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Design and Synthesis of Long-Wavelength Fluorescent Boronic Acid Reporter Compounds

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Three new fluorescent probes based on the 4-amino-1,8-naphthalimide structure were synthesized, and their sugar binding properties were studied by using D-fructose, D-glucose and D-sorbitol in 0.1 M phosphate buffer at pH 7.4. All three compounds showed fluorescence intensity changes upon addition of sugars. In this series, compound **1c** gave the longest emission wavelength (570 nm) and fluorescence intensity increases of up to 2.5-fold after addition of D-fructose.

Introduction

It is now widely recognized that carbohydrates play important roles in a wide variety of biological processes including cell–cell communications, embryo development, immune responses, apoptosis, egg fertilization, etc.[1–4] Furthermore, pathological changes such as the development of malignancy are often associated with changes of cell surface carbohydrates.[5–10] Therefore, there is a need for the development of "tools" for specific and high affinity carbohydrate recognition. Such "tools" can be used in research for the analysis of glycosylation patterns, and as new diagnostics and/or therapeutic agents. Along this line, lectin arrays have been successfully used in carbohydrate analysis.[11,12] However, there are only a limited number of lectins available and they often have cross-reactivity issues. Artificial lectins (or boronolectins) have promising potentials in making a major impact in glycobiology.[13,14] Boronic acids are known to form reversible and tight complexes with diols, and consequently many carbohydrates.[15–17] Therefore, the boronic acid functional group has been explored as a key recognition moiety in developing carbohydrate sensors.[18–30] Our laboratory has been interested in making fluorescent boronic acid-based lectin mimics (boronolectins) for biological applications. Along this line, we have developed a fluorescent boronolectin that can label cells with a high level of sialyl Lewis X.[31,32] In developing fluorescent boronolectins, there is a need for fluorescent boronic acid reporters that (1) change fluorescent properties upon binding, (2) are water-soluble, (3) are chemically and photochemically stable, and (4) emit fluorescence at a long wavelength far beyond the UV region. We have developed a series of fluorescent boronic acid reporters that are water soluble and chemically and photochemically stable.[33–43] Although very useful, most of these boronic acids fluoresce at below 500 nm. We desire to design and synthesize new water-soluble boronic acids that fluoresce at a longer wavelength for biological applications. Herein we report the design and synthesis of three naphthalimide-based boronic acids that (1) change fluorescent properties upon sugar binding and (2) fluoresce at over 550 nm.

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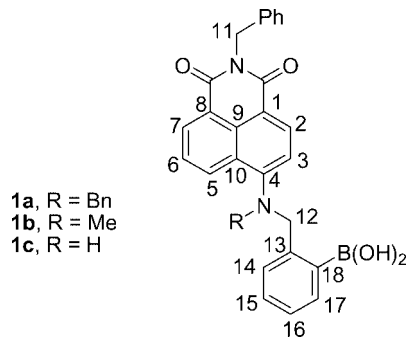
Results and Discussion

The Design

There have been a few fluorescent boronic acid reporter compounds described in the literature that fluoresce at a relatively long wavelength (over 500 nm).[44–52] For example, Takeuchi and co-workers[53] found a modified cyanine diboronic acid sensor, which emits at 579 nm and shows enhanced fluorescence intensity upon addition of a sugar. Kijima[54] synthesized a porphyrin-based diboronic acid as a selective detector for D-lactulose, which fluoresces at 650 nm. However, most of these compounds are very hydrophobic and cannot be used in aqueous solutions. Heagy and co-workers[55,56] synthesized a series of *N*-phenylboronic acid derivatives of 1,8-naphthalimide, some of which can be used in phosphate buffer at pH 7.4. Among these compounds, 3-nitro-*N*-phenylnaphthalimidylboronic acid[57] emits at 550 nm and is water-soluble. One drawback of this compound, however, is its decreased fluorescent intensity upon sugar binding. There are also a few other such boronic acids that are water-soluble, but show quenched fluorescence upon binding. This includes dansylamide boronic acid (510 nm),[45] 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (pyranine) phenyl boronic acid (510 nm),[51]

and 4,7-phenanthrolinium salts phenyl boronic acid (510 nm).[52] To the best of our knowledge, boron-dipyrromethane dyes (BODIPY) phenyl boronic acid is the only reported water-soluble fluorescent probe that (1) fluoresces at over 500 nm (510 nm) and (2) shows fluorescent intensity increases upon sugar binding. However, the fluorescent intensity increase of this compound upon sugar binding was only 20%,[58] which is probably too small to be used in practical applications.

Because the 1,8-naphthalimide fluorophore offers the possibility of long-wavelength emission, and recently it has been reported that addition of a 4-amino group can significantly change the fluorescent properties of the 1,8-naphthalimide fluorophore,[59–63] we were interested in using 4-amino-1,8-naphthalimide as a template for the design of long-wavelength fluorescent boronic acid reporter compounds. One way to induce the desired fluorescent property changes to such a template is to build in a mechanism in which binding of a sugar would perturb the fluorophore's electronic environment. It is well known that sugar binding to an arylboronic acid quite often lowers the pK_a of the boron and consequently causes a change of the hybridization state of the boron atom from the neutral sp^2 form to its anionic sp^3 state at physiological pH.[15–17] It is conceivable that this change in the ionization state of the boron atom can be used to modulate the electronic properties of the fluorophore by either direct attachment of a boronic acid group to the fluorophore or by proximity effect. In order to build flexibility into the system for future modification in preparation of diboronic acid sensors, we are interested in designing compounds that do not have the boronic acid directly attached to the fluorophore. Therefore, by taking advantage of the fact that a boronic acid positioned in a 1,5-relationship to an amino group can affect the nitrogen protonation and electronic state under physiological conditions,[64–67] we designed the compounds **1a**–**c** as potential long-wavelength fluorescent reporters for sugar recognition (Scheme 1). In this design, the boron atom is in a 1,5-relationship with the aniline amino group. We envisioned that complexation with a sugar would change the boron ionization state, which would in turn affect the electronic properties of the fluorophore through interactions with the amino group because of proximity effect, and induce spectroscopic property changes in the fluorophore. At this point, it is important to note that the Shinkai lab developed an anthracene-based system, which relies on proximity effect for switching fluorescent intensities upon sugar binding.[68] The detailed mechanism was initially thought to be due to the strengthening of the B–N bond upon sugar binding.[67] However, our lab has recently shown that the fluorescent intensity changes in the Shinkai system is due to a solvolysis mechanism.[65,66] Recent studies by Anslyn et al. also substantiate this new mechanism.[69]

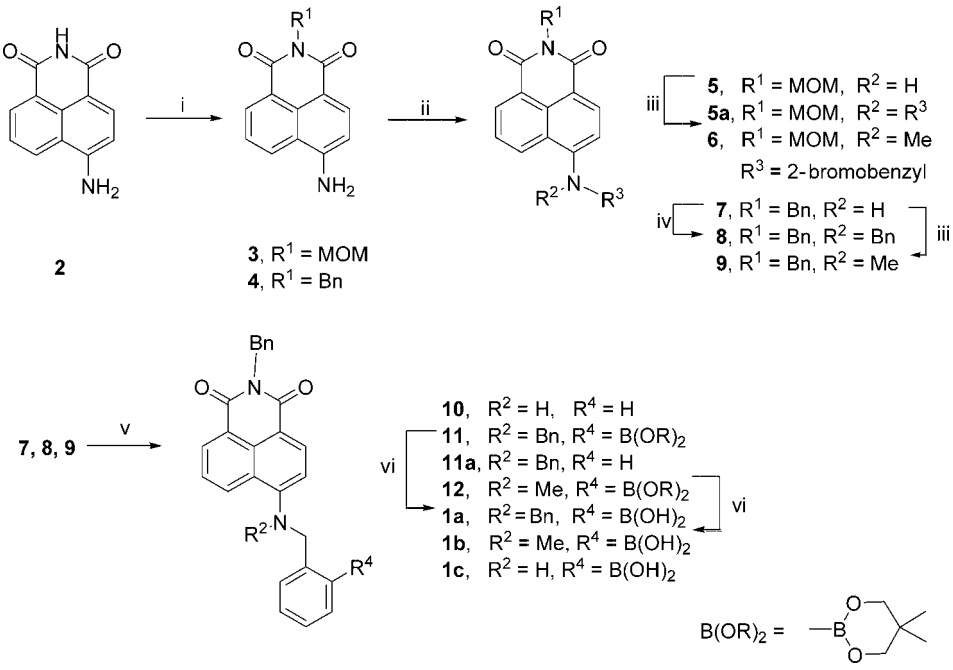


Scheme 1.

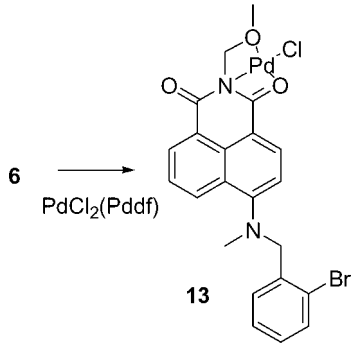
Synthesis

The synthesis started with 4-amino-1,8-naphthalimide (**2**) (Scheme 2). The idea is to attach the arylboronic acid through alkylation of the aniline nitrogen. For this, we would need to first protect the imide nitrogen in order to minimize side reactions. Initially, we used the MOM group for this protection. Therefore, reaction of **2** with methoxydichloromethane gave **3**, which was then reacted with 2-bromobenzyl bromide using sodium hydride as a base to give a mixture of the mono- (**5**) and di-alkylated (**5a**) products. Methylation of the remaining position of aniline gave **6**. The borylation of **5** and **5a** using a palladium catalyst $PdCl_2$(dppf), however, did not yield the desired products. Only the starting material was recovered. This was quite unexpected because this borylation method has been widely used in the literature,[70–73] and our lab has also used this method extensively and successfully.[74,75] Although reaction yields may vary depending on substrate and conditions and side products may form, it is rare to see this kind of lack of product formation and complete recovery of starting materials. Considering the possible reasons for this lack of reactivity, one can envision that the –O–C–N–C=O structural moiety formed between the MOM group and one amide group could complex with Pd (**13**), and therefore inactivate the catalyst (Scheme 3). This reasoning is supported by literature precedents where similar structures are known to recognize transition metals.[76]

If Pd complexation is the reason for the lack of reaction, one would expect that changing the MOM protecting to a benzyl group should solve this problem. Therefore, 4-amino-1,8-naphthalimide was treated with sodium methoxide/methanol in DMF at room temperature, and then reacted with benzyl bromide to give 4-amino-*N*-benzylnaphthalimide (**4**). Compound **4** was then treated with 2-bromobenzyl bromide in DMF at room temperature to afford *N*-benzyl-4-[(2-bromobenzyl)amino]naphthalimide (**7**). Further alkylation of **7** with benzyl bromide or methyl iodide gave *N*-benzyl-4-[benzyl(2-bromobenzyl)amino]naphthalimide (**8**) or *N*-benzyl-4-[(2-bromobenzyl)methylamino]-naphthalimide (**9**), respectively. Borylation of **8** using $PdCl_2$(dppf) gave the expected boronate ester product **11** as well as the de-bromo byproduct **11a**. Boronic acid **12** was similarly prepared from **9**. The deprotection of boronate esters **11** and **12** in 90% trifluoroacetic acid aqueous solution followed by purification using C_{18} RP-HPLC afforded the free boronic acids **1a** (54%) and **1b** (49%), respectively. We



Scheme 2. i, $NaOCH_3/CH_3OH$, R^1X (X = Cl, Br), DMF, room temp. 60%; ii, NaH, DMF, 2-bromobenzyl bromide, room temp. 57–72%; iii, NaH, DMF, MeI, room temp. 88%; iv, NaH, DMF, BnBr, room temp. 84%; v, bis(2,2-dimethylpropylene glycol boronate), $PdCl_2$(dppf), KOAc, DMSO, 90 °C, 50–75%; vi, 90% trifluoroacetic acid, room temp. 50%.



Scheme 3.

also did the borylation with compound **7**. Surprisingly, the free boronic acid **1c** was obtained without the need for an extra deprotection step. In addition, the de-bromo compound **10** was also obtained as a side product. The final product was purified using HPLC to give **1c** in 27% yield.

Fluorescent Binding Studies

Compounds **1a**–**c** were designed as possible fluorescent reporters for carbohydrates. Therefore, their ability to bind to sugars and to change fluorescence upon binding was examined. As the first step in such a study, we examined the water solubility of these three sensors in phosphate buffer solution (PBS) at physiological pH (7.4) by studying their concentration-dependent fluorescent intensity changes. The results (not shown) indicate that **1c** was completely soluble in PBS at the concentration needed for binding studies (5×10^{-5} M). However, compounds **1a**,**b** were not. Therefore, the binding studies for **1a**,**b** were conducted in a mixture of methanol/phosphate buffer (1:1) and for **1c** in both pH 7.4 PBS and a mixture of methanol/PBS (1:1). All three sensors show significant fluorescent property changes upon sugar binding and show emission maxima in the range of 530–570 nm with **1c** showing the longest emission wavelength at 570 nm. Figure 1 is one example spectral set that shows the fluorescent property changes of **1c** upon addition of fructose. It is clear that **1c** in an aqueous environment shows a concentration-dependent fluorescent intensity increase along with a slight blue shift of the emission wavelength upon sugar binding. This general trend is also true with other sugars tested (Figure 2) and in a mixed solvent (buffer and methanol 1:1). However the concentration required for a change in fluorescent intensity of similar magnitude is much higher for glucose (Figure 2) consistent with a lower binding affinity.[16,17] Overall, the maximal fluorescent intensity observed was about 2.5-fold. It should be noted that the fluorescence intensity for all three sensors **1a**–**c** is much higher in methanol-buffer mixture than in PBS buffer alone, which is the reason that different sensor concentrations were used depending on the solvents.

Compound **1b** behaved similarly as **1c** in the presence of sugar when examined in a mixture of methanol and PBS (1:1); it showed fluorescent intensity increases upon sugar addition with the maximal intensity changes being about 0.8-fold (Figure 2, c). Different from **1b** and **1c**, compound **1a** showed a fluorescent intensity decrease with the addition of sugar when examined in a mixture of methanol and PBS (1:1) (Figure 2, d). The magnitude of the fluorescent intensity changes observed was also much smaller (30%).

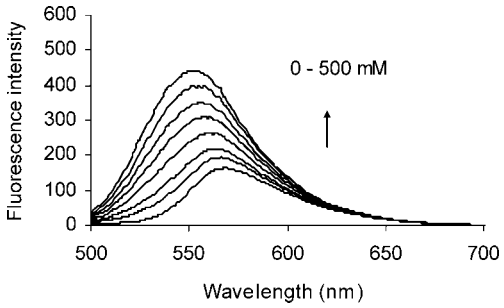


Figure 1. Fluorescence spectra of **1c** (5×10^{-5} M) upon addition of D-fructose (0–500 mM) in 0.1 M phosphate buffer at pH 7.4: λ_{ex} = 493 nm.

In order to achieve a quantitative understanding of the interactions between **1a**–**c** with different sugars, we have also determined their apparent binding constants (Table 1). It was very surprising to find that **1a** has a very low affinity for all three sugars tested with binding constants ranging from 0.1 to 0.3 M^{-1}. Such low binding constants also helps to explain the seemingly linear relationship between sugar concentrations and fluorescent intensity for **1a** (Figure 2, d) because even at the highest concentration tested, the sensor was far from being "saturated." It is not readily clear why this compound has an abnormally low affinity for sugars as compared to other known arylboronic acid.[17] One possible explanation is that the *N*-benzyl group somehow poses a steric hindrance to binding.

Along the same line, compounds **1b**,**c** showed binding constants within the "normal" range for a monoarylboronic acid with **1b** showing higher affinities.[16,17] For example, the binding constants between **1b** and fructose, glucose, and sorbitol are 200, 7, and 340 M^{-1}, respectively. For **1c**, these binding constants are 57, 3, and 111 M^{-1}, respectively, when examined in PBS at pH 7.4. It is also interesting to note that the binding constants for **1c** with various sugars are lower when tested in a mixture of methanol and PBS than in pure PBS (Table 1). We suspect that again con-

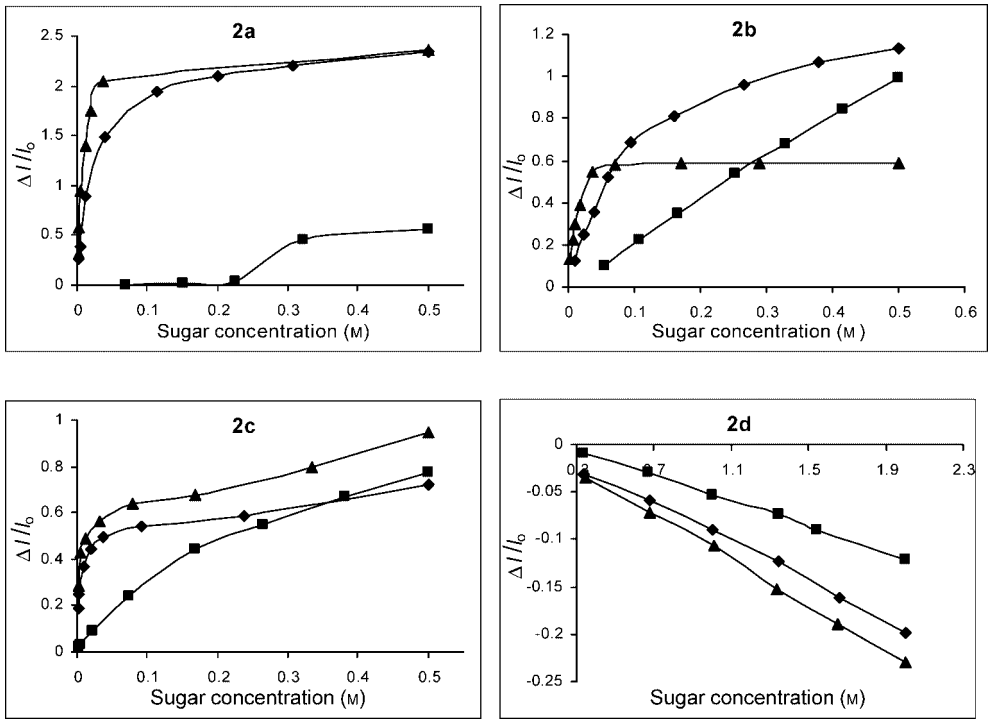


Figure 2. a) Relative fluorescence intensity of **1c** (5×10^{-5} M) in 0.1 M phosphate buffer at pH 7.4 in the presence of D-fructose (♦), D-glucose (■), D-sorbitol (▲): λ_{ex} = 493 nm, λ_{em} = 570 nm. b) Relative fluorescence intensity of **1c** (5×10^{-6} M) in buffer/methanol (1:1) at pH 7.4 in the presence of D-fructose (♦), D-glucose (■), D-sorbitol (▲): λ_{ex} = 493 nm, λ_{em} = 562 nm. c) Relative fluorescence intensity of **1b** (1×10^{-5} M) in a mixture of CH_3OH/PBS (1:1, v/v) at pH 7.4 in the presence of D-fructose (♦), D-glucose (■), D-sorbitol (▲): λ_{ex} = 446 nm, λ_{em} = 543 nm. d) Relative fluorescence intensity of **1a** (1×10^{-5} M) in mixture of CH_3OH/PBS (1:1, v/v) at pH 7.4 in the presence of D-fructose (♦), D-glucose (■), D-sorbitol (▲): λ_{ex} = 432 nm, λ_{em} = 532 nm.

Table 1. Excitation wavelength, emission wavelength and apparent association constants (K_a) of **1a**–**c** with different sugars.

	Ex [nm]	Em [nm]	Fructose K_a [M^{-1}]	ΔI_f[c] (fold)	Glucose K_a [M^{-1}]	ΔI_f (fold)	Sorbitol K_a [M^{-1}]	ΔI_f (fold)
1a[a]	432	532	0.3	0.2	0.1	0.1	0.3	0.16
1b[a]	446	543	200	0.7	7	0.6	340	0.8
1c[a]	493	556	18	0.1	0.1	0.02	60	0.18
1c[b]	493	570	57	2.5	3	1.1	111	2.7

[a] In CH_3OH/buffer (1:1, v/v) solution at pH 7.4; [boronic acid] = 1×10^{-5} M. [b] In 0.1 M phosphate buffer (0.1% CH_3OH) at pH 7.4; [boronic acid] = 5×10^{-5} M. [c] **1a** (intensity decrease); **1b**, **c** (intensity increase). All data are averages of duplicate.

formational changes are the reason for this abnormal behavior since one would expect the intrinsic affinity of a boronic acid being higher in organic solvents than in water.

To examine the relationship between fluorescence intensity changes and the ionization states of the sensor compounds, we have also studied the pH profiles of the fluorescence intensity in the absence and presence of sugars at a fixed sugar concentration (500 mM). When tested in PBS and in the absence of any sugar, the emission intensity of **1c** increased by 9-fold at λ = 570 nm upon changing the pH from 2 to 12, with an apparent pK_a of 6.5, which was assigned to the boronic acid moiety (Figure 3). In the presence of sugars, the fluorescence intensity of **1c** at 570 nm increased by 22-, 22-, and 12-fold with the addition of sorbitol, fructose, and glucose, respectively, when the pH was changed from 2 to 12. The apparent pK_a values observed were of 4.5, 4.5, and 6.0 for esters of sorbitol, fructose and glucose, respectively. In all cases, it seems that the change of the boron atom from the neutral trigonal form to the anionic tetrahedral form is responsible for bringing on the fluorescent intensity changes. The shift to the left for the sorbitol and fructose esters compared with the glucose ester and **1c** alone is consistent with previous findings that sorbitol and fructose esters have lower pK_a values than the free boronic acid and the glucose ester.[16] The pH-dependent fluorescence intensity changes of a control compound **10** have also been examined in order to aid the understanding of how such sensors function (Figure 3). It seems that without the tetrahedral boronic acid moiety, the fluorescent intensity of the control compound **10** never reached the same level as the boronic acid compound **1c** regardless of the pH.

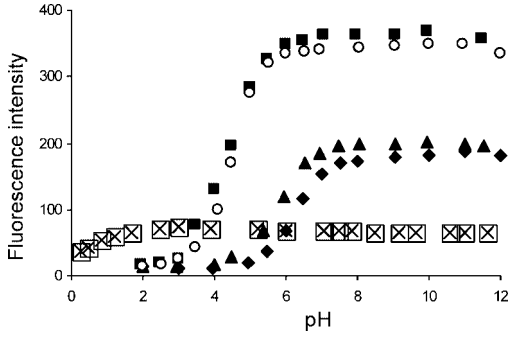


Figure 3. pH profile of the fluorescence intensity of **1c** (5×10^{-5} M) in the absence and presence of sugars in 0.1 M aqueous phosphate buffer, [sugar] = 500 mM, λ_{ex} = 493 nm, λ_{em} = 570 nm. **1c** alone (♦), in the presence of D-fructose (■), in the presence of D-glucose (▲), in the presence of D-sorbitol (o). compound **10** alone (×) (1×10^{-5} M).

One thing that is worth noting is the behavior of **10** in a mixed solvent (PBS/methanol, 1:1) that is quite different from its fluorescent properties in PBS alone (Figure 4). Specifically, **10** was never as fluorescent as **1c** in PBS buffer (Figure 3), and yet it became much more fluorescent than **1c** in a mixed PBS/methanol solvent (1:1). The fluorescence increase seems to correlate with the deprotonation of protonated aniline. Again, the behavior of **1c** in a mixed solvent (PBS/methanol 1:1) is similar to that in PBS. It should also be noted that the fluorescence intensity of **10** is much higher in methanol/buffer mixture than in PBS buffer alone, which is the reason that a lower sensor concentration was used in the mixed solvent.

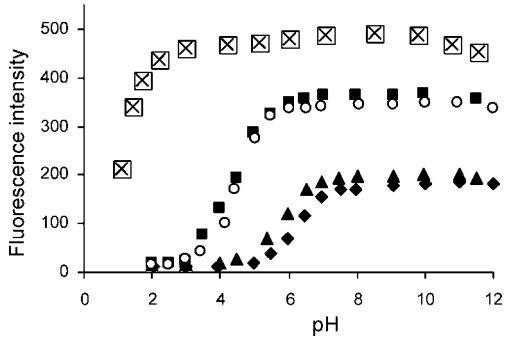


Figure 4. pH profile of the fluorescence intensity of **1c** (5×10^{-5} M) in the absence and presence of sugars in a mixture of 0.1 M PBS and methanol (1:1), [sugar] = 500 mM, λ_{ex} = 493 nm, λ_{em} = 570 nm. **1c** alone (♦), in the presence of D-fructose (■), in the presence of D-glucose (▲), in the presence of D-sorbitol (o), compound **10** alone (×) (1×10^{-6} M).

With compound **1a**,**b**, the situation is very different. Both showed bell-shaped curves when the pH increased from 2 to 12 (Figure 5). The first pK_a seems to correlate with that of the aniline functional group. The mechanistic implication of the fluorescent intensity drop at about pH 11 is not clear because it is very unlikely that this can be correlated to any pK_a values of these two compounds.

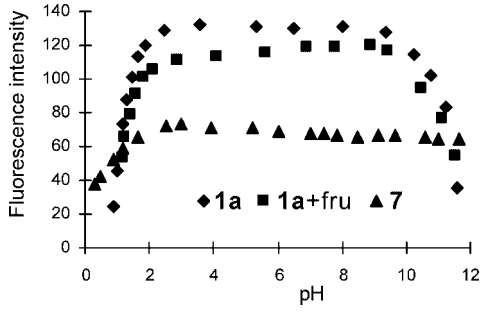


Figure 5. pH profile of the fluorescence intensity of **1a** (1×10^{-5} M) in the absence and presence of 500 mM fructose in MeOH/buffer (1:1), λ_{ex} = 432 nm, λ_{em} = 532 nm. **1a** alone (♦), in the presence of D-fructose (■), compound **7** alone (▲).

Conclusions

In conclusion, three long-wavelength boronic acid fluorescence reporter compounds have been designed, synthesized, and evaluated. Among them, **1c** is very unique in that it is water soluble, increases fluorescent intensities upon sugar binding, and emits at a long wavelength (570 nm). This compound will be useful for the preparation of long-wavelength sensors for sugars.

Experimental Section

4-Amino-*N*-(methoxymethyl)naphthalimide (3): 4-Aminonaphthalimide (**2**) (3 g, 14.1 mmol, 1.0 equiv.) in dry DMF (120 mL) was treated at room temperature with 2 M sodium methoxide in methanol (7.8 mL, 15.5 mmol, 1.1 equiv.) for 10 min followed by methoxydichloromethane (1.2 mL, 15.5 mmol, 1.1 equiv.) addition in a dropwise fashion. The mixture was stirred at room temperature for 5 h. Then the solvent was evaporated under vacuum. Compound **3** (2.2 g, 8.6 mmol, 61%) was obtained as a yellow powder after column chromatography (ethyl acetate). TLC (hexane/ethyl acetate, 1:1, R_f = 0.45). M.p. 214–216 °C. ^{1}H NMR (DMSO, 400 MHz, 25 °C): δ = 8.64 (d, J = 8.4 Hz, 1 H, 7-H), 8.44 (d, J = 7.2 Hz, 1 H, 5-H), 8.22 (d, J = 8.4 Hz, 1 H, 2-H), 7.65 (t, J = 8.0 Hz, 1 H, 6-H), 7.51 (s, 2 H, -NH_2), 6.87 (d, J = 8.4 Hz, 1 H, 3-H), 5.41 (s, 2 H, 11-H), 3.32 (s, 3 H, -OCH_3) ppm. ^{13}C NMR (DMSO, 100 MHz, 25 °C): δ = 164.5 (C=O), 163.4 (C=O), 153.4 (C-4), 134.6, 131.7 (C-2, C-7), 130.6, 130.1 (C-5, C-9), 124.4, 121.9 (C-6, C-8), 119.8 (C-10), 108.7, 107.4 (C-1, C-3), 70.5 (C-11), 57.1 (OCH_3) ppm. MS (ESI+): *m*/*z* (%) = 257 (100) [M^+ + H], 225. $C_{14}H_{12}N_2O_3$ (256.25): calcd. C 65.62, H 4.72, N 10.93; found C 65.42, H 4.65, N 10.68.

4-[(2-Bromobenzyl)amino]-*N*-(methoxymethyl)naphthalimide (5) and 4-[Bis(2-bromobenzyl)amino]-*N*-(methoxymethyl)naphthalimide (5a): Dry DMF (50 mL) was added to a mixture of **3** (1 g, 3.9 mmol, 1.0 equiv.) and sodium hydride (150 mg, 3.9 mmol, 1.0 equiv.) at room temperature. The resulting solution was stirred at room temperature for 10 min, followed by 2-bromobenzyl bromide (975 mg, 3.9 mmol, 1.0 equiv.) addition. The mixture was stirred at room temperature for 5 h before solvent evaporation under vacuum. Compounds **5** (1.2 g, 2.8 mmol, 72%) and **5a** (0.5 g, 0.8 mmol, 21%) were obtained as yellow powders after column chromatography (hexane/ethyl acetate, 1:1).

5: TLC (hexane/ethyl acetate, 1:1, R_f = 0.55), m.p. 148–150 °C. ^{1}H NMR (DMSO, 400 MHz, 25 °C): δ = 8.81 (d, J = 8.4 Hz, 1 H, 7-H), 8.48 (t, J = 7.6 Hz, 2 H, 5-H, 17-H), 8.23 (d, J = 8.4 Hz, 1 H, 2-H), 7.62 (t, J = 8.4 Hz,1 H, 6-H), 7.70 (d, J = 7.6 Hz, 1 H, 14-H), 7.32 (s, 1 H, -NH), 7.25 (br. s, 2 H, 15-H, 16-H), 6.57 (d, J = 8.4 Hz, 1 H, 3-H), 5.40 (s, 2 H, 11-H), 4.67 (d, J = 4.4 Hz, 2 H, 12-H), 3.31 (s, 3 H, OCH_3) ppm. ^{13}C NMR (DMSO, 100 MHz, 25 °C): δ = 164.4 (C=O), 163.4 (C=O), 150.7 (C-4), 136.8 (C-2), 134.8 (C-7), 133.2 (C-5), 131.5 (C-9), 130.2, 129.7, 129.3, 128.8, 128.4, 125.2 (6 C, phenyl), 123.0 (C-6), 122.2 (C-8), 120.7 (C-10), 108.6, 105.0 (C-1, C-3), 70.5 (C-11), 57.1 (OCH_3), 47.0 (C-12) ppm. MS (ESI+): *m*/*z* (%) = 425, 427 (100) [M^+ + H], 393, 395, 214. $C_{21}H_{17}BrN_2O_3$ (425.27): calcd. C 59.31, H 4.03, N 6.59; found C 59.48, H 4.00, N 6.40.

5a: TLC (hexane/ethyl acetate, 1:1, R_f = 0.65), m.p. 156–158 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C): δ = 8.61 (d, J = 7.2 Hz, 1 H, 7-H), 8.53 (d, J = 8.0 Hz, 1 H, 5-H), 8.45 (d, J = 7.2 Hz, 1 H, 2-H), 7.65 (t, J = 7.2 Hz, 1 H, 6-H), 7.58 (d, J = 7.2 Hz, 2 H, 17-H), 7.45 (d, J = 8.8 Hz, 2 H, 15-H), 7.26 (d, J = 7.6 Hz, 2 H, 16-H), 7.15 (m, 3 H, 3-H, 14-H), 5.61 (s, 2 H, 11-H), 4.69 (s, 4 H, 12-H), 3.49 (s, 2 H, OCH_3) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C): δ = 164.5 (C=O), 163.9 (C=O), 154.0 (C-4), 135.8 (2 C, C-13), 133.2 (2 C, C-17), 132.3 (C-2), 131.5 (C-7), 130.5 (C-5), 130.2 (C-9), 129.0 (2 C, C-14), 128.8 (2 C, C-16), 127.6 (2 C, C-15), 126.6 (C-6), 125.9 (2 C, C-18), 123.6 (C-8), 122.9 (C-10), 117.2, 116.0 (C-1, C-3), 70.8 (C-11), 57.9 (OCH_3), 57.6 (C-12) ppm. MS (ESI+): *m*/*z* (%) = 594.8 (100) [M^+ + H], 562, 387, 225, 157, 89. $C_{28}H_{22}Br_2N_2O_3$ (594.29): calcd. C 56.59, H 3.73, N 4.71; found C 56.45, H 3.70, N 4.57.

4-[(2-Bromobenzyl)methylamino]-*N*-(methoxymethyl)naphthalimide (6): To a mixture of **5** (780 mg, 1.8 mmol, 1.0 equiv.) and sodium hydride (110 mg, 2.8 mmol, 1.5 equiv.), dry DMF (40 mL) was added at room temperature. The result solution was stirred for 10 min at room temperature, then methyl iodide (0.18 mL, 2.8 mmol, 1.5 equiv.) was added. The mixture was stirred for 5 h at room temperature. The solvent was evaporated under vacuum. Compound **6** (700 mg, 1.6 mmol, 88%) was obtained as yellow powder after chromatography (hexane/ethyl acetate, 3:1). TLC (hexane/ethyl acetate, 2:1, R_f = 0.45), m.p. 161–163 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C): δ = 8.62 (dd, J = 6.4, 2.4 Hz, 1 H, 7-H), 8.51 (dt, J = 8.4, 1.6 Hz, 2 H, 5-H, 2-H), 7.64 (t, J = 7.2 Hz, 1 H, 6-H), 7.27–7.41 (m, 4 H, phenyl-H), 7.19 (dd, J = 8.0, 1.6 Hz, 1 H, 3-H), 5.66 (d, J = 1.2 Hz, 2 H, 11-H), 4.56 (s, 2 H, 12-H), 3.51 (s, 3 H, OCH_3), 2.99 (s, 3 H, NCH_3) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C): δ = 164.7 (C=O), 164.1 (C=O), 156.5 (C-4), 137.0 (C-2), 132.8 (C-7), 131.4 (C-5), 130.8 (C-9), 128.7, 127.6, 127.5 (6 C, phenyl-C), 125.6 (C-6), 125.3 (C-8), 122.8 (C-10), 115.0, 114.8 (C-1, C-3), 70.8 (C-11), 61.3 (C-12), 57.6 (OCH_3), 41.0 (NCH_3) ppm. $C_{22}H_{19}BrN_2O_3$ (439.30): calcd. C 60.15, H 4.36, N 6.38; found C 60.28, H 4.30, N 6.18.

4-Amino-*N*-benzylnaphthalimide (4): 4-Aminonaphthalimide (**2**) (3 g, 14.1 mmol, 1.0 equiv.) in dry DMF (120 mL) was treated at room temperature with 2 M sodium methoxide in methanol (7.8 mL, 15.5 mmol, 1.1 equiv.) for 10 min, and then benzyl bromide (1.85 mL, 15.5 mmol, 1.1 equiv.) was added dropwise. The mixture was stirred at room temperature for 5 h. Then solvent was evaporated under vacuum. Column chromatography (ethyl acetate) of the residue gave **4** (2.5 g, 8.3 mmol, 59%) as a yellow powder. TLC (hexane/ethyl acetate, 1:1, R_f = 0.45), m.p. 238–240 °C. ^{1}H NMR (DMSO, 400 MHz, 25 °C): δ = 8.65 (d, J = 8.4 Hz, 1 H, 7-H), 8.45 (d, J = 7.6 Hz, 1 H, 5-H), 8.23 (d, J = 8.4 Hz, 1 H, 2-H), 7.64 (t, J = 8.4 Hz, 1 H, 6-H), 7.50 (s, 2 H, -NH_2), 7.22–7.34 (m, 5 H, H-phenyl), 6.87 (d, J = 8.4 Hz, 1 H, 3-H), 5.22 (s, 2 H, 11-H) ppm. ^{13}C NMR (DMSO, 100 MHz, 25 °C): δ = 164.3 (C=O), 163.3 (C=O), 153.4 (C-4), 138.4 (C-2), 134.7 (C-7), 131.7, 130.29 (C-5, C-9), 130.0, 128.7, 127.9, 127.3 (6 C, phenyl), 124.5, 122.1, 119.8 (C-6, C-8, C-10), 108.7, 107.7 (C-1, C-3) ppm. MS (ESI+): *m*/*z* (%) = 303 (100) [M^+ + H], 225, 157, 140. $C_{19}H_{14}N_2O_2$ (302.32): calcd. C 75.48, H 4.67, N 9.27; found C 75.21, H 4.51, N 9.08.

***N*-Benzyl-4-[(2-bromobenzyl)amino]naphthalimide (7):** Dry DMF (50 mL) was added at room temperature to a mixture of **4** (1 g, 3.3 mmol, 1.0 equiv.) and sodium hydride (132 mg, 3.3 mmol, 1.0 equiv.). The resulting solution was stirred at room temperature for 10 min before 2-bromobenzyl bromide (825 mg, 3.3 mmol, 1.0 equiv.) was added. The mixture was stirred at room temperature for 5 h. Then the solvent was evaporated under vacuum. Column chromatography (hexane/ethyl acetate, 1:1) gave **7** (880 mg, 1.9 mmol, 57%) and recovered **4** (250 mg, 0.8 mmol, 25%) as yellow powders. **7:** TLC (hexane/ethyl acetate, 1:1, R_f = 0.55), m.p. 185–186 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C): δ = 8.57 (d, J = 7.2 Hz, 1 H, 7-H), 8.43 (d, J = 8.4 Hz, 1 H, 5-H), 8.10 (d, J = 8.4 Hz, 1 H, 2-H), 7.56–7.63 (m, 2 H, 6-H, 17-H), 7.53 (d, J = 7.6 Hz, 2 H, phenyl-H), 7.37 (d, J = 7.6 Hz, 3 H, phenyl-H), 7.19 (t, J = 6.8 Hz, 2 H, phenyl-H), 6.67 (d, J = 8.4 Hz, 1 H, 3-H), 5.76 (d, J = 4.8 Hz, 1 H, –NH), 5.35 (s, 2 H, 11-H), 4.68 (d, J = 4.2 Hz, 2 H, 12-H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C): δ = 164.5 (C=O), 164.0 (C=O), 148.7 (C-4), 137.7 (C-2), 135.8 (C-7), 134.4 (C-5), 133.2 (C-9), 131.3, 129.7, 129.5, 129.2 (4 C, phenyl-C), 128.8 (2 C, phenyl-C), 128.3 (2 C, phenyl-C), 127.8, 127.2, 125.9, 124.9 (4 C, phenyl-C), 123.5 (C-6), 123.1 (C-8), 120.3 (C-10), 111.0, 105.0 (C-1, C-3), 48.0 (C-11), 43.3 (C-12) ppm. MS (ESI+): *m*/*z* (%) = 471, 473 (80) [M^+ + H], 358 (100), 162. $C_{26}H_{19}BrN_2O_2$ (471.34): calcd. C 66.25, H 4.06, N 5.94; found C 65.99, H 4.03, N 5.64.

***N*-Benzyl-4-[benzyl(2-bromobenzyl)amino]naphthalimide (8):** Dry DMF (30 mL) was added to a mixture of **7** (500 mg, 1.1 mmol, 1.0 equiv.) and sodium hydride (52 mg, 1.3 mmol, 1.2 equiv.) at room temperature. The resulting solution was stirred at room temperature for 10 min, followed by benzyl bromide (0.16 mL, 1.3 mmol, 1.2 equiv.) addition. The mixture was stirred at room temperature for 5 h. Then the solvent was evaporated under vacuum. Column chromatography (hexane/ethyl acetate, 3:1) gave **7** (520 mg, 0.92 mmol, 84%) as yellow powder. TLC (hexane/ethyl acetate, 2:1, R_f = 0.50), m.p. 170–172 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C): δ = 8.59 (t, J = 6.8 Hz, 2 H, 7-H, 5-H), 8.41 (d, J = 8.0 Hz, 1 H, 2-H), 7.67 (d, J = 8.4 Hz, 1 H, 6-H), 7.53 (d, J = 8.8 Hz, 3 H, phenyl), 7.45 (d, J = 7.6 Hz, 1 H, phenyl), 7.18–7.28 (m, 9 H, phenyl), 7.10 (d, J = 8.0 Hz, 2 H, phenyl, 3-H), 5.34 (s, 2 H, 11-H), 4.58 (s, 2 H, 12-H), 4.54 (s, 2 H, 12′-H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C): δ = 164.3 (C=O), 163.8 (C=O), 154.1 (C-4), 137.4 (C-2), 136.5 (C-7), 136.0 (C-5), 133.1 (C-9), 132.1, 131.3, 130.2, 130.1, 129.4, 128.9 (6 C, phenyl-C), 128.8 (2 C, phenyl-C), 128.6 (2 C, phenyl-C), 128.2 (2 C, phenyl-C), 127.7 (2 C, phenyl-C), 127.5, 127.4, 127.2, 126.6 (4 C, phenyl-C), 125.7 (C-6), 123.8 (C-8), 123.2 (C-10), 117.7, 116.3 (C-1, C-3), 58.5 (C-11), 56.6 (C-12), 43.3 (C-12′) ppm. MS (ESI+): *m*/*z* (%) = 561, 563 (100) [M^+ + H], 453, 282. $C_{33}H_{25}BrN_2O_2$ (561.47): calcd. C 70.59, H 4.49, N 4.99; found C 70.37, H 4.43, N 4.76.

***N*-Benzyl-4-[(2-bromobenzyl)methylamino]naphthalimide (9):** Dry DMF (30 mL) was added to a mixture of **7** (500 mg, 1.1 mmol, 1.0 equiv.) and sodium hydride (52 mg, 1.3 mmol, 1.2 equiv.) at room temperature. The resulting solution was stirred at room temperature for 10 min, followed by methyl iodide (80 μL, 1.3 mmol, 1.2 equiv.) addition. The mixture was stirred at room temperature for 5 h. Then the solvent was evaporated under vacuum. Compound **9** (460 mg, 0.95 mmol, 86%) was obtained as yellow powder after column chromatography (hexane/ethyl acetate, 3:1). TLC (hexane/ethyl acetate, 2:1, R_f = 0.50), m.p. 168–169 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C): δ = 8.58 (d, J = 7.2 Hz, 1 H, 7-H), 8.52 (d, J = 8.0 Hz, 1 H, 5-H), 8.33 (d, J = 8.4 Hz, 1 H, 2-H), 7.52–7.63 (m, 5 H, phenyl-H, 6-H), 7.37 (t, J = 8.4 Hz, 1 H, phenyl-H), 7.21–7.29 (m, 5 H, phenyl-H, 3-H), 5.37 (s, 2 H, 11-H), 4.57 (s, 2 H, 12-H), 3.04 (s, 3 H, NCH_3) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C): δ = 164.5 (C=O), 164.0 (C=O), 156.2 (C-4), 137.5 (C-2), 136.2 (C-7), 133.2 (C-5), 132.7 (C-9), 131.3, 130.4, 130.2, 129.1, 128.9 (5 C, phenyl-C), 128.8 (2 C, phenyl-C), 128.3 (2 C, phenyl-C), 127.7, 127.2, 125.6 (2 C, phenyl-C), 125.3 (C-6), 123.7 (C-8), 123.1 (C-10), 115.5, 114.5 (C-1, C-3), 61.23 (C-11), 43.3 (C-12), 41.5 (NCH_3) ppm. MS (ESI+): *m*/*z* (%) = 485, 487 (100) [M^+ + H]. $C_{27}H_{21}BrN_2O_2$ (485.37): calcd. C 66.81, H 4.36, N 5.77; found C 66.52, H 4.13, N 5.57.

2,2-Dimethylpropylene Glycol Boronate Derivatives 11 and 11a. Typical Borylation Procedure: Potassium acetate (88 mg, 0.9 mmol, 3 equiv.) was added to a mixture of **8** (167 mg, 0.3 mmol, 1.0 equiv.), bis(2,2-dimethylpropylene glycol boronate) (115 mg, 0.45 mmol, 1.5 equiv.), and $PdCl_2$(dppf) [dppf = 1,1′-bis(diphenylphosphanyl)ferrocene] (7 mg, 0.009 mmol, 0.03 equiv.) at room temperature under N_2. This was followed by the addition of anhydrous DMSO (2 mL) with a syringe. The solution was heated at 90 °C for 8 h and then cooled to room temperature. Ethyl acetate (15 mL) and water (15 mL) were added into the reaction mixture. The separated organic phase was washed with water (2 × 10 mL). After drying over sodium sulfate and solvent evaporation, the residue was purified by column chromatography (hexane/ethyl acetate, 3:1 to ethyl acetate) to give **11** (89 mg, 0.15 mmol, 50%) and **11a** (43 mg, 0.09 mmol, 30%) as yellow powder.

11: TLC (hexane/ethyl acetate, 1:1, R_f = 0.35), m.p. 190–192 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C): δ = 8.62 (d, J = 6.0 Hz, 2 H, 7-H, 5-H), 8.45 (d, J = 8.0 Hz, 1 H, 2-H), 7.76 (d, J = 7.6 Hz, 1 H, phenyl-H), 7.63 (t, J = 8.0 Hz, 1 H, 6-H), 7.57 (d, J = 7.6 Hz, 2 H, phenyl-H), 7.37 (s, 2 H, phenyl-H), 7.23–7.31 (m, 7 H, phenyl-H), 7.10 (d, J = 8.4 Hz, 1 H, 3-H), 7.05 (d, J = 6.4 Hz, 2 H, phenyl-H), 5.38 (s, 2 H, 11-H), 4.73 (s, 2 H, 12-H), 4.47 (s, 2 H, 12′-H), 3.32 (s, 4 H, OCH_2), 0.81 (s, 6 H, CH_3) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C): δ = 164.5 (C=O), 164.0 (C=O), 155.7 (C-4), 142.4 (C-2), 137.5 (C-7), 136.8 (C-5), 135.0 (C-9), 132.4, 131.2, 130.7, 130.2, 129.9 (5 C), 128.9 (2 C, phenyl-C), 128.3 (2 C, phenyl-C), 128.3 (2 C, phenyl-C), 128.2 (2 C, phenyl-C), 128.1 (2 C, phenyl-C), 127.3, 127.2, 126.7 (3 C, phenyl-C), 126.2 (C-6), 125.4 (C-8), 123.2 (C-10), 117.8, 115.4 (C-1, C-3), 71.9 (C-11), 57.1 (C-12), 56.1 (C-12′), 43.3 (OCH_2), 31.3 [$C(CH_3)_2$], 21.6 (CH_3) ppm. MS (ESI+): *m*/*z* (%) = 595 (100) [M^+ + H], 527, 394, 288.

11a: TLC (hexane/ethyl acetate, 1:1, R_f = 0.55), m.p. 168–170 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C): δ = 9.01 (s, 1 H, phenyl-H), 8.68 (d, J = 8.0 Hz, 1 H, 7-H), 8.58 (d, J = 7.2 Hz, 1 H, 5-H), 7.98 (d, J = 7.6 Hz, 1 H, 2-H), 7.69 (t, J = 8.0 Hz, 1 H, 6-H), 7.58 (d, J = 7.2 Hz, 2 H, phenyl-H), 7.41–7.49 (m, 5 H, phenyl-H), 7.22–7.37 (m, 7 H, phenyl-H), 7.13 (d, J = 7.6 Hz, 1 H, 3-H), 5.41 (s, 2 H, 11-H), 4.25 (s, 4 H, 12-H, 12′-H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C): δ = 164.4 (C=O), 164.1 (C=O), 150.0 (C-4), 137.5 (C-2), 137.4 (C-7), 137.1 (C-5), 131.3 (C-9), 131.1, 130.0 (2 C, phenyl-C), 128.9 (2 C, phenyl-C), 128.8 (2 C, phenyl-C), 128.5, 128.4 (2 C, phenyl-C), 128.4 (2 C, phenyl-C), 128.2, 128.0 (2 C, phenyl-C), 127.8 (2 C, phenyl-C), 127.6, 127.3, 126.8, 126.7 (4 C, phenyl-C), 125.9 (C-6), 123.2 (C-8), 123.1 (C-10), 122.5, 118.0 (C-1, C-3), 57.4, 43.6, 43.5 ppm. MS (ESI+): *m*/*z* (%) = 481 (100) [M^+ – H], 389, 311. $C_{33}H_{26}N_2O_2$ (482.57): calcd. C 82.13, H 5.43, N 5.81; found C 81.29, H 5.40, N 5.55.

2,2-Dimethylpropylene Glycol Boronate Derivative 12: Following the typical borylation procedure described above, with a mixture of **9** (100 mg, 0.21 mmol, 1.0 equiv.), bis(2,2-dimethylpropylene glycol boronate) (69 mg, 0.27 mmol, 1.3 equiv.), $PdCl_2$(dppf)$_2$ [dppf = 1,1′-bis(diphenylphosphanyl)ferrocene] (5 mg, 0.006 mmol, 0.03 equiv.) and potassium acetate (62 mg, 0.63 mmol, 3 equiv.), compound **12** was isolated as yellow powder (85 mg, 0.16 mmol, 78%), after column chromatography (hexane/ethyl acetate, 3:1 to ethyl acetate).

12: TLC (hexane/ethyl acetate, 1:1, R_f = 0.35), m.p. 175–177 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, 25 °C): δ = 8.57 (d, J = 6.8 Hz, 1 H, 7-H), 8.53 (d, J = 8.4 Hz, 1 H, 5-H), 8.38 (d, J = 8.4 Hz, 1 H, 2-H), 7.83 (d, J = 6.8 Hz, 1 H, 17-H), 7.54 (t, J = 6.8 Hz, 4 H, phenyl-H), 7.46 (t, J = 7.2 Hz, 1 H, 6-H), 7.19–7.34 (m, 6 H, phenyl-H, 3-H), 5.38 (s, 2 H, 11-H), 4.79 (s, 2 H, 12-H), 3.43 (s, 4 H, OCH_2), 2.95 (s, 3 H, NCH_3), 0.84 [s, 6 H, $C(CH_3)_2$] ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C): δ = 164.8 (C=O), 164.0 (C=O), 157.3 (C-4), 142.7 (C-2), 137.6 (C-7), 135.2 (C-5), 132.9 (C-9), 131.2, 131.0, 130.3, 130.2 (4 C, phenyl-C), 128.8 (2 C, phenyl-C), 128.3, 127.3, 127.2, 126.7 (3 C, phenyl-C), 125.3 (C-6), 124.9 (C-8), 122.9 (C-10), 114.4, 114.0 (C-1, C-3), 72.0, 60.7, 43.3, 41.7, 31.4, 21.71 ppm. MS (ESI+, in methanol): *m*/*z* (%) = 479 (100) [M^+ – bis(2,2-dimethylpropylene glycol boronate)($C_5H_{12}O_2$) + 2OCH_3], 465, 343, 293.

***N*-Benzyl-4-{benzyl(2-dihydroxyboryl)amino}naphthalimide (1a):** Compound **11** (40 mg, 0.067 mmol) in trifluoroacetic acid (3 mL, 90% in H_2O) was stirred at room temperature for 24 h. After solvent evaporation, the residue was dissolved in methanol (0.3 mL) and purified by HPLC (C_{18} RP column). Elution condition: CH_3CN/H_2O (1 mL/min), 0–20 min (CH_3CN 40–80%), 20–25 min

(CH_3CN, 80%), R_t = 18.3 min; CH_3OH/H_2O (1 mL/min), 0–20 min (CH_3OH, 70–100%), 20–25 min (CH_3OH, 100%), R_t = 15.2 min. **1a** (19 mg, 0.032 mmol, 54%) was obtained as a yellow solid, m.p. 177–179 °C. ^1H NMR ($CDCl_3$, 400 MHz, 25 °C): δ = 8.55–8.62 (m, 2 H, 7-H, 5-H), 8.46 (d, J = 8.0 Hz, 1 H, 2-H), 7.76 (m, 2 H, 6-H, phenyl-H), 7.53 (d, J = 6.4 Hz, 2 H, phenyl-H), 7.18–7.31 (m, 9 H, phenyl-H), 6.81 (d, J = 7.6 Hz, 2 H, phenyl-H, 3-H), 6.66 [s, 1 H, $B(OH)_2$], 5.35 (s, 2 H, 11-H), 4.52 (s, 2 H, 12-H), 4.46 (d, J = 6.0 Hz, 2 H, 12′-H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C): δ = 164.2 (C=O), 163.8 (C=O), 155.0 (C-4), 140.4 (C-2), 137.6 (C-7), 135.5 (C-5), 131.4 (C-9), 131.0, 130.1, 129.9 (3 C, phenyl-C), 129.6 (4 C, phenyl-C), 129.4, 128.9, 128.5 (3 C, phenyl-C), 128.4 (2 C, phenyl-C), 128.3 (2 C, phenyl-C), 128.1, 127.6, 126.4, 125.9 (4 C, phenyl-C), 125.6 (C-6), 123.8 (C-8), 120.6 (C-10), 118.6, 118.2, (C-1, C-3), 60.4 (C-11), 57.4 (C-12), 43.4 (C-12′) ppm. MS (ESI+): m/z (%) = 527 (90) [M^+ + H], 474, 344, 214 (100).

***N*-Benzyl-4-[(2-dihydroxyboryl)methylamino]naphthalimide (1b):** Compound **12** (35 mg, 0.067 mmol) in trifluoroacetic acid (3 mL, 90% in H_2O) was stirred at room temperature for 24 h. After solvent evaporation, the residue was dissolved in methanol (0.3 mL) and purified by HPLC (C_{18} RP column). Elution condition: CH_3CN/H_2O (1 mL/min), 0–20 min (CH_3CN, 40–100%), 20–25 min (CH_3CN 100%), R_t = 11.6 min; CH_3OH/H_2O (1 mL/min), 0–20 min (CH_3OH, 70–100%), 20–25 min (CH_3OH 100%), R_t = 11.6 min. **1b** (15 mg, 0.033 mmol, 49%) was obtained as a yellow solid, m.p. 182–183 °C. ^1H NMR ($CDCl_3$, 400 MHz, 25 °C): δ = 8.57–8.63 (m, 2 H, 7-H, 5-H), 8.43 (d, J = 8.0 Hz, 1 H, 2-H), 7.89 (d, J = 6.4 Hz, 1 H, 17-H), 7.76 (t, J = 8.0 Hz, 1 H, 6-H), 7.60 (d, J = 8.4 Hz, 1 H, phenyl-H), 7.52 (d, J = 7.2 Hz, 2 H, phenyl-H), 7.20–7.38 (m, 6 H, phenyl-H, 3-H), 5.36 (s, 2 H, 11-H), 4.50 (s, 2 H, 12-H), 2.91 (s, 3 H, NCH_3) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C): δ = 164.2 (C=O), 163.7 (C=O), 153.4 (C-4), 140.0 (C-2), 137.2 (C-7), 136.2 (C-5), 132.0 (C-9), 131.5, 131.4, 130.6, 129.8, 129.2 (5 C, phenyl-C), 128.8 (2 C, phenyl-C), 128.4 (2 C, phenyl-C), 128.0, 127.4 (2 C, phenyl-C), 126.8 (C-6), 126.7 (C-8), 123.3 (C-10), 119.1, 117.4 (C-1, C-3), 60.9, 45.5, 43.5 ppm. MS (ESI+): m/z (%) = 451 (100) [M^+ + H], 365, 337.

***N*-Benzyl-4-[(2-dihydroxyboryl)amino]naphthalimide (1c) and *N*-Benzyl-4-(benzylamino)naphthalimide (10):** Following the typical borylation, with a mixture of **7** (300 mg, 0.64 mmol, 1.0 equiv.), bis(2,2-dimethylpropylene glycol boronate) (245 mg, 0.96 mmol, 1.5 equiv.), and $PdCl_2(dppf)_2$ [dppf = 1,1′-bis(diphenylphosphanyl)-ferrocene] (16 mg, 0.02 mmol, 0.03 equiv.), potassium acetate (188 mg, 1.92 mmol, 3 equiv.), compound **10** was obtained (102 mg, 0.34 mmol, 54%) as yellow powder.

10: TLC (ethyl acetate, R_f = 0.65), m.p. 204–206 °C. ^1H NMR ($CDCl_3$, 400 MHz, 25 °C): δ = 8.54 (d, J = 7.6 Hz, 1 H, 7-H), 8.43 (d, J = 8.4 Hz, 1 H, 5-H), 8.01 (d, J = 8.4 Hz, 1 H, 2-H), 7.54 (d, J = 7.2 Hz, 3 H, 6-H, phenyl-H), 7.34–7.41 (m, 5 H, phenyl-H), 7.21–7.29 (m, 3 H, 3-H, phenyl-H), 6.72 (d, J = 8.4 Hz, 1 H, 3-H), 5.57 (s, 1 H, -NH), 5.36 (s, 2 H, 11-H), 4.58 (d, J = 4.8 Hz, 2 H, 12-H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz, 25 °C): δ = 164.5 (C=O), 164.0 (C=O), 149.0 (C-4), 137.7 (C-2), 136.9 (C-7), 134.5 (C-5), 131.2 (C-9), 129.7 (phenyl-C), 129.0 (2 C, phenyl-C), 128.8 (2 C, phenyl-C), 128.3 (2 C, phenyl-C), 128.0 (phenyl-C), 127.6 (phenyl-C), 127.2 (2 C, phenyl-C), 125.9 (phenyl-C), 124.8 (C-6), 123.0 (C-8), 120.2 (C-10), 110.7, 104.9 (C-1, C-3), 47.9 (C-11), 43.2 (C-12) ppm. MS (ESI+): m/z (%) = 393 (100) [M^+ + H], 358, 134. $C_{26}H_{20}N_2O_2$ (392.45): calcd. C 79.57, H 5.14, N 7.14; found C 79.31, H 5.04, N 6.89.

Compound **1c** was partially separated with silica gel column chromatography (ethyl acetate/methanol, 20:1) and further purified by HPLC (C_{18} RP column). Elution condition: CH_3CN/H_2O (1 mL/min), 0–20 min (CH_3CN, 40–80%), 20–25 min (CH_3CN, 80%), R_t = 12.4 min; CH_3OH/H_2O (1 mL/min), 0–20 min (CH_3OH, 70–100%), 20–25 min (CH_3OH, 100%), R_t = 10.0 min. **1c** (75 mg, 0.17 mmol, 27%) was obtained as a yellow solid, m.p. 188–190 °C. ^1H NMR (CD_3OD, 400 MHz, 25 °C): δ = 8.50 (d, J = 6.8 Hz, 1 H, 7-H), 8.43 (d, J = 8.0 Hz, 1 H, 5-H), 7.58 (d, J = 6.8 Hz, 1 H, 2-H), 7.52 (d, J = 7.2 Hz, 2 H, phenyl-H), 7.36–7.45 (m, 5 H, phenyl-H, 6-H), 7.20–7.29 (m, 3 H, phenyl-H), 7.04 (d, J = 8.8 Hz, 1 H, 3-H), 5.31 (s, 2 H, 11-H), 4.46 (s, 2 H, 12-H) ppm. ^{13}C NMR (CD_3OD & $CDCl_3$, 100 MHz, 25 °C): δ = 164.5 (C=O), 164.0 (C=O), 152.0 (C-4), 137.0 (C-2), 136.6 (C-7), 133.89 (C-5), 129.9 (C-9), 128.3 (2 C, phenyl-H), 128.2 (phenyl-C), 127.9 (2 C, phenyl-H), 127.7 (3 C, phenyl-H), 127.6 (3 C, phenyl-H), 127.5 (C-6), 126.6 (2 C, C-8, C-10), 122.1, 106.5 (C-1, C-3), 77.1 (C-11), 42.7 (C-12) ppm. MS (ESI-): m/z (%) = 463 (100) [M^+ + $2OCH_3$], 435 (65) [M^+ – H], 417, 213.

Fluorescence and Absorbance Binding Studies (1c as an example): Solutions of **1c** (5×10^{-5} M) and **1c** (5×10^{-5} M) with sugar (0.5 M) were prepared in 0.1 M phosphate buffer at pH 7.40, respectively. These two solutions were mixed in a 1-cm cuvette. In the solution, the ratio of **1c** to sugar was increased gradually. After shaking for 2 min, the solution was used to test the fluorescence intensity or UV absorbance immediately. Six to eight points were collected for the calculation of apparent binding constant K_a assuming a 1:1 complex formation mechanism. All tests have been duplicated.

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