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Synthesis of a Novel BODIPY Library and Its Application in the Discovery of a Fructose Sensor

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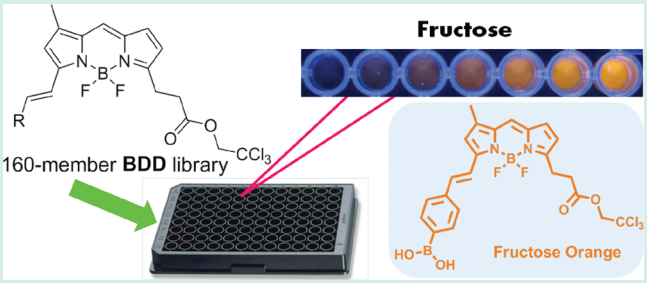
Supporting Information

ABSTRACT: We prepared a new library of 160 compounds by conjugation of a BODIPY core to a collection of aldehydes. This library was screened against 52 biologically relevant analytes and we identified one fluorescent sensor of fructose (Fructose Orange). Fructose Orange showed a 24-fold fluorescence increase upon recognition of fructose and an outstanding selectivity among 24 different saccharides. NMR studies confirmed that five different binding interactions were formed between the sensor and fructose. Furthermore, Fructose Orange was applied to the quantification of fructose in soft drinks, being the most selective fluorescent sensor for fructose reported to date.

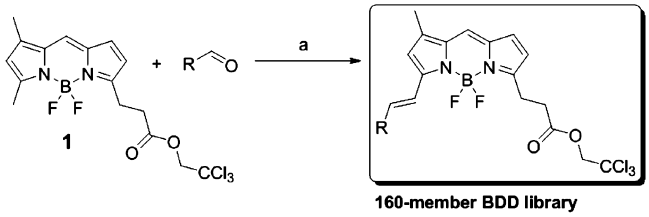


KEYWORDS: *chemosensor, saccharides, fluorescence, molecular recognition*

4,4-Difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) is a versatile fluorophore because of its high quantum yield, tunable fluorescence characteristics, high photostability, and narrow emission bandwidth.[1] Numerous labeling agents derived from the BODIPY scaffold have been reported and even commercialized.[2] On the other hand, chemosensors based on the BODIPY scaffold are relatively rare,[3] and most of the BODIPY derivatives are prepared and tested on a case-by-case basis. We recently reported the first solution and solid-phase syntheses of BODIPY libraries (i.e., BD[3,4] and BDM[5]). The preparation of these combinatorial libraries and their unbiased high-throughput fluorescent screenings accelerated the discovery of new chemosensors, and a number of probes for glucagon, dopamine, bovine serum albumin, and immunoglobulin were discovered. Herein, we report an extended BODIPY library (BDD) and the discovery of a selective and highly sensitive fluorescent sensor of fructose.

Many commercially available BODIPY probes (e.g., LysoTracker Green DND-26, ALDEFLUOR, BODIPY FL C5-ceramide) are prepared from 4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionic acid (BODIPY FL), partially due to its straightforward derivatization. One key intermediate of these probes is the compound **1** (Scheme 1), an activated ester of BODIPY FL.[6] We envisioned that the combinatorial derivatization of **1** may render a suitable toolbox for the discovery of new fluorescent chemosensors, and prepared 160 **BDD** compounds in high purities by Knoevenagel condensation of **1** with structurally diverse aldehydes (Scheme 1 and Supporting Information Table S2). The condensation reaction led to a red shift in the fluorescence emission properties of **1**, due to the extended π-conjugation system in **BDD** compounds.[7] The broad chemical diversity of the aldehyde building blocks gave rise to very diverse spectral properties for **BDD** derivatives: absorption wavelengths ranged from 540 to 627 nm, emission wavelengths from 560 to 795 nm and quantum yields varied from 0.006 to almost 1. This wide range of spectroscopic properties asserted the potential of the **BDD** library as a multicolor (from yellow to near-infrared) and

Scheme 1. General Synthetic Scheme of the BDD Library[a]



[a]Reagents and conditions: (a) pyrrolidine (6 equiv), acetic acid (6 equiv), ACN, 85 °C, 15 min.

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multitype (turn-on or quenching) toolbox for the development of new fluorescent probes.

To discover novel BDD sensors, we examined the fluorescent properties of the whole library after incubation with 52 analytes, classified in seven different categories: (1) pH standard solutions, (2) viscous buffer solutions, (3) nucleotides and nucleosides, (4) nucleic acids, (5) proteins, (6) oxidation−reduction related molecules, and (7) miscellaneous analytes. Four different concentrations of analytes were used to study the dose-dependence of the fluorescence emission. This high-throughput screening resulted in the identification of a turn-on sensor with a high response and selectivity for fructose, and we named it Fructose Orange (Figure 1).

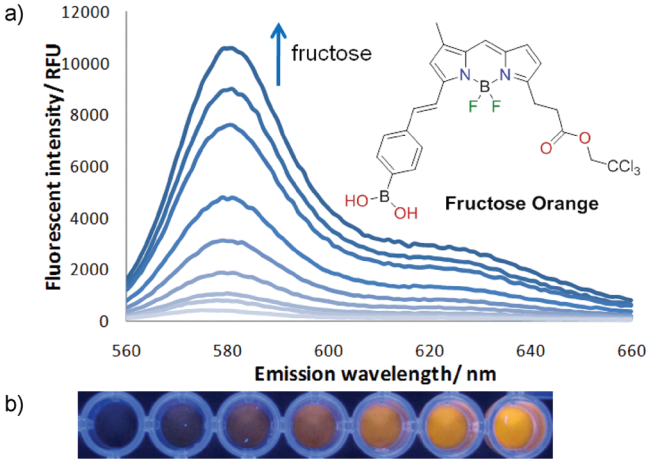

Figure 1. (a) Fluorescent spectra of Fructose Orange (10 μM) after incubation with fructose (0, 1, 2, 5, 10, 20, 50, 100, and 200 mM) in HEPES buffer (20 mM, pH = 7.4) under excitation at 530 nm. (b) Pictures of Fructose Orange (10 μM) solutions containing fructose (from left to right: 0, 5, 10, 20, 50, 100, and 200 mM) under irradiation with a hand-held 365-nm lamp.

Fructose Orange contained a boronic acid motif, which is a well-known sugar binding group.[8] Although several boronic acid-based sensors for saccharides have been reported,[9] highly selective fluorescent fructose sensors are limited. The most selective sensor for fructose reported to date was a tetrathiafulvaleneanthracene boronic acid, which showed a 5-fold fluorescence increase upon fructose recognition.[10] Fructose Orange exhibited a remarkable 24-fold fluorescence enhancement (i.e., its quantum yield changed from 0.01 in the absence of fructose to 0.27 in the presence of 200 mM fructose) with a dissociation constant (K_d) of 30 mM and an excellent selectivity among a large collection of 24 saccharides (Figure 2).

Next we evaluated the solubility and pH-dependence of Fructose Orange. Spectral measurements confirmed that the dye is soluble in HEPES buffer (20 mM, pH = 7.4) with 1% DMSO in a broad concentration range (i.e., 1 to 100 μM) (Supporting Information, Figure S3), and that a consistent fluorescent response to fructose was observed within a pH range from 6 to 9 (Supporting Information, Figure S4). To determine structure-fluorescence relationships, we compared the fluorescence response of Fructose Orange to **BD-187**, a BODIPY derivative containing a phenylboronic acid but without the trichloroethyl ester moiety. As depicted in Figure

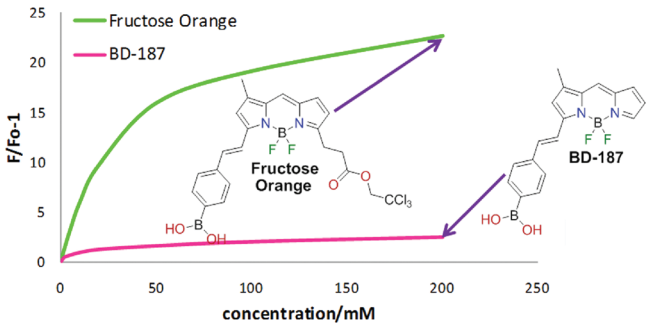

Figure 3. Chemical structures and fluorescence responses of Fructose Orange and **BD-187** (10 μM) after incubation with fructose (from 0 to 200 mM).

3, **BD-187** only showed a 2.5-fold fluorescence increase to fructose, which proved that the selectivity of Fructose Orange toward the saccharide was due to both the boronic acid and the ester groups. We further prepared six new derivatives (**FO1−6**) in which we functionalized the alkyl moiety with different esters and amides. While all **FO1−6** showed a dose-dependent fluorescent enhancement upon incubation with fructose, Fructose Orange displayed the highest fold change, indicating the importance of the trichloroethyl ester for the molecular recognition. **FO2** exhibited a better selectivity profile than **BD-**

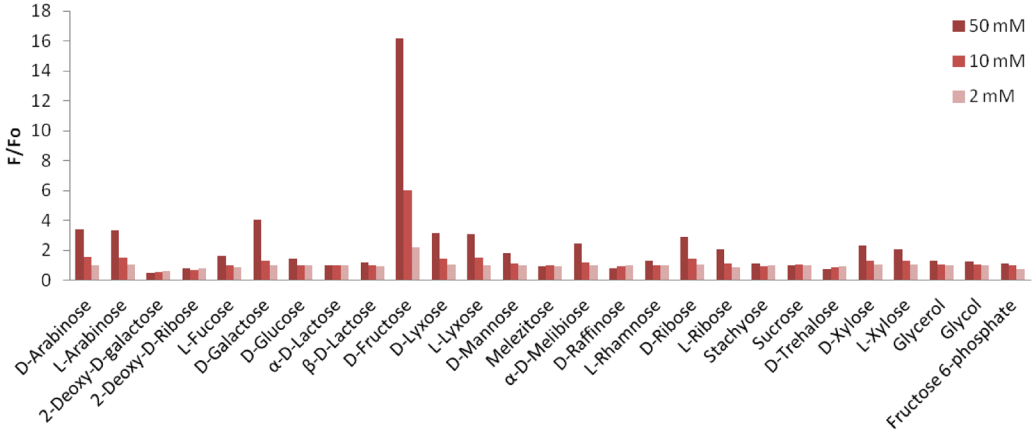

Figure 2. Selectivity of Fructose Orange (10 μM) against 24 different sugars, glycerol, glycol and fructose 6-phosphate at 3 different concentrations in HEPES buffer (20 mM, pH = 7.4). Excitation wavelength: 530 nm.

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187 (Supporting Information, Figure S1), which corroborated the implication of the ethyl ester group in the binding process.

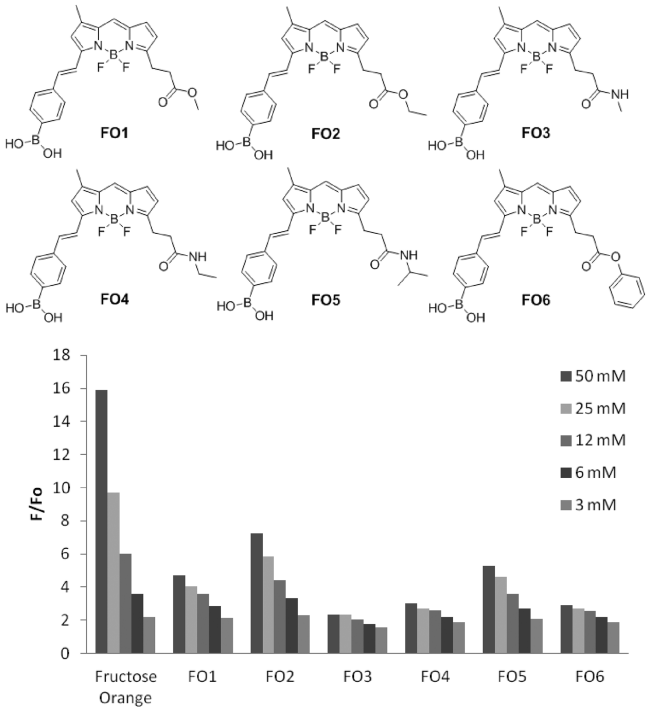

Figure 4. Structures of **FO1–6** and fluorescent response of Fructose Orange and **FO1–6** (10 μM) toward fructose in HEPES buffer (20 mM, pH = 7.4).

Eggert and co-workers reported spectroscopic evidence of the complexes formed by fructose and *p*-tolylboronic acid using ^{13}C NMR to determine the $^{1}J_{CC}$ coupling constants.[11] To investigate the binding mode of Fructose Orange we performed a similar analysis using ^{13}C-fructose. In DMSO-d_6 fructose presents three major (i.e., β-fructofuranose, β-fructopyranose and α-fructofuranose) and two minor isomeric forms (i.e., α-fructopyranose and fructoketose).[12] The signals of these five isomers were detected by ^{13}C NMR and assigned according to the literature (Supporting Information,Table S4).[11,12] After mixing fructose with Fructose Orange in a 1:1 ratio, five different complexes (**2–6**, Figure 5) were formed. The most

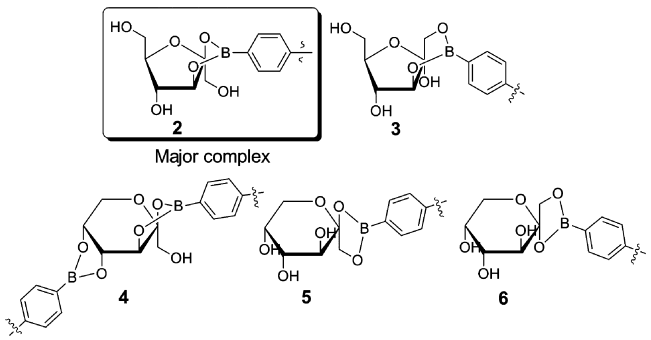

Figure 5. Structures of the complexes detected in DMSO-d_6 upon interaction of Fructose Orange and fructose.

abundant complex was **2**, as proven by the 2D-COSY spectra and the decrease on the signal corresponding to the C-2 hydroxyl group (Supporting Information, Figure S7).

In view of the sensitivity and selectivity of Fructose Orange, we examined its potential for the quantification of fructose in real samples. First, we observed that the fluorescent intensity of Fructose Orange showed a good linear correlation with the concentration of fructose up to 10 mM in both HEPES and 1% fructose-free Coca Cola solutions (Figure 6). These results

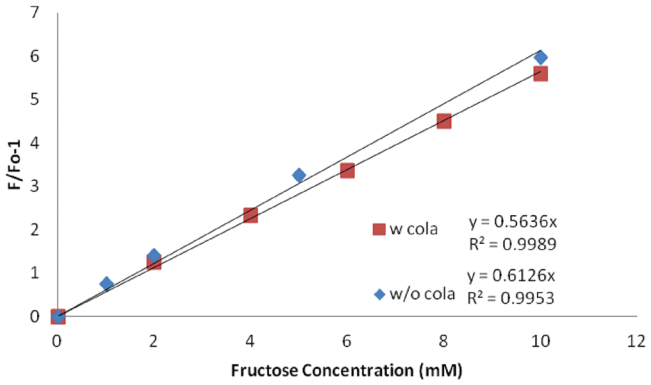

Figure 6. Application of Fructose Orange in real samples. A series of fructose solutions were mixed without and with 1% fructose-free Coca Cola in HEPES buffer (20 mM, pH 7.4). Emission intensity values of Fructose Orange at 580 nm were plotted against the total concentration of fructose. Excitation wavelength: 530 nm.

confirmed that the matrix of soft drinks did not interfere in the fluorescence response of Fructose Orange. Second, we applied our sensor to determine the concentration of fructose in regular Coca Cola and compared our results to a conventional method. The concentration of fructose in regular Coca Cola determined by Fructose Orange was 272 mM, which matched well with the results obtained by HPLC quantification (i.e., 245 mM) (Supporting Information, Figure S8). With this data, we validated the use of Fructose Orange to quantify the levels of fructose in real samples.

In summary, we synthesized a new fluorescent library (BDD) based on a trichloroethyl ester-containing BODIPY scaffold and its derivatization with aldehyde building blocks. A high-throughput unbiased screening of the library enabled the identification of a selective turn-on fructose sensor (Fructose Orange). The binding mode of the interaction was studied by fluorescence spectroscopy and NMR, and we proved that Fructose Orange can be applied to the quantification of fructose in soft drinks. Further applications of BDD compounds will be reported in due course.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental and synthetic procedures, characterization data, and additional spectroscopic information. This material is available free of charge via the Internet at http://pubs.acs.org.

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