ABSTRACT: L-Glucose has recently been investigated as an artificial sweetener, but no facile method is established for the measurement of L-glucose. The commercial probe Eversense employs a fluorescent diboronate in a small device for the optical monitoring of D-glucose in people with diabetes. Being achiral, the Eversense probe should be able to detect L-glucose as well as native D-glucose, but the probe is designed for fixation under the skin, and our attempts to use the probe at laboratory conditions failed, as the probe was resetting when moved between compartments. We thus designed a water-soluble anthracene diboronate similar to the fluorophore used in Eversense and found it to respond well to L-glucose and other carbohydrates and artificial sweeteners, thus enabling measurements of L-glucose with the limit of quantification of 12 μM. Notably, the fluorescent signal of diboronate was largely quenched in buffers with the physiological concentration of albumin (0.5 mM), so the given analytical method would need more optimization to be useful for measuring L-glucose and other carbohydrates in blood samples. We suspect that other diboronate fluorophores from the literature may be similarly quenched if applied in the presence of albumin.

INTRODUCTION

Artificial sweeteners are commonly used as low-calorie replacement for added sugars in food, replacing mainly D-sucrose, D-glucose, and D-fructose. Some people have hesitations toward “artificial” sweeteners, and although this is poorly grounded from a scientific viewpoint, there is a drive toward finding “natural” low-calorie sweeteners. L-glucose has been investigated in this regard, and we took interest in measuring L-glucose in aqueous buffers and blood samples. Monitoring of blood D-glucose is important for the treatment of diabetes, and various devices are available for fast D-glucose measurements, using “strips” with handy glucometers for spot checks of D-glucose in small blood samples (finger pricks), or using small electrodes that are inserted under the skin, for continuous D-glucose readings over days or weeks. However, most of such devices use glucose oxidase or other enzymes, which respond to D-glucose, but are inert to the L-enantiomer. The exception is Eversense, which is a small probe to be inserted under the skin and which employs a fluorescent anthracene diboronate for the optical monitoring of D-glucose in the interstitial fluid continuously over weeks/months. Being achiral (nonenzymatic), the Eversense diboronate should respond to L-glucose as well as D-glucose, so theoretically, L-glucose concentrations could be measured as [total glucose value] minus [D-glucose value], with D-glucose measured using glucometers as mentioned above. We tried to use the Eversense sensor for the measurements of L-glucose in simple buffers, but we found that the sensor was not suited for laboratory works. The sensor is connected to a mobile phone app and must be calibrated daily against glucometer readings. When carefully moved between vials, the sensor was resetting constantly, thus requiring repeated time-consuming and erratic recalibrations. We thus decided to make the Eversense fluorophore in an isolated form, without the device. Notably,
the diborane in the Eversense probe is immobilized in the sealed probe via polymerization of alkene 9 with the matrix components, so the Eversense fluorophore is not directly suited for use in aqueous solution. We therefore redesigned the Eversense diborane for better water solubility, replacing the alkene with carboxylate, so that the fluorophore can be used in simple aqueous buffer and potentially with blood samples. Our diborane fluorophore 8 is similar to Shinkai and James’ classical anthracene diborane 10 (Chart 1). However, like Eversense Fluorophore 9 (Before Immobilization in Probe), Chart 1. Shinkai/James Diboronate Fluorophore 10, Eversense Fluorophore 9 (Before Immobilization in Probe), and Water-Soluble Fluorophore 8

most diboranes from the literature, the classical diborane 10 is not well soluble in water. For this reason, prior studies on diboranes for carbohydrate measurements often employ partial organic solvents (typically 50% methanol). Diborane 8 is well soluble in water and thus directly applicable to measurements in aqueous solutions. However, when tried with buffers including albumin in native concentration (0.5 mM), we found the fluorescent signal to be largely quenched.

## EXPERIMENTAL SECTION

Unless stated otherwise, reagents and solvents were sourced from commercial suppliers and were used directly as received. LCMS apparatus was Waters Acquity UPLC SQD 2000. NMR apparatus was Bruker Avance III HD 300 MHz. Fluorometer was a Tecan Spark multimode microplate reader.

### Synthesis of Diamino Anthracene 3

9,10-Bis-(chloromethyl)anthracene 1 (2.75 g, 10.0 mmol) and tert-butyl 3-aminoopropanoate-methane hydrochloride 2 (14.5 g, 80.0 mmol) were dissolved in N,N-dimethylformamide (75 mL). N,N-Diisopropylethylamine (17.0 mL, 100 mmol) was added to the reaction mixture, which was stirred for 20 h at room temperature. The mixture was diluted with ethyl acetate (500 mL) and washed with 10% aqueous solution of sodium sulfite (2 × 300 mL) and saturated aqueous solution of sodium chloride (1 × 100 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo. The residue was partially purified by flash column chromatography (silica gel, 0.040–0.063 mm; eluent: cyclohexane/ethyl acetate 3:1), affording di-tert-butyl-3,3’-((anthracene-9,10-diylbis(methylene))bis-(azanediyl))dipropionate-methane 3 as an impure yellow solid. Compound 3 was further purified by HPLC (Gemini C18 column, 5 μM, 250 mm × 50 mm, acetonitrile/water 5:95 during 20 min, 5:95 to 35:65 during 90 min, 35:65 to 55:45 during 60 min + 0.05% AcOH) to give acetate salt as the pure product. To release the free amines, the material was dissolved in dichloromethane (200 mL) and washed with 5% aqueous solution of sodium carbonate (1 × 200 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo to give di-tert-butyl-3,3’-((anthracene-9,10-diylbis(methylene))bis-(azanediyl))dipropionate-methane 3 as yellow crystals. Yield: 3.08 g (62%).

1H NMR spectrum (300 MHz, CDCl3, δH): 8.40 (dd, J = 6.9 and 3.3 Hz, 4 H); 7.54 (dd, J = 6.9 and 3.2 Hz, 4 H); 4.73 (s, 4 H); 3.16 (t, J = 6.5 Hz, 4 H); 2.54 (t, J = 6.5 Hz, 4 H); 1.70 (s, 2 H); 1.42 (s, 18 H). LCMS m/z: 493.2 (M + H)−.

### Synthesis of CF3 Toluene Pinacol Boronate 5

(2-Methyl-5-(trifluoromethyl)phenyl)boronic acid 4 (5.82 g, 28.5 mmol) and pinacol (3.37 g, 28.5 mmol) were dissolved in ethyl acetate (60 mL). The reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated, and the residue was co-evaporated with dichloromethane (3 × 100 mL). The residue was crystallized from hot methanol (30 mL). The fine precipitate was collected by filtration, washed with chilled (0 °C) methanol/water mixture (7:3, 1 × 100 mL) and then washed with chilled (0 °C) methanol (1 × 100 mL), dried with suction (40 mL). The organic layer was separated, washed with 10% aqueous solution of sodium chloride (1 × 100 mL) at room temperature for 2 h. Afterward, the reaction mixture was diluted with methyl tert-butyl ether (40 mL) and water (40 mL). The organic layer was separated, washed with 10% aqueous solution of sodium chloride (1 × 100 mL), dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo, affording a mixture of product 6 and 2-(2-(dibromomethyl)-5-(trifluoromethyl)phenyl)-4,4,5,5-tetramethyl-2-(2-methyl-5-(trifluoromethyl)phenyl)-1,3,2-dioxaborolane 5 as pale yellow crystals. Yield: 5.75 g (71%).

1H NMR spectrum (300 MHz, CDCl3, δH): 8.01 (s, 1 H); 7.65–7.45 (m, 1 H); 7.27 (d, 1 H); 2.59 (s, 3 H); 1.37 (s, 12 H).

### Synthesis of Bromomethyl CF3 Phenyl Pinacol Boronate 6

A mixture of 4,4,5,5-tetramethyl-2-(2-methyl-5-(trifluoromethyl)phenyl)-1,3,2-dioxaborolane 5 (4.86 g, 17.0 mmol), N-bromosuccinimide (3.33 g, 18.7 mmol), and 2,2-azobis(2-methylbutyronitrile) (VAZOTM 67, 326 mg, 1.70 mmol) in acetonitrile (40 mL) was stirred at 80 °C for 2 h, and then it was cooled to room temperature. The reaction mixture was diluted with methyl tert-butyl ether (40 mL) and water (40 mL). The organic layer was separated, washed with 10% aqueous solution of sodium chloride (1 × 100 mL), dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo. The residue was partially purified by HPLC (Gemini C18 column, 5 μM, 250 mm × 50 mm, acetonitrile/water 5:95 during 20 min, 5:95 to 35:65 during 90 min, 35:65 to 55:45 during 60 min + 0.05% AcOH) to give acetate salt as the pure product. To release the free amines, the material was dissolved in dichloromethane (200 mL) and washed with 5% aqueous solution of sodium carbonate (1 × 200 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo to give di-tert-butyl-3,3’-((anthracene-9,10-diylbis(methylene))bis-(azanediyl))dipropionate-methane 3 as yellow crystals. Yield: 3.08 g (62%).

1H NMR spectrum (300 MHz, CDCl3, δH): 8.40 (dd, J = 6.9 and 3.3 Hz, 4 H); 7.54 (dd, J = 6.9 and 3.2 Hz, 4 H); 4.73 (s, 4 H); 3.16 (t, J = 6.5 Hz, 4 H); 2.54 (t, J = 6.5 Hz, 4 H); 1.70 (s, 2 H); 1.42 (s, 18 H). LCMS m/z: 493.2 (M + H)−.

### Synthesis of 9,10-Bis-(chloromethyl)anthracene 1

(2.75 g, 10.0 mmol) and tert-butyl 3-aminoopropanoate-methane hydrochloride 2 (14.5 g, 80.0 mmol) were dissolved in N,N-dimethylformamide (75 mL). N,N-Diisopropylethylamine (17.0 mL, 100 mmol) was added to the reaction mixture, which was stirred for 20 h at room temperature. The mixture was diluted with ethyl acetate (500 mL) and washed with 10% aqueous solution of sodium sulfite (2 × 300 mL) and saturated aqueous solution of sodium chloride (1 × 100 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo. The residue was partially purified by HPLC (Gemini C18 column, 5 μM, 250 mm × 50 mm, acetonitrile/water 5:95 during 20 min, 5:95 to 35:65 during 90 min, 35:65 to 55:45 during 60 min + 0.05% AcOH) to give acetate salt as the pure product. To release the free amines, the material was dissolved in dichloromethane (200 mL) and washed with 5% aqueous solution of sodium carbonate (1 × 200 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo to give di-tert-butyl-3,3’-((anthracene-9,10-diylbis(methylene))bis-(azanediyl))dipropionate-methane 3 as yellow crystals. Yield: 3.08 g (62%).

1H NMR spectrum (300 MHz, CDCl3, δH): 8.40 (dd, J = 6.9 and 3.3 Hz, 4 H); 7.54 (dd, J = 6.9 and 3.2 Hz, 4 H); 4.73 (s, 4 H); 3.16 (t, J = 6.5 Hz, 4 H); 2.54 (t, J = 6.5 Hz, 4 H); 1.70 (s, 2 H); 1.42 (s, 18 H). LCMS m/z: 493.2 (M + H)−.
dried by suction in air and then in vacuo to give 2-(2-(bromomethyl)-5-(trifluoromethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane 6 as a white powder. Yield: 4.83 g (78%). 1H NMR spectrum (300 MHz, CDCl₃, δH): 8.08 (s, 1 H); 7.66 (dd, J = 8.0 and 1.5 Hz, 1 H); 7.51 (d, J = 8.1 Hz, 1 H); 4.92 (s, 2 H); 1.40 (s, 12 H).

**Synthesis of Pinacol Diboronate Anthracene 7.** Di-tert-butyl-3,3′-(anthracene-9,10-diylbis(methylene))bis[(azanediyl)-dipropionate-methane]-((anthracene-9,10-diylbis(methylene))bis[(azanediyl))-dipropionic acid (TFA salt, 1 mM) in 0.1 M phosphate buffer pH 7.4 was determined by measuring the integrated emission area of the fluorescence spectrum (λexc = 347 nm) and comparing with the area measured for quinine sulfate (25 μM in HClO₄, 0.1 M, ϕ = 0.59, λexc = 347 nm). The equation used is the following:

\[ \frac{\phi_r}{\phi_s} = \frac{n_s^2}{n_r^2} \times \frac{\int_{0}^{\infty} I_r(\lambda_{em})d\lambda_{em}}{\int_{0}^{\infty} I_s(\lambda_{em})d\lambda_{em}} \times \frac{A_r(\lambda_{exc})}{A_s(\lambda_{exc})} \]

R stands for reference and S for sample, ϕ is the quantum yield, n is the refractive index of the excitation, I is the fluorescence intensity, and A is the absorbance at the excitation wavelength (λexc). Emission scan was performed at 385–700 nm.

**Fluorescence Polarization (Anisotropy) Experiment for the Measurement of Dissociation Constant (Kₜ) of Albumin.** Anisotropy (r) is defined as the ratio between the fluorescence intensity emitted parallel and perpendicular divided by the total intensity. First, a solution of the highest albumin concentration and fluorophore 8 at 25 μM in 0.1 M phosphate buffer pH 7.4 was prepared and diluted in 1:1 ratio with 25 μM of 8 into a 96-well black flat-bottom plate at a final working volume of 150 μL. The fluorescence measurements were carried out at room temperature using excitation wavelength at 380 ± 10 nm and emission at 430 ± 15 nm, with optimal gain and G-factor as calibrated from the reference and blank well, 1.697. Polarization data were plotted versus the concentration of HSA (logarithmic scale) and fitted (Prism 9.0.1, GraphPad) with the nonlinear regression function “log(agonist) vs response-variable slope (four parameters)” to obtain the Kₜ value for the binding of diboronate 8 to each type of sugar.

**Measurement of Limit of Quantification for L-Glucose.** The calibration curves were handled by semilog plots, as generally recommended for a nonlinear correlation between signal readout and analyte concentration.14,15 Lower and upper limits of quantification (LLOQ and ULOQ, respectively) were determined by the two plateau regions of the observed S-shaped curves and represent the assay range. Every point of the calibration curve is measured in triplicate, and standard deviations are represented by error bars. The analytical sensitivity of the method is expressed by the LLOQ value according to FDA guidelines for method validation. Linearity is evaluated by the R² value of the linear regression.

**Measurement of Relative Fluorescence Quantum Yield for Diboronate 8.** The quantum yield of fluorophore 8 (25 μM) in 0.1 M phosphate buffer pH 7.4 ± 0.5 mM nonglycated albumin was determined by measuring the integrated emission area of the fluorescence spectrum (λexc = 347 nm) and comparing with the area measured for quinine sulfate (25 μM in HClO₄, 0.1 M, ϕ = 0.59, λexc = 347 nm). The equation used is the following:

\[ \frac{\phi_r}{\phi_s} = \frac{n_s^2}{n_r^2} \times \frac{\int_{0}^{\infty} I_r(\lambda_{em})d\lambda_{em}}{\int_{0}^{\infty} I_s(\lambda_{em})d\lambda_{em}} \times \frac{A_r(\lambda_{exc})}{A_s(\lambda_{exc})} \]

R stands for reference and S for sample, ϕ is the quantum yield, n is the refractive index of the excitation, I is the fluorescence intensity, and A is the absorbance at the excitation wavelength (λexc). Emission scan was performed at 385–700 nm.

Fluorophore 8 (25 μM) was dissolved in 0.1 M phosphate buffer, pH 7.4, and left in an open vial versus shielded vial (aluminum foil). Samples were analyzed daily by LCMS (column 1.7 μC18 100 A 2.1 × 50 mm, detector: PDA: 210–400 nm, scanning range: 100–1500). The chromatographic method used a linear gradient of 10 to 90% B for a total runtime of 4 min, with a flow rate of 0.3 mL/min and column temperature of 40 °C. Solvent A, 0.1% TFA in water; solvent B, 0.1% TFA in CH₃CN.

**Fluorescence Measurement of Dissociation Constant (Kₜ) of Fluorophore 8 toward Carbohydrates and Other Relevant Compounds.** A stock solution of fluorophore 8 (TFA salt, 1 mM) in 0.1 M phosphate buffer pH 7.4 was prepared. The sugars were dissolved at the highest concentrations needed for their individual titrations, in buffer containing 25 μM fluorophore, and titrated in triplicate into 96-well black flat-bottom plates (Thermo-Fisher Scientific). The fluorescence analysis was carried out at room temperature, with the excitation wavelength at 360 ± 10 nm and emission scan at 385–520 ± 10 nm. Data obtained for fluorescence intensity were plotted versus sugar concentration (logarithmic scale) and fitted (Prism 9.0.1, GraphPad) with the nonlinear regression function “log(agonist) vs response-variable slope (four parameters)” to obtain the Kₜ value for the binding of diboronate 8 to each type of sugar.

**Stability of Diboronate 8 in Aqueous Solution at Neutral pH.** Fluorophore 8 (25 μM) was dissolved in 0.1 M phosphate buffer, pH 7.4, and left in an open vial versus shielded vial (aluminum foil). Samples were analyzed daily by LCMS (column 1.7 μC18 100 A 2.1 × 50 mm, detector: PDA: 210–400 nm, scanning range: 100–1500). The chromatographic method used a linear gradient of 10 to 90% B for a total runtime of 4 min, with a flow rate of 0.3 mL/min and column temperature of 40 °C. Solvent A, 0.1% TFA in water; solvent B, 0.1% TFA in CH₃CN.
sigmoidal function to obtain the $K_d$ value for the binding of the fluorophore 8 to HSA.

## RESULTS AND DISCUSSION

Carboxy anthracene diboronate 8 was synthesized, as outlined in Scheme 1. Compounds with free boronic acids tend to dehydrate to several species during handling, which can complicate NMR analysis, and so forth. For this reason, the boronates were protected as pinacols. It is allegedly difficult to remove pinacol, but we found that pinacol was easily removed during RP-HPLC under aqueous acidic conditions, as employed for purification in the final step, providing 8 as a free diboronic acid TFA salt.

Diboronates are fragile and can degrade during handling and storage of their aqueous solutions, especially when exposed to light. To investigate this point, aqueous solutions of fluorophore 8 at neutral pH were stored in open vials, compared to vials shielded from light using aluminum foil. By LCMS analysis over 7 days, fluorophore 8 was found to be stable when kept in the dark, whereas solutions of 8 exposed to ambient light showed significant degradation over a few days (>30%). During normal laboratory handling (preparation of titration series, etc.), no shielding from light was found necessary.

Fluorophore 8 can be excited at 380 nm, and its maximum emission wavelength is 430 nm. However, in order to eliminate the cross talk between excitation and emission, we recorded the fluorescent readings with the excitation wavelength of 360 nm. When sugar is added to 8, the emission spectra show two peaks with maximum at 405 and 430 nm, respectively (Figure 1).

Dissociation constants ($K_d$) for 8 toward 1-glucose and other substrates were measured by plotting data from the titration series versus the employed sugar concentrations and reading out $K_d$ using GraphPad Prism. Titration plots and curve fits can be seen in Figures 1 and 2. $K_d$ of 8 toward both D- and L-glucose was found to be 0.44 mM, with the minimum quantification limit for both enantiomers being 12 μM. L-glucose calibration curve is shown in Figure 3 (semilog plot). Measurements of D-glucose concentrations by using 8 were compared to values using a commercial glucometer (Abbott FreeStyle Precision Neo). As mentioned, the glucometer could not measure L-glucose and gave an error message.

Dissociation constants of other investigated carbohydrates and related compounds are listed in Table 1. Overall, the affinities follow rankings, as known from other mono- and diboronates. D-Fructose binds weaker than glucose to diborionate 8 ($K_d$, 2.5 mM and LOQ, 160 μM), but it is noteworthy that fructose never reaches mM concentrations in human circulation due to first-pass liver extraction. Lactate, 

![Scheme 1. Synthesis of Fluorophore 8 as TFA Salt](image)

![Figure 1. Fluorescence emission of 8 when titrated with 1-glucose ($\lambda_{ex}$, 360 nm) in 0.1 M phosphate buffer pH 7.4 at room temperature.](image)

![Figure 2. GraphPad plot of fluorescence signal vs 1-glucose logC (arbitrary units) for the determination of $K_d$ ($\lambda_{ex}$, 360 nm and $\lambda_{em}$, 430 nm) in 0.1 M phosphate buffer pH 7.4 at room temperature. See plots for other substrates in the Supporting Information. Inset: photograph of titration series taken under a UV lamp (285 nm).](image)

![Figure 3. Calibration curve for 1-glucose ($\lambda_{ex}$, 360 nm and $\lambda_{em}$, 430 nm) in 0.1 M phosphate buffer pH 7.4 at room temperature.](image)
which can reach >10 mM values in human blood during intense exercise,\textsuperscript{22} binds weakly to 8 with \( K_d \) of 22 mM. The strong binding of 8 to catechol (\( K_d \) 4 \( \mu \)M) has precedence from other diborons, but catechol is not found in such concentrations physiologically. Glucosides such as \( \alpha \)-sucrose and methyl mannopyranoside, as well as glucose-1-phosphate, bind very weakly to 8 as the main binding motif of sugars, 1,2-cis-diol, is blocked in these substrates.

The mechanism of sugar binding to boronates can involve mutarotation of anomers toward the furanose form, as reported by Norrild et al. using NMR experiments.\textsuperscript{23} We observed that pure alpha-\( \alpha \)-glucose and common \( \alpha \)-glucose, which consist of a mixture of anomers with beta-\( \beta \)-pyranose as the main component (67\%), gave the same \( K_d \) value, indicating that glucose during contact with diboronate is quickly pulled to a common anomer mixture, irrespective of the starting configuration. The given readouts were recorded within minutes of mixing, and we observed no change in readings over time, thus indicating that the mutarotation of glucose in contact with diboronate 8 reaches completion within minutes, whereas the mutarotation of neat glucose anomers takes approximately 30 min to reach equilibrium.\textsuperscript{24}

Notably, the sensitivity of fluorophore 8 to glucose was found to decrease in solutions containing human serum albumin (HSA, nonglycated). It has previously been described that nonfluorescent diboronates can bind to albumin,\textsuperscript{25,26} and this also happens with fluorophore 8, whereby the fluorescence signal is largely quenched. Human and animal blood as well as interstitial liquid contains albumin in concentrations near 0.5 mM, and albumin is known to bind many small molecules.\textsuperscript{27,28}

The exact binding constants of albumin are generally difficult to determine due to multiple binding pockets in the protein, meaning that titration curves rarely give well-defined \( K_d \) values. We tried to measure \( K_d \) of 8 of nonglycated albumin by simple titration with fluorescence readouts, but the plot did not show a well-defined affinity value, probably due to the binding of 8 to multiple albumin-binding pockets with overlapping \( K_d \) values. Fluorescence polarization (anisotropy) was shown to be a better approach, and by titration of albumin against a fixed concentration of fluorophore 8 (5 \( \mu \)M), we saw a sigmoidal curve, with \( K_d \) reading out as 2 \( \mu \)M (Figure 4). By the Job plot analysis (see Supporting Information),\textsuperscript{29} the binding stoichiometry was found to be 4:1, which makes sense considering the multiple binding pockets in albumin. The displacement of fluorophore 8 from albumin by warfarin (Sudlow site 1) and ibuprofen (site 2) was also studied by anisotropic fluorescence. Quantitative data could not be collected, likely due to the mixed binding of 8 to several HSA-binding pockets, but qualitatively, 8 appears to bind at least to site 1 and site 2 (see Supporting Information).

Finally, we measured the relative fluorescence quantum yields (\( \phi \)) of 8 in phosphate buffer pH 7.4 compared to the solution with 0.5 mM nonglycated albumin, using quinine sulfate in 0.1 M HClO\textsubscript{4} as reference (\( \phi = 0.59 \)).\textsuperscript{30,31} Albumin quenched fluorescence of 8 with an eightfold decrease in quantum yield, from 0.16 in simple buffer to 0.02 in albumin-containing solution. Accordingly, more work would be required in order to use 8 for measuring carbohydrates in blood samples, with albumin present. The reason Eversense probe can work in vivo despite the presence of albumin is probably the use of a membrane that hinders contact between diboronate and albumin. Unfortunately, Eversense probe is not suited for laboratory use, as discussed above.

\section*{CONCLUSIONS}

In conclusion, the water-soluble diboronate fluorophore 8 was synthesized by a facile five-step route, and 8 was found to be stable to long-term storage in aqueous solutions when shielded from ambient light. The fluorophore could be used to measure \( i \)-glucose, showing \( K_d \) of 0.44 mM and LOQ of 12 \( \mu \)M. The physiological concentration of albumin (0.5 mM) largely quenched the fluorescence signal of 8; hence, using 8 for

\begin{table}[h]
\centering
\caption{\textbf{\( K_d \) Values of Fluorophore Diboronates 8 toward Sugars and Other Substrates.} See LLOD/ULOQ Values in Supporting Information}
\begin{tabular}{|l|l|}
\hline
substrate & \( K_d \) (mM) \\
\hline
\( i \) glucose & 0.44 \\
\( \alpha \) glucose & 0.43 \\
\( \alpha \) d-glucose & 0.43 \\
\( \alpha \) glucose with HSA 0.05 mM & 3.5 \\
\( \alpha \) glucose with HSA 0.5 mM & 20 \\
catechol & 0.037 \\
\( \alpha \) sorbose & 0.27 \\
\( \alpha \) tagatose & 1.5 \\
\( \alpha \) glucorionate sodium & 1.6 \\
\( \alpha \) sorbitol & 2.3 \\
\( \alpha \) fructose & 2.5 \\
\( \alpha \) galactose & 7.2 \\
\( \alpha \) ribose & 8.2 \\
\( \alpha \) mannose & 14.4 \\
sodium \( i \) lactate & 22.9 \\
\( \alpha \) mannitol & 64.0 \\
\( \alpha \) d-lactose & 68.0 \\
2-deoxy-\( \alpha \) ribose & 77.0 \\
\( \beta \) maltose & 142 \\
glycerol & 158 \\
methyl-\( \alpha \) mannopyranoside & 160 \\
erithritol & 339 \\
N-acetyl-\( \alpha \) glucosamine & 575 \\
\( \alpha \) sucrose & binding not saturated at 1 M \\
saccharin sodium & no binding \\
\( \alpha \) d-glucose-1-phosphate & no binding \\
sucrose & no binding \\
\hline
\end{tabular}
\end{table}
measurements of l-glucose in blood samples would require more work. It seems likely that other fluorescent diboronates from the literature can have similar interactions with albumin, and this should be considered for future design and evaluation of diboronates for sugar detection.

**ASSOCIATED CONTENT**

**Supporting Information**
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c02519.

The Supporting Information is available free of charge on the ACS Publications website. LLOQ, ULOQ, and linearity ($R^2$) for all given substrates; fluorescence excitation spectra ± albumin, with MS spectrum of diborionate 8 and LCMS chromatograms for monitoring the stability of 8 ± ambient light; Job plot and analysis; albumin site marker analysis; comparison of glucose measurements using 8 to glucometer readings; and binding curves and calibration plots for all given substrates; NMR spectra of compounds 3 to 8 (PDF)

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**Author Contributions**
V.T., T.K., and T.H.J. designed and synthesized the fluorophore 8. K.S., G.I., and T.H.J. planned the fluorescence experiments, and K.S. performed the experimental work. T.H.J. wrote the main text with contributions from all authors, and K.S. and T.H.J. prepared the Supporting Information. The final text is approved by all authors.

**Notes**
The authors declare no competing financial interest.

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