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Heteroditopic Ruthenium(II) Bipyridyl Receptor with Adjacent Saccharide and Phosphate Binding Sites

Martin J. Deetz and Bradley D. Smith*

Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556-5670, USA.

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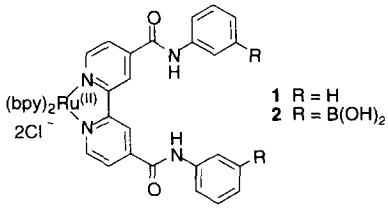
Abstract: A luminescent ruthenium(II) bipyridyl receptor with pendant boronic acids is synthesized and shown to bind phosphorylated sugars in water. The association constant for fructose-6-phosphate is log K_a = 3.1 M^{-1} at 298 °K. The receptor simultaneously binds saccharide/phosphate with positive cooperativity.

Recent designs of anion receptors have included acyclic ruthenium(II) bipyridyl derivatives, such as **1**, which bind phosphate anions in polar aprotic solvents by a combination of electrostatic interactions and hydrogen bonding with the receptor amide residues.[1,2] This paper describes the luminescent ruthenium(II) bipyridyl receptor **2** which contains pendant boronic acids. Since boronic acids form covalent complexes with the diol groups in saccharides,[3,4] we reasoned that **2** would act as a heteroditopic receptor capable of binding saccharides,[5] and phosphate groups at two adjacent sites (Figure 1). Here we report the synthesis of **2** and show that it is capable of: (a) binding and sensing phosphorylated sugars in aqueous solution, and (b) binding saccharides and phosphate ions with positive cooperativity.[6]



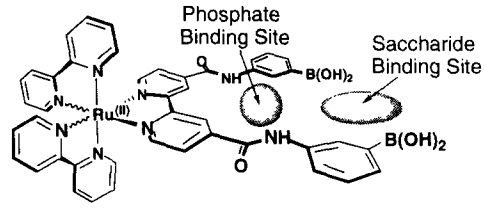


Figure 1. Heteroditopic binding of saccharide and phosphate by racemic receptor **2**.

Compounds **1**[1] and **2**[7] were prepared in straightforward fashion by treating $Ru(bpy)_2Cl_2.2H_2O$ with the appropriate bipyridyl ligand in ethylene glycol. Compound **2** exhibits the optical properties expected for a ruthenium(II) bipyridyl derivative (MLCT absorption band at 479 nm in water (ε = 1.1 x 10^4 M^{-1} cm^{-1}), emission at 637 nm). The electronic absorption spectrum of **2** in aqueous solution hardly changes upon treatment with phosphorylated sugars. However, the emission intensity increases with fructose-6-phosphate producing the largest change (Figure 2)[8]. Titration of **2** with fructose-6-phosphate in a range of solvents produces isotherms that fit nicely to a 1:1 binding model.[9] The association constants listed in Table 1 were extracted from the luminescence titration curves by iterative curve fitting methods.[10] The 1:1 binding stoichiometry was confirmed by: (i) ^{31}P NMR titration experiments in water which showed a peak due to the **2**/fructose-6-phosphate complex in slow exchange and 1.2 ppm upfield of free fructose-6-phosphate, and (ii)

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mass spectral analysis (positive ion electrospray) of a mixture of **2** and fructose-6-phosphate in water/acetonitrile which showed a peak for the 1:1 complex (*m/z* 582 [**2** + disodium fructose-6-phosphate - $2H_2O]^{2+}$) with no other stoichiometry observed.

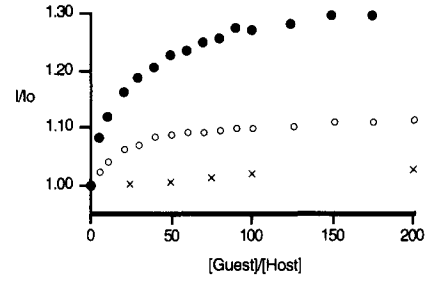


Figure 2. Change in emission for **2** (30 μM) upon addition of: (•) fructose-6-phosphate in water, (o) fructose in sodium phosphate solution (10 mM, pH 7.3), and (x) fructose in water; λ_{ex} 470 nm, λ_{em} 637 nm.

Table 1. Association Constants for **2** and Various Saccharides in Different Solvents.

Entry	Saccharide	Solvent[a]	log K_a /M^{-1} (I/I_0)[b]
1	Fructose-6-phosphate[c]	water	3.1 (1.34)
2	Fructose-6-phosphate[c]	water/methanol (1:1)	3.8 (1.51)
3	Fructose-6-phosphate[c]	water/methanol (1:4)	4.8 (1.40)
4	Fructose-6-phosphate[c]	10 mM NaH_2PO_4/Na_2HPO_4	2.7 (1.21)
5	Fructose-6-phosphate[c]	10 mM NaCl	3.0 (1.39)
6	Fructose-6-phosphate[c]	10 mM $NaClO_4$	3.0 (1.32)
7	Fructose-1,6-diphosphate[d]	water	3.1 (1.15)
8	Glycerol-2-phosphate[c]	water	2.8 (1.23)
9	Glucose-6-phosphate[c]	water	e
10	Glucose-1-phosphate[c]	water	3.1 (1.13)
11	Galactose-6-phosphate[c]	water	2.8 (1.22)
12	Fructose	10 mM NaH_2PO_4/Na_2HPO_4	3.2 (1.12)
13	Fructose	10 mM NaCl	1.5 (1.30)
14	Fructose	10 mM $NaClO_4$	e
15	Mannose	10 mM NaH_2PO_4/Na_2HPO_4	1.4 (1.20)
16	Maltose	10 mM NaH_2PO_4/Na_2HPO_4	0.6 (1.45)
17	Ethylene glycol	10 mM NaH_2PO_4/Na_2HPO_4	-0.3 (1.65)
18	Xylulose	10 mM NaH_2PO_4/Na_2HPO_4	4.2 (1.13)
19	Sorbitol	10 mM NaH_2PO_4/Na_2HPO_4	3.3 (1.13)

[a]pH 7.3. [b]Errors estimated to be ≤ 5 %. I/I_0 is fluorescence enhancement at receptor saturation, T= 298 °K. [c]Disodium salt. [d]Trisodium salt. [e]No fluorescence enhancement observed.

Molecular modeling and literature precedence[1,3,11] indicates that the most likely 1:1 complex is the structure shown in Figure 3 and the following results are consistent with this structure. The association constant for **2**/fructose-6-phosphate increases as the solvent is made less polar (compare Entries 1, 2 and 3 in Table 1), whereas the inclusion of competing salts in the titration solvent leads to decreased binding (compare Entries 1 and 4). The non-boron control **1** shows no response to fructose-6-phosphate in water, proving that the boronic acid/saccharide diol interaction is crucial for good binding and fluorescence enhancement. The nature of the phosphate/amide interaction is still under investigation, but the data in hand suggests that hydrogen bonding is an

important factor. For example, fructose-6-phosphate has the same affinity as the more negatively charged fructose-1,6-diphosphate (Entries 1 and 7), a result that does not correlate with a binding model based solely on electrostatic attraction, but does agree with the postulated hydrogen bonded structure shown in Figure 3.

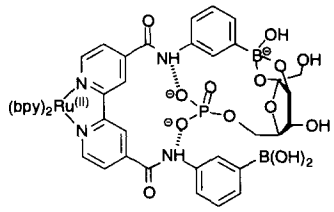
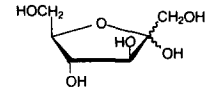
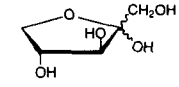


Figure 3. Postulated 1:1 complex for **2**/fructose-6-phosphate.



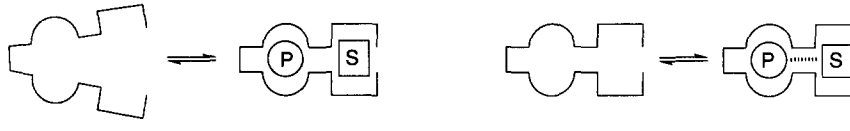
fructose



xylulose

Evidence that a phosphate group is needed for good saccharide binding to **2** is the observation that all the non-phosphorylated saccharides listed in Table 1 exhibit log $K_a \leq 1.2$ in water or water/methanol (data not listed but see Figure 2). Furthermore, the association constants for certain saccharides increase by two orders of magnitude when the titrations are conducted in sodium phosphate buffer (Entries 12, 18 and 19). The order of saccharide binding affinities in phosphate solution (xylulose > sorbitol ~ fructose > mannose > maltose > ethylene glycol) matches the usual affinity order for boronic acids,[3,4] indicating that the phosphate is simply enhancing the ability of the saccharide to bind to the boronic acid groups in **2**. The sodium salts of other anions such as chloride or perchlorate do not induce such a strong enhancing effect (Entries 12, 13 and 14). Thus, phosphate anion must associate with the heteroditopic receptor **2** in a way that stabilizes simultaneous saccharide binding.

There are two most likely explanations for this apparent cooperativity and both require the phosphate to form hydrogen bonds with the amide groups in **2** (Figure 4). One possibility is an induced fit mechanism, where a phosphate anion bridges the amide groups (see Figure 1 for a schematic view) and preorganizes the two pendant boronic acids so they can more effectively form a 1:1 cyclic saccharide-bis(boronate) structure. At present there are two pieces of evidence against this binding mechanism. First, mass spectral analysis (positive ion electrospray) of mixtures of **2** and fructose in water/acetonitrile shows that the 1:1 acyclic complex (*m/z* 1075 [**2**+fructose-2H_2O-Cl]$^+$, 520 [**2**+fructose-2H_2O-2Cl]$^{2+}$) is the major species, with the 1:2 acyclic complex (*m/z* 1219 [**2** + 2fructose-4H_2O-Cl]$^+$, 592 [**2** + 2fructose-4H_2O-2Cl]$^{2+}$) as a minor component. No ion corresponding to the 1:1 cyclic fructose-bis(boronate) is observed. Second, xylulose (structure shown above), a saccharide that is incapable of forming a 1:1 cyclic saccharide-bis(boronate), has nonetheless a high affinity for **2** in sodium phosphate solution (Entries 12 and 19).



Induced Fit **Stabilizing Interaction Between Guests**

Figure 4. Two possible mechanisms for positive saccharide/phosphate binding cooperativity.

The alternative rationalization of the observed binding cooperativity involves a stabilizing interaction between the two guests (Figure 4). This explanation is supported by molecular modeling which shows that if HPO_4^{2-} associates with **2** by accepting hydrogen bonds from the two amide groups,[1,2] then it is in close enough proximity to the saccharide bound at the neighboring boronic acid to donate a hydrogen bond to the saccharide ring oxygen.

Efforts to elucidate the detailed structures of these supramolecular complexes and to better understand the reasons for the binding cooperativity are continuing and will be reported in due course.

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References and Notes

1. Szemes, F.; Hesek, D.; Chen, Z.; Dent, S. W.; Drew, M. G. B.; Goulden, A. J.; Graydon, A. R.; Grieve, A.; Mortimer, R. J.; Wear, T.; Weightman, J. S.; Beer, P. D. *Inorg. Chem.* **1996**, *35*, 5868. Beer, P. D.; Dent, S. W.; Wear, T. J. *J. Chem. Soc., Dalton Trans.* **1996**, *11*, 2341. Beer, P. D.; Szemes, F.; Balzani, V.; Salà, C. M.; Drew, M. G. B.; Dent, S. W.; Maestri, M. *J. Am. Chem. Soc.* **1997**, *119*, 11864.

2. Watanabe, S.; Onogawa, O.; Komatsu Y.; Yoshida, K. *J. Am. Chem. Soc.* **1998**, *120*, 229.

3. Smith, B. D. *Supramol. Chem.* **1996**, *7*, 55. Paugam, M.-F.; Riggs J. A.; Smith, B. D. *Chem. Commun.* **1996**, 2539.

4. James, T. D.; Sandanayake K. R. A. S.; Shinkai, S. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1910.

5. Near the end of our study a paper appeared describing a neutral rhenium(I) analogue of **2** and its ability to bind hexoses in mixed aqueous/organic solvent. Yam, V. W.; Kai, A. S. *Chem. Commun.* **1998**, 109.

6. For recent examples of cooperative binding using neutral rhenium(I) receptors see: Beer, P. D.; Cooper, J. B. *Chem. Commun.* **1998**, 129. Redman, J. E.; Beer, P. D.; Dent, S. W.; Drew, M. G. B. *Chem. Commun.* **1998**, 231.

7. The bipyridyl ligand was prepared by treatment of 4,4'-bis(chlorocarbonyl)-2,2'-bipyridine with 3-aminophenylboronic acid in THF with Et_3N. The ligand was then heated at 100°C overnight in ethylene glycol with $Ru(bpy)_2Cl_2 \cdot H_2O$. The ethylene glycol was evaporated and the product redissolved in water which was also removed at reduced pressure. Spectroscopic data for compound **2**. ^1H NMR (300 MHz, CD_3OD) δ 9.51 (s, 2H), 8.75 (d, 4H, *J*=7.5Hz), 8.19-7.84 (m, 16H), 7.54 (t, 4H, *J*=6.5Hz), 7.42 (d, 2H, *J*= 7.5Hz), 7.35 (t, 2H, *J*=6.5Hz) ppm. ^{13}C NMR (125 MHz, CD_3OD) 164.0, 159.2, 158.5, 158.5, 153.5, 153.1, 152.8, 144.8, 139.8, 138.8, 131.5, 129.4, 129.4, 129.3, 129.2, 127.4, 127.2, 125.9, 124.0, 123.7 ppm. MS (FAB^+, glycerol matrix) *m/z* 1008 $[M+2Glycerol-2Cl]^+$; (ESI^+) *m/z* 931 $[M-Cl]^+$, 448 $[M-2Cl]^{2+}$. Observed high resolution isotope cluster patterns at *m/z* 931 and 448 unambiguously match calculated patterns. IR (KBr) 3370, 1670, 1604, 1549, 1426, 1340 cm^{-1}.

8. Degassing the titration solutions by bubbling nitrogen resulted in absolute luminescence intensities that were 10-15% higher; however, I/I_0 values and extracted binding constants remained unchanged. Therefore, all titrations were carried out in non-degassed solutions.

9. Conners, K. *Binding Constants: The Measurement of Molecular Complex Stability*, Wiley: New York, 1987.

10. Hughes, M. P.; Smith, B. D. *J. Org. Chem.* **1997**, *62*, 4492.

11. Imada, T.; Kijima, H.; Takeuchi, M.; Shinkai, S. *Tetrahedron Lett.* **1995**, *36*, 2093.