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

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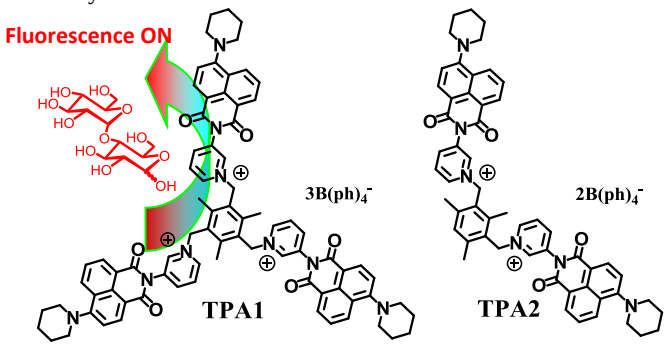
New aminonaphthalimide-based pyridinium podands as "turn-on" fluorescent probes were designed and synthesised for selectively sensing maltose in aqueoues media and living cells.

The design of artificial carbohydrate sensors operating via non-covalent interactions remains a subject of the current research, due to the broad utility in wide-ranging applications from the food and cosmetic industries to medicinal and academic arenas.[1] While advances in this areas have been given insight intervene into biologically important recognition phenomena and facilitated the development of therapeutic chemosensors, challenges are still remain in developing probes for saccharides which meet the criteria necessary for the application in real-world settings.[2] To realize the applications of these sensors in an industrial setting include their use in the quality control of food and dietary products and monitoring the fermentation of beverages and extracts.[3] They must be relatively easy to synthesize, operate at constant physiological pH in aqueous media, and target multiple saccharides and its derivatives with high selectivity.[4]



Scheme 1. Structures of **TPA1** and **TPA2**.

On the other hands, difficulties on developing saccharides sensors also arise from the lack of a spectroscopic handle, such as a chromophore or fluorophore, whose modulation could be harnessed in a sensing scheme.[5] While fluorescence sensing by means of reversible formation of covalent bonds from diol units and boronic acid has been relatively successful and exhibits good selectively between disaccharides,[6] there are few effective systems for luminescence sensing natural saccharides by using non-covalent bonds relevant to biological carbohydrate recognition.[7] As a continuation of our research work on the tripodal receptors,[8] by incorporating 1,8-naphthalimide fluorophore onto the pyridinium-based tripodands,[9] herein, we report the syntheses and carbohydrate binding properties of new pyridinium-based receptor for fluorescent discriminating of natural saccharides in aqueous media. Interestingly the green (λ~540-550 nm) with high quantum yields (Φ_f)[10] and visible-light excitation of aminonaphthalimide groups provide an opportunity to luminescent image natural saccharides in living cells.

TPAs were synthesized by the reaction of 4-(piperidin-1-yl)-1-(pyridin-3-yl)-1,8-naphthalimide with the respectively two and three arms bromomethylbenzene derivatives, and followed by an anion exchange reaction with $NaB(C_6H_5)_4$. ESI-MS spectra of **TPA1** (1 mM) exhibited an intenses peak at about *m/z* = 410.22, corresponding to the trivalent species of **TPA1** receptor, confirming the formation of tripodal pyridinium-based receptor (see supporting materials). ^{1}H NMR spectra of the receptor **TPA1** (1 mM) consistent well with the simulated chemical shifts. Interestingly, 2D NOESY of **TPA1** exhibited significant cross peaks corresponding to H4-H7 and H5-H6 (Fig. 1), demonstraing the possible intra- and/or intermolecular stacking interactions between these 1,8-naphthalimide groups. But no obvious of these nuclear overhauser effect (NOE) signal could be observed from the 2D NOESY spectra of **TPA2** (Fig. S10, ESI†).

TPAs exhibited a 1,8-naphthalimide characteristic absorption band cenerted at 425 nm (log ε = 5.33) in CH_3CN: H_2O (9:1/v:v) solution. Upon excitation at 468 nm, **TPA2** and **TPA1** exhibited emissions at 570 nm and 590 nm, respectively (Fig. 2). The higher energy band in the solution of **TPAs** was safely ascribed to aminonaphthalimide emission,[11] whereas, the obvious red-shift of emmision bands with the increasing number of arms might be attributed to the possible excimer emission caused by the intra- or/and intermolecular interactions of the luminophores.[12]

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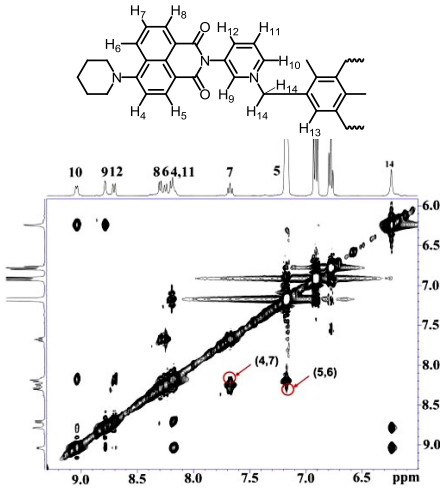


Fig. 1 Partial ^{1}H-^{1}H NOESY spectra of **TPA1** (1 mM) in DMSO-d^6 showing the possible intra- and/or intermolecular interactions between H_4 and H_7, H_5 and H_6 (marked in red cycles).

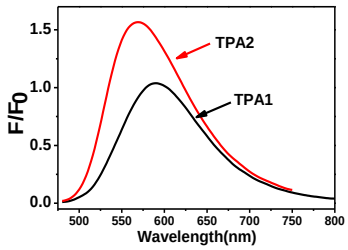


Fig. 2 Fluorescence spectra of **TPA2** (30 uM) and **TPA1** (20 uM) in CH_3CN:H_2O (9:1/v:v) solution. Excitation at 468 nm.

Upon addition of maltose to the solution containing **TPA1** (20 μM), a fluorescence enhancement of the 1,8-naphthalimide characteristic emission was observed (Fig. 3). And the titration curve showed a steady and smooth increase until a plateau was reached (Φ_f = 0.15).[13] The nonlinear fitting of the titration curve suggested a 2:3 stoichiometry of the host-guest complexation species with the association constant (log K_{ass})[14] calculated as 12.76 (Fig. S11**,** ESI†). Under the same conditions no significant fluorescence enhancements of **TPA1** were observed in the presence of a variety of tested saccharides (0.15 mM) (*D*-Galactose, Erythrose, Mannose, Fructose, Xylose, Glucose, Lactose, Sucrose, Maltose). These results suggested that **TPA1** was a useful probe for the selectively fluorescent sensing of maltose (Fig. 4).

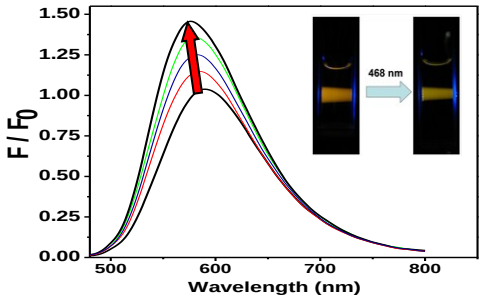


Fig. 3 Emission spectra and visual change (insert) of **TPA1** (20 *u*M) upon addition of different concentration of maltose in CH_3CN:H_2O = 9:1 (v:v), up to 0.25 mM, excitation at 468 nm.

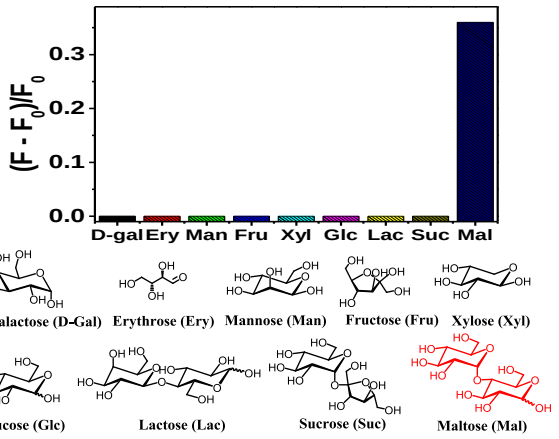


Fig. 4 Fluorescent responses (576 nm) of **TPA1** (20 uM) upon the addition of 0.2 mM of saccharides interested in CH_3CN:H_2O (9:1/v:v) solution. excitation at 468 nm.

ESI-MS of **TPA1** in the presence of maltose exhibited new peaks at about *m/z* 524.66, 638.71 and 752.77, assignable [**TPA1**+mal]$^{3+}$, [**TPA1**+2mal]$^{3+}$ and [**TPA1**+3mal]$^{3+}$, respectively (Fig. 5), supporting the formation of Mal–**TPA1** host-guest comlexation species. ^{1}H NMR spectra of the receptor **TPA1** (1 *m*M) upon addition of maltose (3 *m*M) exhibited small but significant downfield shifts of these hydroxyl protons (Fig. S10, ESI†), suggesting the possible interactions corresponding to these hydroxyl groups. Most importantly, beside the fluorecence enhancement of **TPA1**, the addition of maltose also caused significant blue-shift (about 15 *nm*) of the emission band. Such that the emission spectrum of the Mal–**TPA1** host-guest comlex was quite similar to that of the free **TPA2** (Fig. 2). And no obvious emission spectral changes of **TPA2** upon addition of maltose in the same condition **(**Fig. S3**,** ESI†**)**.

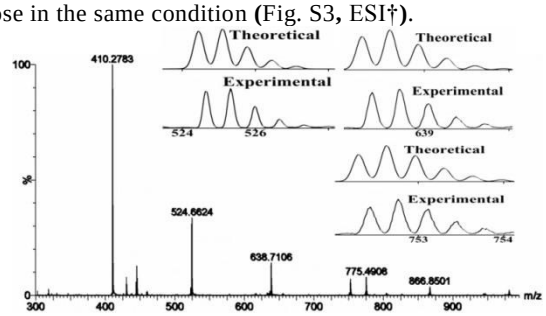
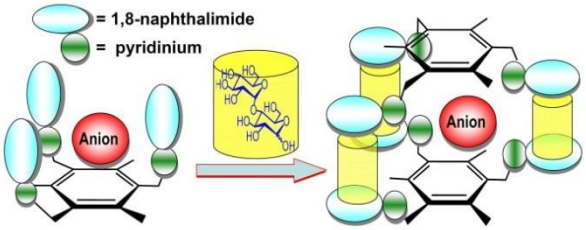


Fig. 5 Mass spectrum of **TIA1**+nMaltose in CH_3CN:H_2O (9:1/v:v) solution.

From a mechanitic view of point, the fluorescence enhancement with the blue-shift is partly due to the absence of efficient intramolecular interactions between these 1,8-naphthalimide groups in this podands system.[15] In fact, the disappeared H_4–H_7 and H_5–H_6 NOE signal (Fig. S9, ESI†) in 2D NOESY of **TPA1** in the presence of maltose agreed well with the hypothesis. At the mean time, the selectivity of the response with maltose over other saccharides was ascribed the suitable hydrogen bonds location of the **TPA1** and the special stairs type of maltose. Since the maltose-binding did not change the absorbance spectra significantly (Fig. S2**,** ESI†), the maintenance of the emission wavelength with the significant luminescence enhancement possibly suggested a PET mechanism. It seemed that the enhancement of the fluorescence was due to a reduced electron-charge density at the pyridinium site after the binding with maltose, which reduced the "push-pull" nature of the IC

excited state of **TPA1** (caused by the electron-donating amine and the electron-withdrawing pyridinium). [16]



Scheme 2. Presumptive binding-scheme showing the 2:3 stoichiometry of the host-guest complexation species of **TPA1** with maltose.

We further investigate the biological application of **TPA1** in cultured cell (HeLa cells). HeLa cells incubated with **TPA1** (10 μM) for 30 minutes at room temperature showed a weak yellow green intracellular fluorescence, which suggested that **TPA1** was cell permeable (Fig. 6a). The cells remained viable and no apparent toxicity and side effects were observed throughout the imaging experiments. When cells stained with compound **TPA1** were further incubated with maltose (1 mM) in phosphate-buffered saline (PBS) for 30 minutes and washed, a remarkable enhancing of the green fluorescence intensity (Fig. 6b) and a long wavelength blue shifted phenomena (corresponding the fluorescence titration) was observed, suggesting the successful application in the maltose stain experiments.

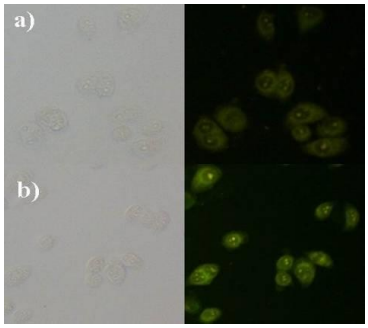


Fig. 6 Blue emission (485-550 *nm*) images of HeLa cells incubated with **TPA1** (10 uM) (a) and their images after further incubated with maltose (1 mM) (b).

Conclusions

In summary, we have reported a new type chemical sensor **TPA1** for maltose. **TPA1** exhibits a selective "turn-on" fluorescent property for maltose over other saccharides in aqueoues media. Fluorescence, NMR and MS spectra demonstrate the strong binding propertity may be an important factor influencing the fluorescence response to maltose. The sensor is also successfully applied to the cells imaging for the maltose.

Acknowledgements

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