ORGANIC LETTERS

1999
Vol. 1, No. 2
331−334

Simple and Rapid Visual Sensing of Saccharides

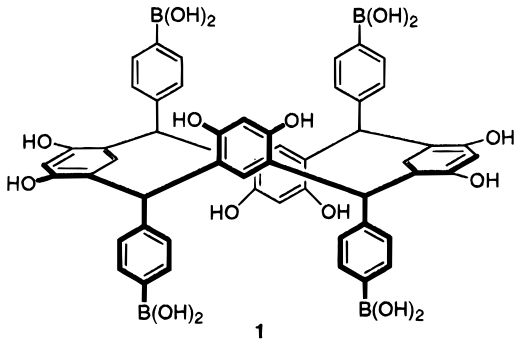
Claude J. Davis, Patrick T. Lewis, Matthew E. McCarroll, Mark W. Read, Rafael Cueto, and Robert M. Strongin*

Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803

rob.strongin@chem.lsu.edu

Received May 25, 1999

ABSTRACT



1

Solutions of compound 1 exhibit dramatic, characteristic color changes in response to sugar analytes. Structurally related saccharides including glucose phosphates and amino and carboxylic acid sugars can be readily distinguished by visual inspection. These findings should promote the design of unique color sensory materials based on readily available, functional macrocyclic hosts.

Facile methods for detecting and monitoring saccharides are of immense importance to medical diagnostics and industry. A current challenge in this area is the fabrication of readily accessible, stable artificial receptors that promote fast, sensitive, and selective detection.[1] Such materials could lead to improved sensors relative to degradable enzyme-based systems or to those requiring complex and expensive syntheses or instrumentation. Herein we report that a tetraarylboronic acid resorcinarene macrocycle, obtained on large scale in one step and easily purified,[2] promotes the most versatile visible detection of sugars observed to date. Characteristic and dramatic solution color changes are attained for carbohydrates, glucose phosphates, amino sugars, and sialic and uronic acids.

The visual determination of saccharides has been of great interest for more than a century. In 1887 Seliwanoff reported a resorcinol color test that was specific for ketoses.[3] Other resorcinol-based color tests for sugars were later developed.[4,5] In this decade great progress has been made toward the enhanced selective visible detection of saccharides via the pioneering studies of Shinkai and co-workers, based mainly on chromophore-functionalized arylboronic acids[1] or the affinity of nitrogen-containing chromophores for arylboronic acids.[6] A recent review underscored the lack of sugar receptors that promote dramatic color changes in the presence of individual analytes.[1] Powerful color receptors could be of practical utility for monitoring disease states or the products of fermentation processes.

We have previously reported a facile synthesis of macrocycles which embody both arylboronic acid and resorcinol moieties.[2] One of these, compound **1**, is obtained as a white solid which forms a colorless solution when dissolved in DMSO at room temperature. After standing in solution for

(1) James, T. D.; Samankumara Sandanayake, K. R. A.; Shinkai, S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1910−1922.
(2) Lewis, P. T.; Davis, C. J.; Saraiva, M.; Treleaven, W. D.; McCarley, T. D.; Strongin, R. M. *J. Org. Chem.* **1997**, *62*, 6110−6111.
(3) Seliwanoff, T. *Chem. Ber.* **1887**, *20*, 181−182.
(4) Bial, M. *Dtsch. Med. Wehschr.* **1902**, *28*, 253−254.
(5) Kulka, R. G. *Biochem J.* **1956**, *63*, 542−548.
(6) Koumoto, K.; Takeuchi, M.; Shinkai, S. *Supramol. Chem.* **1998**, *9*, 203-210.

10.1021/ol990105a CCC: $18.00
Published on Web 07/01/1999

several hours or upon heating at 90 °C for 1 min, the solution develops a pinkish-purple color. These color changes can be monitored in the UV−vis spectrum of **1** via the appearance of a new absorption at 536 nm. The fluorescence spectrum of colorless solutions of **1** exhibits only short-wavelength excitation and emission bands at 350 and 410 nm, respectively. The colored solutions additionally display long-wavelength excitation at 525 nm and emission at 570 nm. When stored as a white solid over a period of several months, no change in the UV−vis or fluorescence spectrum of **1** is observed.

Heating colorless solutions of **1** (5.2 mM, 10:1 DMSO:H_2O, 1 mL) and 3.0 equiv of D-(−)-fructose, α-D-glucose, or sucrose at 90 °C for 1 min results in selective coloration. The fructose solution is deep yellow and the glucose solution pink-yellow. The solution containing sucrose, which exhibits the weakest binding to boronic acids in this series,[7,8] is purple and easily distinguished from glucose and fructose. It is similar in appearance to the solution of **1** heated by itself. Upon heating in the presence of excess (16 mg) Na_2SO_4, which we previously employed to promote boronate ester formation of **1** with diols,[2] the colors of the glucose and fructose/**1** solutions become more distinct, turning peach-colored and yellow, respectively (Figure 1).[9] The decrease in relative absorbances for the sugars, measured at 536 nm, qualitatively mirrors the known relative binding affinity of fructose (A = 0.057) > glucose (A = 0.067) > sucrose (A = 0.10) for arylboronic acids.[7,8] This trend is confirmed by ^{1}H NMR via the successive decrease of the integral areas of the boronic acid protons of solutions of **1** containing these added carbohydrates.

D-Glucose-6-phosphate and α-D-glucose-1-phosphate are two intermediates in glycogen biosynthesis.[10] Heating colorless solutions of these sugars (3 equiv) in the presence of **1** (5.2 mM) in 10:1 DMSO:H_2O at 100 °C for 1.5 min results in a very bright crimson color for α-D-glucose-1-phosphate disodium salt hydrate (λ_{max} = 495 nm, A = 1.1 and 536 nm, A = 0.49, Figure 1) and a dark reddish brown for the D-glucose-6-phosphate monosodium salt solution (λ_{max} = 471 nm, A = 1.8). The same respective colors are observed for glucose-1-phosphate monosodium salt and glucose-6-phosphate disodium salt. The results of the sugar/**1** color experiments are reproducible as evidenced by visual inspection and spectrophotometric measurements. The average relative error in the measurements of three separate thermolyses of the five sugar/**1** complexes is 4.6%.

Room-temperature fluorescence anisotropy measurements of colored solutions of **1** reveal that the species associated with the long-wavelength fluorescence has a much slower rotational correlation time (r = 0.189) than that associated with the short-wavelength fluorescence (r = 0.010). Saccharides appear to perturb an aggregation−deaggregation equilibrium; added analytes change the relative intensities of the long- and short-wavelength fluorescence. Importantly,

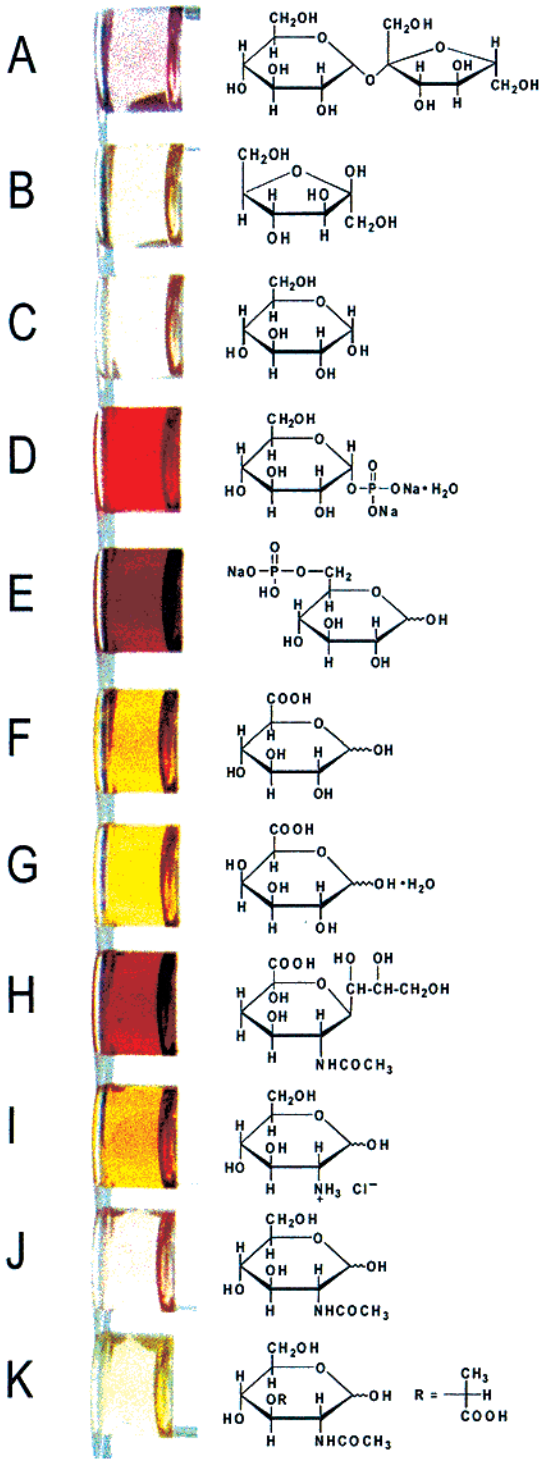


Figure 1. Heated 5.2 mM DMSO solutions of **1** and 3 equiv of (i) carbohydrates, **A** = sucrose, **B** = D-(−)-fructose, **C** = α-D-glucose; (ii) glucose phosphates, **D** = α-D-glucose-1-phosphate disodium salt hydrate, **E** = D-glucose-6-phosphate monosodium salt; (iii) carboxylic acid and amino sugars, **F** = D-glucuronic acid, **G** = D-galacturonic acid monohydrate, **H** = sialic acid, **I** = D-glucosamine hydrochloride, **J** = *N*-acetyl-D-glucosamine, **K** = (+)-*N*-acetylmuramic acid.

(7) James, T. D.; Samankumara Sandanayake, K. R. A.; Shinkai, S. *Supramol. Chem.* **1995**, *6*, 141−157.
(8) Smith, B. D. *Supramol. Chem.* **1996**, *7*, 55−60.
(9) Reheating colored solutions of **1** with 3 equiv of each of the three saccharides produces the same solution colors observed when heating colorless solutions of **1** initially with the sugars.
(10) Fontana, J. D. *FEBS Lett.* **1980**, *109*, 85−92.

this property is sugar-specific. In addition, each heated saccharide/**1** solution exhibits a characteristic rotational correlation time (Table 1).

Table 1. Fluorescence Properties of **1** (5.2 mM in DMSO) Heated Alone and in the Presence of 3 equiv of Various Saccharides Showing the Maximum Excitation and Emission of the Long-Wavelength Fluorescence, the Fluorescence Anisotropy (r) of the Long-Wavelength Species, and the Intensity of the Long-Wavelength Fluorescence Relative to the Short-Wavelength Fluorescence (I_{long}/I_{short})

added sugar	λ_{ex} (nm)	λ_{em} (nm)	anisotropy (r)	I_{long}/I_{short}
none	543	575	0.189	3.2
α-D-glucose	535	573	0.255	3.3
glucose-1-phosphate	550	585	0.155	5.2
glucose-6-phosphate	490	580	0.161	5.7
fructose	492	572	0.100	1.6
sucrose	540	578	0.129	4.4

A significant blue shift in λ_{ex} of 60 nm is observed for D-glucose-6-phosphate compared to α-D-glucose-1-phosphate. This complements recent efforts involving the fluorescent sensing of these molecules. In the prior studies, glucose-1- and -6-phosphate were differentiated by observed changes in ^{31}P NMR and CD spectra[11] or variations in fluorescence emission intensities upon receptor binding.[12]

It is intriguing that **1** does not have extended π-conjugation and yet heated solutions of **1** display spectrophotometric absorption as well as fluorescence excitation and emission in the visible region. The ^{1}H and ^{13}C NMR spectra of DMSO-d_6 solutions of **1**, heated for 3 min as above, exhibit no change in chemical shifts or peak area integrals compared to colorless samples. After prolonged (12−24 h) heating of air-saturated solutions of **1** in DMSO at 180 °C, no evidence of carbonyl formation by ^{13}C NMR or FT-IR spectroscopy is observed; moreover, the ^{11}B NMR spectra reveal no evidence of boronate ester formation or boronic acid hydrolysis. This does not, however, allow us to rule out the presence of trace amounts of highly colored oxidized material appearing at levels too low to detect.

If trace amounts of oxidized products are responsible for the coloration, then altering the concentrations of the oxidized materials should afford variable color schemes. To test this hypothesis we heated solutions of **1** alone (183 °C) and in the presence of 3 equiv of the five saccharides for 3 min (control set, affording the same visible color schemes as noted above but with increased absorbance intensities due to greater heating), in the dark, and with N_2 or air saturation. Upon N_2 saturation, every solution is observably fainter in color than in the control case, with absorbance decreases of 39−82%. Importantly, the solution of **1** alone exhibits a significant absorbance decrease of 61% (536 nm). Upon air saturation, the two glucose phosphate solutions begin to change color within seconds and are the most darkened, with glucose-6-phosphate showing a >7.5-fold increase and glucose-1-phosphate exhibiting a >2-fold increase in absorbance intensity. Upon heating in the dark, the glucose and sucrose solutions are the faintest in color, displaying respective absorbance decreases of 33% and 26% compared to the control case. Light and oxygen[13] are therefore clearly factors in the coloration process. We ascribe the coloration of solutions of **1** as due to the production of trace amounts of highly absorbing, oxidized (quinone) derivatives of **1**.

Oxidation of either an arylboronic acid or resorcinol moiety must result in sp^2 hybridization of a macrocycle methine carbon. This would place the resultant quinone in π-conjugation with two aromatic rings. Only partial oxidation of **1** at low levels would occur due to the strain imparted to the macrocyclic ring upon introduction of sp^2 hybridization. We propose that binding with the respective analytes could then afford selective coloration via sugar-specific perturbations in torsion angles and/or aggregation−deaggregation equilibrium (as evidenced by fluorescence anisotropy, vide supra).[14] The presence of strongly π-accepting, oxidized aromatics enhances intermolecular π-donor/π-acceptor interactions, thereby contributing to the order of magnitude change in rotational correlation time observed for the colored solutions of **1**.

The visual detection of saccharides promoted by **1** is versatile, not limited to the carbohydrates and glucose phosphates described above. D-Glucuronic acid and D-galacturonic acid promote monosaccharide oxidation and vitamin C biosynthesis.[15] Sialic acid is an important antigenic cell-surface residue.[16] Colorless DMSO solutions of **1** (5.2 mM) and 3.0 equiv of each of these three structurally related carboxylic acid sugars, upon heating in the presence of excess Na_2SO_4 for 2 min until reflux, are bright gold (λ_{max} = 450 nm, A = 0.20), faint yellow (λ_{max} = 456 nm, A = 0.10), and reddish-orange (λ_{max} = 440 nm, A = 0.80), respectively.

D-Glucosamine and *N*-acetyl-D-glucosamine (NAG) are the major constituents of chitin, one of the most abundant

(11) Imada, T.; Kijima, H.; Takeuchi, M.; Shinkai, S. *Tetrahedron* **1995**, *36*, 2093−2096.

(12) Deetz, M. J.; Smith, B. D. *Tetrahedron Lett.* **1998**, *19*, 6841−6844.

(13) The selective coloration is solvent sensitive. Boiling 5.2 mM solutions (3 min) of **1** in DMF with added H_2O and Na_2SO_4 produces a very pale pink tint solution coloration. This color is also observed when heating **1** in the presence of 3 equiv of added sucrose and glucose. In the presence of fructose, the solution color is pale peach. Boiling 5.2 mM solutions (3 min) of **1** in triglyme with added H_2O and Na_2SO_4 produces only a very faint yellow tint which is also observed via heating in the presence of 3 equiv added glucose, fructose, and sucrose.

(14) Heated 5.2 mM solutions (for 5 min to reflux) of resorcinol or phenylboronic acid either alone or together in DMSO containing H_2O and Na_2SO_4 are colorless. Heating these solutions with added sucrose produces no color change. In the presence of glucose, only the mixed resorcinol/$PhB(OH)_2$ solution develops a faint yellow tint. Added fructose results in a yellow coloration of the mixed resorcinol/$PhB(OH)_2$ solution and a very faint yellow coloration of the solutions containing resorcinol or $PhB(OH)_2$. These experiments demonstrate that the macrocyclic structure is a prerequisite for attaining the dramatic color changes observed. We are investigating the possibility of the role of sugar (beyond simple boronate ester formation and hydrogen bonding; e.g., furfural or other sugar-derived aldehyde condensation) reactions with **1** or oxidized, potentially ring-opened **1**. In addition, comparative studies of the coloration process employing **1** and simple aliphatic and aromatic diols vs other hydoxy compounds, reducing vs nonreducing sugars, and ketoses vs aldoses in buffered solutions are in progress.

(15) Touster, O. *Annu. Rev. Biochem.* **1962**, *31*, 407−450.

(16) Kobata, A. *Acc. Chem. Res.* **1993**, *26*, 319−324.

polysaccharides on earth.[17] (+)-*N*-Acetylmuramic acid (NAM) is a component of bacterial cell walls.[18] Colorless solutions of **1** (5.2 mM) containing 3 equiv of these amino sugars, upon heating in the presence of excess Na_2SO_4 for 2 min until reflux, are pale pink ($\lambda_{max} = 540$ nm, $A = 0.13$), pale yellow ($\lambda_{max} = 460$ nm, $A = 0.43$), and very dark orange ($\lambda_{max} = 425$ nm, $A = 3.1$) for NAG, NAM, and D-glucosamine hydrochloride, respectively.

In conclusion, solutions of receptor **1** containing added saccharides exhibit dramatic, characteristic solution color changes that are readily distinguished by visual inspection. The color selectivities can be tuned by altering simple parameters such as added water or a drying agent. This unique methodology is rapid and reproducible, with the solution colors remaining stable by visual inspection for periods lasting typically up to several days. Since tetraarylboronic acid resorcinarenes are amenable to a variety of synthetic modifications,[2,19] this research should lead to the fabrication of new classes of color-sensing materials. Our current efforts include the synthesis and study of **1** and congeners and related oxidized model resorcinol- and/or boronic acid-derived compounds. The detailed characterization and analysis of the saccharide−receptor complexes employing a variety of solvents and conditions, including competitive binding studies of sugar mixtures and other polar biomolecules, is also in progress.

Acknowledgment. We gratefully acknowledge the Arnold and Mabel Beckman Foundation for support of this research through the Beckman Young Investigator Award Program. We also acknowledge partial support from the donors of the Petroleum Research Fund of the American Chemical Society, administered by The American Chemical Society. We thank Professors Isiah Warner, Donald Matteson, and David Creed for helpful discussions and Professors George Stanley and Andrew Maverick for help in the preparation of this manuscript.

Supporting Information Available: UV−vis spectra of solutions of **1** and added saccharide analytes. This material is available free of charge via the Internet at http://pubs.acs.org.

OL990105A

(17) Muzzarelli, R. A. *Chitin*; Pergamon Press: New York, 1977.
(18) Park, J. T. *J. Biol. Chem.* **1952**, *194*, 885−895.
(19) Lewis, P. T.; Strongin, R. M. *J. Org. Chem.* **1998**, *63*, 6065−6067.