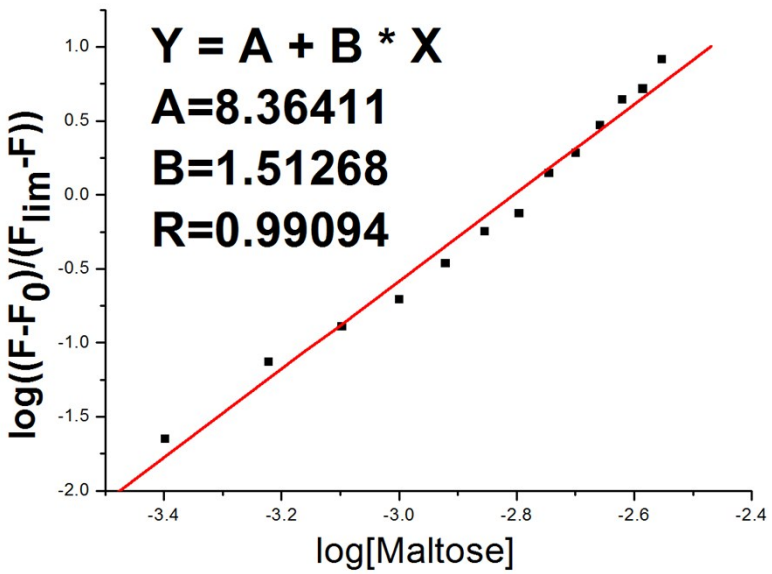


12. Figure S10 Partial ^{1}H NMR spectra for pure maltose (a), **TPA1**+maltose (b) and **TPA1** (c).



13. Figure S11 Fluorescence spectra of **TPA1** (20 μM) in aqueous solution upon addition of increasing concentrations of maltose with an excitation wavelength at 468 nm. Scan slit:2 nm. liner of log $((F-F_0)/(F_{lim}-F)$ vs. log [maltose]. (A present fluorescence of **TPA1** at 590 nm).





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-3.3757	-1.70234

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-3.21558	-1.22484
-3.1622	-1.06568
-3.10883	-0.90651
-3.05545	-0.74735
-3.00208	-0.58818
-2.9487	-0.42902
-2.89533	-0.26985
-2.84195	-0.11069
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