Published in final edited form as:
Tetrahedron. 2007 June 18; 63(25): 5427–5436. doi:10.1016/j.tet.2007.04.035.

Effect of extended conjugation with a phenylethynyl group on the fluorescent properties of water-soluble arylboronic acids

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Abstract

Boronic acids that change fluorescent properties upon sugar binding are very important reporter units for the development of small molecule lectin mimics (boronolectins). Aimed at developing long wavelength fluorescent boronic acid reporter compounds, we have designed and synthesized a series of boronic acid analogs **2a-d** with an extended π conjugation. Such designs are based on earlier fluorescent boronic acids that change fluorescent properties upon sugar binding. Compared with the corresponding parent chromophores, these new compounds with extended conjugations show longer excitation and emission wavelengths as designed. The patterns of fluorescent changes for the new compounds are also different from that of the corresponding parent compounds.

Keywords

Boronic acids; π Conjugated system; Fluorescence; Sensing; Sensor

1. Introduction

Lectin-carbohydrate binding plays very important roles in biological and pathological processes such as cell-cell adhesion,[1-4] inflammation reactions,[5-7] cancer metastasis,[8,1,9-13] etc. Therefore, small molecule mimics of lectins that can recognize certain carbohydrate biomarkers have the potential to be developed as therapeutic and diagnostic agents. Along this line, we have reported the first lectin mimic, termed as boronolectin,[14] which can recognize cell-surface carbohydrate biomarkers for cell labelling and recognition applications.[15,16] In preparing small molecule lectin mimics, the boronic acid functional group is especially useful because of its intrinsic affinity for the diol functional group,[17-20] which is commonly found on carbohydrates. The intrinsic affinity of boronic acids for the hydroxyl and diol groups has been used in the preparation of boronic acid-based sugar sensors,[21-33] carbohydrate transporters,[34-36] drug delivery materials,[37,38] and other pharmaceutical agents.[39,40,14]

In the preparation of boronolectins, water-soluble boronic acids that change fluorescent properties upon diol binding are very useful for two reasons.[14] First, the fluorescent property changes upon binding are of tremendous help in screening combinatorial libraries. Second, they allow for the development of new boronic acid-based sensors and diagnostics without the need of incorporating a separate signalling moiety. Along this line, we[41,42,14,43,44,40,45,

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[46] and others[24] have developed several such boronic acid fluorescent reporters. In our continuing effort to develop boronolectins and fluorescent boronolectins, we are interested in examining whether adding conjugation to such known boronic acid fluorescent reporters would result in more favorable spectroscopic properties in terms of wavelength and magnitude of fluorescent property changes. For this, we have selected four representative compounds (**1a-d**, Figure 1). Three of them are naphthylboronic acids (**1a-c**) that are positional isomers of each other and one is a quinolinylboronic acid (**1d**). Out of these four, **1a** shows fluorescence intensity increases upon sugar binding;[41] **1b** shows ratiometric fluorescent intensity changes at two wavelengths;[47] **1c** shows fluorescent intensity decreases upon sugar binding;[47] and **1d** did not show significant fluorescent property changes upon sugar binding. These compounds were chosen for their (1) water soluble nature, (2) known fluorescent property changes upon binding(except for **1d**), (3) chemical stability, and (4) anticipated ease of synthetic manipulation. With these reporters having diverse patterns of spectroscopic changes upon sugar binding, we reasoned that the results should allow us to do some representative sampling of what could be expected by extending the conjugation of similar boronic acid fluorescent reporters. Specifically, we are interested in learning the effect of such extended conjugations on (1) the excitation and emission wavelengths, (2) quantum yields of both the free boronic acids and their corresponding esters, (3) the pattern of fluorescent property changes, and (4) mechanism of fluorescent property changes upon sugar binding.

Phenylethynyl and ethynyl groups have received a great deal of attention as a linker for new materials with potential applications ranging from chemical sensing to molecular electronics.[48-54] Such elongation affords the needed conjugation and only introduces one more rotational degree of freedom. Therefore such compounds are expected to have good fluorescent properties. After considering the ease of synthesis, potential effect on water solubility, chemical stability of the extended product, and the desire to minimize the degree of freedom in the new extended conjugation system, we decided to use an alkyne group as a way to extend the conjugation. Therefore, four new compounds (**2a-d**) with their conjugation extended by an alkyne group were designed (Figure 1). The results show that extended conjugation not only resulted in increased emission and excitation wavelength as expected, but also improved fluorescent property changes upon binding with interesting mechanistic implications.

2. Results and discussions

2.1. Synthesis

The designed 4-arylethynylphenyl boronic acids **2a-d** were synthesized using the Sonogashira coupling reaction of arylbromides with 2,2-dimethylpropane-1,3-diyl-4-ethynylphenyl boronate (**4**) as the building block under microwave irradiation followed by the deprotection of neopentyl group with TFA in water. Specifically, Sonogashira coupling reaction of 2,2-dimethylpropane-1,3-diyl-4-bromophenyl boronate with trimethylsilyl acetylene under microwave irradiation gave 2,2-dimethylpropane-1,3-diyl[4-trimethylsilylethynylphenyl] boronate (**3**) in 98% isolated yield. This yield was much higher than that without using microwave.[55] Deprotection of the trimethylsilyl group (TMS) of **3** with potassium carbonate afforded **4**.[55] It should be noted that the two key reactions for the synthesis of **3** and **5a-d** were both microwave-facilitated Sonogashira coupling reaction and no Suzuki coupling side reactions were observed. In addition, the use of microwave was found to significantly shorten the reaction time.

2.2. Fluorescence studies

The fluorescent properties of compounds **2a-d** in the presence and absence of sugars were examined in phosphate buffer (0.1 M, pH 7.4). Compound **2a** showed an 8-fold fluorescence intensity increase upon addition of 50 mM D-fructose (Figure 2). It also showed significant

fluorescence intensity increases upon addition of other sugars. Compared with compound **1a** (λex = 300 nm and λem = 445 nm),[41] the λex of **2a** increased by about 40 nm to 340 nm and the λem increased by only about 10 nm to 454 nm (Table 1).

Binding constants for **2a** as well as the other sensors (**2b-d)** with fructose and three other sugars were determined at pH 7.4 by fitting $1/\Delta I_f$ *vs.* 1/Con. into a 1:1 binding model. In all cases, R^2 were > 0.99, indicating an excellent fit into a 1:1 binding model (Figure 3).

The affinity trend with **2a** followed that of **1a**[17,18] in the order of sorbitol > fructose > galactose > glucose with *Ka* (M^{-1}) of 619, 293, 8.1 and 2.5, respectively (Table 1). As has been addressed before in the literature, sorbitol can engage in trivalent binding, which is the reason for its high affinity relative to other sugars.[57,58,14]

The fluorescence quantum yields (Φ_F) of **2a** were determined in 0.1 M phosphate buffer (pH 7.4) in the absence and presence of 50 mM fructose, with 8-quinolinc boronic acid (Φ_F = 0.58 in 12 M H_2SO_4) as a reference compound.[59] The results (Table 1) show that the Φ_F (0.013) of **2a** in the absence of a sugar is similar to that of **1a** (0.01). However, after addition of 50 mM fructose, the Φ_F of **2a** increase to 0.096, which is lower than the Φ_F (0.42) of **1a** under the same conditions. Therefore, it seems that the increased wavelength came at the expense of a decreased quantum yield possibly due to the increased degree of freedom of rotation which allows for the irradiative decay of the excited state.

The pH induced fluorescence spectral changes of **2a** in the absence and presence of fructose at a fixed concentration (50 mM) were studied to examine the relationship between the fluorescence intensity changes and the different ionization states. The fluorescence intensity of **2a** at 370 nm decreased when pH increased from 1.57 to ~5.0, and did not show further changes beyond 5.0. Almost no fluorescence was observed at 454 nm in the pH range 1.57-5.0, while a new peak increased very significantly at 454 nm as pH increased from 5.0 to 11.5 (Figures 4 and 5).

The pH-induced spectral changes at 370 nm in the absence of a sugar were similar to that in the presence of 50 mM fructose. A decrease in intensity (more than 99%) at 370 nm was observed when pH increased from 1.57 to 5.0. Plotting the intensity changes at 370 nm against pH revealed the first apparent p*K*a of 3.6 for **2a** in the absence of sugar and 3.5 in the presence of fructose. The pH profiles of the fluorescence intensity of **2a** at 454 nm revealed the second apparent p*K*a of 9.4 in the absence of a sugar and 6.8 in the presence of fructose (50 mM). It is reasonable to assign the first p*K*a to the aniline group and the second p*K*a to the boronic acid. Therefore, one can use Scheme 2 to show the various ionization states of **2a**, which helps to understand the assignment of each fluorescent peak to a particular species.

The pH-induced fluorescence changes of **2a** and **1a** have a similar trend. The free forms of **2a** and **1a** are weakly fluorescent and only the protonated form (370 nm) and the boron ionized form (454 nm) are fluorescent.

With compound **2b**, its affinity trend also followed that of its parent compound, **1b**, in the order of sorbitol > fructose > galactose > glucose with *Ka* (M^{-1}) of 311, 178, 7.5 and 7.0, respectively (Table 1). However, compared with the situation for **2a**, the extension of the conjugation of 5-*N*,*N*-dimethylaminonaphthylboronic acid (**1b**) gave some very noticeably different outcomes. Compound **2b** exhibited very different fluorescence properties compared with its parent, **1b**. Addition of fructose (50 mM) to a solution of **1b** induced a 61% fluorescence intensity decrease at the longest wavelength, 513 nm, and a 36-fold increase at 433 nm.[47,56] In contrast, **2b** showed a 2.5-fold fluorescence intensity increase upon addition of 50 mM D-fructose (Figure 6) at 531 nm. With the added conjugation, the excitation wavelength of **2b** increased by 40 nm to 340 nm and the highest emission wavelength changed from 513 to 531 nm. It should be

noted that numerous literature precedents have shown that substitution patterns make a great difference in dictating the fluorescent properties of a boronic acid compound.[41,56,47,46] Therefore, it is not surprising that positional isomers behave somewhat differently in their fluorescent responses upon elongated conjugation.

The fluorescence quantum yields (Φ_F) of **2b** were determined in the presence and absence of a sugar using the same method as with **2a**. The results (Table 1) show that Φ_F of compounds **2b** in the absence of a sugar was 0.027, which increased to 0.12 after addition of 50 mM fructose.

Figures 7 and 8 show the fluorescence intensity changes of **2b** at different pH in the absence and presence of fructose (50 mM). It can be seen that the fluorescence intensity of **2b** at 375 nm decreased when pH increased from 1.39 to ~6.0, and did not show further changes beyond 6.0, while a new peak increased very significantly at 531 nm as pH increased from 4.0 to 11.0.

The pH-induced spectral changes at 375 nm in the absence of a sugar are similar to those in the presence of 50 mM fructose. A decrease in intensity (more than 99%) at 375 nm was observed when pH increased from 1.57 to 5.0. Plotting the intensity changes at 375 nm against pH revealed the first apparent p*K*a for **2b** as 4.0 both in the absence and in the presence of fructose. The pH profiles of the fluorescence intensity of **2b** at 531 nm revealed the second apparent p*K*a of **2b** as 9.8 in the absence of a sugar and 6.3 in the presence of fructose. It is reasonable to assign the first p*K*a to the aniline group and the second p*K*a to the boronic acid. Scheme 2 shows the various ionization states of **2b** and their relationship to various fluorescent peaks.

Compound **2c** is similar to **2b**, where elongation of the conjugation resulted in more than just an increase in excitation and emission wavelengths. It displayed different patterns of fluorescence intensity changes compared with its parent compound, **1c**. With the addition of fructose to the solution of **1c**, a significant 80% decrease in fluorescent intensity was observed at 432 nm.[47] In contrast, **2c** showed a 6-fold fluorescence intensity increase upon addition of 50 mM D-fructose (Figure 9) at 448 nm. The affinities of **2c** for various sugars are in the order of fructose > sorbitol > galactose > glucose with *Ka* (M^{-1}) of 132, 11.1, 0.2 and 0.1, respectively (Table 1). This order of affinity strength is different from that of its parent compound, **1c**. It is very interesting to note the relatively low affinity of **2c** for sorbitol, galactose, and glucose. It is not readily clear why this is the case. It should be noted that we have conducted an extensive evaluation of various factors that affect boronic acid-diol binding constants. Though pKa and pH values are two major factors, there are other factors that are not well understood at the present time. [17,18]

The fluorescence quantum yields (Φ_F) of **2c** were determined in a similar fashion as for **2a** and **2b**. After addition of 50 mM fructose, the fluorescence quantum yield (Φ_F) of **2c** increased from 0.025 to 0.058 at 448 nm. As a comparison, the fluorescence quantum yield (Φ_F) of **1c** in the absence of a sugar was 0.89, and the quantum yield (Φ_F) decreased after the addition of 50 mM fructose.

Figures 10 and 11 shows the fluorescence intensity changes of **2c** at different pH in the absence and presence of fructose (50 mM). It can be seen that the fluorescence intensity of **2c** at 363 nm decreased when pH increased from 1.52 to ~6.0, while a new peak increased in intensity at 448 nm as pH increased from 4.0 to 11.0.

The pH-induced spectral changes of **2c** at 363 nm in the absence of a sugar are similar to that in the presence of 50 mM fructose. A more than 200-fold intensity decrease at 363 nm was observed when pH increased from 1.52 to 6.0. Plotting the intensity changes at 363 nm against pH revealed the first apparent p*K*a as 3.8 for **2c** both in the absence and presence of fructose.

The pH profiles of the fluorescence intensity of **2c** at 448 nm revealed the second apparent p*K*a of **2c** as 8.7 in the absence of a sugar and 7.1 in the presence of fructose. It is reasonable to assign the first p*K*a to the aniline group and the second p*K*a to the boronic acid. Scheme 2 shows the various ionization states of **2c** and their relationship to various fluorescent emissions.

It is also interesting to note that the pH-induced fluorescence changes of **2c** are very different from that of **1c.** The free form of **2c** is only weakly fluorescent and only the protonated form and the boron ionized form are fluorescent (Scheme 2). In contrast, the free form of **1c** is the fluorescent species, and neither the protonated form nor the boron ionized form of **1c** is fluorescent.

The effect of conjugation elongation was most prominent with compound **2d**, which showed a significant fluorescence intensity decrease upon addition of D-fructose (Figure 12) at 371 nm, while its parent compound, **1d**, showed no significant fluorescence intensity changes upon addition of D-fructose and sorbitol.[60] The affinity trend of **2d** followed that of **1a-c**, **2a** and **2b** in the order of sorbitol > fructose > galactose > glucose with *Ka* (M^{-1}) of 822, 427, 49, and 18, respectively (Table 1). Compound **2d** also showed the highest binding affinity with different sugars compared with all other water-soluble boronic acid sensors described in this study. This situation is very similar to 3-pyridine boronic acid, which also showed a high affinity for various sugars. The key reason seems to be due to the formation of zwitterionic complex, which favors binding.[61]

The fluorescence quantum yields (Φ_F) of **2d** were also determined in 0.1 M phosphate buffer (pH 7.4) in the absence of a sugar and in the presence of 50 mM fructose in a similar fashion as with **2a-c**. The quantum yield of **2d** slightly decreased from 10% to 8% after addition of 50 mM fructose.

The pH induced fluorescence spectral changes of **2d** in the absence of any sugar (Figures 13 and 14) showed only one apparent p*K*a at 3.3, while in the presence of 50 mM fructose, two apparent p*K*a values were found for **2d** at 3.3 and 10. If the situation is similar to 3-pyridineboronic acid, the first p*K*a of the complex could be the boron and the second one could be due to the deprotonation of the quinoline nitrogen.

3. Conclusion

A series of boronic acid analogs **2a-d** with increased conjugation have been synthesized in four steps from the corresponding arylbromides and 2,2-dimethylpropane-1,3-diyl-4-ethynylphenylboronate. The key reaction was a microwave-facilitated selective Sonogashira reaction, which gave 47% ~ 67% yields. Detailed fluorescent studies show that extending the conjugation with an alkynyl group (1) decreased quantum yield, (2) increased the excitation and emission wavelength by 20-40 and 9-16 nm respectively, (3) resulted in alterations in the patterns of the fluorescent property changes upon sugar binding, and (4) altered the pH profiles and thus the mechanism through which fluorescent properties change upon sugar addition. The results suggested that extending the conjugation of a known fluorescent boronic acid with an alkynyl moiety results in more than a simple increase in excitation and emission wavelength.

4. Experimental

4.1. General

All reagents were purchased from Acros and Aldrich. Microwave heating was performed in the single-mode microwave cavity of a Discover Synthesis System (CEM Co.), and all microwave-irradiated reactions were conducted in a heavy-walled glass vials sealed with Teflon septa. ^{1}H-NMR, ^{13}C-NMR and DEPT spectra were recorded at 400 and 100 MHz,

respectively, on a Bruker Avance 400 NMR spectrometer. Mass spectra were performed by the Mass Spectrometry Facilities at Georgia State University. Fluorescence spectra were recorded on a Shimadzu RF-5301 PC spectrofluorometer. Absorption spectra were recorded on a Shimadzu UV-1700 UV-Visible spectrophotometer. All pH values were determined by a UB-10 Ultra Basic Benchtop pH meter (Denver Instrument).

4.2. Synthesis

(6-Bromo-naphthalen-2-yl)-methylamine,[62] (6-bromo-naphthalen-2-yl)-dimethylamine,[47] (4-bromo-naphthalen-1-yl)-dimethylamine,[63] and (5-bromo-naphthalen-1-yl)-dimethylamine[56] were synthesized following literature procedures.

2,2-dimethylpropane-1,3-diyl [4-trimethylsilylethynylphenyl] boronate (3).[55]—A mixture of 2,2-dimethylpropane-1,3-diyl-4-bromophenyl boronate (0.24 g, 0.9 mmol), Pd$(PPh_3)_2Cl_2$ (32 mg, 0.05 mmol), CuI (9 mg, 0.05 mmol), triphenylphosphine (48 mg, 0.18 mmol), trimethylacetylene (0.14 mL, 1 mmol), and dimethylamine (1.5 mL, 13.6 mmol) in dimethylformamide (DMF, 0.5 mL) was irradiated with microwave at 120 °C for 20 min in a heavy-walled glass vial sealed with a Teflon septum. After irradiation, the solvent was concentrated under reduced pressure and the residue was purified by flash column chromatography to give 0.25 g (98%) of 2,2-dimethylpropane-1,3-diyl [4-trimethylsilylethynylphenyl] boronate (**3**). ^{1}H-NMR ($CDCl_3$): 7.65 (d, J = 8.0 Hz, 2H), 7.37 (d, J = 8.0 Hz, 2H), 3.67 (s, 4H), 0.93 (s, 6H), 0.17 (s, 9H). ^{13}C-NMR ($CDCl_3$): 133.5 (d), 131.0 (d), 125.1 (s), 105.4 (s), 95.0 (s), 72.3 (t), 31.8 (s), 21.9 (q), -0.05 (q). GC-MS (EI): 286 $[M]^+$.

2,2-Dimethylpropane-1,3-diyl-4-ethynylphenyl boronate (4).[55]—To a solution of 2,2-dimethylpropane-1,3-diyl [4-trimethylsilylethynylphenyl] boronate (**3**) (0.14 g, 0.5 mmol) in methanol (10 mL), was added potassium carbonate (0.14 g, 1 mmol). The resulting mixture was stirred at room temperature for 0.5 h and then filtered. The filtrate was concentrated under reduced pressure. The crude residue was purified by flash column chromatography to afford 0.22 g (80%) of 2,2-dimethylpropane-1,3-diyl-4-ethynylphenyl boronate (**4**). ^{1}H-NMR ($CDCl_3$): 7.82 (d, J = 8.0 Hz, 2H), 7.54 (d, J = 8.0 Hz, 2H), 3.81 (s, 4H), 3.18 (s, 1H), 1.06 (s, 6H). ^{13}C-NMR ($CDCl_3$): 133.6 (d), 131.1 (d), 124.1 (s), 83.9 (s), 78.0 (d), 72.3 (t), 31.8 (s), 21.8 (q). GC-MS (EI): 214 (M^+). HRMS (ESI^-) calcd for $C_{18}H_{26}BO_4$ [M+$C_5H_{12}O_2$ (neopental glycol)-H]$^-$: 317.1924. Found: 317.1929.

General procedure[64,55] for the synthesis of compounds 5a-d by microwave-assisted Sonogashira coupling—A mixture of aryl bromide (0.9 mmol), Pd$(PPh_3)_2Cl_2$ (0.05 mmol), CuI (0.05 mmol), triphenylphosphine (0.18 mmol), diethylamine (13.6 mmol) and 2,2-dimethylpropane-1,3-diyl-4-ethynylphenylboronate (1 mmol) was irradiated under microwave at 120 °C for 25 min. in a heavy-walled glass vial sealed with a Teflon septum. Then the reaction mixture was filtered and washed with dichloromethane. The filtrate was concentrated under reduced pressure and the residue was purified by flash column chromatography (silica gel 60) to give compound **5**.

{4-[4-(5,5-Dimethyl[1,3,2]dioxaborinan-2-yl)-phenylethynyl]naphthalen-1-yl} dimethylamine (5a)—Yield: 51%. ^{1}H-NMR ($CDCl_3$): 8.44 (dd, J = 0.8 and 8.0 Hz, 1H), 8.22 (dd, J = 1.2 and 8.0 Hz, 1H), 7.81 (d, J = 8.0 Hz, 2H), 7.67 (d, J = 7.6 Hz, 1H), 7.61 (d, J = 8.4 Hz, 2H), 7.57 (m, 1H), 7.52 (m, 1H), 7.00 (d, J = 7.6 Hz, 1H), 3.78 (s, 4H), 2.92 (s, 6H), 1.03 (s, 6H). ^{13}C-NMR ($CDCl_3$): 151.7 (s), 134.5 (s), 133.7 (d), 130.8 (d), 130.5 (d), 128.3 (s), 126.8 (d), 126.5 (d), 125.9 (s), 125.5 (d), 124.6 (d), 114.9 (s), 113.3 (d), 93.6 (s), 89.1 (s), 72.3 (t), 45.0 (q), 31.9 (s), 21.9 (q). GC-MS (EI): 383 $[M]^+$.

{5-[4-(5,5-Dimethyl[1,3,2]dioxaborinan-2-yl)-phenylethynyl]naphthalen-1-yl} dimethylamine (5b)—Yield: 49%. ^{1}H-NMR ($CDCl_3$): 8.27 (d, *J* = 8.0 Hz, 1H), 8.16 (d, *J* = 8.0 Hz, 1H), 7.84 (d, *J* = 8.0 Hz, 2H), 7.75 (dd, *J* = 0.8 and 7.2 Hz, 1H), 7.63 (d, *J* = 8.0 Hz, 2H), 7.51 (m, 1H), 7.46 (m, 1H), 7.13 (d, *J* = 7.2 Hz, 1H), 3.80 (s, 4H), 2.91 (s, 6H), 1.05 (s, 6H). ^{13}C-NMR ($CDCl_3$): 151.3 (s), 134.7 (s), 133.8 (d), 130.7 (d), 130.4 (d), 128.7 (s), 126.7 (d), 125.6(s), 125.1 (d), 124.4 (d), 121.1 (s), 121.0 (d), 114.6 (d), 94.4 (s), 89.0 (s), 72.3 (t), 45.3 (q), 31.9 (s), 21.9 (q). GC-MS (EI): 383 $[M]^+$.

{6-[4-(5,5-Dimethyl[1,3,2]dioxaborinan-2-yl)-phenylethynyl]naphthalen-2-yl} dimethylamine (5c)—Yield: 47%. ^{1}H-NMR ($CDCl_3$): 7.90 (s, 1H), 7.78 (d, *J* = 8.4 Hz, 2H), 7.67 (d, *J* = 9.2 Hz, 1H), 7.59 (d, *J* = 8.4 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 2H), 7.46 (dd, *J* = 1.2 and 8.4 Hz, 1H), 7.16 (dd, *J* = 2.4 and 9.2 Hz, 1H), 6.87 (d, *J* = 2.4 Hz, 1H), 3.78 (s, 4H), 3.06 (s, 6H), 1.03 (s, 6H). ^{13}C-NMR ($CDCl_3$): 149.1 (s), 134.6 (s), 133.7 (d), 131.3 (d), 130.6 (d), 128.9 (d), 128.7 (d), 126.12 (d), 126.09 (s), 125.8 (s), 116.5 (d), 116.1 (s), 106.0 (d), 91.6 (s), 88.9 (s), 72.3 (t), 40.6 (q), 31.9 (s), 21.9 (q). GC-MS (EI): 383 $[M]^+$.

3-[4-(5,5-Dimethyl[1,3,2]dioxaborinan-2-yl)phenylethynyl]quinoline (5d)—Yield: 67%. ^{1}H-NMR ($CDCl_3$): 9.00 (d, *J* = 2.0 Hz, 1H), 8.30 (d, *J* = 1.6 Hz, 1H), 8.10 (d, *J* = 8.4 Hz, 1H), 7.83~7.78 (m, 3H), 7.72 (m, 1H), 7.59~7.54 (m, 3H), 3.78 (s, 4H), 1.03 (s, 6H).^{13}C-NMR ($CDCl_3$): 152.1 (d), 146.8 (s), 138.3 (d), 133.8 (d), 130.8 (d), 130.0 (d), 129.4 (d), 127.6 (d), 127.3 (d), 124.5 (s), 117.5 (s), 93.0 (s), 87.5 (s), 72.3 (t), 31.9 (s), 21.9 (q). GC-MS (EI) : 341 $[M]^+$. HRMS (ESI^+) calcd for $C_{22}H_{21}BNO_2$ $[M+H]^+$: 342.1665. Found: 342.1670.

General procedure for the preparation of compounds 2a-d by deprotection of the neopentyl group—A mixture of **5** (0.5 mmol) in 70% TFA was stirred at 60~70 °C overnight. The reaction mixture was neutralized with saturated Na_2CO_3 and extracted with dichloromethane. The combined organic solution was dried over Na_2SO_4. After evaporation of solvent, the crude was purified on silica gel by flash column chromatography to afford **2**.

Compound 2a—Yield: 43%. ^{1}H-NMR ($CDCl_3$): 8.48 (dd, *J* = 0.8 and 8.4 Hz, 1H), 8.25~8.23 (m, 3H), 7.76~7.72 (m, 3H), 7.60 (m, 1H), 7.54 (m, 1H), 7.03 (d, *J* = 7.6 Hz, 1H), 2.95 (s, 6H). ^{13}C-NMR ($CDCl_3$): 152.0 (s), 135.5 (d), 134.6 (s), 131.1 (d), 130.9 (d), 128.3 (s, two carbon overlap), 126.73 (d), 126.68 (d), 125.5 (d), 124.7 (d), 114.6 (s), 113.3 (d), 93.4 (s), 90.5 (s), 45.0 (q). MS (ESI^-): 314.05 $[M-H]^-$. HRMS (ESI^-) calcd for $C_{20}H_{17}BNO_2$ $[M-H]^-$: 314.1352. Found: 314.1360.

Compound 2b—Yield: 36%. ^{1}H-NMR ($CDCl_3$): 8.30~8.27 (m, 2H), 8.18 (d, *J* = 8.4 Hz, 1H), 7.81~7.75 (m, 3H), 7.66 (m, 1H), 7.56 ~ 7.46 (m, 2H), 7.16 (d, *J* = 7.2 Hz, 1H), 2.90 (s, 6H). MS (ESI^-): 314.10 $[M-H]^-$. HRMS (ESI^-) calcd for $C_{20}H_{17}BNO_2$ $[M-H]^-$: 314.1352. Found: 314.1342.

Compound 2c—Yield: 41%. ^{1}H-NMR ($CDCl_3$): 8.22~8.19 (m, 2H), 7.95 (s, 1H), 7.70~7.67 (m, 3H), 7.62 (d, *J* = 8.8 Hz, 1H), 7.51 (dd, *J* = 1.6 and 8.4 Hz, 1H), 7.16 (dd, *J* = 1.6 and 8.8 Hz, 1H), 6.88 (s, 1H), 3.08 (s, 6H). ^{13}C-NMR ($CDCl_3$): 149.2 (s), 135.5 (d), 134.7 (s), 131.6 (d), 130.9 (d), 128.9 (d), 128.1 (s), 126.2 (d), 126.1 (s), 116.6 (d), 115.8 (s), 106.0 (d), 93.0 (s), 88.7 (s), 40.7 (q). HRMS (ESI^-) calcd for $C_{20}H_{17}BNO_2$ $[M-H]^-$: 314.1352. Found: 314.1351.

Compound 2d—Yield: 47%. ^{1}H-NMR (MeOD): 8.96 (s, 1H), 8.54 (s, 1H), 8.07 (d, *J* = 8.4 Hz, 1H), 7.99 (d, *J* = 8.0 Hz, 1H), 7.85~7.82 (m, 3H), 7.69 (m, 1H), 7.59 (d, *J* = 7.6 Hz, 2H).^{13}C-NMR ($CDCl_3$): 151.8, 146.3, 138.9, 138.8, 134.5, 133.5, 131.0, 130.4, 129.0, 127.7, 127.5, 92.9, 87.6. HRMS (ESI^+) calcd for $C_{17}H_{13}BNO_2$ $[M+H]^+$: 274.1039. Found: 274.1028.

4.3. Procedure for sugar-binding studies

The solutions of the sensors (1.0×10^{-5} or 1.0×10^{-6} M) were prepared in 0.1 M phosphate buffer at pH 7.4 and the solutions of sugars (1.0×10^{-1} M) were prepared in above solutions of the sensors at pH 7.4. Then, 3 mL of mixtures were prepared by mixing a sensor solution with a different ratio of sugar solution. After stirring for 20 min, the mixture was transferred into a 1 cm quartz cell and the fluorescence intensity was recorded immediately.

Acknowledgments

Financial support from the National Institutes of Health (CA123329, CA113917, CA88343, and NO1-CO-27184), the Georgia Cancer Coalition through a Distinguished Cancer Scientist Award, and the Georgia research Alliance through an Eminent Scholar endowment and an Eminent Scholar challenge grant is gratefully acknowledged.

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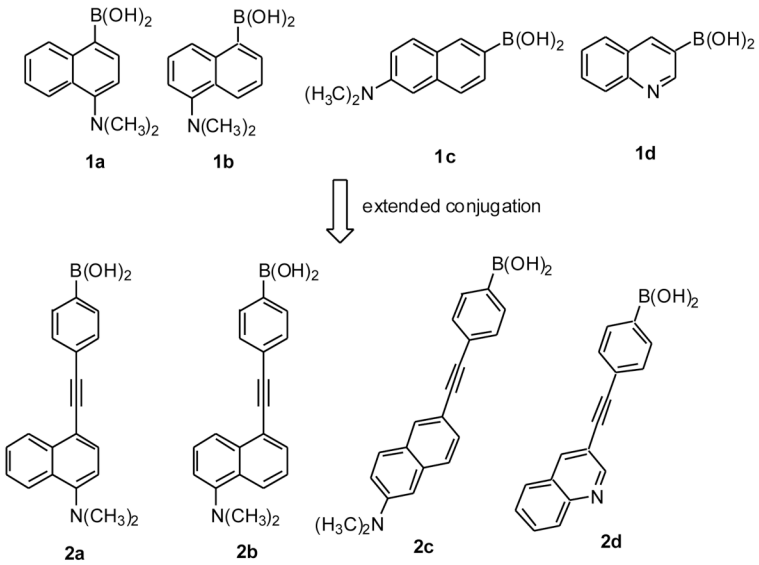


Figure 1.
Design of compounds **2a-d** from **1a-d**.

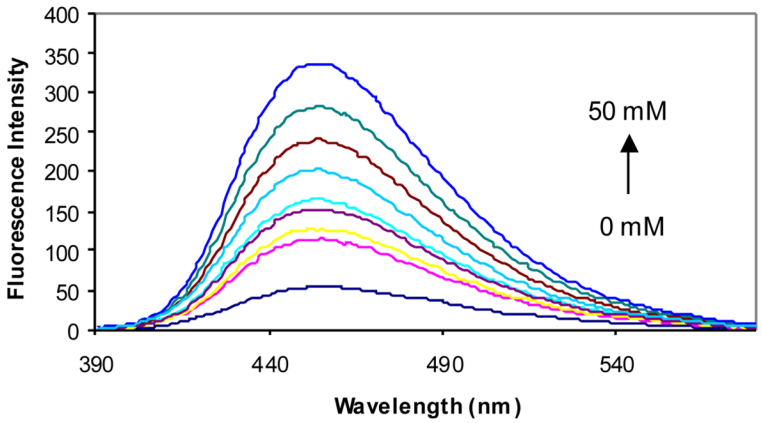


Figure 2.

Fluorescent spectral changes of **2a** (1.0×10^{-5} M) with different concentrations of D-fructose (0-50 mM) in 0.1 M aqueous phosphate buffer at pH 7.4, λex = 340 nm.

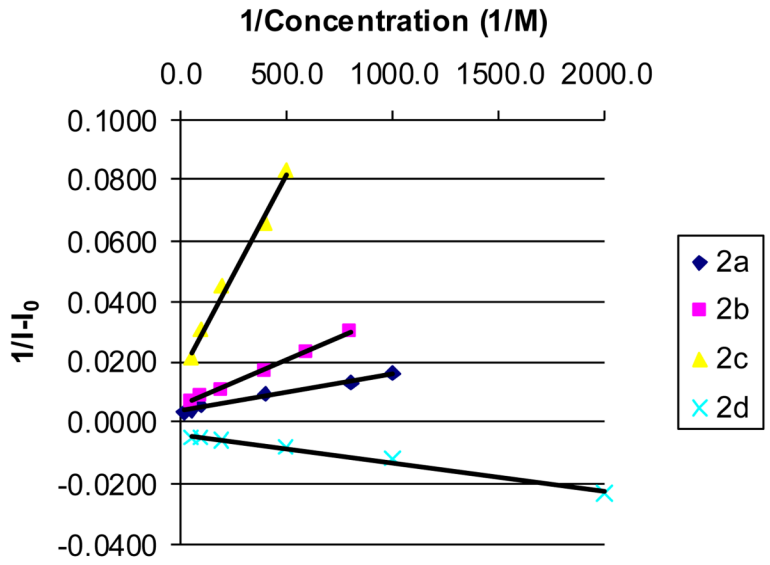
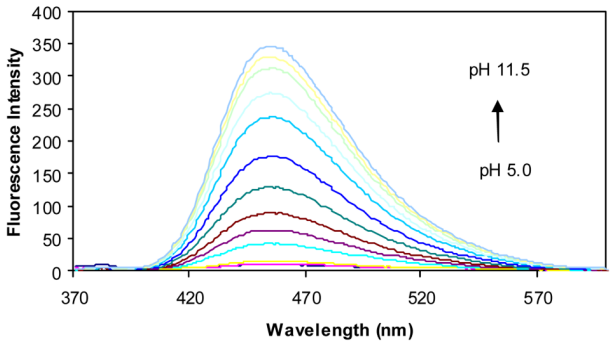
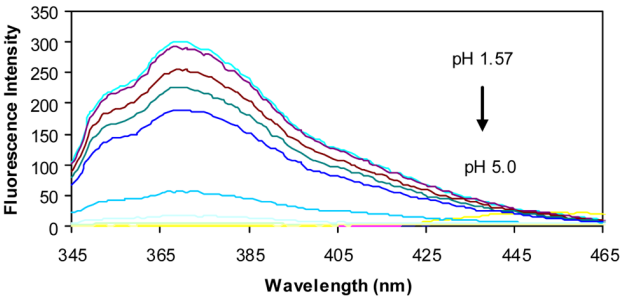


Figure 3.
Determination of the *Ka* for **2a-d** with fructose in 0.1 M phosphate buffer at pH 7.4; $R^2 > 0.99$ in all cases.



(A)



(B)

Figure 4.

Fluorescence spectral changes of **2a** (1.0×10^{-5} M) in 0.1 M aqueous phosphate buffer at different pH, λex = 340 nm. (A) fluorescent intensity changes at 454 nm; (B) fluorescent intensity changes at 370 nm.

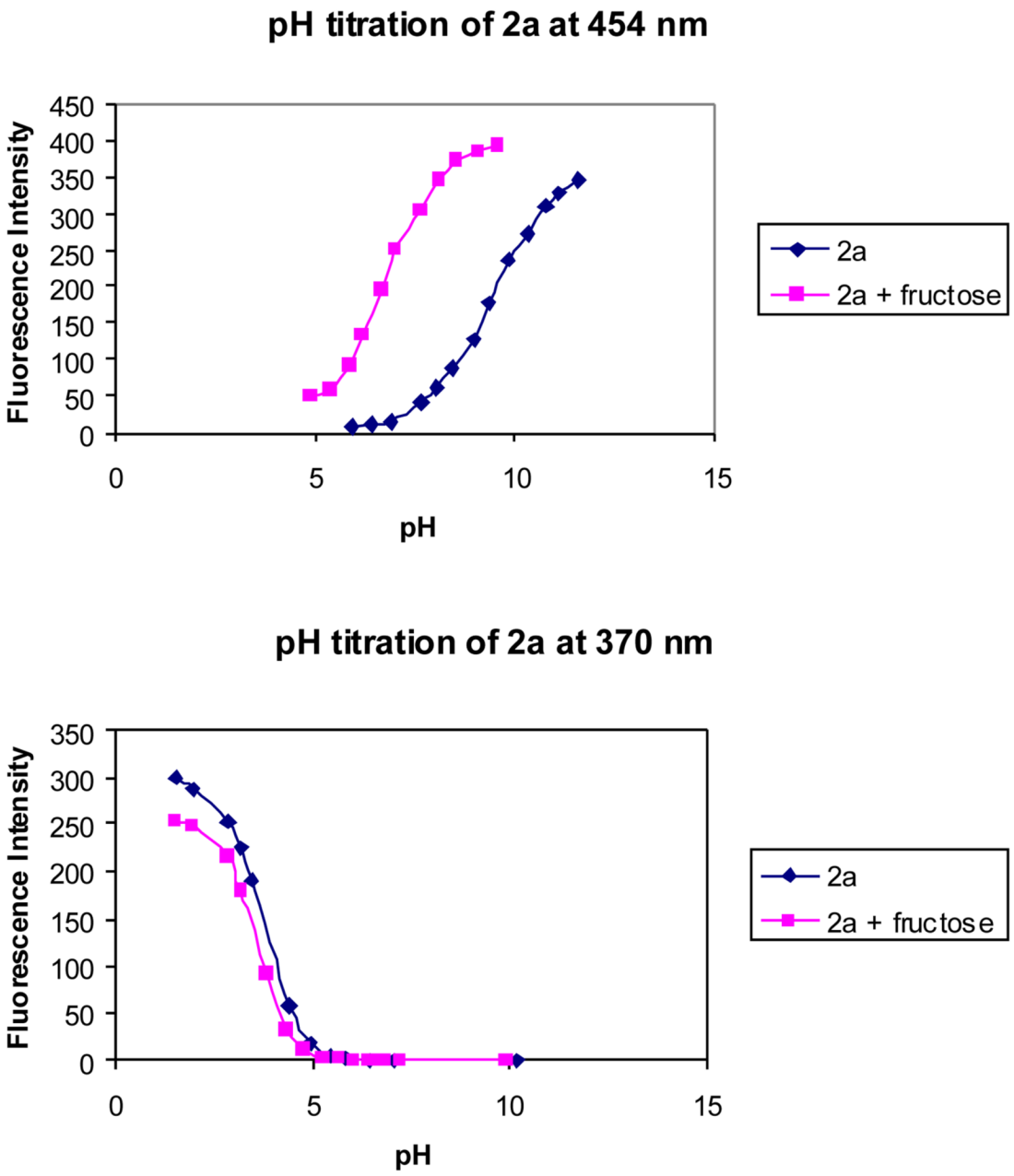


Figure 5.
pH titration of **2a** (1.0×10^{-5} M) with or without sugar (50 mM fructose) in 0.1 M aqueous phosphate buffer at pH 7.4, λex = 340 nm.

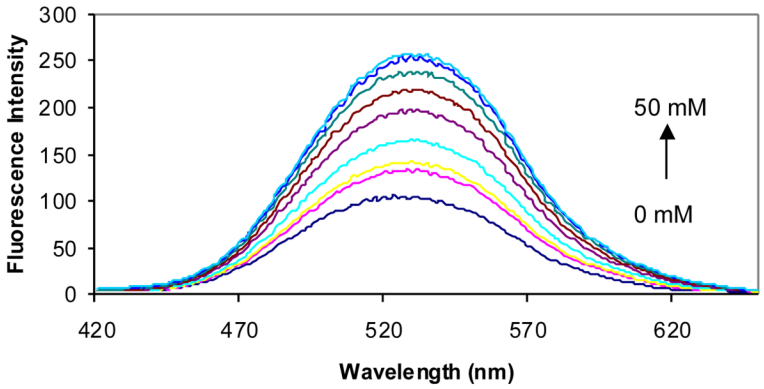


Figure 6.

Fluorescent spectral changes of **2b** (1.0×10^{-5} M) with different concentrations of D-fructose (0-50 mM) in 0.1 M aqueous phosphate buffer at pH 7.4, λex = 340 nm.

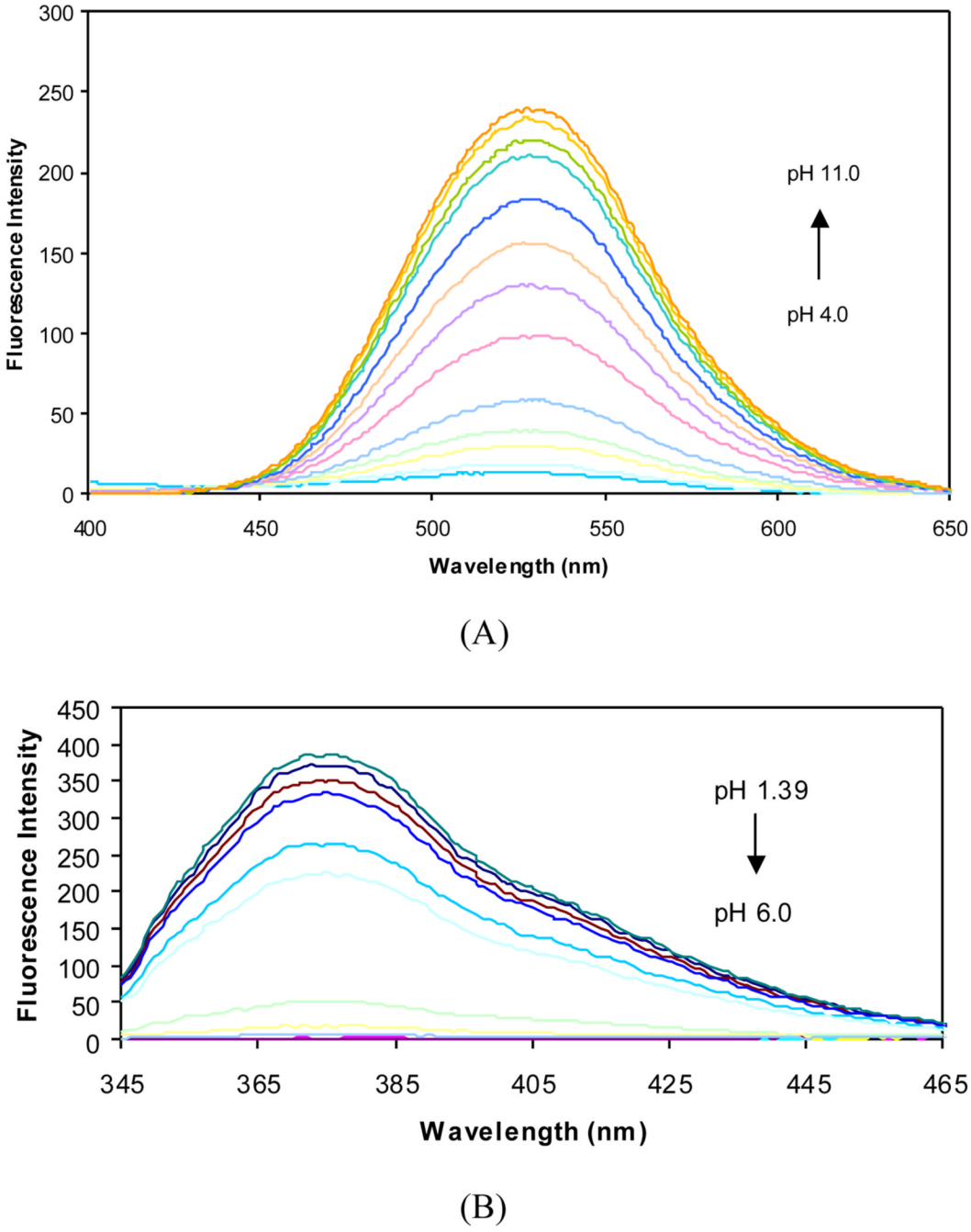


Figure 7.
Fluorescent spectral changes of **2b** in 0.1 M aqueous phosphate buffer (1.0×10^{-5} M) in the absence of sugar at different pH, λex = 340 nm. (A) changes at 531 nm; (B) changes at 375 nm.

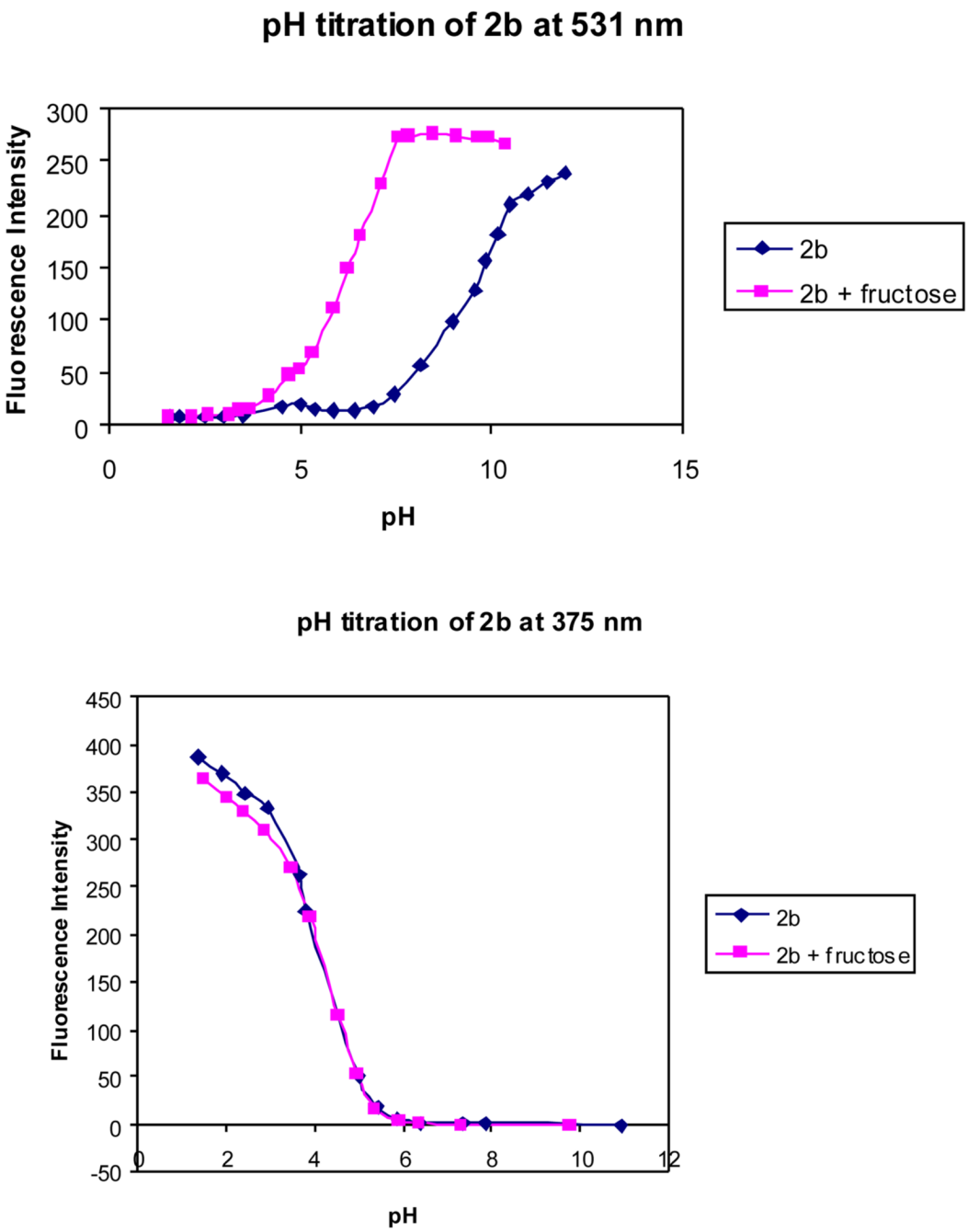


Figure 8.
pH titration of **2b** (1.0×10^{-5} M) with or without fructose sugar (50 mM) in 0.1 M aqueous phosphate buffer, λex = 340 nm.

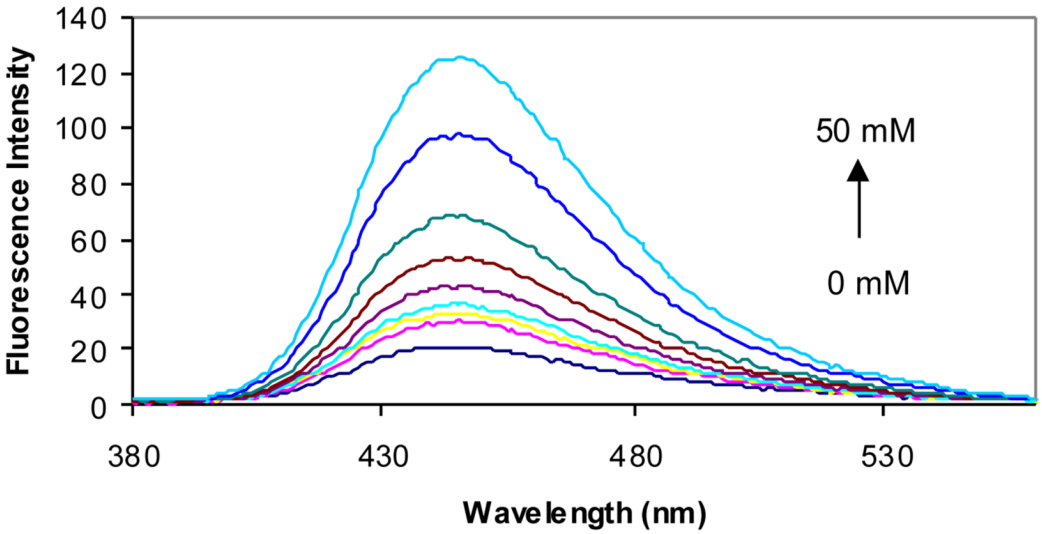


Figure 9.
Fluorescence spectral changes of **2c** (1.0×10^{-6} M) with different concentrations of D-fructose (0-50 mM) in 0.1 M aqueous phosphate buffer at pH 7.4, λex = 330 nm

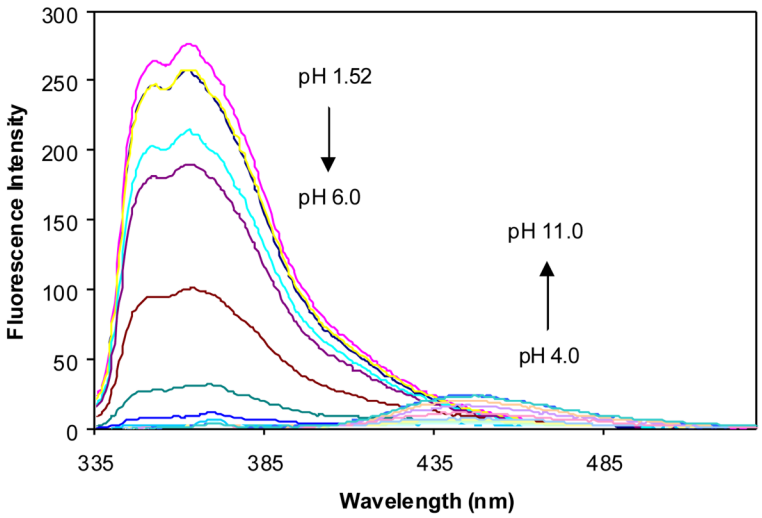


Figure 10.
Fluorescence spectral changes of **2c** (1.0×10^{-6} M) in 0.1 M aqueous phosphate buffer at different pH, λex = 330 nm.

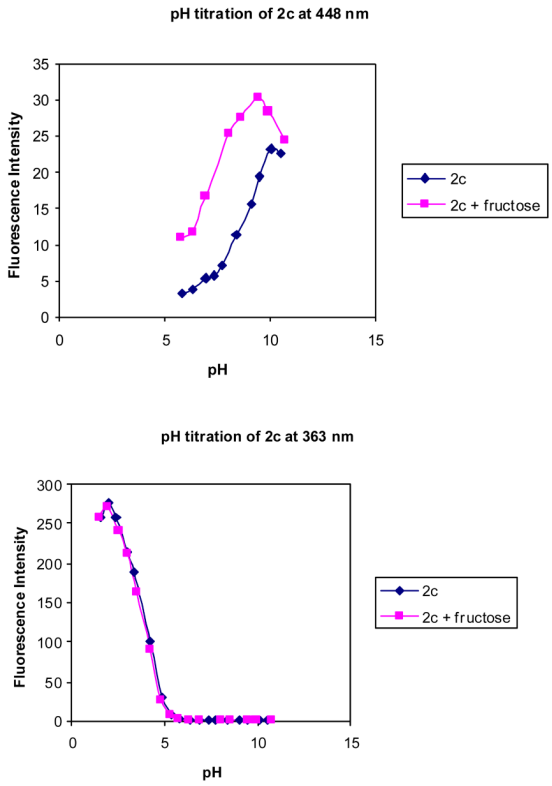


Figure 11.
pH titration of **2c** (1.0×10^{-6} M) with and without fructose (50 mM) in 0.1 M aqueous phosphate buffer at pH 7.4, λex = 330 nm.

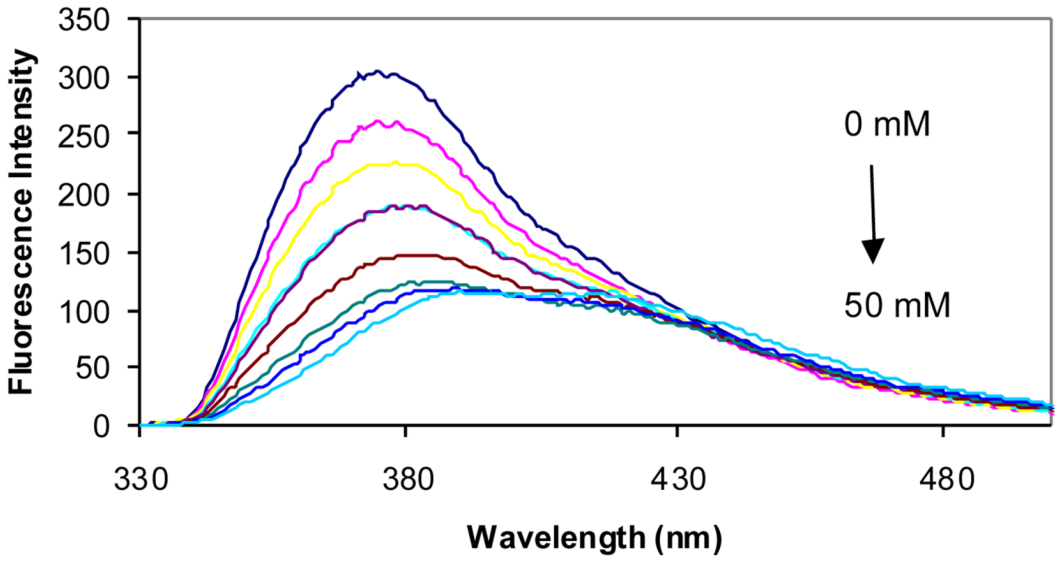


Figure 12.
Fluorescence spectral changes of **2d** (1.0×10^{-5} M) with different concentrations of D-fructose (0-50 mM) in 0.1 M aqueous phosphate buffer at pH 7.4, λex = 310 nm

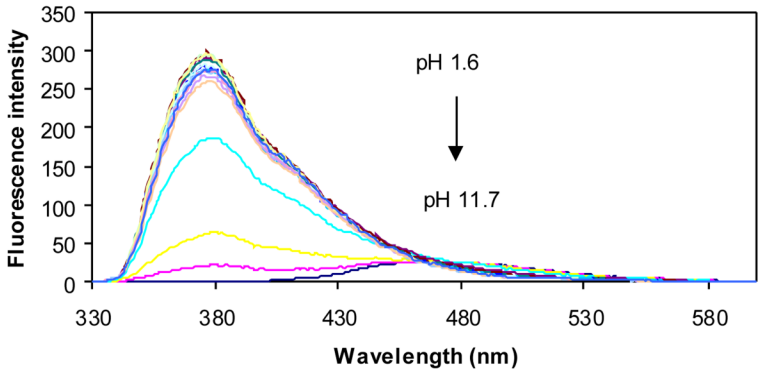


Figure 13.

Fluorescence spectral changes of **2d** (1.0×10^{-5} M) in the absence of sugar at different pH, in 0.1 M aqueous phosphate buffer, λex = 310 nm

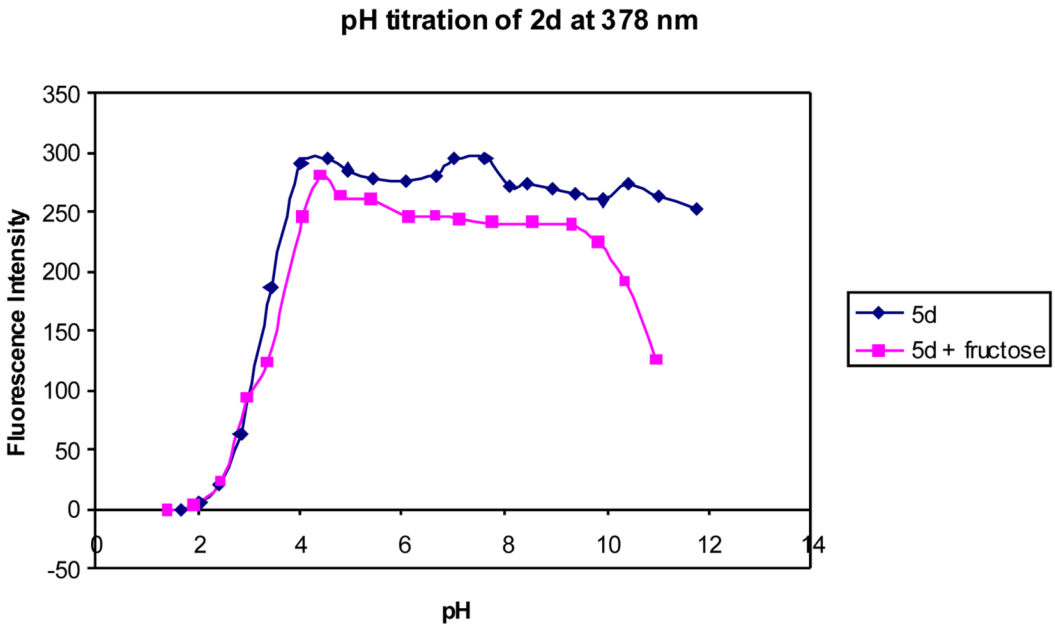
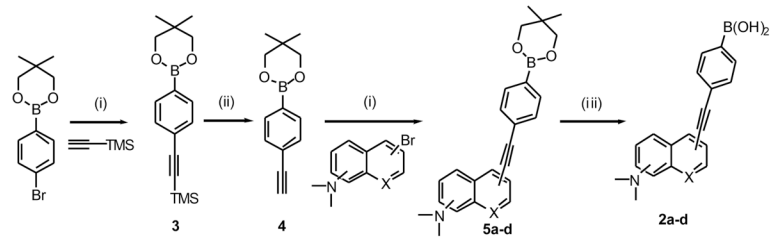
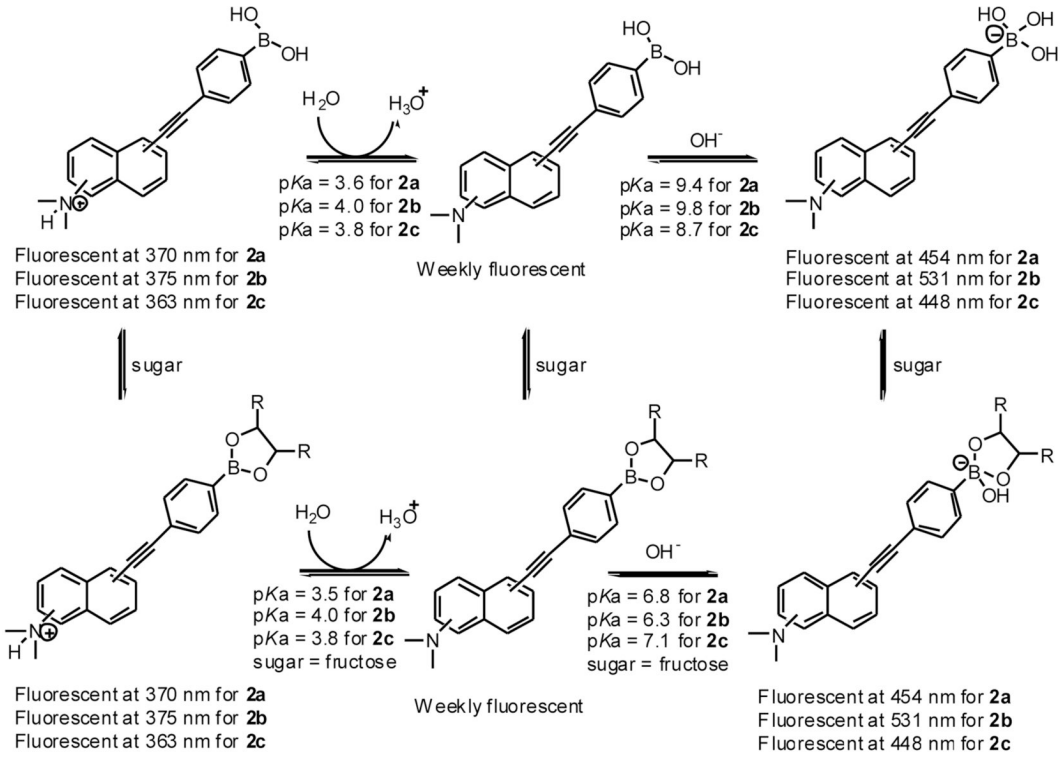


Figure 14.
pH titration of **2d** (1.0×10^{-5} M) with or without a sugar (50 mM) in 0.1 M aqueous phosphate buffer at pH 7.4, λex = 310 nm.



Scheme 1.

Synthesis of 4-aryethynylphenylboronic acids **2a-d**: (i) $PdCl_2(Ph_3P)_2$, CuI, Ph_3P, 120 °C, microwave irradiation, 98% for **3** and 47% ~ 67% for **5a-d**; (ii) K_2CO_3, MeOH, rt, 80%; (iii) TFA, H_2O, 36% ~ 47%.



Scheme 2.

Fluorescent properties of **2a, 2b** and **2c** in different ionization states in the absence and presence of a sugar in 0.1 M aqueous phosphate buffer

Table 1

Association constants[a] and fluorescent properties of **1a-c** and **2a-d** with and without added sugar (50 mM fructose)

Compd	λ_{ex}	Without sugar		With Fructose		*Ka* (M^{-1})			
	[nm]	λ_{em} [nm]	Φ_F	λ_{em} [nm]	Φ_F	sorbitol	fructose	galactose	glucose
1a[41]	300	445	0.01	445	0.42	226 ± 12	207 ± 8	12.0 ± 3.6	4.0 ± 2.1
2a	340	454	0.013	454	0.096	619 ± 16	293 ± 19	8.1 ± 1.4	2.5 ± 1.6
1b[56]	300	513, 433	0.07	433	0.23	337 ± 11	311 ± 13	23.0 ± 5.2	3.6 ± 1.8
2b	340	531	0.027	531	0.12	311 ± 9	178 ± 2.1	7.5 ± 0.8	7.0 ± 2.0
1c[47]	310	432	0.89	-[b]	-[b]	227 ± 10	120 ± 6	8.0 ± 1.5	2.4 ± 1.1
2c	330	448	0.025	448	0.058	11.1 ± 0.3	132 ± 5	1.8 ± 0.6	0.1 ± 0.05
1d	310	-[b]	-[b]	412 (weak)	0.034	-[b]	-[b]	-[b]	-[b]
2d	310	371	0.1	371, 430	0.08	822 ± 17	427 ± 18	49 ± 6.0	18 ± 3.0

[a] Constants determined by fitting into a 1:1 binding model. Errors reported are two standard deviations; $R^2 > 0.99$ in all cases.

[b] Not applicable.