Electronic Supplementary Information (ESI)

A 2:2 Stilbeneboronic acid/γ-Cyclodextrin Fluorescent Ensemble Highly Selective for Glucose in Aqueous Solutions

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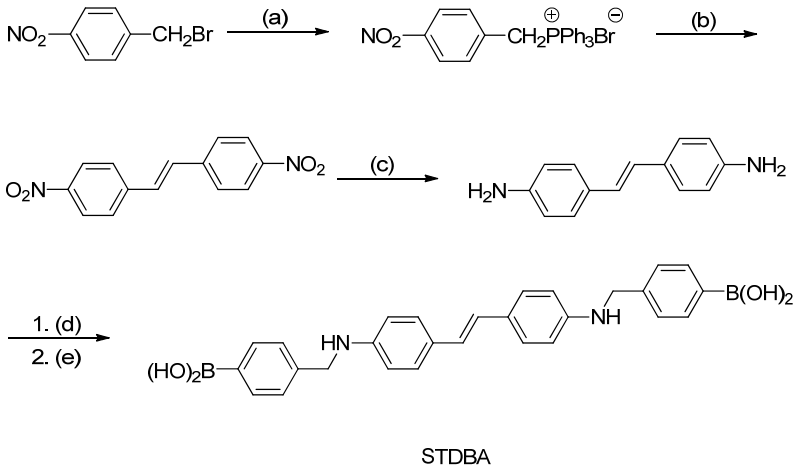
1. Materials and Instrumentation

4-Nitrobenzyl bromide (99%), 4-nitrobenzaldehyde (AR) and potassium *tert*-butoxide (CP) were used as received from Aladdin reagent. 4-Formylphenylboronic acid (97%) was purchased from Matrix Scientific. Dimethyl sulfoxide (HPLC) and mannose (AR) were purchased from Alfa Aesar. β-Cyclodextrin from Shanghai Chemical Reagent Company was recrystallized twice in water prior to use. γ-Cyclodextrin from Tokyo Chemical Industry (TCI) was used as received. Triphenylphosphine at chemical-pure grade and sodium borohydride, D-glucose, D-fructose, D-galactose and other chemicals at analytical grade were products of Sinopharm Chemical Reagent Co. Ltd.

^{1}H and ^{13}C NMR were obtained on a Bruker Avance 500 NMR spectrometer. High-resolution mass spectra (HRMS) were taken on a Bruker En Apex ultra 7.0T FT-MS mass spectrometer. Absorption spectra were recorded on a Thermo Evolution 300 UV-Vis spectrophotometer. Fluorescence spectra were recorded on a Hitachi F-4500 spectrofluorometer. Circular dichroism (CD) spectra were recorded on a Jasco J-810 circular dichroism spectropolarimeter.

2. Synthesis and Characterization of STDBA



STDBA

Scheme S1 Synthesis of STDBA. (a) PPh_3, toluene; (b) *p*-nitrobenzaldehyde, *t*-BuOK, *t*-BuOH, THF; (c) $SnCl_2 \cdot 2H_2O$, EtOH; (d) *p*-formylphenylboronic acid, MeOH; (e) $NaBH_4$, MeOH.

Trans-4,4'-dinitrostilbene was synthesized from 4-nitrobenzyl bromide and 4-nitrobenzaldehyde according to a reported procedure.[1] It was then reduced by stannous chloride to give *trans*-4,4'-diaminostilbene.[1] To a solution of *trans*-4,4'-diaminostilbene (0.11 g, 0.5 mmol) in 20 mL MeOH was added 4-formylphenylboronic acid (0.15 g, 1 mmol). The yellow cloudy mixture was stirred at room temperature for 24 hours. The reaction mixture was cooled to 0 °C and sodium borohydride (0.19 g, 5 mmol) was slowly added. The reaction mixture was stirred at room temperature for 7 hours as it gradually became clear. The mixture was again cooled to 0 °C, and sodium borohydride (0.76g, 45 mmol) was added in small portions and the reaction mixture continued to be stirred at room temperature for 3 hours. The solvent was removed under reduced pressure. Brine (20 mL) was added to the residue resulting in a blue cloudy mixture. pH was adjusted to 7-8 using hydrochloric acid. The yellow mixture was then extracted with ethyl acetate (20 mL×2) and the organic phase was filtered to remove the precipitate, washed with brine (15 mL) and dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo* and the crude product was purified by trituration with ethyl acetate to give STDBA as a brownish yellow powder (0.14 g, 58%).^{1}H NMR (500 MHz, DMSO-d_6): δ (ppm) 7.95 (s, 4H, $B(OH)_2$), 7.72 (d, J = 8.0 Hz, 4H, ArH), 7.32 (d, J = 8.0 Hz, 4H, ArH), 7.18 (d, J = 8.5 Hz, 4H, ArH), 6.71 (s, 2H, CH=CH), 6.53 (d, J = 8.5 Hz, 4H, ArH), 6.34 (t, J = 6.0 Hz , 2H, NH), 4.27 (d, J = 6.0 Hz , 4H, CH_2). ^{13}C NMR (126 MHz, DMSO-d_6): δ (ppm) 148.20, 142.61, 134.63, 127.21, 126.68, 126.37, 124.32, 112.93, 46.99. HRMS (ESI-TOF, *m/z*): $[M\text{-}H_2O\text{-}H]^-$ calcd for $C_{28}H_{25}B_2N_2O_3^-$, 459.2055; found, 459.2059.

3. Procedures for Saccharide Sensing

Procedures for Saccharide Sensing in Buffer Solution. A stock solution of 1 mM STDBA was prepared by dissolving STDBA in DMSO. The stock solution was stored in a refrigerator at 4 °C before use. Standard stock solutions of saccharides (0.1 M and 1 M) were prepared by dissolving an appropriate amount of saccharides in deionized

water. Mixture of STDBA/γ-CyD inclusion complex and saccharide was prepared by adding desired amount of the saccharide stock solution to 1.98 mL of carbonate buffer containing 1.0 mM γ-CyD followed by addition of 20 μL of DMSO stock solution of STDBA, which was subject to spectral measurements after 30 min.

Procedures for Saccharide Sensing in Urine. An artificial urine was prepared according to a literature recipe[2] that contained urea, lactic acid, citric acid, $NaHCO_3$, $CaCl_2$, NaCl, $MgSO_4$, KH_2PO_4, K_2HPO_4, Na_2SO_4, and NH_4Cl. The final samples for spectral measurements were prepared in a similar method to that reported by Tang *et al.*[3] that contained 25% (v%) of the original artificial urine. To 0.5 mL of the artificial urine was added desired amount of glucose stock solution, 1.48 mL of 0.1 M carbonate buffer (pH 10.5) containing 1.33 mM γ-CyD and 20 μL of DMSO stock solution of STDBA.

4. Effects of DMSO Content and Cyclodextrins on Fluorescence Spectra of STDBA

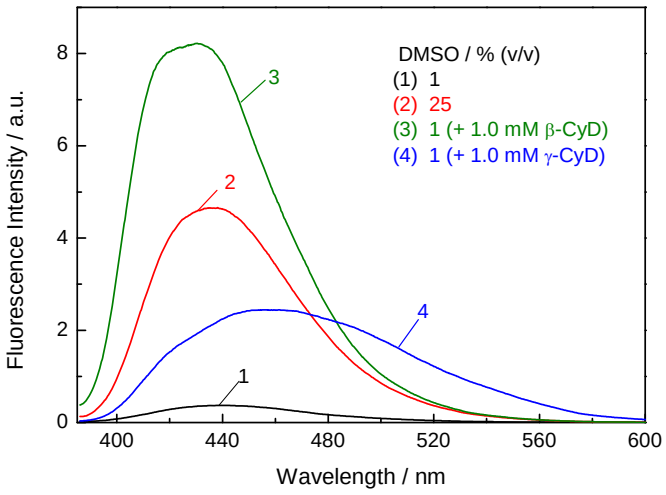


Fig. S1. Fluorescence spectra of STDBA in aqueous DMSO solutions with or without cyclodextrins. [STDBA] = 10 μM, I = 0.1 M with NaCl, pH 10.5 buffer of 10 mM $NaHCO_3$-Na_2CO_3. λ_{ex} = 376 nm.

5. Optimization of pH and γ-CyD Concentration

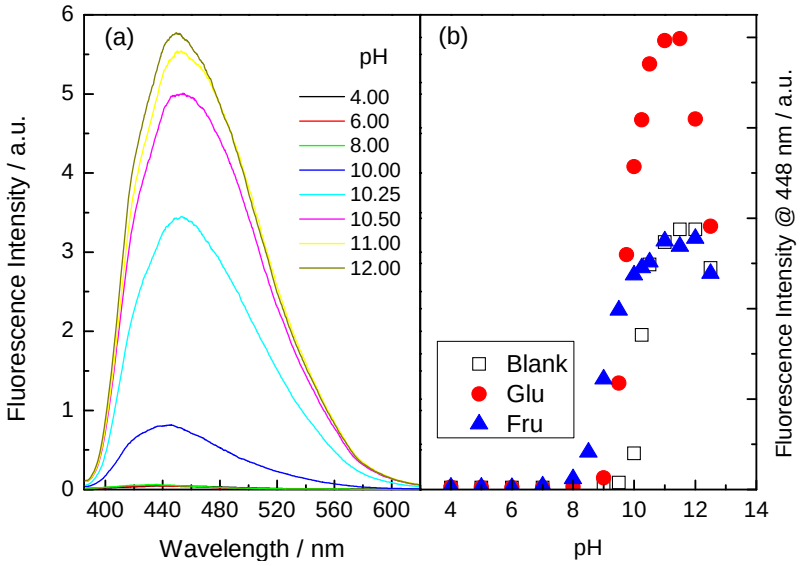


Fig. S2 (a) Fluorescence spectra of STDBA at varying pH in the absence of saccharide and (b) pH profile of the relative fluorescence intensity of STDBA in 1% DMSO aqueous γ-CyD solutions in the absence and presence of saccharides at 5 mM. [STDBA] = 10 μM, [γ-CyD] = 1.0 mM, I = 0.1 M with NaCl. λ_{ex} = 376 nm.

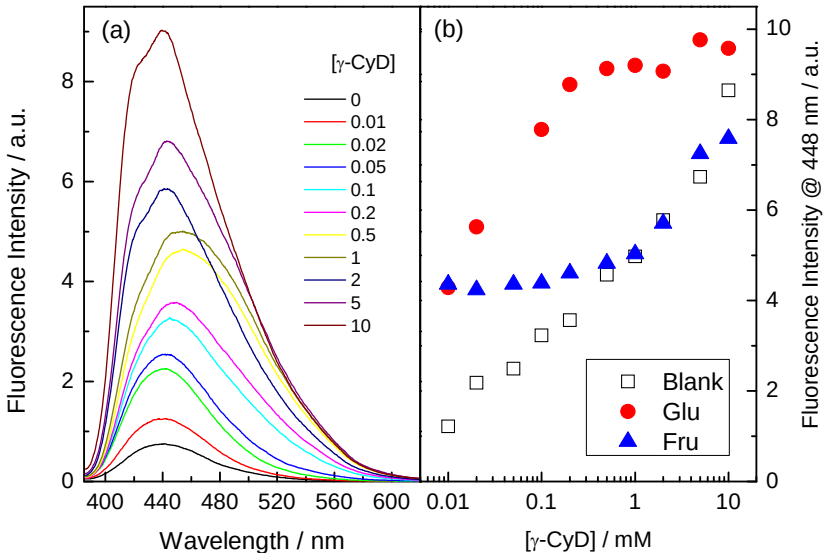


Fig. S3 (a) Fluorescence spectra of STDBA in the presence of γ-CyD of increasing concentration in the absence of saccharide and (b) relative fluorescence intensity of STDBA versus γ-CyD concentration in the absence and presence of saccharides in 1% DMSO aqueous γ-CyD solutions buffered at pH 10.5. [STDBA] = 10 μM. λ_{ex} = 376 nm.

6. Fluorescence Responses of STDBA/γ-CyD Complex toward Galactose and Mannose

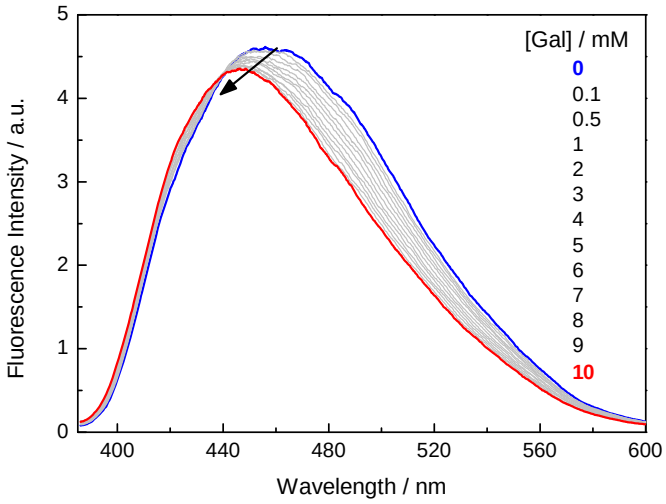


Fig. S4 Fluorescence spectra of STDBA in the presence of galactose of increasing concentration in 1% DMSO aqueous γ-CyD solutions buffered at pH 10.5. [STDBA] = 10 μM, [γ-CyD] = 1.0 mM. λ_{ex} = 376 nm.

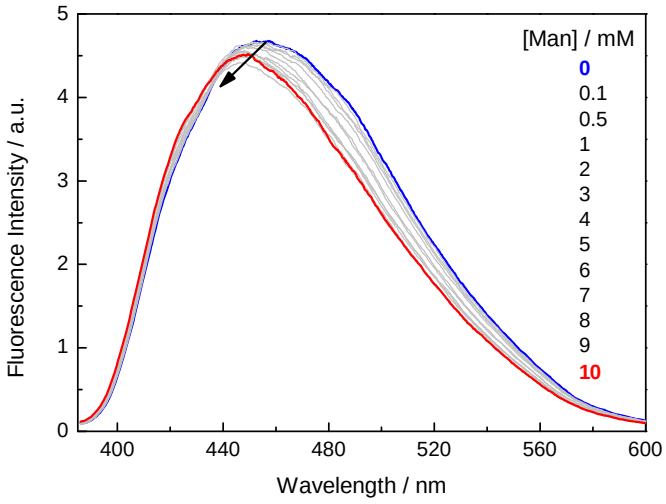


Fig. S5 Fluorescence spectra of STDBA in the presence of mannose of increasing concentration in 1% DMSO aqueous γ-CyD solutions buffered at pH 10.5. [STDBA]= 10 μM, [γ-CyD] = 1.0 mM. λ_{ex} = 376 nm.

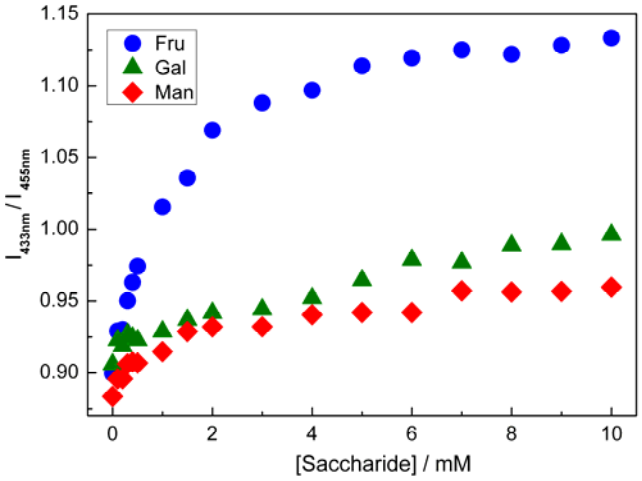


Fig. S6 Fluorescence intensity ratio (I_{433nm}/I_{455nm}) of STDBA as a function of saccharide concentration in 1% DMSO aqueous γ-CyD solutions buffered at pH 10.5. [STDBA] = 10 μM, [γ-CyD] = 1.0 mM. λ_{ex} = 376 nm.

7. Fluorescence Responses of STDBA/β-CyD Complex toward Saccharides

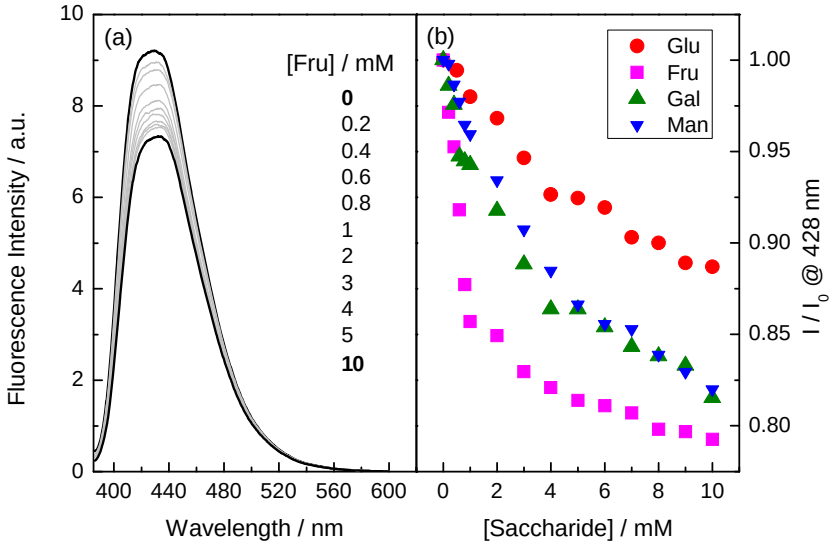


Fig. S7 (a) Fluorescence spectra of STDBA in the presence of fructose of increasing concentration and (b) extent of quenching of STDBA fluorescence at 428 nm as a function of the concentration of glucose, fructose and galactose in 1% DMSO aqueous β-CyD solutions buffered at pH 10.5. [STDBA] = 10 μM, [β-CyD] = 1.0 mM. λ_{ex} = 376 nm.

8. Job Plot for STDBA/γ-CyD Complex in the Presence of Glucose

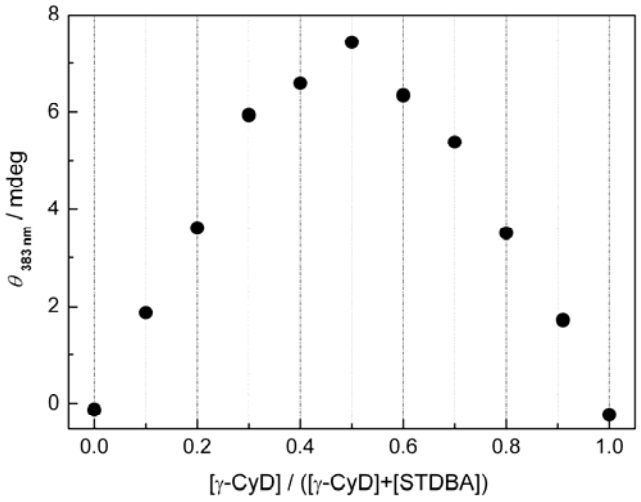


Fig. S8 Job plot for the STDBA/γ-CyD complex created from CD intensity at 383 nm in 1% DMSO aqueous solutions buffered at pH 10.5 in the presence of 10 mM glucose. [γ-CyD] + [STDBA] = 50 μM.

9. Evaluation of Binding Constants

The binding constant of glucose with STDBA/γ-CyD inclusion complex was determined from the titration of fluorescence at 448 nm. Equations (1) and (2) were used for fitting the data in 1:1 and 2:1 models, respectively:

$$I/I_0 = 1 + k_{\Delta HG}\frac{[H]_0 + [G]_0 + 1/K_{11} - \sqrt{([H]_0 + [G]_0 + 1/K_{11})^2 - 4[H]_0[G]_0}}{2I_0} \quad (1)$$

$$I/I_0 = 1 + \frac{k_{\Delta HG}[H]_0[G] + k_{\Delta HG2}[H]_0 K_1 K_2 [G]^2}{I_0(1 + K_1[G] + K_1K_2[G]^2)} \quad (2)$$

in which H, G and HG denote host, guest and the host-guest complex, *i.e.* the 2:2 STDBA/γ-CyD inclusion complex ($[H]_0 = [STDBA]_0 / 2$), glucose, and glucose/STDBA/ γ-CyD complex, respectively. K is the binding constant, and $k_{\Delta HG}$ and $k_{\Delta HG2}$ are linear proportional constants.

Under 1:1 model, K_{11} was determined to be 1048 ± 64 M^{-1} (Fig. S9). Fitting according to 2:1 model gives K_1 and K_2 values of 775 ± 61 M^{-1} and 21 ± 102 M^{-1}, respectively (Fig. S10).

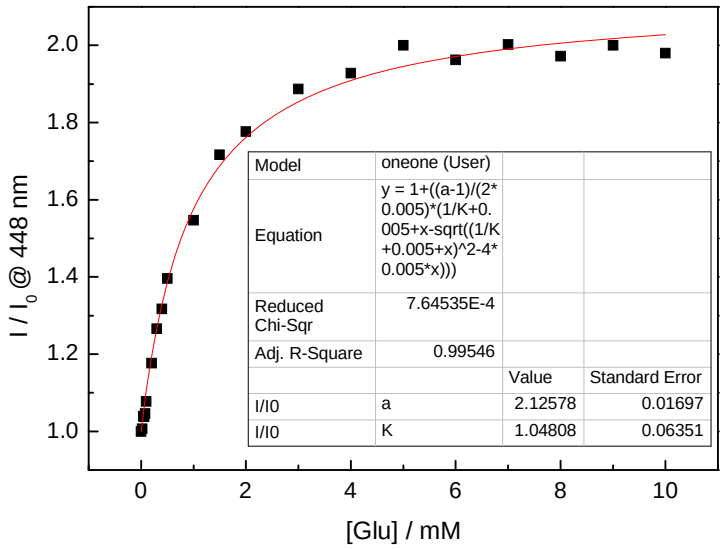


Fig. S9 Titration and fitted curves for the determination of binding constant of glucose with 2:2 STDBA/γ-CyD ensemble under 1:1 model

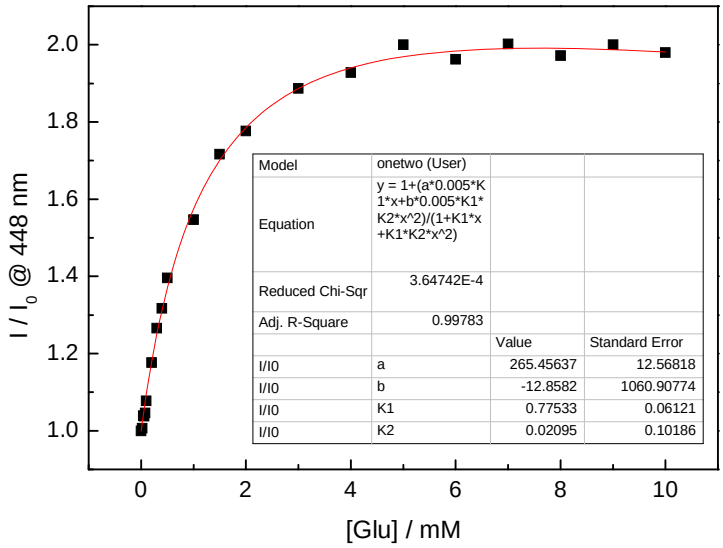


Fig. S10 Titration and fitted curves for the determination of binding constant of glucose with 2:2 STDBA/γ-CyD ensemble under 2:1 model

The binding constant of fructose with the STDBA/γ-CyD ensemble cannot be calculated with the abovementioned models since fructose binding results in the disassembly of the 2:2 ensemble. According to Scheme 1, the chemical reaction between the STDBA/γ-CyD ensemble and fructose can be described in (3),

$$[B_2 - CyD_2] + 2F + 2CyD \overset{K}{\Leftrightarrow} 2[BF - CyD_2] \quad (3)$$

in which B is STDBA, F is fructose, and K is the equilibrium constant of this reaction.

Since γ-CyD is in large excess, its concentration is assumed constant during the titration. Equation (3) is thus simplified into (4),

$$[B_2 - CyD_2] + 2F \overset{K'}{\Leftrightarrow} 2[BF - CyD_2] \quad (4)$$

in which K' is now an apparent equilibrium constant that contains K and the concentration of γ-CyD. K' can be recognized as the binding constant of STDBA/γ-CyD inclusion complex with fructose.

K' was fitted to be 10 ± 1 M^{-1} (Fig. S11) based on the variation of fluorescence intensity at 438 nm versus fructose concentration following Equation (5):

$$I / I_0 = 1 + k_{\Delta HG} \frac{-K'[G]^2 / 2 + \sqrt{K'^2 [G]^4 / 4 + 4K'[H]_0[G]^2}}{2I_0} \quad (5)$$

in which H is the STDBA/γ-CyD inclusion complex, G is fructose, HG is the STDBA/fructose/γ-CyD complex, and $k_{\Delta HG}$ is a proportional constant.

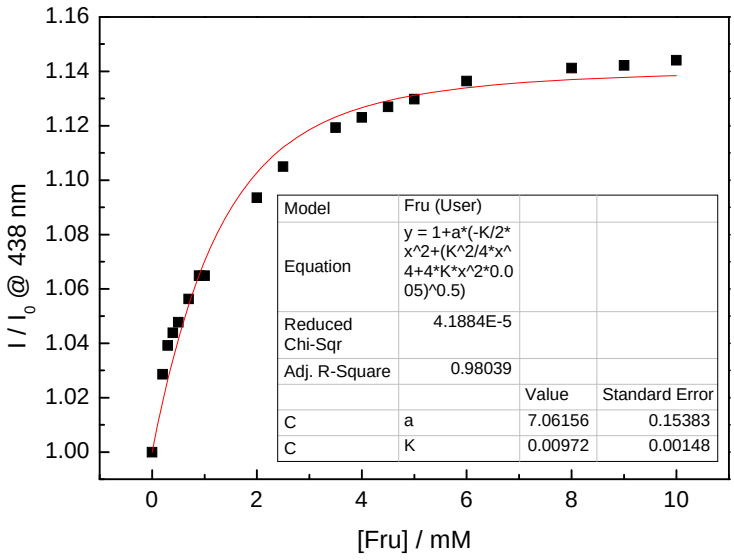


Fig. S11 Titration and fitted curves for the determination of binding constant of fructose with STDBA/γ-CyD inclusion complex

In Table S1, an apparent 1:1 binding constant of fructose with STDBA/γ-CyD inclusion complex is given. It should be noted that in the case of binding of fructose, galactose and mannose with STDBA/γ-CyD inclusion complex, the reaction does not actually proceed in a 1:1 stoichiometry and the apparent binding constants are shown only for comparison.

Table S1 1:1 binding constants of saccharides with STDBA/β-CyD and STDBA/γ-CyD inclusion complexes

Saccharides	STDBA/β-CyD K / M^{-1} (R^2)	STDBA/γ-CyD K / M^{-1} (R^2)
D-Glucose	94 ± 18 (0.989)	1048 ± 64 (0.995)
D-Fructose	1144 ± 152 (0.973)	789 ± 47 (0.993)
D-Galactose	343 ± 41 (0.987)	—[a]
D-Mannose	155 ± 12 (0.997)	—[a]

[a] Spectral changes too small to allow a credible fitting of the binding constant.

10. NMR Spectra of STDBA

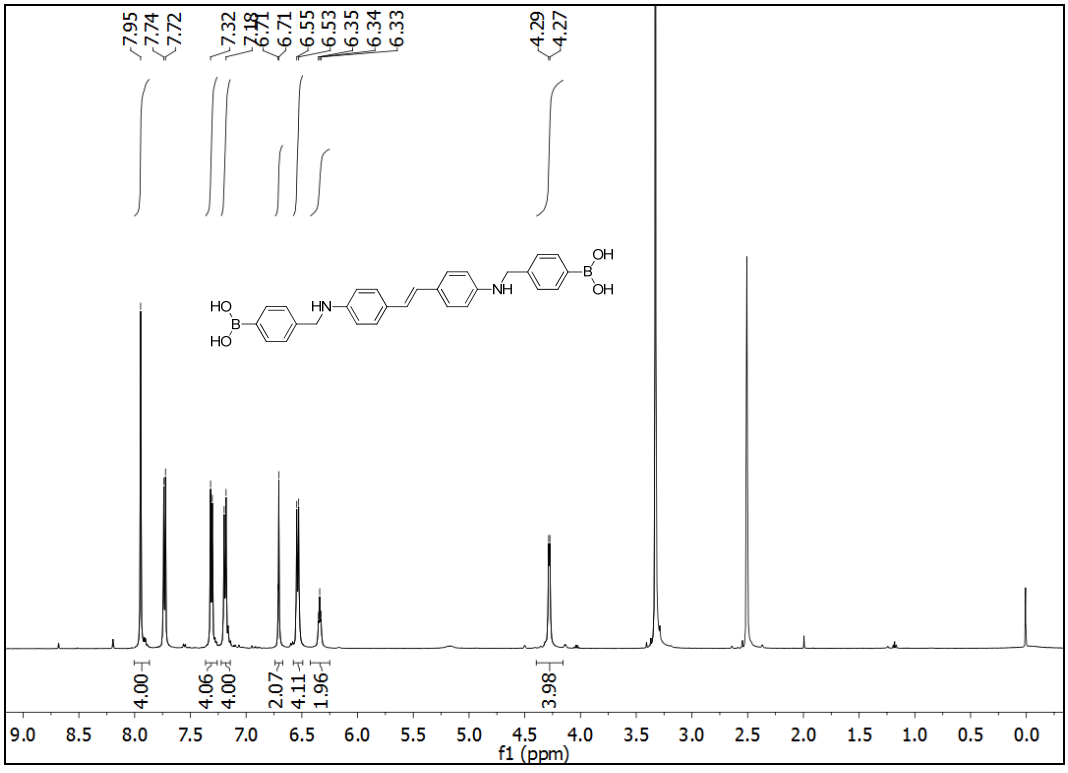


Fig. S12 ^{1}H NMR spectrum of STDBA in DMSO-d_6

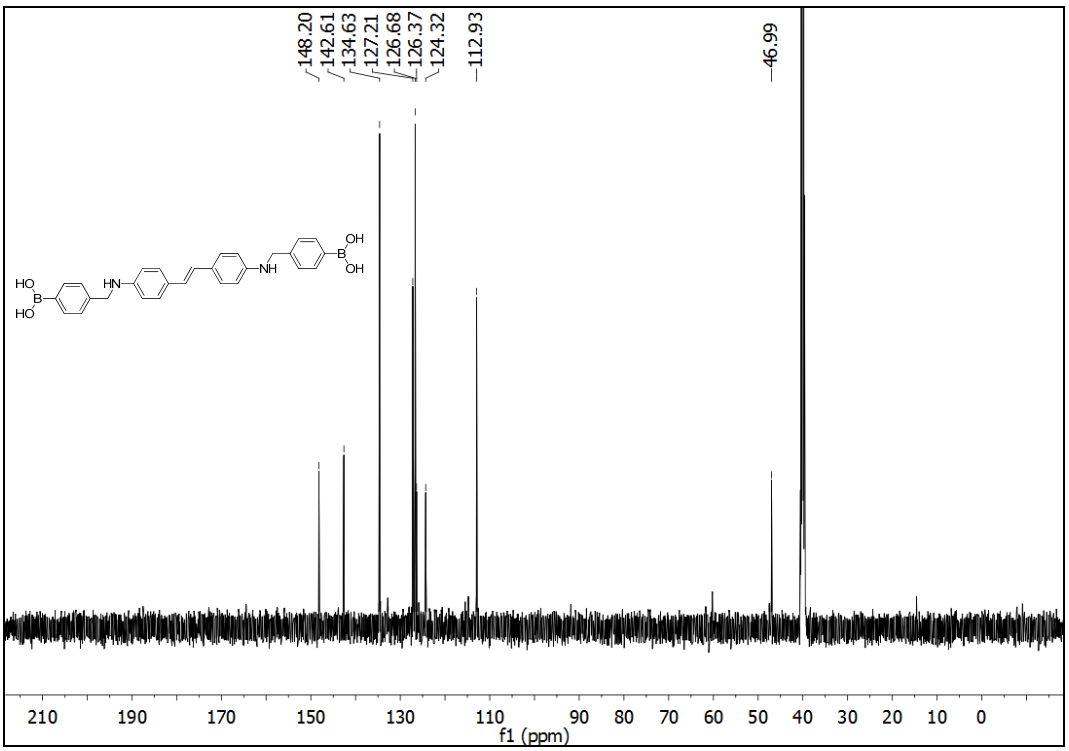


Fig. S13 ^{13}C NMR spectrum of STDBA in DMSO-d_6

References

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3 Y. Liu, C. Deng, L. Tang, A. Qin, R. Hu, J. Z. Sun and B. Z. Tang, *J. Am. Chem. Soc.*, 2010, **133**, 660-663.