ORGANIC LETTERS

2000
Vol. 2, No. 5
589−592

Visual Sensing of Saccharides Promoted by Resorcinol Condensation Products

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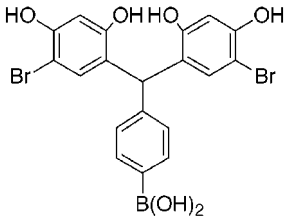
Received December 10, 1999

ABSTRACT



3

Heating aqueous DMSO solutions of five saccharides in the presence of 1–3 reveals that each receptor promotes solution colors characteristic for each sugar. New compound 3 allows the direct correlation of sugar concentration with visible region absorbance and/or fluorescence intensities.

The specific recognition of saccharides by synthetic hosts is of great current interest.[1] Classical colorimetric methods for the nonenzymatic detection of sugars are typically limited to discriminating between, for instance, reducing and nonreducing sugars.[2] Recent progress toward improved synthetic visual receptors has been reported based on the affinity of nitrogen-containing arylboronic acid compounds.[3]

In 1872 von Baeyer studied the condensation of benzaldehyde and resorcinol.[4] He found that a red-colored product was formed which changed color to violet in the presence of base. We recently reported the discovery that **1** promotes the visual differentiation of specific saccharide solutions by simple color changes.[5] Our methodology, involving heating aqueous DMSO solutions of **1**[6] containing saccharides, produces characteristic color changes for specific carbohydrates, glucose phosphates, amino sugars, and sialic and uronic acids.[5] Herein, as part of our ongoing investigations aimed at fully elucidating the mechanism, scope, and limitations of this selective new coloration process we detail our findings employing receptor **2**[7] and new compound **3** as the sensing agents. We observe colorimetric properties with each resorcinol condensation product. In addition, compound **3** affords linear correlations between sugar concentrations and visible region absorbance and/or fluorescence intensities.

Compound **2** is a readily available prototypical crown stereoisomeric resorcinarene.[8] Heating 5.2 mM **2** (colorless after several recrystallizations from MeOH) in 1 mL of 9:1 DMSO:H_2O for 3 min to a gentle reflux followed by cooling to rt leads to a purple solution. The color formation can be monitored via a new absorption appearing at 536 nm. This result is consistent with the same experiment employing **1**.[5]

(1) Reviews: (a) James, T. D.; Samankumara Sandanayake, K. R. A.; Shinkai, S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1910. (b) Davis, A. P.; Wareham, R. S. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 2979.

(2) Chaplin, M. F. In *Carbohydrate Analysis. A Practical Approach*; Chaplin, M. F., Kennedy, J. F., Eds.; Oxford University Press: Oxford, 1994; pp 2−8.

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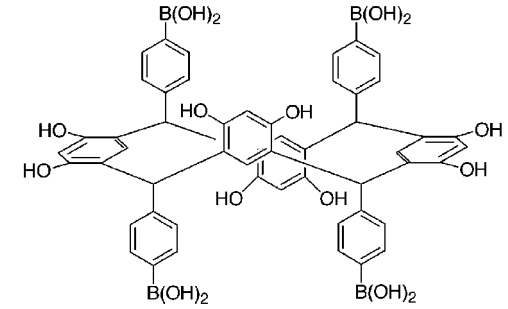
10.1021/ol9903990 CCC: $19.00
Published on Web 02/09/2000



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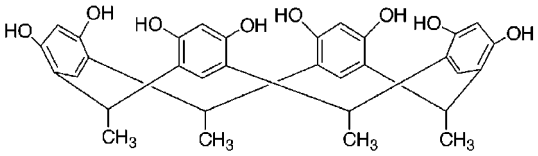

2

Heating colorless solutions of **2** and 3.0 equiv of D-(−)-fructose, α-D-glucose, sucrose, or the glucose phosphates (Table 1) results in colors selective for each sugar; however, by visual inspection, the colors appear paler and less distinctive as compared to those from sensing experiments with **1**. The experiments with **2** indicate that the visual color sensing of saccharides appears to be general to the resorcinarenes, including those without boronic acids.

To confirm that the characteristic solution colors are due to the resorcinol hydroxyls, we synthesized the known octaacetate of **2**.[7b] Colorless 5.2 mM 9:1 DMSO:H_2O solutions of the octaacetate, heated at a gentle reflux for 3 min, remain colorless. When heated in the presence of 3 equiv of sucrose, glucose, or fructose, the ocataacetate solution colors are identical to those of the sugars heated without octaacetate (sucrose is colorless; glucose and fructose solutions are light yellow).

Previously we reported that heating resorcinol and phenylboronic acid alone or as a mixture in the presence of added sugars did not produce the dramatic, characteristic solution colors observed with **1**.[5] To extend the scope of the sensing process with a simpler receptor, we synthesized the new triaryl compound **3**.[9] This compound embodies a (bromine-containing) substructure of **1**.[10,11] Compound **3** (5.2 mM in 1 mL of 9:1 DMSO:H_2O) affords the most vivid solution colors we have observed to date (Table 1). The colors are highly characteristic for each sugar and much more brilliant in appearance than the solutions containing **1** or **2**. This color brilliance can be further enhanced by prolonged heating (to 8 min).

Previously we found that oxygen promoted the color development of solutions of **1** alone or in the presence of sugars.[5] Heating N_2-saturated samples of **2** or **3** with or without the five saccharides listed in Table 1 for 3 min results in a significant decrease in visible region absorbance intensity (decrease by 3−40% [536 nm] and decrease by 30−76% [532 nm], respectively) as compared to that of samples heated without protection from air. This supports our prior proposal that color formation is promoted by oxygen.[5,12]

We also decided to examine the effect of acid and base on the colorimetric properties of **1**−**3**. The solution colors of resorcinol condensation products have long been known to exhibit pH dependence.[4] If adding sugars to the receptor solutions is producing color changes solely via sugar-induced absorbance changes resulting from pH effects, we should be able to correlate acid−base titration-induced absorbance changes with sugar-induced absorbance changes. Figure 1 shows an expansion of the plot of absorbance intensity vs

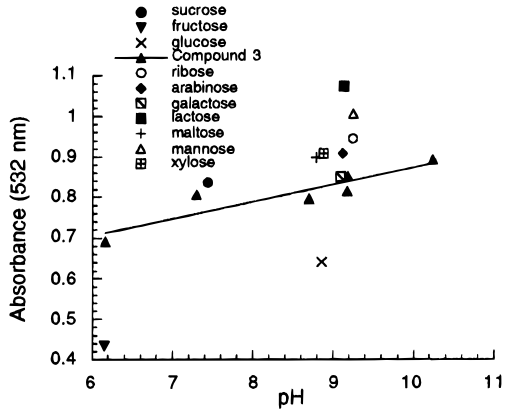

Figure 1. Expansion of pH[13] vs absorbance plot for **3** overlayed with pH/absorbance data for 10 carbohydrates. Samples were heated 7 min (5 mL of 9:1 DMSO:H_2O, 2.6 mM **3**, and 3 equiv of sugar). Glucose 1- and 6-phosphate data were off scale (A = 2.17 at pH 9.9 and A = 1.73 at pH 9.1, respectively).

Table 1. Monitored λ, Corresponding Absorbances (A), and Solution Color Observed upon Heating 9:1 DMSO:H_2O 5.2 mM Solutions of **2** or **3** and 3 equiv of Added Saccharides to a Gentle Reflux for 3 min. Values Represent Averages of Three Runs

	2			**3**		
added sugar	λ (nm)	A	color	λ (nm)	A	color
none	536	0.472	purple	532	0.807	deep pink
sucrose	536	0.329	purple	532	0.801	deep pink
α-D-glucose	536	0.172	yellow-orange	532	0.642	bright peach
D-(−)-fructose	536	0.128	peach	532	0.407	deep yellow
α-D-glucose 1-phosphate disodium salt hydrate	409	2.25	orange-brown	532	2.16	bright reddish-orange
	536	0.536				
D-glucose 6-phosphate monosodium salt	452	0.680	yellow-brown	532	1.73	bright brownish-auburn
	536	0.274				

increased [OH^-] for heated solutions of **3**. The pH[13] vs absorbance data for 10 solutions containing **3** and a specific sugar are overlayed. The data show a poor correlation between pH[13] with the solutions of **3** vs the solutions with **3** and a specific sugar. These results suggest that the sugar color sensing mechanism involves more than just pH effects.

To determine whether the sensing process is amenable to controlled pH conditions, we derived a correlation plot of fluorescence intensity versus sugar concentration (Figure 2)

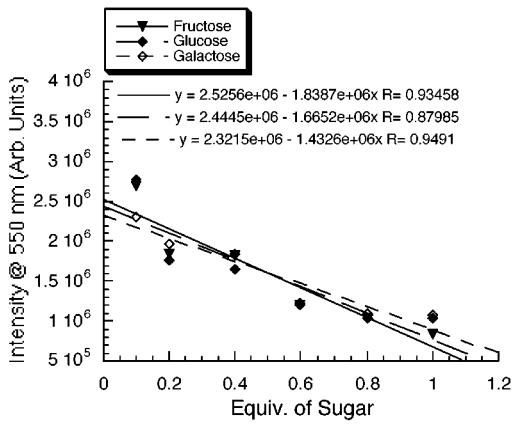

Figure 2. Changes in fluorescence intensity in the emission spectra (λ_{ex} = 533 nm, λ_{em} = 550 nm) of 9:1 DMSO:H_2O heated (8 min) solutions (6 mL) of **3** [2.6×10^{-5} M] and D-(−)-fructose, α-D-glucose, or D-(+)-galactose and K_2CO_3 buffer [5.0×10^{-2} M].

in the presence of carbonate buffer and **3**. We observe a steady decrease in fluorescence intensity upon increased saccharide concentration. At constant pH, compound **3** promotes fluorescence,[1,14−16] monitoring of μM levels of glucose, fructose, and galactose, within the range of their concentrations found in blood.[1a]

We also observe a correlation of visible region fluorescence intensity with μM changes in glucose concentration in the presence of a fixed amount of fructose [52 μM] and **3** [52 μM] (Figure 3). Similarly, a linear correlation of

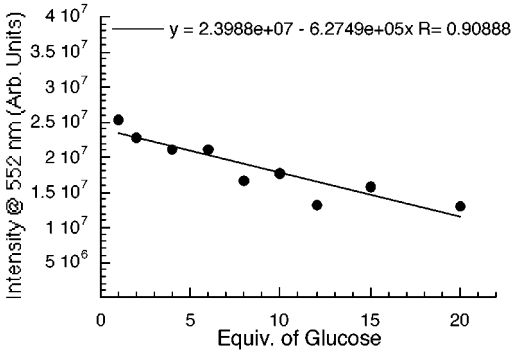

Figure 3. Changes in fluorescence intensity in the emission spectra (λ_{ex} = 533 nm, λ_{em} = 552 nm) of 9:1 DMSO:H_2O solutions (6 mL) of heated (8 min) **3** [5.2×10^{-5} M] with varied amounts of α-D-glucose in the presence of a fixed concentration of D-(−)-fructose [5.2×10^{-5} M] and no buffer.

fluorescence intensity with varied fructose concentration in the presence of glucose [52 μM] and **3** [52 μM] is observed. The slope for the fructose binding decreases approximately twice as rapidly as that for glucose (-1.132×10^6, R = 0.90643). This indicates that **3** is more sensitive to fructose than glucose. In addition, **3** promotes a correlation of sugar concentration with UV−vis absorbance intensities in the mM range. Figure 4 shows an excellent linear correlation of glucose concentration with visible region absorbance intensity.

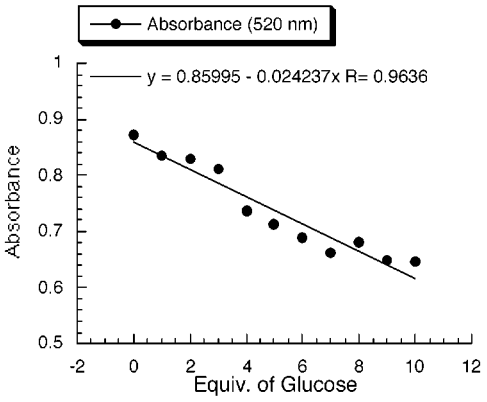

Figure 4. Changes in visible region absorbance intensity promoted by **3** [5.2 mM] heated for 8 min in 2 mL of 9:1 DMSO:H_2O and varying concentrations of α-D-glucose.

(8) (a) Timmerman, P.; Reinhoudt, D. N. *Tetrahedron* **1996**, *52*, 2263. (b) **2** has been recently studied as a receptor for a variety of polar molecules: Iwanek, W.; Frohlich, R.; Urbaniak, M.; Nather, C.; Mattay, J. *Tetrahedron* **1998**, *54*, 14031. (c) Binding of sugars by related alkyl-substituted resorcinarenes in apolar media: Aoyama, Y.; Tanaka, Y.; Sugihara, S. *J. Am. Chem. Soc.* **1989**, *111*, 5397.

(9) 4-Bromoresorcinol (2.00 g, 10.6 mmol) and 4-formylphenylboronic acid (0.793 g, 5.29 mmol) are stirred in EtOH (30 mL) followed by addition of concentrated HCl (15 mL) dropwise. The mixture is heated at 70 °C for 3 h. After cooling to rt, the mixture is neutralized with $NaHCO_3$, extracted into EtOAc, and refluxed with decolorizing carbon to remove the solution color for sensing studies. Upon filtration and recrystallization from MeOH, filtration, and drying of the precipitate, **3** (1.64 g,61%) is obtained: mp >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.70 (s, 1H), 6.50 (s, 2H), 6.53 (s, 2H), 6.91 (d, J = 8.0 Hz, 2H), 7.96 (bs, 2H), 9.92 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 41.9, 97.5, 103.6, 123.2, 127.9, 131.6, 132.4, 134.0, 145.7, 152.8, 155.0; UV λ_{max} = 286 nm (DMSO); MALDI MS *m*/*z* calcd 509.94, obsd 510.77 M^+.

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(11) Our synthesis is based directly on similar molecules: Rumboldt, G.; Böhmer, V.; Botta, B.; Paulus, E. V. *J. Org. Chem.* **1998**, *63*, 9618.

(12) Compound **3** is more soluble in organic solvents compared to **1** and **2** and is apparently more amenable to flash column chromatography. To date, we have isolated small amounts (>0.5 mg) of a purple solid which appears to form in enriched amounts upon heating aqueous DMSO solutions of **3**. The purple material exhibits a strong absorbance at 535 nm and remains on the baseline of the flash column after elution of **3** in 5:1 $CHCl_3$:MeOH. Efforts are ongoing in our laboratory aimed at isolating and studying colored materials produced upon heating **1**−**3**.

In conclusion, the visual sensing of saccharides is applicable to a variety of readily available resorcinol condensation products.[8a] In addition, changing the receptor framework can alter the color patterns observed for the various sugars. O_2 consistently promotes the solution color formation with **1**−**3**. The resorcinol hydroxyls play a key role in the color formation of the receptor solutions,[12] as demonstrated by control experiments with the octaacetate derivative of **2**. Compound **3** enables direct correlations between concentration and fluorescence and/or absorbance intensities in the visible region, leading to facile monitoring of sugar levels.

Ongoing studies are aimed at further probing the mechanism and scope of biomolecule visual sensing with resorcinol condensation products. Our efforts include examining the role of pH, aggregation−deaggregation,[1a,5] oxidation[5,12,17] and covalent[1a,18] and/or noncovalent[1b,8] analyte/receptor interactions.[1,8,19,20] We will report further details of the color sensing mechanism in due course.

Acknowledgment. We thank the Arnold and Mabel Beckman Foundation for support through the Beckman Young Investigator Award Program and Professor Isiah Warner and Dr. Rafael Cueto for the use of fluorescence equipment.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of **3**. This information is available free of charge via the Internet at http://pubs.acs.org.

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(13) The pH values for Figure 1 were obtained via a pH meter. They therefore represent relative nominal pH readings (to display trends for the present study), not precise pH values.

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(16) Selective glucose fluorescent sensing with a synthetic receptor: (a) Bielecki, M.; Eggert, H.; Norrild, J. C. *J. Chem. Soc., Perkin Trans. 2* **1999**, 449. (b) Eggert, H.; Frederiksen, J.; Morin, C.; Norrild, J. C. *J. Org. Chem.* **1999**, *64*, 3846.

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(19) Extensive NMR studies of the binding of sugars to boronic acids: (a) Norrild, J. C.; Eggert, H. *J. Am. Chem. Soc.* **1995**, *117*, 1479. (b) Norrild, J. C.; Eggert, H. *J. Chem. Soc., Perkin Trans. 2* **1996**, 2583.

(20) Addition of 3 equiv of sucrose, glucose, or fructose to colored (preheated) 10% aqueous DMSO solutions containing 5.2 mM **1** affords characteristic color changes (purple, peach, and yellow for sucrose, glucose, and fructose, respectively, as described in ref 5) upon standing at rt overnight. Interestingly, addition of preheated (6 min at gentle reflux) 10% aqueous DMSO solutions containing 3 equiv of each of the three sugars to colored (preheated) solutions of **1** also affords solution colors similar to those reported in ref 5, but within 1 min at room temperature. The absorbance intensity monitored at 538 nm diminishes corresponding to the previously observed order: sucrose (A = 0.546) > glucose (A = 0.493) > fructose (A = 0.174).