Tetrahedron Letters 49 (2008) 6054–6057

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Dual optical and electrochemical saccharide detection based on a dipyrido[3,2-a:2′3′-*c*]phenazine (DPPZ) ligand

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ARTICLE INFO

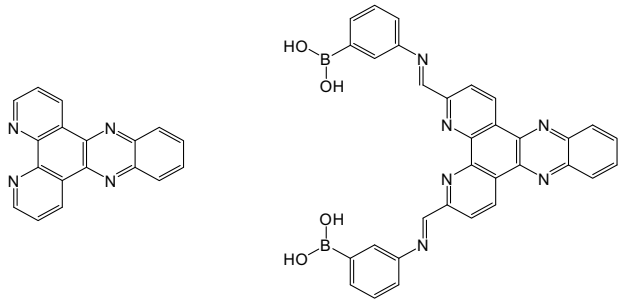
Article history:
Received 31 December 2007
Revised 27 July 2008
Accepted 28 July 2008
Available online 31 July 2008

ABSTRACT

We report a new molecular sensor based on dipyrido[3,2-a:2′3′-*c*]phenazine (DPPZ) functionalized with boronic acid groups. The sensor binds to saccharides and upon binding results in changes in fluorescence intensity as well as cathodic shifts in the sensor's formal potential. The ability of the new DPPZ-based sensor to provide both electrochemical and optical signal outputs demonstrates the viability of this family of molecules to be developed as dual-signal detectors. Measurements to determine the stability constants with four saccharides are shown.

There is a need for sensors that operate using simultaneous optical and electrochemical means of signal transduction.[1–3] Such sensors allow for versatile detection of analytes and reduce the possibilities of false positives. Ligands based on dipyrido[3,2-a:2',3'-*c*]phenazine (DPPZ (**1**)) have been widely used in the coordination of various transition metals and the resulting complexes have been studied in DNA intercalation,[4] optical probes,[5] and in dye-sensitized solar cells.[6] Surprisingly, little has been done towards developing DPPZ-based ligands as detection units, though their optical[7,8] and electrochemical properties[8] provide a unique approach to tailor these ligands as tunable sensors. We report a new ligand (**2**) which can function as both an optical and an electrochemical sensor for saccharides. **2** is a DPPZ ligand coupled to phenylboronic acid groups via an ethylidenemethylamine linker. We demonstrate that **2** undergoes independent optical and electrochemical signals upon binding to saccharides.

The necessity for saccharide detection and monitoring arises from its use in industrial, medical and biotechnological applications.[9,10] Recently, there have been several reports on the development of boronic acid-based saccharide sensor that provide either fluorescence or electrochemical signal outputs.[11] Most of these sensors are based on the strong covalent interactions between a boronate anion and hydroxyl groups on glucose thus forming a cyclic boronate ester.[12] The primary problem with boronic acid-based saccharide sensors is that basic conditions are typically required to deprotonate the boronic acid to the corresponding boronate anion.[13] The pH limitation can be overcomed by incorporating a tertiary amine in close proximity to the boronic acid group. As a result, the amine neighbouring group participant (NGP) induces boronate anion formation in turn allowing for complexation of the boronate anion with glucose under non-basic conditions.[14] We demonstrate that **2**, in which the boron atom does not form an initial B–N bond is capable of binding to saccharides, and upon binding provides independent optical and electrochemical signal outputs.



1 **2**

These results demonstrate that analogues of DPPZ could in principle be designed to enable dual signal transductions for various analytes of interest.

The synthesis of **2** is shown in Scheme 1. Commercially available 2,9-dimethyl-1,10-phenanthroline **3** was oxidized to the corresponding dione (**4**) using H_2SO_4/HNO_3 in the presence of KBr following a well-established literature procedure.[15] Reaction of **4** with 1,2-phenylenediamine in ethanol under reflux conditions produced **5** in 72% yield.[16] Compound **5** was then oxidized using SeO_2 in 4% aqueous 1,4-dioxane to the carboxaldehyde compound **6**. **2** was synthesized from **6** through reaction with 3-aminophenylboronic acid in ethanol at room temperature to yield a light yellow powder in 85% yield. The sensor **2** consists a boronic acid functional group that serves as the receptor site and the DPPZ

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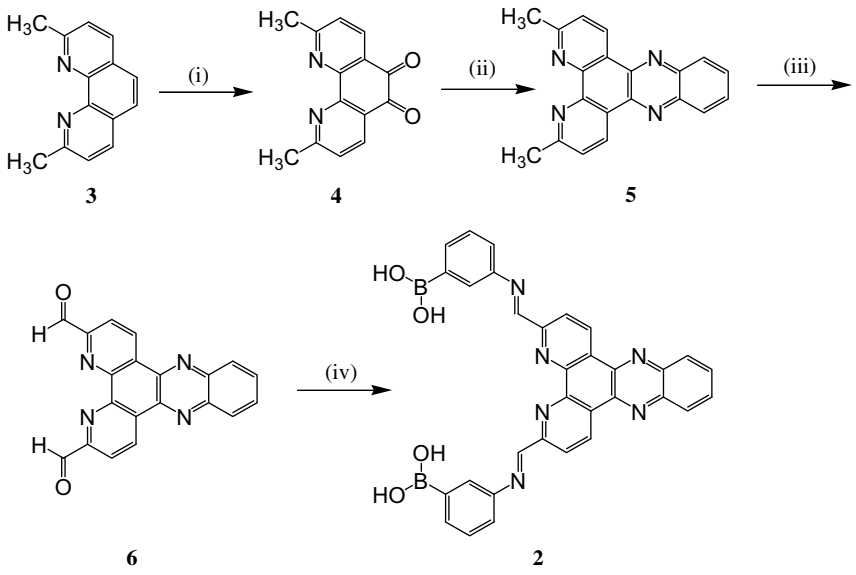
doi:10.1016/j.tetlet.2008.07.154



Scheme 1. Synthesis of DPPZ-$(B(OH)_2)_2$ (**1**): Reagents and conditions: (i) H_2SO_4, HNO_3, KBr, 80 °C, 90%; (ii) EtOH, 1,2-phenylenediamine, 78 °C, 72%; (iii) 1,4-dioxane (4% aq), SeO_2, 95 °C, 48%; (iv) EtOH, 3-aminophenylboronic acid, rt, 85%.

ligand which is an electron-rich aromatic molecule with optical and electrochemical properties.

Figure 1 shows the UV–vis absorbance spectrum of **2** (*spectrum represented by the dashed line*) measured in a 1:4 v/v DMSO/H_2O solution. **2** exhibits two absorbance peaks at 350 nm and 375 nm, with molar absorptivity ε values of 1557 M^{-1} cm^{-1} and 1437 M^{-1} cm^{-1}, ($\log\varepsilon$ values = 3.19 and 3.15), respectively. The UV-visible spectrum of **2** with excess D-glucose, D-galactose, D-mannose or D-fructose was also measured and showed no changes with either saccharide as shown in Figure 1.

For all emission measurements of **2**, 265 nm was used as the excitation wavelength and the sensor was prepared in a 1:4 v/v DMSO/H_2O solution at a concentration of 1.67×10^{-4} M. **2** alone exhibits two emission peaks at 375 nm and 420 nm, with the ratio of the intensity of the emission peaks of $\lambda_{375\ nm}$ to $\lambda_{420\ nm}$ being 1:2. Figure 2 represents the emission spectral changes of **2** when titrated with D-glucose. The emission intensity of **2** at 375 nm and 420 nm increased with increase in D-glucose concentration. The emission intensity of the 375 nm peak increased substantially relative to the intensity of the 420 nm peak, resulting in a shift in the ratio of the emission intensity of the $\lambda_{375\ nm}$ to $\lambda_{420\ nm}$ at saturation concentrations of D-glucose to 2:1. Similar trends were observed when D-fructose, D-galactose and D-mannose were added to **2**. These observations can be explained as follows: emission of the unbound sensor is quenched by energy transfer from the enimine nitrogen to the phenylboronic acid. Upon complexation with saccharides, the boronic acid no longer acts as an electron acceptor which results in changes in emission due to agitation of the charge transfer of the excited state.[17] At a 1:1 saccharide-**2** ratio, the intensities of the $\lambda_{375\ nm}$ to $\lambda_{420\ nm}$ are comparable, however, increase in saccharide concentration results in a gradual shift in the ratio of the emission intensities at $\lambda_{375\ nm}$ to $\lambda_{420\ nm}$. The data suggest that these changes may arise as a result of saccharide binding to one of the boronic acid groups to form a 1:1 saccharide-**2** complex, but with increase in saccharide concentration, a 2:1 saccharide-**2** complex is formed.

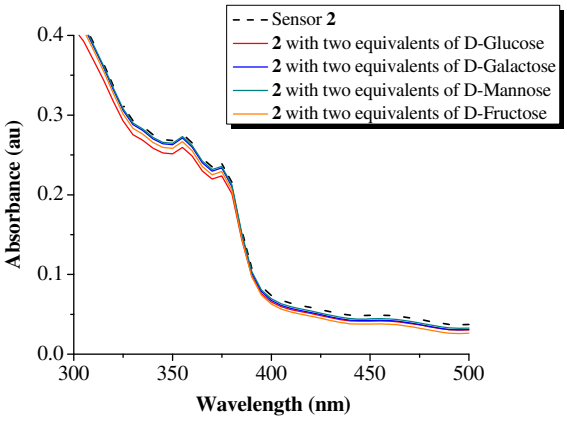


Figure 1. UV–vis absorbance spectrum of **2** (dashed line), with 2 equiv of the saccharides D-glucose, D-galactose, D-mannose and D-fructose. The UV–vis absorbance of **2** remains unchanged with addition of either saccharide.

The formation of the cyclic boronate ester caused by binding of the boronic acid with the saccharide diols, results in fluorescence enhancement since the energy transfer is unfavourable. Thus the binding process results in the negation of the fluorescence quenching mechanism associated with the excited state and thus allows for increased fluorescence.[18]

The electrochemical detection of glucose through the enzymatic activity of glucose oxidase is widely utilized in glucose sensing.[19] However, there is a great demand for non-enzymatic glucose sensors that overcome the limitations of enzymes, that is, stability, reproducibility, simplicity and oxygen interference.[20] Electrochemical signal transduction has been shown to occur in boronic acid-based glucose sensors in which the interaction of a boronic acid and neighbouring tertiary amine is strengthened upon binding to a saccharide.[21]

We investigated the effect of the changes in electrochemical signal of **2** upon binding with D-glucose. Solutions of **2** (1.67×10^{-4} M) and D-glucose (1.67×10^{-4} M) were prepared in 0.1 M $TBAPF_6$ in DMSO. Sweep cyclic voltammograms were recorded for 1.67×10^{-4} M **2** with D-glucose (0–6.68×10^{-4} M) in 0.1 M $TBAPF_6$ in DMSO in a N_2 saturated atmosphere. Figure 2 shows that the formal potential of **2** (−995 mV vs Ag/AgCl) and gradually becomes more negative (−1044 mV vs Ag/AgCl) with increase in D-glucose concentration accompanied by changes in current. However, there is no change in reduction potential for additions of more than 2 equiv of D-glucose. Boronic acid binding

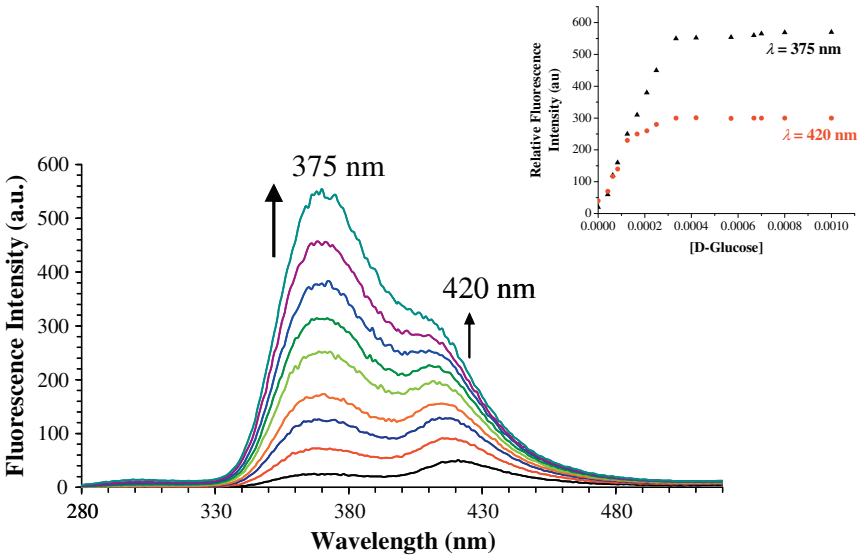


Figure 2. Fluorescence spectral changes of 1.67×10^{-4} M of **2** (excited at 265 nm) titrated with D-glucose. From bottom to top, [D-glucose] = 0, 4.18×10^{-5} M, 6.26×10^{-5} M, 8.35×10^{-5} M, 1.25×10^{-4} M, 1.67×10^{-4} M, 2.09×10^{-4} M, 2.50×10^{-4} M, 3.34×10^{-4} M. The inset shows a typical saturation curve in which increase in the D-glucose concentration results in an overall increase in emission intensity but these increases at more drastic at 375 nm relative to the 420 nm peak.

with D-glucose results in the formation of the cyclic boronate ester, which in turn causes an increase in electron density around the enimine nitrogen. The nitrogen lone pairs conjugate with the π system thus increasing the stability of the complex and thus causing a more cathodic over potential of the overall complex. We note that a similar experiment in which the CV of **1** with increasing concentrations of saccharide was measured. There were no changes in the CV of **1** (Fig. 3).

The method of continuous variation was used to determine the stoichiometry between **2** and the saccharides. It was found that one ligand binds to two saccharide molecules. The 1:2 interaction of the sensor and analyte was further supported by the electrochemistry data. Stability constants were calculated based on 1:2 sensor to saccharide ratio.[22] The binding constant (K) was calculated from the fluorescence emission titration of **2** (1.67×10^{-4} M in DMSO) with D-glucose (1.18×10^{-2} M in DMSO) at the 370 nm peak. The resulting values were averaged and reported with the respective standard deviation. A correlation coefficient (r^2) was also determined (using MINITAB v. 14.0) between the binding constant and the D-glucose concentration. Binding constants were calculated for **2** with D-fructose, D-glucose, D-galactose and D-mannose and found to be $(12.7 \pm 0.8) \times 10^{-4}$ M^{-2}, $(9.0 \pm 0.9) \times 10^{-4}$ M^{-2}, $(3.7 \pm 1.2) \times 10^{-4}$ M^{-2} and $(0.4 \pm 0.1) \times 10^{-4}$ M^{-2}, respectively. This trend is consistent with other literature reports.[10]

In conclusion, we have shown that a new DPPZ-based ligand with boronic acid functional groups is capable of binding to saccharides and upon binding provides independent electrochemical and optical signal outputs. Such ligands can in principle be applied towards various analytes of interest to offer sensors with dual signal transductions and minimize false positives.

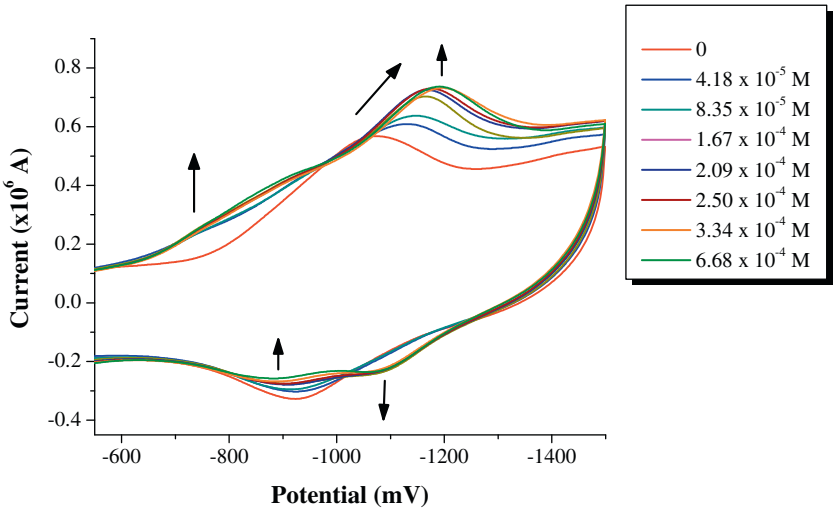


Figure 3. Cyclic voltammograms of **2** (1.67×10^{-4} M) with different concentrations of D-glucose concentration (0–6.68×10^{-4} M) in 0.1 M $TBAPF_6$ in DMSO. Increase in D-glucose concentration results in cathodic shifts in up to 2 equiv, after which no changes are observed. (In the direction of the arrows), [D-glucose] = 0, 4.18×10^{-5} M, 8.35×10^{-5} M, 1.67×10^{-4} M, 2.09×10^{-4} M, 2.50×10^{-4} M, 3.34×10^{-4} M, 6.68×10^{-4} M, corresponding to 0, 0.25, 0.50, 1.00, 1.25, 1.50, 2.00 and 4.00 equiv, respectively. The area of the glassy carbon electrode is 0.28 cm^2. Increase in [D-glucose] results in a new complex that has a formal potential = −1044 mV versus Ag/AgCl. Voltammetric parameters are as follows: Scan rate = 50 mV/s, reference electrode = Ag/AgCl.

Acknowledgements

We thank the National Science Foundation under grant 0811026 for funding. We also thank Western Michigan University for financial support of this project. DSB thanks the Western Michigan University Lee's Honors and the College of Arts and Science for funding through the Undergraduate Research and Creative Activities Award.

Supplementary data

Supplementary data (synthetic procedures and NMR results associated with compounds **1**–**6**). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.07.154.

References and notes

1. Anzenbacher, P.; Palacios, M. A.; Jursikova, K.; Marquez, M. *Org. Lett.* **2005**, *22*, 5027.
2. Beer, P. D. *Chem. Commun.* **1996**, 689.
3. Beer, P. D. *Coord. Chem. Rev.* **2000**, *205*, 131.
4. (a) Zeglis, B. M.; Pierre, V. C.; Barton, J. K. *Chem. Commun.* **2007**, 4565. and references therein; (b) Phillips, T.; Haq, I.; Meijer, A. J. H. M.; Adams, H.; Soutar, I.; Swanson, L.; Sykes, M. J.; Thomas, J. A. *Biochemistry* **2004**, *43*, 13657.
5. Xu, W.; Wittich, F.; Banks, N.; Zink, J.; Demas, J. N.; DeGraff, B. A. *Appl. Spectrosc.* **2007**, *61*, 1238; Bedoya, M.; Dıez, M. T.; Moreno-Bondi, M. C.; Orellana, G. *Sens. Actuators, B* **2006**, *113*, 573; Fantacci, S.; De Angelis, F.; Sgamellotti, A.; Marrone, A.; Re, N. *J. Am. Chem. Soc.* **2005**, *127*, 14144; Keller, C. E.; Pollard, C.; Yeung, L. K.; Plessinger, W. D.; Murphy, C. J. *Inorg. Chim. Acta* **2000**, *298*, 209.
6. Mitsopoulou, C. A.; Veroni, I.; Philippopoulos, A. I.; Falaras, P. *J. Photochem. Photobiol.* **2007**, *191*, 6; Onozawa-Komatsuzaki, N.; Kitao, O.; Yanagida, M.; Himeda, Y.; Sugihara, H.; Kasuga, K. *New J. Chem.* **2006**, *30*, 689.
7. Obare, S. O.; Murphy, C. J. *Inorg. Chem.* **2001**, *40*, 6080.
8. Guo, W.; Obare, S. O. *Tetrahedron Lett.* **2008**, *49*, 4933.
9. Palleschi, G.; Cubadda, R. *Ital. J. Food Sci.* **2001**, *13*, 137.
10. Heller, A. *Annu. Rev. Biomed. Eng.* **1999**, *1*, 153.
11. (a) Arimori, S.; Consiglio, G. A.; Phillips, M. D.; James, T. D. *Tetrahedron Lett.* **2003**, *44*, 4789; (b) Arimori, S.; Ward, C. J.; James, T. D. *Tetrahedron Lett.* **2002**, *43*, 303; (c) Arimori, S.; Bosch, L. I.; Ward, C. J.; James, T. D. *Tetrahedron Lett.* **2002**, *43*, 911; (d) Arimori, S.; Bell, M. L.; Oh, C. S.; Frimat, K. A.; James, T. D. *J. Chem. Soc., Perkin Trans. I* **2002**, 802; (e) Arimori, S.; Bell, M. L.; Oh, C. S.; Frimat, K. A.; James, T. D. *Chem. Commun.* **2001**, 1836; (f) James, T. D.; Sandanayake, K. R. A. S.; Iguichi, R.; Shinkai, S. *J. Am. Chem. Soc.* **1995**, *117*, 8982.
12. Nagai, Y.; Kobayashi, K.; Toi, H.; Aoyama, Y. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 2965.
13. Yoon, J.; Czarnik, W. *J. Am. Chem. Soc.* **1992**, *114*, 5874.
14. Sandanayake, K. R. A. S.; James, T. D.; Shinkai, S. *Pure Appl. Chem.* **1996**, *6*, 1207.
15. Dietrich-Buchecker, C. O.; Marno, P. A.; Sauvage, J.-P. *Tetrahedron Lett.* **1982**, *23*, 5291.
16. Yamada, M.; Tanaka, Y.; Yoshimoto, Y.; Kuroda, S.; Shimao, I. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 1006.
17. DiCesare, N.; Lackowicz, J. R. *J. Biomed. Opt.* **2002**, *7*, 538.
18. Lackowicz, J. R. *In Principles of Fluorescence Spectroscopy*, 3rd ed.; Springer, 2006.
19. (a) Schuhmann, W.; Schmidt, H.-L. *Adv. Biosensors* **1992**, *2*, 79; (b) Heller, A. *Acc. Chem. Res.* **1990**, *23*, 128; (c) Degani, Y.; Heller, A. *J. Phys. Chem.* **1987**, *91*, 1285.
20. Park, S.; Boo, H.; Chung, T. D. *Anal. Chim. Acta* **2006**, *556*, 46.
21. Arimori, S.; Ushiroda, S.; Peter, L. M.; Jenkins, A. T. A.; James, T. D. *Chem. Commun.* **2002**, 2368.
22. See Supplementary data.