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Spectroscopic and Photophysical Characterization of Fluorescent Chemosensors for Monosaccharides Based on *N*-Phenylboronic Acid Derivatives of 1,8-Naphthalimide

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Spectroscopic and photophysical properties of two fluorescent probes for monosaccharides are presented. Probes are based on the *N*-phenyl-1,8-naphthalimide structure having the boronic acid group [R-$B(OH)_2$] in *ortho* in one case, and *meta* in the other case, positions of the *N*-phenyl group. Formation of the anionic form of the boronic acid group [R-$B(OH)_3^-$] induced a substantial decrease of the steady-state fluorescence of both compounds. Because no change in the fluorescence lifetime from the neutral to the anionic forms is observed, static quenching resulting from photoinduced electron transfer from the anionic form of the boronic acid is used to explain the decrease of the emission intensity. Both compounds show substantial decreases of their fluorescence intensity in the presence of sugars. In addition, this decrease of the fluorescence intensity is associated with an increase of the fluorescence lifetime for the *ortho* derivative while no effect on the lifetime is observed for the *meta* derivative. Both photoinduced electron transfer and steric hindrance are discussed to correlate the observed results.

KEY WORDS: Boronic acids; glucose sensing; *N*-phenyl-1,8-naphthalimide; fluorescence sensing.

INTRODUCTION

The development of molecular probes has been a longstanding goal for chemists. In recent decades, many research groups have investigated the use of chelator groups and fluorophores for the development of materials for the recognition and signaling of various analytes [1–8]. The use of the fluorescence spectroscopy is an attractive way to detect and monitor a specific analyte. Fluorescence spectroscopy is well known for its sensitivity and specificity. In addition, it is a non-consuming method, allowing the development of implantable devices [9]. Chemosensors can be divided into three important parts. The first is the chelator group for the recognition step; the second, the fluorophore giving the spectroscopic characteristics, and the third; the mechanism producing the perturbation of the fluorophore following the chelation of the analyte.

Among chelator groups, boronic acids have attracted a lot of interest in the last decade for their ability to interact with sugars [10–15]. The boronic acid group forms covalent bonds with diols characterized by a fast and reversible interaction in aqueous media [16]. The boronic acid group [$-B(OH)_2$] is a Lewis acid possessing a planar trigonal geometry with an sp^2 hybridized boron atom [17]. On the other hand, the anionic form of the boronic acid [$-B(OH)_3^-$] possesses a tetrahedral conformation with an sp^3 hybridized boron atom. In addition

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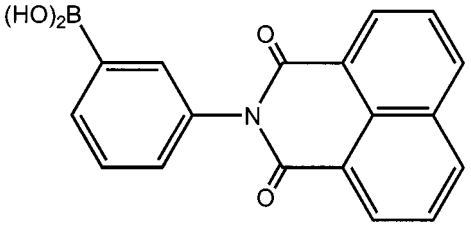
to a conformational change, the electrophilicity of the boron group increases after binding sugars. The pK_a of the phenylboronic acids is typically around 9, it decreases to around 6 in the presence of sugars [18–21]. Many fluorescent probes for sugars using boronic acids are based on these conformational and electrophilicity changes. The photoinduced electron transfer (PET) mechanism has been widely investigated using amino-fluorophore containing a phenylboronic acid [10,22–23]. In this case, change in the acido-base interaction in the presence of sugar between the boron atom and the nitrogen of the amino group is used to induce spectral changes. Probes for sugars using intramolecular charge transfer in the excited state have also been developed [15,21]. In this case, the change between the neural form of the boronic acid (electron-withdrawing group) and its anionic form (electron-donating group) is used to induce spectral changes. Among other mechanisms, molecular rigidification [24–25] and excimer formation [26] have also be investigated.

N-Substituted naphtalimides show interesting spectroscopic and photophysical properties. They have been used, for example, for photocleavage of double-helix DNA [27], as brightening agents for polymers [28], as laser dyes [29], and as fluoroionophores [30]. The properties of the first singlet excited state of *N*-alkyl-1,8-naphthalimide derivatives is dominated by a high intersystem crossing quantum yield (π_{isc}) because of the presence of close-lying upper triplet excited state with n,π^* character, resulting in low fluorescent quantum yields and short fluorescent lifetimes [31]. On the other hand, the photophysical properties of *N*-phenyl-1,8-naphthalimide derivatives are governed by a pseudo–Jahn-Teller effect resulting from the interaction of close-lying singlet excited states [32,33]. In this case, an efficient internal conversion process to the ground state occurs, resulting in low fluorescence quantum yields and short lifetimes [32,33]. Substitution on the *N*-phenyl ring by a methyl and/or electron-withdrawing group has almost no effect on the spectroscopic and photophysics parameters in comparison with the unsubstitute *N*-phenyl-1,8-naphthalimide [32,33]; in this case, both the ground and excited states have a twisted conformation. The incorporation of electron-donating group on the *N*-phenyl ring results in the formation of a long-wavelength emission and longer fluorescence lifetime [32,33]. In this case, the long-wavelength emission results from a conformational relaxation to a more planar conformation of the molecule in the excited state, as well as changes in the relative position of the locally excited state (π,π^*) and the charge transfer excited states.

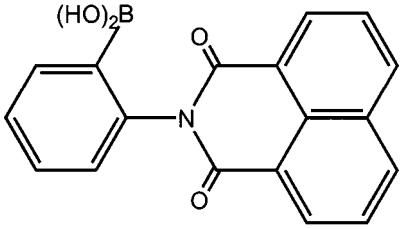
Recently, we reported the synthesis and sugar effects of an *N*-phenyl-1,8-naphthalimide derivative possessing a boronic acid group in *meta* position of the *N*-phenyl ring (*m*-pbani of Fig. 1) [34]. Important decrease of the fluorescence intensity was observed in the presence of sugar. We report in this study the spectroscopic and photophysical properties of m-pbani as well as the *ortho* analogue, *o*-pbani (Fig. 1). As observed for the *meta* compound, the *ortho* derivative shows a large decrease of the fluorescence intensity at high pH and/or in the presence of sugar. Because no fluorescence lifetime changes were observed as a function of the pH, a static quenching from the anionic form of the boronic acid is used to explain the intensity decrease observed at high pH. In contrast, an increase of the fluorescence lifetime of the *ortho* derivative was observed in the presence of sugar, while no effect for the *meta* derivative was obtained. Results are presented and discussed in terms of future development of chemosensors for sugars.

EXPERIMENTAL

D-Glucose, D-galactose and D-fructose were purchased from Sigma and used as received. All solvents used were HPLC grade and purchased from Aldrich. The



***m*-pbani**



***o*-pbani**

Fig. 1. Molecular structure of the two probes investigated.

synthesis of *N*-(3′-boronophenyl)-1,8-naphthalimide (*m*-pbani) has been described previously [34].

N-(2′-boronophenyl)-1,8-naphthalimide (o-pbani). A 50-ml round-bottom flask equipped with Dean-Stark condenser and receiver was charged with 2-aminophenylboronic (40 mg, 0.29 mmol), 1-8-naphthalenedicarboxylic acid anhydride (0.48 mg, 0.24 mmol), 4 Å molecular sieve, 11 mg zinc acetate along with 5 ml toluene and 10 ml pyridine. The reaction mixture was allowed to reflux with stirring for 24 h under a nitrogen atmosphere. Pyridine was removed by distillation and replaced with dichloromethane. The dichloromethane solution was concentrated by rotary evaporator, and the crude solid was purified over a plug of silica gel using 80:10:10 hexane:CH_2Cl_2:acetone solvent system. The filtrate was shaken (2 × 10 ml) with a 2N HCl solution to remove excess aminophenylboronic acid. The reaction afforded a tan powder (0.35 mg, 45% yield) mp 325°C (dec). ^{1}H NMR (300 MHz, CD_3SOCD_3) δ: 8.49 (m, 4H), 7.90 (m, 3H), 7.78 (s, 2H, OH), 7.56 (t, J = 7.5 Hz, 1H), 7.44 (t, J = 7.4 Hz, 1H), 7.28 (d, J = 7.71 Hz, 1H); ^{13}C NMR (75 MHz, CD_3SOCD_3) δ: 154.7, 150.7, 135.2, 133.5, 132.5, 131.5, 119.9, 116.5, 115.0, 114.8. Anal. Calcd. for $C_{18}H_{12}BNO_4 \cdot H_2O$: C, 64.51; H, 4.21; N, 4.18. Found: C, 64.12; H, 4.24; N, 3.74.

Absorption spectra were recorded with a Cary 50 UV-VIS spectrophotometer from Varian. Emission spectra were recorded with an Eclipse spectrofluorometer from Varian. In both case, the measurements were taken at room temperature in a 1-cm quartz cuvette. For all measurements, the absorbancies of the solutions were about 0.1, corresponding to a concentration range of 7–8 × 10^{-6} M of the fluorophore. Fluorescence quantum yields were measured against anthracene in air equilibrated cyclohexane solution (ϕ_F = 0.36) [35].

Titration curves against pH were measured in buffer solutions: acetate buffer for pH 4.0–5.5, phosphate buffer for pH 6.0–9.0, and carbonate buffer for pH 10.0–11.0. Titration curves were fitted and pK_a ($pK_a = -\log K_a$) values were obtained using the equation

$$I = \frac{10^{-pH} I_{acid} + K_a I_{base}}{K_a + 10^{-pH}} \quad (1)$$

where I_{acid} and I_{base} are the intensity limits in the acid and basic regions, respectively. Titration curves against sugar were fitted, and apparent dissociation constant (K_D) values were obtained using the equation

$$I = \frac{I_o + I_f K_D^{-1}[C]}{1 + K_D^{-1}[C]} \quad (2)$$

where I_o and I_f are the initial (no sugar) and final (plateau) intensities of the titration curves. In all case, the spectral response to sugar was immediately observed after the addition of the sugar into the solution.

Frequency-domain (FD) measurements were performed using the instrumentation described previously [36]. Excitation was provided by a rhodamine 6G dye laser at ~310 nm. Emission was observed through a combination of a cut-off (KV 389) and glass (Corning 7-59) filters to remove scattered and Raman scattered light. The measurements were taken in a 1-cm cuvette. The frequency intensity profiles were analyzed by nonlinear least squares in terms of the multiexponential model:

$$I(t) = \sum_i \alpha_i \exp(-t/\tau_i) \quad (3)$$

where α_i are the preexponential factors associated with the decay time τ_i, with $\Sigma_i \alpha_i = 1.0$. The mean lifetime is given by:

$$\bar{\tau} = \frac{\sum \alpha_i \tau_i^2}{\sum \alpha_i \tau_i} = \sum f_i \tau_i \quad (4)$$

where f_i are the fractional steady-state intensities of each lifetime component:

$$f_i = \frac{\alpha_i \tau_i}{\sum_j \alpha_j \tau_j} \quad (5)$$

Errors of 0.5 and 0.01 on the phase angle and modulation have been used, respectively, for calculation of the goodness-of-fit parameter χ_R^2.

RESULTS AND DISCUSSION

Absorption and Emission Properties

Absorption and emission spectra of both compounds are displayed in Fig. 2 and spectroscopic parameters are listed in Table I. Both compounds show similar absorption and emission maximum in phosphate buffer at pH 7.5. Solvent polarity has little effect on the absorption and emission maxima of the *meta* derivative. For this derivative, a red shift of 16 nm is observed in the absorption band while a blue shift of 15 nm is observed in the emission band, from cyclohexane to the phosphate buffer solution. This solvent effect is similar to those observed for the unsubstituted *N*-phenyl derivative of the 1,8-naphthalimide [33]. The ground state of the *N*-phenyl-1,8-naphthalimide derivatives is characterized by a twisted conformation of the phenyl ring, and then absorption spectra show weak effect in function of the substitution

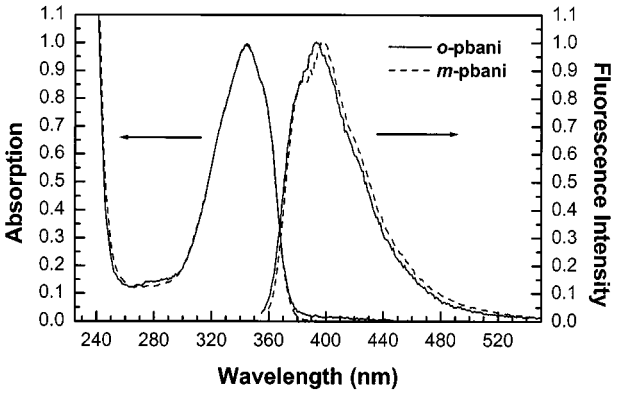


Fig. 2. Absorption and emission spectra of the investigated probes, measured in phosphate buffer (100 m*M*) pH 7.5 at room temperature (λ_{ex} = 345 nm).

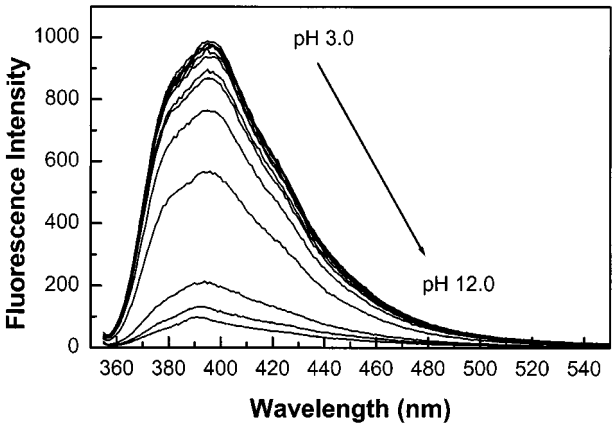


Fig. 3. Effect of the pH on the emission spectrum of *o*-pbani, measured at room temperature (λ_{ex} = 345 nm).

and polarity of the solvent [32,33]. The absorption spectrum of the *ortho* derivative shows shift similar to that of the *meta* derivative as the polarity of the solvent increased, λ_{abs} = 326 and 344 nm from cyclohexane to phosphate buffer solution, respectively. On the other hand, the *ortho* derivative shows very weak emission in cyclohexane. A large decrease (~100-fold) of the fluorescence quantum yield was also observed for an electron-withdrawing ($-CF_3$) substitute in the para position of the *N*-phenyl ring of the 1,8-naphthalimide [32]. Both derivatives investigated do not show the presence of long-wavelength emission bands as observed for some naphthalimide derivatives [32,33]. Long-wavelength emission bands are associated with a charge transfer excited state and conformation change to the excited state.

pH and Sugar Effects on the Steady-State Fluorescence

Figure 3 shows the pH effect on the emission of the *ortho* derivative. Similar results were obtained for the *meta* derivative (not shown). As the pH increases, we observed an important decrease (~90%) of the fluorescence intensity, although no significant shift could be observed. The equilibrium involved with pH change is schematically presented in Scheme 1. Previous work on the formation of the anionic form of the boronic acid group linked to a fluorophore has shown that the anionic form could lead to a quenching of the emission by photo-induced electron transfer [18,37] and that the anionic form could act as electron-donating group leading to the formation of an intramolecular charge transfer excited state [15,21]. Because the incorporation of an electron-donating group on the *N*-phenyl ring of the 1,8-naphthalimide leads to a large red shift (~236 nm) in the emission spectrum and no apparent change in the fluorescence quantum yield, in comparison with the unsubstitute *N*-phenyl derivative, the pH effect on the emission intensity of the *meta* and *ortho* derivatives seems to be caused by a quenching process similar to that observed with previous boronic acid fluorophores [18,37]. In addition, the fluorescence lifetime does not show any significant effect with the pH (see next section) suggesting a static quenching.

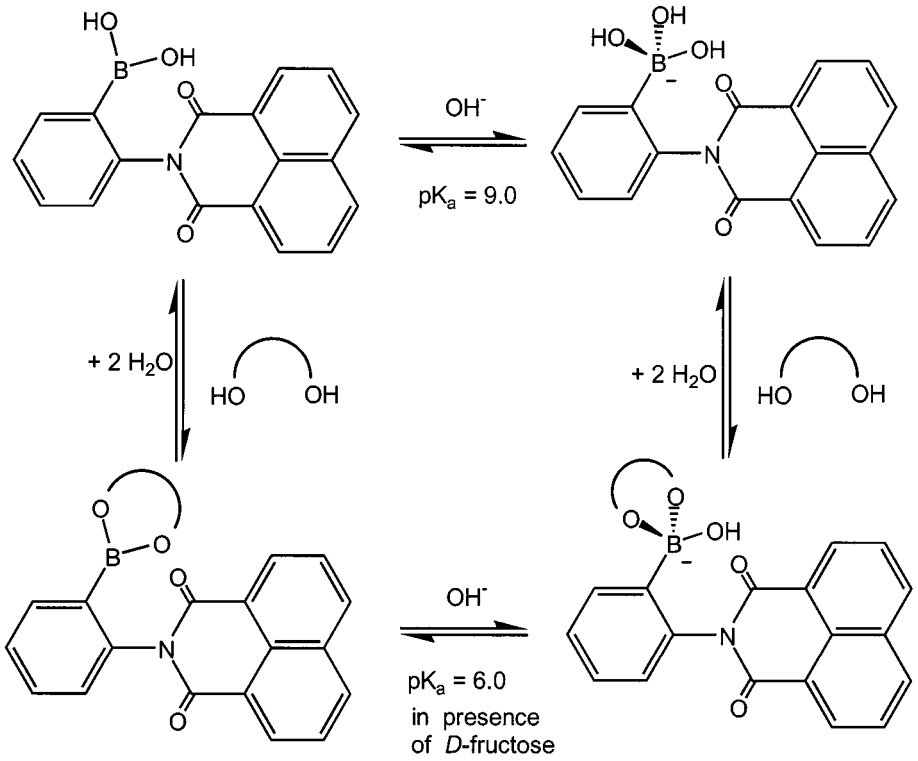
Figure 4 displays the titration curves of *o*-pbani in absence and presence of sugars. The pK_a values are listed in Table II. As generally observed with boronic acids, the titration curves are shifted to the lower pH range in the presence of sugars. Maximum intensity change in the titration curves appears at pH 7.5 for D-fructose and 8.0 for D-glucose. A single pH was chosen (pH of 7.5) for the sugar measurements to have comparable data. The pK_a of the *o*-pbani derivative (9.0) is larger than that of the *meta* derivative (7.7). Electronic repulsion between the negative charge of the boron group and the nitrogen and/or oxygen atoms and/or steric hindrance could explain this change in the electrophilicity of the boron group in the *ortho* position.

Figure 5 shows the effect of D-fructose on the emission spectrum of *o*-pbani. As observed for the pH effect,

Table I. Spectroscopic Parameters for the Two Probes Investigated; Measured in Phosphate Buffer Solution, pH 7.5, at Room Temperature

Probes	λ_{abs} (nm)	ε (M^{-1} cm^{-1})	λ_F (cm)	ϕ_F
***o*-pbani**	345	13 100	393	0.01
***m*-pbani**	345	—	397	0.01[a]

[a] From ref. 34.



Scheme 1. Equilibrium in the pH and sugar titration.

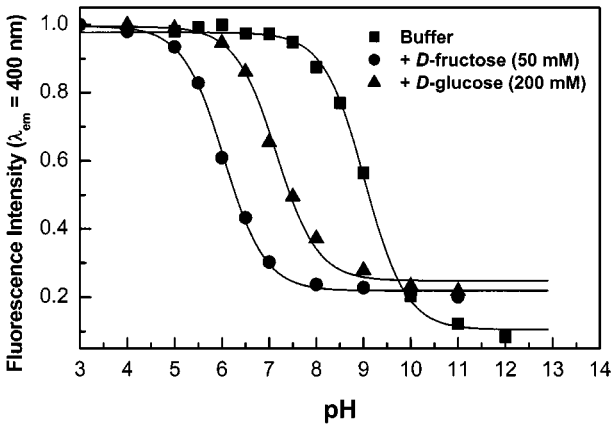


Fig. 4. Titration curves against the pH for *o*-pbani, measured at room temperature (λ_{ex} = 345 nm).

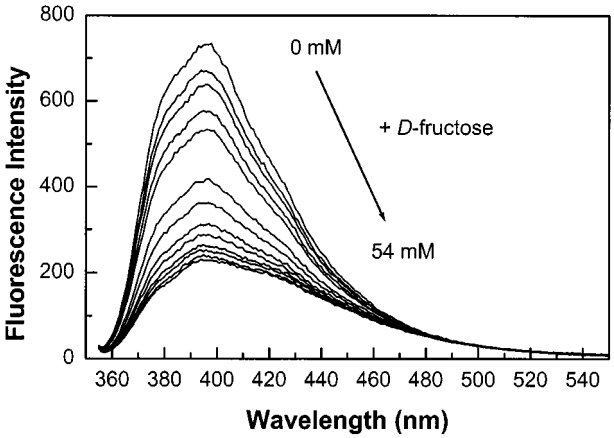


Fig. 5. Effect of *D*-fructose on the emission spectrum of *o*-pbani, measured in phosphate buffer (100 m*M*) pH 7.5 at room temperature (λ_{ex} = 345 nm).

Table II. pK_a and Apparent Dissociation Constants (K_D) for the Two Probes Investigated

	pK_a			K_D (measured at pH 7.5)		
Probes	Buffer	+ D-fructose (50 m*M*)	+ D-glucose (200 m*M*)	D-fructose	D-galactose	D-glucose
***o*-pbani**	9.0	6.0	7.2	1.6 ± 0.1	17.9 ± 0.9	57 ± 3
***m*-pbani**	7.7[a]	~5.7	–	1.3 ± 0.2	12.2 ± 0.8	46 ± 4

[a] From ref. 34.

we observed a decrease of the fluorescence intensity but no apparent shift as the sugar concentration increases. The equilibrium involved in the sugar interactions are displayed in Scheme 1. At pH 7.5, *o*-pbani is presented in its neutral form, whereas in the presence of sugar, it is present under its anionic form because of the decrease of the pK_a of the probe:sugar complex. The sugar effect is similar to the pH effect, thus, the same conclusion could be obtained: PET quenching instead of charge transfer.

Titration curves against sugar are displayed in Fig. 6, and apparent dissociation constants are listed in Table II. Both *ortho* and *meta* derivatives show similar apparent dissociation constants. As observed for the majority of monoboronic acid groups, the affinity is higher for D-fructose and decreases for D-galactose and D-glucose, respectively.

pH and Sugar Effects on the Fluorescence Decays

The pH effect on the fluorescence lifetime of *o*-pbani is shown in Fig. 7, and decay parameters are listed in Table III. The fluorescence lifetime of the *ortho* derivative in aqueous solution is around 1 ns and does not show significant change with pH. Similar results were observed for the *meta* derivative (not shown). Fluorescence decays are multiexponential, characterized by a short component of a few picoseconds and a long component of a few nanoseconds. Fluorescence lifetimes of both compounds are longer than the fluorescence lifetime reported for *N*-phenyl derivative of 1,8-naphthalimide [32,33]. The combination of a large decrease of the fluorescence intensity at high pH observed for both derivatives with the invariability of the fluorescence lifetime lead to a conclusion that a static quenching is at the origin of the important decrease of the fluorescence intensity. It seems that the static quenching is due to a PET mechanism.

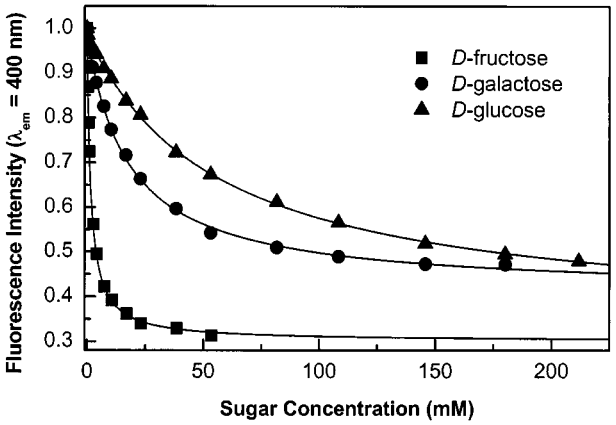


Fig. 6. Titration curves against sugars for *o*-pbani, measured in phosphate buffer (100 m*M*) pH 7.5 at room temperature (λ_{ex} = 345 nm).

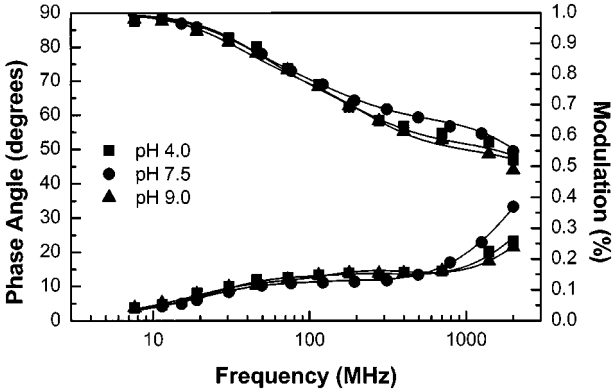


Fig. 7. Effect of the pH on the frequency decay profile of *o*-pbani, measured at room temperature (λ_{ex} = 310 nm).

In contrast to the effect of pH, the presence of sugar induces a significant increase of the fluorescence lifetime of the *ortho* derivative (Fig. 8). The fluorescence lifetime changes are characterized by a decreased contribution of the short component and an increased contribution of the long component. Fluorescence decay results on the *ortho* derivative in the presence of sugar do not correlate a static quenching as observed for the pH effect. As shown in Table III, sugar effects on the fluorescence lifetime of the *meta* derivative are small. From this result, it seems that the position of the boronic acid group on the *N*-phenyl ring plays a role in the sugar response, but not in the pH response. This could suggest the presence of steric hindrance and/or the participation of a charge transfer excited state. Because the photophysics of the *N*-phenyl derivatives of 1,8-naphthalimide are governed by a pseudo–Jahn-Teller effect, resulting in an important nonradiative internal conversion process, the presence of a sugar molecule associate to the boronic acid group at the *ortho* position could lead to a rigidification of the molecule and thus to the decrease of the internal conversion process. On the other hand, the formation of the electron donor anionic form of the boronic acid group in the *ortho* position could also lead to a longer fluorescence lifetime [32,33], but in this case a similar effect with pH is expected. In all cases, increase of the fluorescence lifetime should be associated with an increase of the fluorescence intensity. Because we observed a decrease of the fluorescence intensity, we believe that more than one process is involved in the photophysics of the probe in the presence of sugars.

CONCLUSION

The incorporation of the boronic acid group in the *meta* or *ortho* position does not lead to significant spectro-

Table III. Fluorescence Intensity Decay Parameters for the Two Probes Investigated

	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)	τ_4 (ns)	α_1	α_2	α_3	α_3	f_1	f_2	f_3	f_4	$\bar{\tau}_F$ (ns)	χ_R^2
***o*-pbani**														
PH 4.0	0.032	0.72	3.70	–	0.983	0.01	0.004	–	0.57	0.17	0.26	–	1.11	2.46
PH 7.5	0.049	0.98	4.30	–	0.986	0.01	0.004	–	0.64	0.14	0.22	–	1.11	0.39
PH 9.0	0.027	0.75	4.8	–	0.982	0.02	0.003	–	0.54	0.23	0.23	–	1.30	1.47
+ fructose[a] (50 m*M*)	0.042	1.05	5.22	–	0.967	0.021	0.012	–	0.32	0.18	0.50	–	2.83	1.49
+ glucose[a] (200 mM)	0.054	1.18	5.92	–	0.976	0.019	0.005	–	0.51	0.21	0.28	–	1.91	1.37
***m*-pbani**														
PH 4.0	0.048	0.53	5.40	–	0.987	0.011	0.002	–	0.74	0.09	0.17	–	0.97	1.31
PH 7.5	0.062	1.25	6.83	–	0.993	0.005	0.007	–	0.74	0.008	0.18	–	1.36	0.64
PH 9.0	0.008	0.07	1.50	7.14	0.894	0.148	0.002	0.005	0.30	0.43	0.12	0.15	1.31	1.78
+ fructose[a] (50 m*M*)	0.057	1.63	6.58	–	0.993	0.005	0.003	–	0.69	0.10	0.21	–	1.61	1.28

[a] Phosphate buffer, pH 7.5.

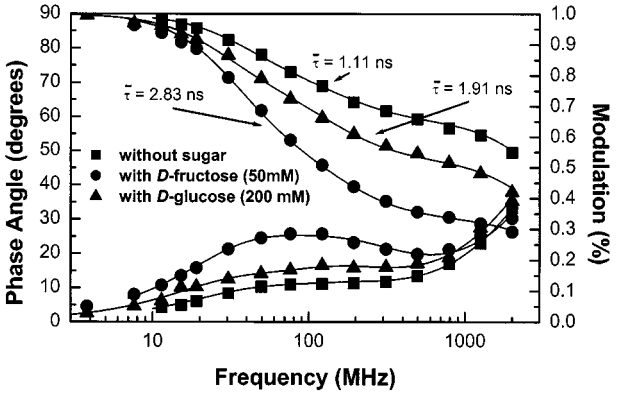


Fig. 8. Effect of sugar on the frequency decay profile of *o*-pbani, measured in phosphate buffer (100 m*M*) pH 7.5 at room temperature (λ_{ex} = 310 nm).

scopic and photophysical changes in comparison with the unsubstitute *N*-phenyl analogue. Both derivatives show larger decreases of their fluorescence emission at high pH while no effect of the pH was observed on the fluorescence lifetime. This suggests the presence of a static quenching resulting from the formation of the anionic form of the boronic acid group. Both compounds show also important decrease of their fluorescence intensity in the presence of sugar, suggesting they could be used as on-off probes in analytical devices for sugar signaling. A significant increase of the fluorescence lifetime was observed for the *ortho* derivative in the presence of sugar while relatively small effect was obtained for the *meta* derivative. The increase of the fluorescence seems to be correlated to a steric hindrance effect. Important change in the phase angle and modulation in the presence of sugar show the potential of the *ortho* derivative for its use as a sugar probe for fluorescence lifetime–based sensing.

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