Supporting Information

Selective Glucose Recognition by Boronic Acid Azoprobe/γ -Cyclodextrin Complexes in Water

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1. Apparatus

UV-vis absorption spectra were recorded on a Hitachi U-3000 spectrophotometer with a 1.0-cm quartz cell. The absorption spectra of each sample were obtained by subtraction of the spectra of γ-CD solution containing 1% DMSO-99% water (v/v) in the absence of probe. ICD spectra were obtained on a JASCO J-820 spectrophotometer with a 1.0-cm quartz cell. ^{1}H NMR spectra were obtained using a JEOL-GX-500MHz.

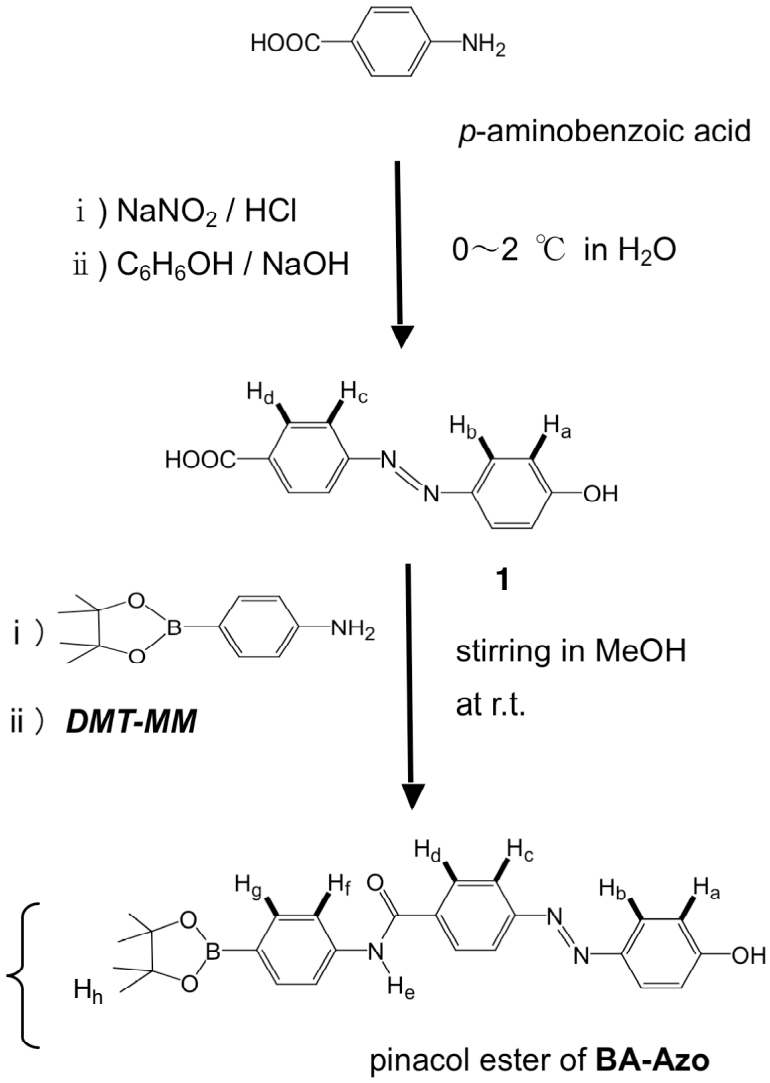
2. Syntheses of BA-Azo and B-Azo

Synthesis of BA-Azo. Scheme 4.1 shows the synthesis procedure for **BA-Azo**. First, **1** was synthesized by diazo-coupling: 5.01 g (36.5 mmol) *p*-aminobenzoic acid was dissolved in 100 cm^3 of water, and 10 cm^3 of concentrated hydrochloric acid was added, and then the solution was stirred and cooled in the water bath with ice. 2.53 g (36.7 mmol) of sodium nitrite in 15 cm^3 of water was slowly added into the cooled solution, and the mixture was stirred for an hour. The other mixture of 3.50 g (37.2 mmol) of phenol in 10 cm^3 of water and 10 cm^3 of 5 M sodium hydroxide was also

prepared and cooled. The latter solution was slowly added into the former solution and stirred for an hour (pH 2). The resultant precipitate was filtered. The filtrate was cooled to obtain theadditional precipitate and the mixture of 3.5 g (37.2 mmol) of phenol in 10 cm^3 of water and 10 cm^3 of 5 M sodium hydroxide was added again. This mixture was stirred for 30 minutes (pH 9). The obtained precipitate was filtered. The two precipitates were recrystallized together from methanol with water. The yield of **1** was 77.3% (6.84 g, 28.2 mmol). The structure of **1** was confirmed by ^{1}H NMR and EI-MS. ^{1}H NMR (500 MHz、CD_3OD) δ (ppm) 6.93 (d, 2H, H_a,J_{ab} = 9.0), 7.87 (d, 2H, H_b,J_{ab} = 9.0), 7.89 (d, 2H, H_c, J_{cd} = 9.0), 8.16 (d, 2H, H_d, J_{cd} = 9.0). EI-MS, *m/z* 242.

Second, **1** (0.500 g, 2.06 mmol) and 0.502 g (2.29 mmol) of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) aniline were dissolved in 100 cm^3 of methanol and the mixture was stirred at room temperature. The 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl- morpholinium chloride (***DMT-MM,*** 0.724 g, 2.30 mmol) in 25 cm^3 of methanol was added into the mixture and this solution was stirred for 6 hours at r.t. The reaction mixture was evapolated *in vacuo*. The residue was dissolved in chloroform, and washed with 1% acetic acid aqueous solution. The organic solution was dried over anhydrous magnesium sulfate, filtered and evaporated. The residue was dissolved in chloroform and purified by SEC (size exclusion chromatography). The yield was 59.3% (0.543 g, 1.22 mmol) as orange crystal. The structure of the product was confirmed as pinacolyl ester of **BA-Azo** by ^{1}H NMR, negative-ion ESI-MS, and elemental analyses. ^{1}H NMR (500 MHz、DMSO-d_6) δ (ppm) 1.30 (s, 12H, H_h), 6.97 (d, 2H, H_a, J_{ab} = 8.5), 7.68 (d, 2H, H_g, J_{fg} = 8.5), 7.86 (m, 4H, $H_{b,f}$), 7.93 (d, 2H, H_c, J_{cd} = 8.5), 8.14 (d, 2H, H_d, J_{cd} = 8.5), 10.49 (s, 1H, H_e). Negative-ion ESI-MS, *m/z* 442. Anal. Calculated for $C_{25}H_{26}BN_3O_4 \cdot 0.7H_2O$, C, 65.86; H, 6.06; N, 9.22, Found, C, 66.12; H, 6.17; N, 8.90.

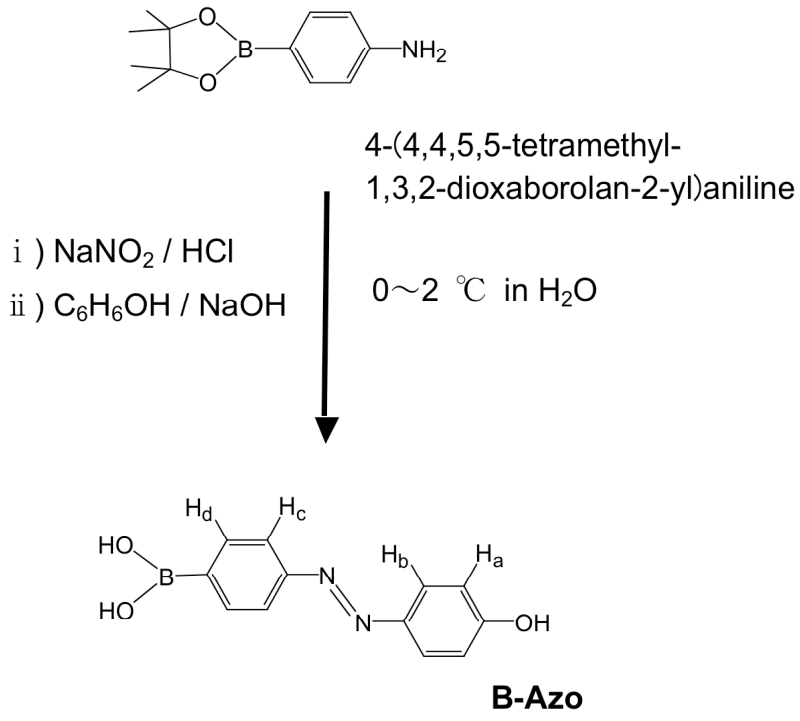
The pinacol ester of **BA-Azo** was used for the measurement in this study, because the pinacol protection group is known to be easily deprotected in water.



Scheme S1 Synthesis procedure for **BA-Azo**.

Synthesis of B-Azo. Scheme S2 shows the synthesis procedure for **B-Azo**. A mixture of 1.00 g (4.56 mmol) of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline, 30 cm^3 of water, and 0.86 cm^3 of concentrated hydrochloric acid were stirred and cooled in a water bath with ice. Sodium nitrite (0.315 g, 4.57 mmol) in 10 cm^3 of water was slowly added into the cooled solution, and the mixture was stirred for an hour. The other mixture of 0.429 g (4.56 mmol) of phenol in 10 cm^3 of water and 1 cm^3 of 5 M sodium hydroxide was also prepared and cooled. The latter solution was

slowly added into the former solution and the combined mixture was stirred for an hour (pH 4). The red-brown precipitate was filtered. The filtrate was cooled to obtain additional precipitate, and the mixture of 0.425 g (4.52 mmol) of phenol in 10 cm^3 of water and 0.5 cm^3 of 5 M sodium hydroxide were added again, and this mixture was stirred for 30 minutes (pH 8). The obtained precipitate was filtered. The two precipitates were recrystallized together from methanol with water. The product yield was 36.7% (0.405 g, 1.67 mmol). The structure was confirmed as **B-Azo** by ^{1}H NMR , EI-MS, and elemental analyses. ^{1}H NMR (500 MHz, CD_3OD) δ (ppm) 6.92 (d, 2H, H_a, J_{ab} = 8.5), 7.82 (m, 6H, $H_{b,c,d}$). EI-MS, *m/z* 242. Anal. Calculated for $C_{12}H_{11}BN_2O_3 \cdot 0.14H_2O$, C, 58.92; H, 4.65; N, 11.45, Found, C, 59.00; H, 4.42; N, 11.59.



Scheme S2 Synthesis procedure for **B-Azo**

3. ICD and UV-Vis spectral response of B-Azo and BA-Azo

Figure S1 shows the ICD spectra (a and c) and the UV-Vis spectra (b and d) of the **B-Azo** and the **BA-Azo**. No significant spectral responses were noted for both azoprobes upon addition of 30 mM D-fructose and D-glucose.

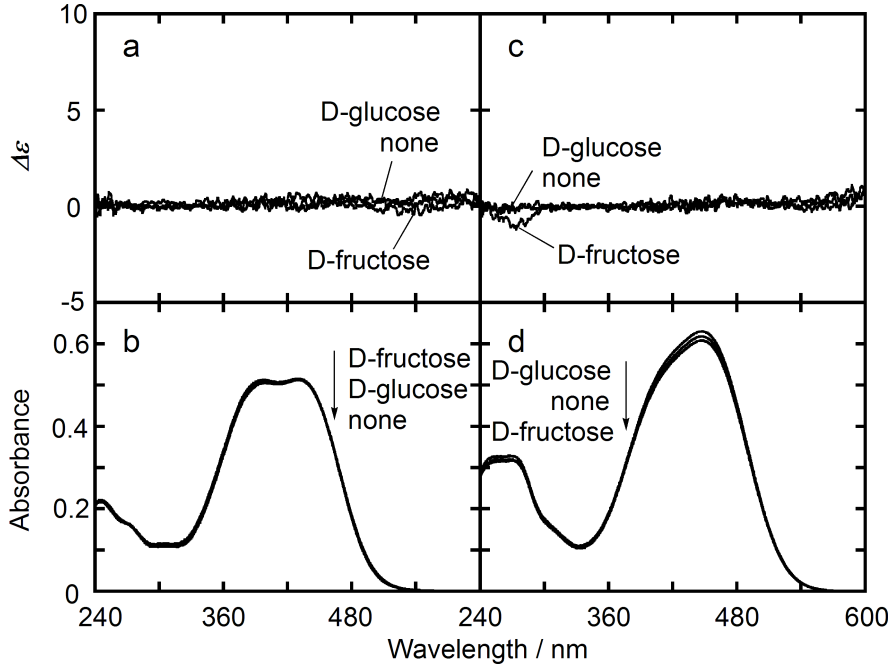


Fig. S1 (a) ICD and (b) UV-Vis spectra of **B-Azo**, (c) ICD and (d) UV-Vis spectra of **BA-Azo** in 1% DMSO - 99% water (v/v). [Azoprobe] = 5.0 x 10^{-5} M (a, c), 2.5 x 10^{-5} M (b, d), [saccharide] = 30 mM, I = 0.1 M with NaCl, pH 10.0 adjusted by Na_2CO_3/HCl buffer, 25°C.

4. ICD and UV-Vis spectral response of B-Azo/β-CD and BA-Azo/β-CD complexes

Figure S2 shows the ICD spectra (a and c) and the UV-Vis spectra (b and d) of the **B-Azo**/β-CD and the **BA-Azo**/β-CD complexes. Similar to Fig. S1, no significant spectral responses were noted for both azoprobes upon addition of 30 mM D-fructose and D-glucose.

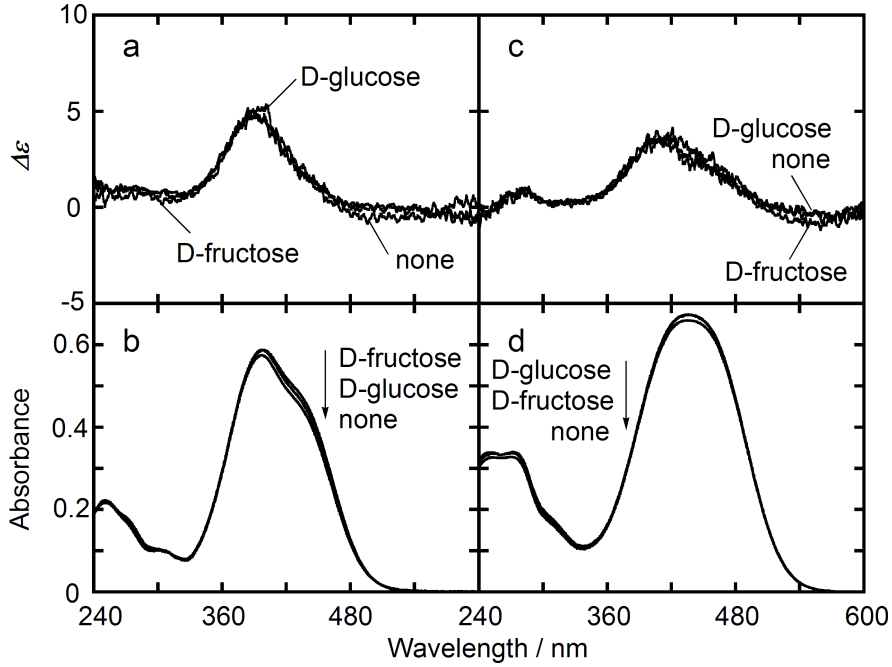


Fig. S2 (a) ICD and (b) UV-Vis spectra of **B-Azo**/β-CD complex, (c) ICD and (d) UV-Vis spectra of **BA-Azo**/β-CD complex in 1% DMSO - 99% water (v/v) containing 3.0 mM β-CD. [Azoprobe] = 5.0 x 10^{-5} M (a, c), 2.5 x 10^{-5} M (b, d), [saccharide] = 30 mM, I = 0.1 M with NaCl, pH 10.0 adjusted by Na_2CO_3/HCl buffer, 25°C.

5. Inclusion complex formation of BA-Azo with γ-CD

To clarify the 2:1 inclusion complex formation of **BA-Azo** with γ-CD in the presence of D-glucose, the effect of γ-CD concentration on the absorbance from the UV-vis spectra for **BA-Azo** was examined. With an increase in γ-CD concentration, the absorbance ratio (A_{436}/A_{446}) for **BA-Azo** increased in the presence of D-glucose (Fig. 2). When it is assumed that the absorbance change is only induced by the formation of a 2:1complex (L_2CD) between **BA-Azo** (L) and γ-CD (CD), the absorbance ratio is expressed by the following equations:

$$\frac{A_{436}}{A_{446}} = \frac{4\dfrac{\varepsilon_{L_{436}}}{\varepsilon_{L_{446}}} + \dfrac{\varepsilon_{(L_2CD)_{436}}}{\varepsilon_{L_{446}}}\left(-1+\sqrt{1+8K[CD][L]_t}\right)}{4+\dfrac{\varepsilon_{(L_2CD)_{446}}}{\varepsilon_{L_{446}}}\left(-1+\sqrt{1+8K[CD][L]_t}\right)} \qquad (1)$$

$$K = \frac{[L_2CD]}{[CD][L]^2} \qquad (2)$$

where $[L]_t$ is the total concentration of **BA-Azo**, and ε_{L436} and ε_{L446} are the molar absorptivity for **BA-Azo** at 436 and 466 nm, respectively. Similarly, $\varepsilon_{(L2CD)436}$ and $\varepsilon_{(L2CD)436}$ are the molar absorptivity for the 2:1 complex at 436 and 466 nm respectively. The observed results in Fig. 2 were fitted well with eq. 1 (solid line), and the binding constant (K) for the 2:1 inclusion complex of **BA-Azo** with γ-CD was calculated as (2.7 ± 0.4) × 10^7 M^{-2}. The binding constants without saccharide and D-fructose were not calculated due to their low spectral responses.

6. Binding constant of BA-Azo/γ-CD complex for D-glucose.

The apparent 2:1 binding constant (K_{21}) of **BA-Azo** with D-glucose in the presence of 3.0 mM γ-CD was determined from the changes in UV-vis spectra. With an increase in D-glucose concentration, the absorbance ratio (A_{436}/A_{446}) for **BA-Azo** increased (Fig. S3). On the assumption that the absorbance change is only induced by the formation of a 2:1complex (L_2CD) between **BA-Azo** (L) and D-glucose (S), the absorbance ratio can be expressed by the following equations:

$$\frac{A_{436}}{A_{446}} = \frac{4\frac{\varepsilon_{L_{436}}}{\varepsilon_{L_{446}}} + \frac{\varepsilon_{(L_2S)_{436}}}{\varepsilon_{L_{446}}}(-1+\sqrt{1+8K_{21}[S][L]_t})}{4 + \frac{\varepsilon_{(L_2S)_{446}}}{\varepsilon_{L_{446}}}(-1+\sqrt{1+8K_{21}[S][L]_t})} \qquad (3)$$

$$K_{21} = \frac{[L_2S]}{[S][L]^2} \qquad (4)$$

where $[L]_t$ is the total concentration of **BA-Azo**, and ε_{L436} and ε_{L446} are the molar absorptivity for **BA-Azo** at 436 and 466 nm, respectively. Similarly, $\varepsilon_{(L2S)436}$ and $\varepsilon_{(L2S)436}$ are the molar absorptivity for the 2:1 complex at 436 and 466 nm respectively.

From eq. (3), the binding constant for the 2:1 inclusion complex of **BA-Azo** with D-glucose was calculated as $(3.0 \pm 0.5) \times 10^7\ M^{-2}$.

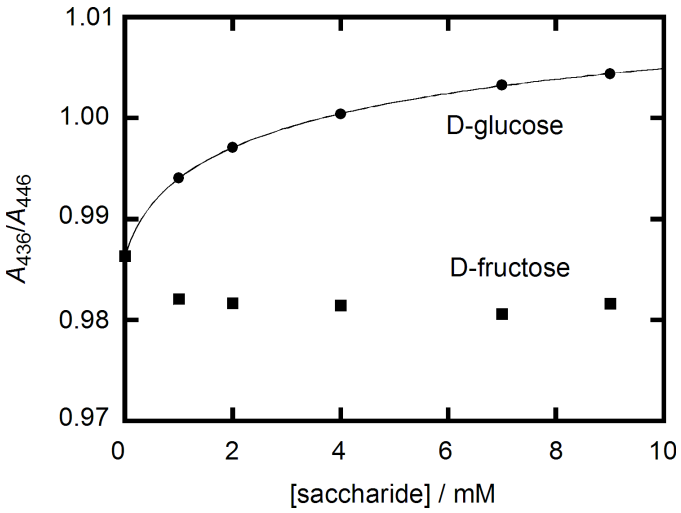


Fig. S3 Absorbance ratio (A_{436}/A_{446}) of **BA-Azo** as a function of saccharide concentration of D-glucose and D-fructose in the presence of 3.0 mM γ-CD. [**BA-Azo**] = 2.5 x 10^{-5} M in 1% DMSO–99% water (v/v), I = 0.1 M with NaCl, pH 10.0 adjusted by Na_2CO_3/HCl buffer, 25°C.

7. ^{1}H NMR analysis

^{1}H NMR analysis was carried out for **BA-Azo** and **BA-Azo**/γ-CD complex. To enhance **BA-Azo** concentration at 2.0 mM for ^{1}H NMR analysis, 80% D_2O-20% DMSO-d_6 (v/v) was used as solvent. Although the response efficiency was significantly reduced in 80% D_2O-20% DMSO-d_6 (v/v) solution, the additional peaks of phenyl protons appeared at the upfield region of each peaks for **BA-Azo**/γ-CD complex in the presence of D-glucose. This result supports the dimer formation of **BA-Azo** with D-glucose inside γ-CD.

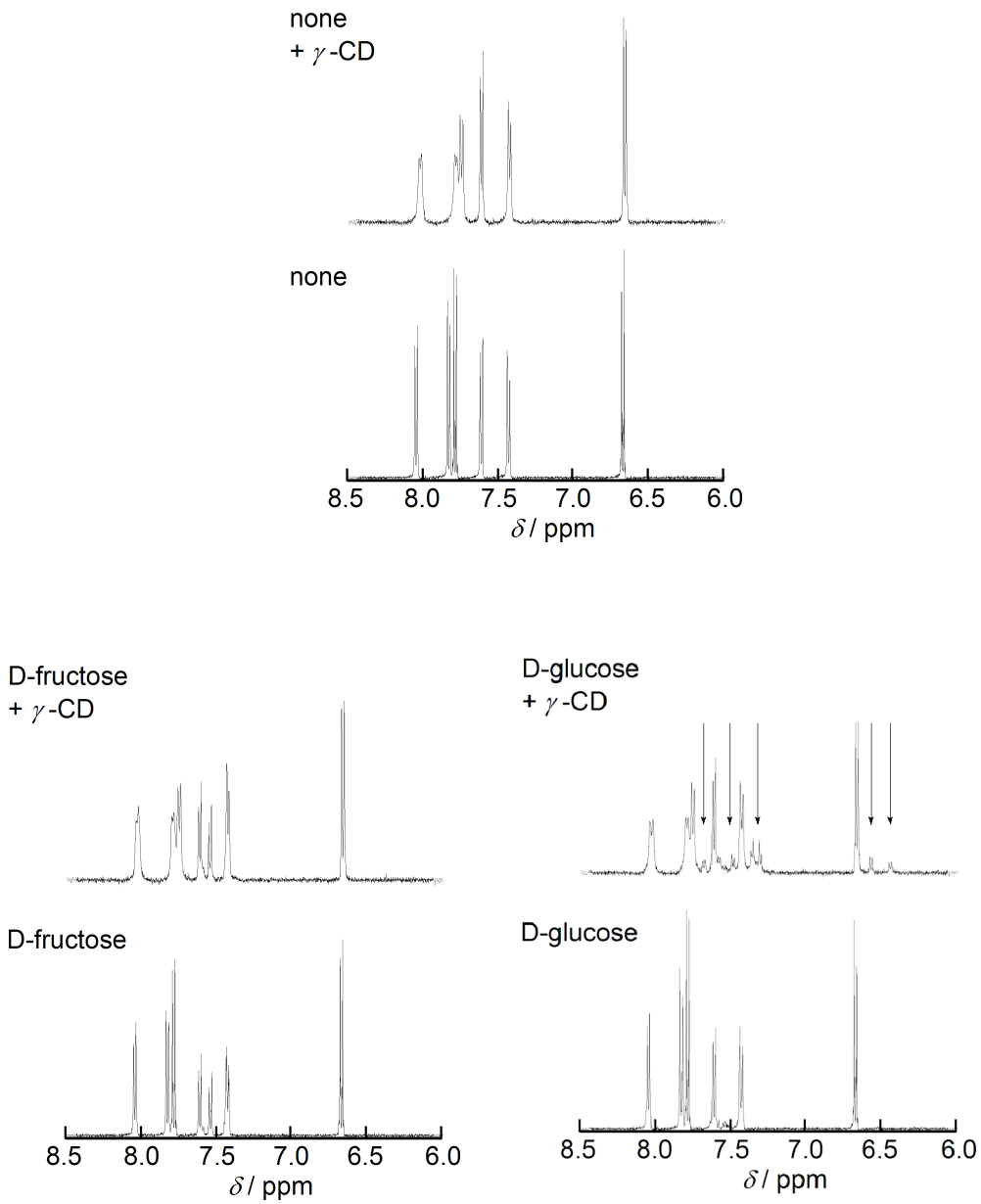


Fig. S4 ^{1}H NMR spectra of **BA-Azo** and **BA-Azo**/γ-CD complex in 80% D_2O-20% DMSO-d_6 (v/v). [**BA-Azo**] = 2.0 mM, [saccharide] = 1.0 mM, and [γ-CD] = 4.0 mM. pD = 11.4 adjusted by Na_2CO_3 buffer, 25°C.