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Water-Soluble Fluorescent Boronic Acid Compounds for Saccharide Sensing: Substituent Effects on Their Fluorescence Properties

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Abstract: Four new naphthalene-based boronic acid compounds (**1**–**4**) were synthesized. The effect of various carbohydrates on their fluorescence properties has been studied in aqueous phosphate buffer at pH 7.4. Different substitutions on the aniline group of the naphthalene ring resulted in significant differences in fluorescence properties for these four compounds. Compound **1** shows ratiometric fluorescence changes upon addition of a sugar. Compounds **2** and **3** do not show ratiometric fluorescence changes but show very large fluorescence intensity changes (about 70-fold fluorescence intensity increase). In addition to the quantifiable fluorescence property changes upon sugar addition, the fluorescence color changes of **1**–**3** are also visible to the naked eye. However, amidation of the aniline nitrogen atom significantly diminishes the fluorescence intensity of compound **4**. The crystal structure of one boronic acid provided some insight into the structural features that are important for the fluorescence properties of these compounds.

Keywords: boronic acid · carbohydrates · fluorescence · lectins · sensors

Introduction

Recent years have seen a great deal of interest in using boronic acids as recognition moieties for the synthesis of artificial receptors for compounds that contain two adjacent Lewis base functional groups. Such compounds may include carbohydrates,[1–3] catechols,[4] α-hydroxy acids,[5] amino acids,[4f] etc. The use of the boronic acid moiety is based on the formation of tight complexes between boronic acid and compounds that have two adjacent nucleophiles, such as diols.[1] Our laboratory group has a long-standing interest in the development of boronic acid based artificial lectins for the recognition of oligosaccharides.[6a,b] We term these recognition moieties "boronolectins". These boronolectins have the potential to be developed as medicinal, imaging, and diagnostic agents.[1e,2d,g,6,7] Their ability to recognize cell-surface carbohydrate biomarkers also allows them to be used as targeting vectors for the delivery of other medicinal and imaging agents and as research tools for the identification of cells based on their surface biomarkers. Furthermore, fluorescent boronolectins can also be used as sensors for various analytical applications.[3]

In the preparation of boronolectins, regardless of whether fluorescence is required for the eventual application, the availability of fluorescent boronic acid reporter compounds is very important. For the preparation of fluorescent boronolectins for analytical applications, the change in fluorescence properties provides a reporting event in response to binding. For other applications where fluorescence properties are not essential, the use of fluorescent boronic acids allows the quick screening of libraries of compounds for the identification of boronolectins of interest. It is for these reasons that we and others have been interested in the development of fluorescent boronic acid reporter compounds[8] that 1) change fluorescence properties upon binding to a diol, 2) are chemically and photochemically stable, and 3) are water soluble. Such reporters can be used for the preparation of combinatorial libraries of potential boronolectins.

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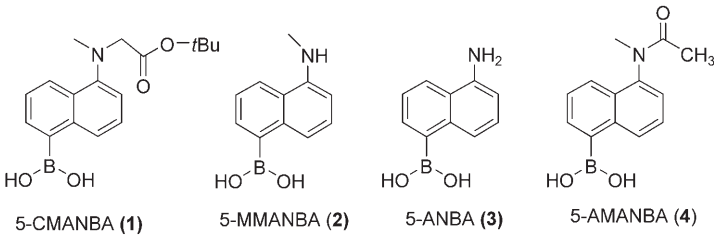
Supporting information for this article (^{1}H and ^{13}C NMR spectra of **1**–**4** and the intermediates, crystal data for compound **10**·HCl) is available on the WWW under http://www.chemeurj.org/ or from the author.

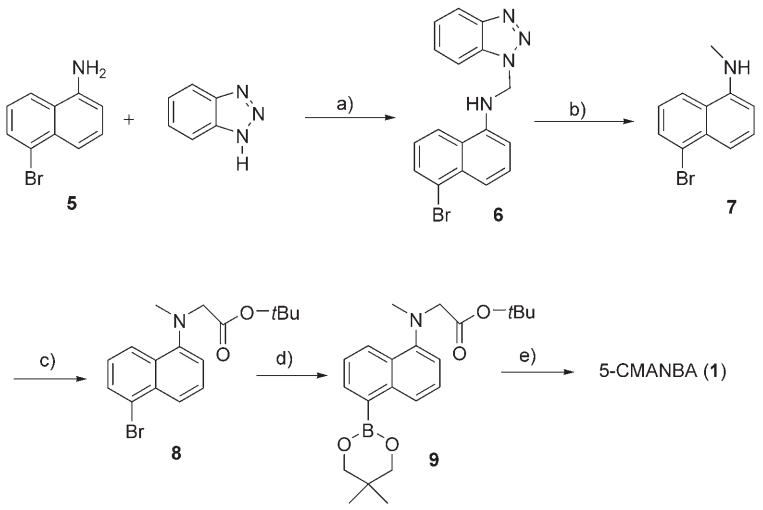
Recently, we reported two new water-soluble boronic acid sensors: 4-(dimethylamino)-naphthalene-1-boronic acid (4-DMANBA) and 5-(dimethylamino)-naphthalene-1-boronic acid (5-DMANBA).[8a,b] 5-DMANBA is a ratiometric sensor, while 4-DMANBA is an off–on sensor. In order for these reporter compounds to be useful for our bisboronic acid combinatorial-library construction in the search for boronolectins specific for saccharides of biological interest, certain "handles" need to be installed so that the boronic acids can be "conjugated". For this, one can think of introducing an alkyl group, which contains a functional group for conjugation, on the aniline nitrogen atom. Another possibility is to use the aniline nitrogen atom for amide formation as a way to achieve conjugation. However, it is well known that the fluorescence properties of a compound can be affected by various factors. In this study we wanted to see whether amidation chemistry, the introduction of an alkyl group bearing an ester moiety, or a change in the number of alkyl group on the aniline nitrogen atom would affect the fluorescence properties of 5-DMANBA. Such information would be very useful for the application of such fluorescent reporters in our combinatorial-library work. Therefore, we designed and examined a series of new fluorescent boronic acid compounds (**1**–**4**), which have different substituents on the aniline amino group (Scheme 1). These derivatives show somewhat different fluorescence properties to 5-DMANBA depending on the specific structural features that are introduced. Some are detrimental to their application (**4**) and others (**1**–**3**) have no effect.



Scheme 1. The structures of the fluorescent boronic acid compounds synthesized in this study.

Results and Discussion

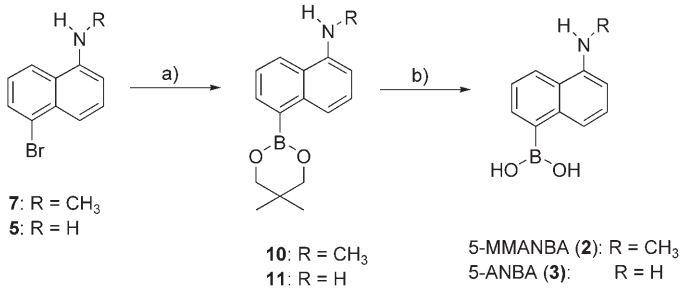
Synthesis: The synthesis of 5-(*tert*-butoxycarbonylmethyl-methylamino)-naphthalene-1-boronic acid (5-CMANBA, **1**) is shown in Scheme 2. The starting material 5-amino-1-bromonaphthalene (**5**) was synthesized as described previously.[8b] 5-(Monomethylamino)-1-bromonaphthalene (**7**) was synthesized by the reaction of compound **5** with formaldehyde in the presence of 1*H*-benzotriazole and then reduction with $NaBH_4$ following a procedure similar to that in the literature.[9] Alkylation of compound **7** with *tert*-butyl bromoacetate gave compound **8**. The protected boronate compound **9** was synthesized through the reaction of **8** with bis(neopentyl glycolato)diboron with [$PdCl_2$(dppf)] as the catalyst.[10] The final boronic acid compound **1** was obtained through deprotection of compound **9** by using a mixture of acetone and aqueous HCl solution.



Scheme 2. Synthesis of 5-CMANBA (**1**): a) HCHO, EtOH, 92%; b) $NaBH_4$, THF, 50°C, 98%; c) *tert*-butyl bromoacetate, K_2CO_3, DMF, 80°C, 70%; d) bis(neopentyl glycolato)diboron, KOAc, [$PdCl_2$(dppf)], DMSO, 80°C, 64%; e) acetone/H_2O, HCl, 30%. THF = tetrahydrofuran, DMF = *N*,*N*-dimethylformamide, dppf = 1,1′-bis(diphenylphosphanyl)ferrocene, DMSO = dimethyl sulfoxide.

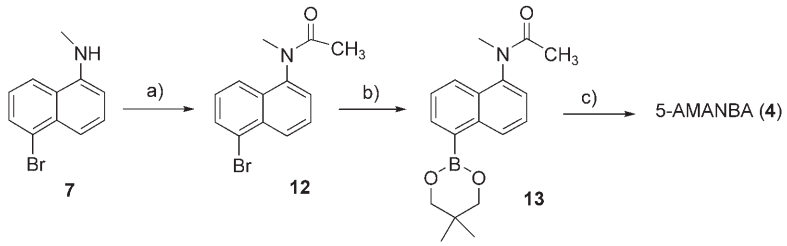
5-(Monomethylamino)-naphthalene-1-boronic acid (5-MMANBA, **2**) and 5-Amino-naphthalene-1-boronic acid (5-ANBA, **3**) were readily synthesized from compounds **7** and **5**, respectively, by catalytic borylation with bis(neopentyl glycolato)diboron followed by deprotection (Scheme 3).

The synthesis of 5-(acetylmethylamino)-naphthalene-1-boronic acid (5-AMANBA, **4**) started with the amidation of **7** by using acetyl chloride to give **12**. The boronic acid com-



Scheme 3. Synthesis of 5-MMANBA (**2**) and 5-ANBA (**3**): a) bis(neopentyl glycolato)diboron, KOAc, [$PdCl_2$(dppf)], DMSO, 80°C, 71% for **10** and 82% for **11**; b) acetone/H_2O, HCl, 39% for **2** and 36% for **3**.

pound 5-AMANBA (**4**) was obtained through catalytic borylation followed by deprotection (Scheme 4).



Scheme 4. Synthesis of 5-AMANBA (**4**): a) acetyl chloride, iPr$_2$NEt, toluene, 94%; b) bis(neopentyl glycolato)-diboron, KOAc, PdCl$_2$(dppf), DMSO, 80°C, 70%; c) acetone/H$_2$O, HCl, 45%.

Fluorescence property examinations: Earlier, we reported 5-DMANBA to be a ratiometric fluorescent sensor.[8b] It shows very significant fluorescence intensity changes at 2 wavelengths, 513 and 433 nm, upon addition of a sugar. For example, addition of fructose (50 mM) to the solution of 5-DMANBA induced a 61% fluorescence intensity decrease at 513 nm and a 36-fold increase at 433 nm.[8b] We are interested in examining the effect of various structural modifications on its fluorescence properties. Such modifications include a) alkylation of the aniline amino group with an alkyl group bearing another functional group (an ester), which is useful for conjugation (**1**), b) removal of one (**2**) or both (**3**) methyl groups from the aniline nitrogen atom, and c) conversion of the aniline nitrogen atom into an amide group (**4**). The fluorescence properties of compounds **1**–**3** were studied in 0.1 M aqueous phosphate buffer at pH 7.4. The effect of various carbohydrates on the fluorescence properties of these compounds was examined under similar conditions. It was found that a change in the number and/or nature of the alkyl groups on the aniline nitrogen atom (as in compounds **1**–**3**) resulted in some changes in the fluorescence properties of 5-DMANBA, changes that do not affect the application of these compounds as fluorescent reporter compounds. These compounds show very large fluorescence property changes upon sugar binding (Figure 1). However, when the aniline nitrogen atom was masked in an amide group, compound **4** became only weakly fluorescent (Figure 1). These results are discussed in detail in the subsequent sections.

5-CMANBA (**1**) is very similar to its parent compound, 5-DMANBA, in that it has two alkyl groups attached to the aniline nitrogen atom. The only difference is that one of these two alkyl groups has a protected carboxylic acid in **1**. Compound **1** shows similar ratiometric fluorescence changes (Figure 1A) upon addition of a sugar to those of 5-DMANBA.[8b] However, compound **1** shows a shorter emission maximum (at 490 nm versus 513 nm for 5-DMANBA) and a longer absorption maximum (at 320 nm versus 300 nm for 5-DMANBA) in 0.1 M aqueous phosphate buffer at pH 7.4, with a Stokes shift of 170 nm. Addition of carbohydrate resulted in a significant decrease in fluorescence intensity at 490 nm and an increase with a greater magnitude at 440 nm. For example, addition of fructose (50 mM) to the solution of **1** induced a 4.2-fold fluorescence intensity increase at 440 nm and a 32% fluorescence intensity decrease at 490 nm (Figure 1A and Table 1). An isosbestic point was observed at 478 nm. The effect of other sugars, such as sorbitol, tagatose, galactose, and glucose, on the fluorescence properties of **1** was also examined. Ratiometric fluorescence changes were observed with all the sugars tested (Table 1). Such results indicate that the introduction of an ester functional group on the side chain does

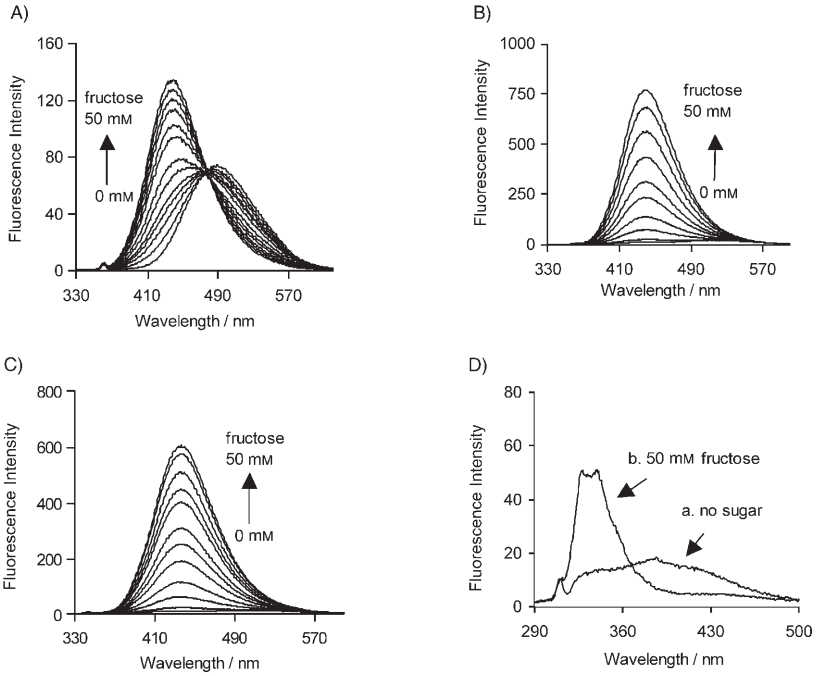


Figure 1. Fluorescence spectral changes of boronic acid compounds **1**–**4** with different concentrations of D-fructose (0–50 mM) in 0.1 M aqueous phosphate buffer at pH 7.4. A) 5-CMANBA (**1**; 1.0×10^{-5} M), $\lambda_{ex}=320$ nm; B) 5-MMANBA (**2**; 4×10^{-6} M), $\lambda_{ex}=320$ nm; C) 5-ANBA (**3**; 1.0×10^{-5} M), $\lambda_{ex}=307$ nm; D) 5-AMANBA (**4**; 1.0×10^{-5} M), $\lambda_{ex}=280$ nm.

Table 1. Apparent association constants (K_a) and maximal fluorescence intensity changes ($\Delta I/I_0$) of compounds **1–3** with different sugars.

Sugar	5-CMANBA (**1**)			5-MMANBA (**2**)		5-ANBA (**3**)	
	K_a [M^{-1}]	$\Delta I/I_0$ (sugar conc. [M])		K_a [M^{-1}]	$\Delta I/I_0$ (sugar conc. [M])	K_a [M^{-1}]	$\Delta I/I_0$ (sugar conc. [M])
		440 nm	490 nm				
sorbitol	263 ± 4	4.6 (0.05)	−0.33 (0.05)	268 ± 4	71 (0.05)	288 ± 4	69 (0.05)
fructose	159 ± 3	4.2 (0.05)	−0.32 (0.05)	180 ± 3	71 (0.05)	190 ± 3	66 (0.05)
tagatose	170 ± 3	3.7 (0.05)	−0.34 (0.05)	159 ± 2	66 (0.05)	185 ± 3	58 (0.05)
galactose	16.3 ± 0.2	3.3 (0.50)	−0.37 (0.50)	23 ± 0.3	54 (0.50)	20 ± 0.3	49 (0.50)
glucose	4.5 ± 0.2	0.79 (0.50)	−0.35 (0.50)	5.5 ± 0.1	30 (0.50)	5.0 ± 0.1	28 (0.50)

not abolish the ability of the fluorophore to show a ratiometric response to binding with a sugar. This is very important for the preparation of di- or multiboronic acid compounds for sensing and other applications.

5-MMANBA (**2**) has one fewer methyl group on the nitrogen than the parent compound, 5-DMANBA. It shows an emission maximum at 515 nm and a smaller shoulder emission peak at 438 nm before addition of a sugar (Figure 1B). After addition of a sugar, the fluorescence intensity at 515 nm remained about the same but the intensity at 438 nm increased significantly. For example, addition of fructose (50 mM) to the solution of **2** induced a 71-fold fluorescence intensity increase at 438 nm (Figure 1B and Table 1). This represents the largest fluorescence change among all the fluorescent boronic acids we have developed. To the best of our knowledge, such a large fluorescence change has never been reported in the literature. Theoretically, this compound also gives ratiometric changes upon addition of a sugar. However, because of the much lower fluorescence intensity in the absence of any sugar, the peak at 515 nm cannot be used for ratiometric sensing in an accurate fashion (Figure 1B).

The situation with 5-ANBA (**3**), which has both methyl groups removed from the aniline group of 5-DMANBA, is similar to that of **2**. Compound **3** shows an emission maximum at 513 nm and a smaller shoulder emission peak at 437 nm before addition of a sugar (Figure 1C). After addition of sugar, the fluorescence intensity at 513 nm remained about the same, but the intensity at 437 nm increased significantly. For example, addition of fructose (50 mM) to the solution of **3** induced a 66-fold fluorescence intensity increase at 437 nm (Figure 1C and Table 1). Similarly to **2**, compound **3** does not allow ratiometric sensing of sugars in an accurate fashion because of the large difference in quantum yields between the peaks at 513 and 437 nm after the addition of a sugar, a difference that does not allow for baseline resolution of the two peaks. Therefore, although both **2** and **3** show ratiometric fluorescence property changes after addition of a sugar, neither can be used accurately for ratiometric sensing. This is in direct contrast to the fluorescence properties of 5-DMANBA. However, compounds **2** and **3** are both very good off–on fluorescent sensors because of their dramatic fluorescence increases upon binding of a sugar. Overall, all three analogues with either a free aniline or an alkylated aniline show large fluorescence intensity changes upon sugar addition.

5-AMANBA (**4**) is in a unique category by itself. The aniline group in **4** has been masked as an amide. It is well known that the lone-pair electrons of the nitrogen atom in an amide are not readily available due to resonance stabilization. Since the participation of the nitrogen lone-pair electrons is very important for the fluorescence property changes upon sugar binding,[8,11] one would expect compound **4** to behave differently from compounds **1–3**. This is indeed the case. Compound **4** shows much a shorter emission wavelength and weaker fluorescence than **1–3**. The emission spectrum change of **4** in the absence and presence of fructose at a fixed concentration (50 mM) in 0.1 M aqueous phosphate buffer solution (pH 7.4) is shown in Figure 1D. We did not examine the binding of **4** with additional carbohydrates because of its weak fluorescence properties.

The fluorescence quantum yields (ϕ_F) of **1–3** were determined in 0.1 M phosphate buffer (pH 7.4) in the absence of a sugar and in the presence of 50 mM fructose, with 8-quinoline boronic acid ($\phi_F = 0.58$ in 12 M H_2SO_4) as a reference compound.[12] The results are summarized in Table 2. A good quantum yield ($\phi_F = 0.26$) was observed for compound **1** at 490 nm in the absence of a sugar. Upon addition of 50 mM of fructose, the emission wavelength changed to 440 nm and the quantum yield at this shorter wavelength increased to 0.37. The fluorescence quantum yields of compounds **2** and **3** in the absence of a sugar were below 10%, but they increased dramatically after addition of 50 mM fructose. The fluorescence quantum yield of compound **4** was not studied because of its low fluorescence intensity.

Table 2. The excitation and emission maxima and fluorescence quantum yields (ϕ_F) of compounds **1–3** with and without sugar (50 mM fructose).

	λ_{ex} [nm]	Without sugar		With sugar	
		λ_{em} [nm]	ϕ_F	λ_{em} [nm]	ϕ_F
1	320	490	0.26	440	0.37
2	320	515 and 438	0.056	438	0.72
3	307	513 and 437	0.041	437	0.89

In addition to the quantifiable fluorescence property changes upon sugar addition, the fluorescence color changes of **1–3** are visible to the naked eye. Figure 2 shows the fluorescence color changes of these sensor solutions in the absence and presence of a sugar (50 mM fructose) under a 365 nm UV light. The solutions of these compounds without a sugar clearly show greenish colors under the commonly used 365 nm UV light although their excitation maximums are in the range of 300–320 nm. Addition of a sugar (for example, 50 mM fructose) causes a clearly visible color change

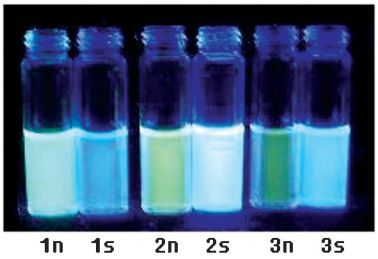


Figure 2. Fluorescence colors of compounds **1**–**3** in the absence and presence of 50 mM fructose in 0.1 M aqueous phosphate buffer (pH 7.4). **1n**, **2n**, and **3n** are the solutions of compounds **1**–**3**, respectively, without sugar; **1s**, **2s**, and **3s** are the solutions of compounds **1**–**3**, respectively, with 50 mM fructose. The pictures were taken under irradiation of the solutions (5×10^{-5} M) under a 365 nm UV light.

in the sensor solutions. Therefore, these compounds also have the potential to be used as color sensors.

The apparent binding constants, K_a, of these boronic acid compounds (**1**–**3**) with five carbohydrates (sorbitol, fructose, tagatose, galactose, and glucose) were determined under the same conditions with the assumption of the formation of a 1:1 complex.[13] These binding constants are similar to those observed with phenylboronic acid.[1c,d] As expected, the affinity trend of various sugars with these three sensors (**1**–**3**) also followed that of simple monoboronic acid, in the order sorbitol > fructose ≈ tagatose > galactose > glucose (Table 1). The different binding properties of these sugars are attributed to the different dihedral angles of the diols. The substitution pattern on the aniline nitrogen atom has a significant effect on the fluorescence properties of these compounds but not on the selectivity of these sensors for different sugars. The sensors **1**–**3** show similar selectivity for the five tested sugars. The results are consistent with other boronic acids reported. Usually, selectivity can be bestowed by the incorporation of a secondary or tertiary binding site.

X-ray crystallography: We were also interested in examining the crystal structures of the boronic acids synthesized, in the hope of gaining some insight into the structural features that are important for the fluorescence properties of these compounds. Along this line, we successfully grew single crystals of the protected form of 5-MMANBA (**2**), that is, [5-(5,5-dimethyl-[1,3,2]dioxaborinan-2-yl)-naphthalen-1-yl]-methylamine (**10**·HCl). These single crystals were grown in methanol and the crystal structures were analyzed by X-ray diffraction (Figure 3).[14]

It is important to note that the fluorescence changes of 4- and 5-DMANBA are believed to be through the regulation of an internal charge-transfer process, which involves the lone-pair electrons of the aniline nitrogen atom and the open shell of the boron atom. In order for this to happen, both the lone-pair electrons and the boron open shell need to be in conjugation with the aromatic system. The crystal structure studies indicate that the dihedral angles formed between the methyl group on the aniline nitrogen atom and the naphthalene ring are within 5° of coplanarity, so the nitrogen lone-pair electrons are in conjugation with the naphthalene ring. A similar dihedral angle measurement indicates that the boron open shell is within 9° of the naphthalene plane, which would result in good conjugation for the system. Such structural results are consistent with the proposed internal charge-transfer mechanism for fluorescence changes upon sugar binding.

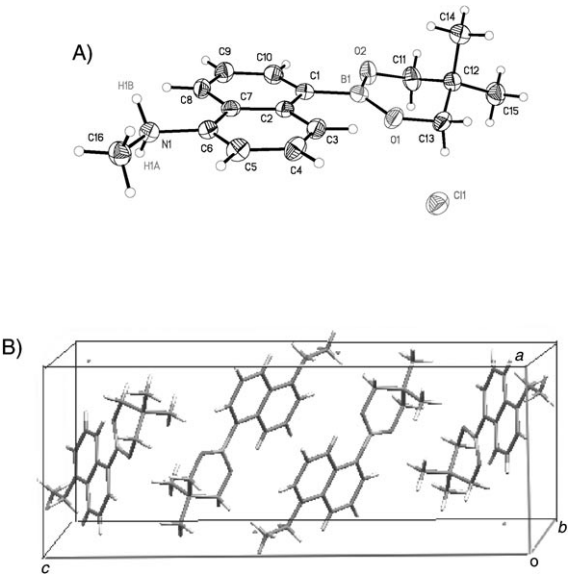


Figure 3. A) The crystal structure of compound **10**·HCl;[14] B) packing diagram for molecules in the unit cell.

Conclusion

Four water-soluble naphthalene-based fluorescent saccharide sensors, 5-CMANBA (**1**), 5-MMANBA (**2**), 5-ANBA (**3**), and 5-AMANBA (**4**), have been synthesized and their fluorescence properties have been studied. Although these compounds are analogues of 5-DMANBA, which we reported previously, they show very different fluorescence changes after modification of the aniline group. The substitution pattern on the aniline nitrogen atom has a significant effect on the fluorescence properties of these compounds. 5-CMANBA (**1**) shows ratiometric fluorescence changes upon binding of a sugar, so it can be used for the synthesis of ratiometric di- or multiboronic acid compounds after removal of the *tert*-butyl protection group and a coupling reaction with a di- or multiamine linker. 5-MMANBA (**2**) and 5-ANBA (**3**) show dramatic fluorescence increases upon binding of a sugar, so they are very good off–on fluorescent sensors for sugars. In addition to the quantifiable fluorescence property changes upon sugar addition, the fluorescence color changes of **1**–**3** are also visible to the naked eye. Therefore, they can also be used as color sensors. The crystal structure of one boronic acid provided some insight into the structural features important for the fluorescence properties of these compounds.

Experimental Section

General: ^{1}H and ^{13}C NMR spectra were recorded at 300 or 400 MHz and 75 or 100 MHz, respectively, with tetramethylsilane as the internal standard. Elemental analyses and mass spectrum analyses were performed by the Georgia State University Analytical Facility. Fluorescence spectra were recorded on a Shimadzu RF-5301 PC spectrofluorometer. Absorption spectra were recorded on Shimadzu UV-1700 UV/Vis spectrophotometer. All pH values were determined with a UB-10 Ultra Basic Benchtop pH meter (Denver Instruments). Column chromatography was performed by using silica gel (200–400 mesh) from Aldrich. THF was distilled from Na and benzophenone.

Benzotriazol-1-ylmethyl-(5-bromonaphthalen-1-yl)-amine (6): An aqueous solution of formaldehyde (40% wt, 0.68 mL, 9.0 mmol) was added to a solution of compound **5** (2.0 g, 9.0 mmol) and 1*H*-benzotriazole (1.1 g, 9.0 mmol) in EtOH (40 mL). The reaction mixture was stirred vigorously overnight under N_2 at room temperature. The precipitate was filtered and recrystallized from ethanol to give compound **6** as a white solid (3.25 g, 92%): ^{1}H NMR (400 MHz, DMSO): $\delta = 8.23$ (s, 1H), 8.15 (s, 1H), 7.99 (m, 2H), 7.46 (m, 5H), 7.09 (1H), 6.30 ppm (s, 1H); ^{13}C NMR (100 MHz, DMSO): $\delta = 146.1$, 142.3, 133.2, 132.7, 130.6, 128.6, 127.7, 125.6, 124.9, 124.5, 123.0, 122.0, 119.6, 116.5, 111.6, 106.4, 57.7 ppm; EI-MS: *m*/*z* calcd for $C_{17}H_{13}BrN_4$: 354.2 [M^+]; found: 354.1; elemental analysis calcd (%) for $C_{17}H_{13}BrN_4$: C 57.81, H 3.71, N 15.86; found: C 57.76, H 3.65, N 15.81.

(5-Bromonaphthalen-1-yl)-methylamine (7): $NaBH_4$ (1.5 g, 40 mmol) was added to a solution of compound **5** (2.8 g, 8.0 mmol) in THF (40 mL). The reaction mixture was stirred at 50°C overnight. After evaporation of solvent, the residue was dissolved in CH_2Cl_2 (50 mL); the solution was then washed with 5% $NaHCO_3$ (3×30 mL) and dried over Na_2SO_4. After filtration and evaporation of solvent, the crude product was purified on a silica gel column, with elution with CH_2Cl_2/hexanes (1:3), to give compound **7** as a colorless oil (1.86 g, 98%): ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.70$ (d, $J = 1.2$ Hz, 1H), 7.57 (t, $J = 6.3$ Hz, 2H), 7.38 (t, $J =$ 8.4 1H), 7.09 (t, $J = 7.2$ Hz, 1H), 6.53 (d, $J = 7.5$ Hz, 1H), 4.21 (s, 1H), 2.87 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 144.9$, 132.9, 130.2, 128.4, 124.8, 124.7, 123.9, 120.0, 116.3, 104.8, 31.2 ppm; EI-MS: *m*/*z* calcd for $C_{11}H_{10}BrN$: 235.0 [M^+]; found: 235.0; elemental analysis calcd (%) for $C_{11}H_{10}BrN$: C 55.96, H 4.27, N 5.93; found: C 55.96, H 4.19, N 5.93.

***tert*-Butyl [(5-bromonaphthalen-1-yl)-methylamino]acetate (8)**: K_2CO_3 (2.0 g, 15 mmol) was added to a solution of compound **7** (0.40 g, 1.7 mmol) and *tert*-butyl bromoacetate (1.20 mL, 7.4 mmol) in DMF (6 mL). The reaction mixture was stirred at 80°C for 2 days. After evaporation of solvent, the residue was dissolved in CH_2Cl_2 (30 mL); the solution was then washed with 5% $NaHCO_3$ (3×20 mL) and dried over Na_2SO_4. After filtration and evaporation of solvent, the crude product was purified on a silica gel column, with elution with CH_2Cl_2/hexanes (1:3), to give compound **8** as a yellow oil (0.41 g, 70%): ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.25$ (d, $J = 8.4$ Hz, 1H), 7.95 (d, $J = 8.4$ Hz, 1H), 7.75 (d, $J = 7.4$ Hz, 1H), 7.47 (t, 1H), 7.28 (t, 1H), 7.21 (d, $J = 7.2$ Hz, 1H), 3.78 (s, 2H), 2.98 (s, 3H), 1.48 ppm (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 170.1$, 149.5, 133.5, 130.4, 127.2, 125.8, 124.1, 123.4, 122.6, 117.3, 81.5, 59.8, 42.2, 28.3 ppm; HR ESI-MS: *m*/*z* calcd for $C_{17}H_{21}BrNO_2$: 350.0756 [M+H]$^+$; found: 350.0771.

***tert*-Butyl {[4-(5,5-dimethyl-[1,3,2]dioxaboronan-2-yl)naphthalen-1-yl]methylamino}acetate (9)**: KOAc (30 mg, 0.30 mmol) was added to a solution of compound **8** (35 mg, 0.10 mmol), bis(neopentyl glycolato)diboron (51 mg, 0.20 mmol), and $PdCl_2$(dppf) (4 mg, 0.005 mmol) in DMSO (3 mL). The reaction mixture was stirred at 80°C overnight. The mixture was dissolved in CH_2Cl_2 (30 mL); the solution was then washed with 5% $NaHCO_3$ (4×20 mL) and dried over Na_2SO_4. After filtration and evaporation of solvent, the crude product was purified on a silica gel column, with elution with CH_2Cl_2/hexanes (2:1), to give compound **9** as a white powder (150 mg, 64%): ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.46$ (d, $J =$ 19.5 Hz, 2H), 8.03 (d, $J = 6.3$ Hz, 1H), 7.44 (m, 2H), 7.18 (d, $J = 7.2$ Hz, 1H), 3.89 (s, 4H), 3.81 (s, 2H), 3.00 (s, 3H), 1.45 (s, 9H), 1.10 ppm (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 170.2$, 149.2, 138.0, 134.2, 128.8, 126.5, 125.5, 124.5, 123.8, 115.5, 81.0, 72.5, 59.8, 42.0, 31.7, 28.1, 21.9 ppm; EI-MS: *m*/*z* calcd for $C_{22}H_{30}BNO_4$: 383.2 [M^+]; found: 383.0; elemental analysis calcd (%) for $C_{22}H_{30}BNO_4$: C 68.94, H 7.89, N 3.65; found: C 69.42, H 8.14, N 3.33.

5-(*tert*-Butoxycarbonylmethyl-methylamino)-naphthalene-1-boronic acid (5-CMANBA, 1): Compound **9** (0.5 g, 1.3 mmol) was deprotected by stirring in acetone/water (9:1, 10 mL), with aqueous 12 N HCl (3 drops), at RT for 4 h. After evaporation of solvent, the mixture was dissolved in CH_2Cl_2 (30 mL); the solution was then washed with 5% $NaHCO_3$ (2× 20 mL) and dried over Na_2SO_4. The crude product was purified on a silica gel column, with elution with CH_2Cl_2/MeOH (30:1), to give **1** as a white powder (24 mg, 30%): ^{1}H NMR (300 MHz, CD_3OD): $\delta = 8.23$ (d, $J = 7.5$ Hz, 1H), 7.48 (m, 4H), 7.18 (d, $J = 8.1$ Hz, 1H), 3.79 (s, 2H), 2.96 (s, 3H), 1.43 ppm (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 170.9$, 149.5, 136.4, 129.8, 128.5, 125.6, 124.6, 124.4, 123.4, 115.7, 81.3, 72.5, 59.6, 41.2, 27.2 ppm; HR ESI-MS: *m*/*z* calcd for $C_{17}H_{23}BNO_4$: 316.1720 [M+H]$^+$; found: 316.1733.

[5-(5,5-Dimethyl-[1,3,2]dioxaborinan-2-yl)-naphthalen-1-yl]-methylamine (10): Compound **10** was synthesized from **7** by following equivalent reaction procedures to those used for the synthesis of **9**. The crude product was purified on a silica gel column, with elution with CH_2Cl_2/hexanes (2:1), to give compound **10** as colorless crystals (71%): ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.13$ (d, $J = 7.8$ Hz, 1H), 7.99 (d, $J = 6.9$ Hz, 1H), 7.82 (d, $J = 7.5$ Hz, 1H), 7.39 (m, 2H), 6.56 (d, $J = 7.2$ Hz, 1H), 3.81 (s, 4H), 2.90 (s, 3H), 1.02 ppm (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta =$ 161.2, 144.9, 137.6, 134.1, 126.8, 123.9, 122.5, 117.9, 103.7, 72.6, 31.8, 22.2 ppm; EI-MS: *m*/*z* calcd for $C_{16}H_{20}BNO_2$: 269.1 [M^+]; found: 269.0; see Figure 3 for the crystal structure.

5-(Methylamino)-naphthalene-1-boronic acid (5-MMANBA, 2): Compound **2** was synthesized from **10** by following the deprotection procedure as that used for the synthesis of **1**. The crude product was purified on a silica gel column, with elution with CH_2Cl_2/MeOH (30:1), to give **2** as a white powder (39%): ^{1}H NMR (300 MHz, CD_3OD): $\delta = 8.0$ (d, $J =$ 8.1, 1H), 7.4–7.2 (m, 3H), 7.03 (d, $J = 8.4$, 1H), 6.54 (d, $J = 7.5$,1H), 2.94 ppm (s, 3H); ^{13}C NMR (75 MHz, CD_3OD): $\delta = 145.9$, 135.7, 129.3, 126.8, 123.6, 123.5, 121.4, 116.4, 103.2, 103.1, 29.75 ppm; HR ESI-MS: *m*/*z*: calcd for $C_{11}H_{13}BNO_2$: 202.1039 [M+H]$^+$; found: 202.1043.

5-(5,5-Dimethyl-[1,3,2]dioxaboronan-2-yl)-naphthalen-1-yl]-amine (11): Compound **11** was synthesized from **5** by following equivalent reaction procedures to those used for the synthesis of **9**. The crude product was purified on a silica gel column, with elution with CH_2Cl_2/hexanes (2:1), to give compound **11** as a white powder (82%): ^{1}H NMR (300 MHz, CD_3OD): $\delta = 8.21$ (d, $J = 8.4$ Hz, 1H), 8.00 (d, $J = 5.7$ Hz, 1H), 7.88 (d, $J = 8.4$ Hz, 1H), 7.41 (t, 1H), 7.29 (t, 1H), 6.74 (d, $J = 7.5$ Hz, 1H), 3.84 (s, 4H), 1.05 ppm (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 142.5$, 137.8, 134.4, 134.1, 126.6, 124.2, 123.9, 123.6, 119.7, 109.8, 72.7, 22.3 ppm; HR ESI-MS: *m*/*z* calcd for $C_{15}H_{19}BNO_2$: 256.1509 [M+H]$^+$; found: 256.1514.

5-Aminonaphthalene-1-boronic acid (5-ANBA, 3): Compound **3** was synthesized from **11** by following the same deprotection procedure as that used for the synthesis of **1**. The crude product was purified on a silica gel column, with elution with CH_2Cl_2/MeOH (30:1), to give **3** as a white powder (36%): ^{1}H NMR (300 MHz, CD_3OD): $\delta = 8.13$ (d, $J = 8.7$, 1H), 7.51 (t, $J = 6.9$, 1H), 7.36 (d, $J = 6.9$, 1H), 7.01 (t, $J = 7.5$, 1H), 6.81 (d, $J =$ 7.5, 1H), 6.66 ppm (d, $J = 8.4$, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 145.8, 137.9, 131.5, 128.4, 125.7, 125.6, 124.2, 120.1, 111.5 ppm; HR ESI-MS: *m*/*z* calcd for $C_{10}H_{11}BNO_2$: 188.0883 [M+H]$^+$; found: 188.0887.

***N*-(5-Bromo-naphthalen-1-yl)-*N*-methylacetamide (12)**: Acetyl chloride (21.5 mg, 0.27 mmol) was added to a solution of compound **7** (50 mg, 0.18 mmol) and (iPr)$_2$EtN (94 mg, 0.55 mmol) in toluene (15 mL). The reaction mixture was stirred at 35°C overnight. The mixture was dissolved in CH_2Cl_2 (30 mL); the solution was then washed with 5% $NaHCO_3$ (2× 20 mL) and dried over Na_2SO_4. After filtration and evaporation of solvent, the crude product was purified on a silica gel column, with elution with CH_2Cl_2/MeOH (60:1), to give compound **12** as a colorless oil (56 mg, 94%): ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.32$ (d, $J = 8.4$, 1H), 7.87 (d, $J = 7.5$, 1H), 7.81 (d, $J = 9.3$, 1H), 7.63 (t, 1H), 7.43 (m, 2H), 3.36 (s, 3H), 1.76 ppm (s, 3H); ^{13}C NMR (75 MHz, CD_3OD): $\delta = 141.1$, 133.4, 131.7, 131.8, 123.1, 128.1, 127.9, 127.4, 126.4, 123.8, 122.4, 37.3, 22.3 ppm;

HR ESI-MS: m/z calcd for $C_{13}H_{13}BrNO$: 278.0181 $[M+H]^+$; found: 278.0190.

***N*-[5-(5,5-Dimethyl-[1,3,2]dioxaboronan-2-yl)-naphthalen-1-yl]-*N*-methylacetamide (13)**: Compound **13** was synthesized from **12** by following the equivalent reaction procedure to that used for the synthesis of **9**. The crude product was purified on a silica gel column, with elution with CH_2Cl_2/hexanes (2:1), to give compound **13** as a white powder (70%): ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.81$ (d, $J = 8.7$, 1H), 8.11 (d, $J = 6.9$, 1H), 7.89 (d, $J = 8.4$, 1H), 7.62–7.52 (m, 2H), 7.36 (d, $J = 7.2$, 2H), 3.94 (s, 4H), 3.38 (s, 3H), 1.78 (s, 3H), 1.15 ppm (s, 6H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 171.5$, 140.8, 137.9, 135.0, 130.0, 129.1, 128.6, 126.6, 125.8, 124.7, 122.2 ppm; EI-MS: m/z calcd for $C_{18}H_{22}BNO_3$: 311.2 $[M^+]$; found: 311.0.

5-(Acetyl-methylamino)-naphthalene-1-boronic acid (5-AMANBA, 4): Compound **4** was synthesized from **15** by following the same deprotection procedure as that used for the synthesis of **1**. The crude product was purified on a silica gel column, with elution with CH_2Cl_2/MeOH (30:1), to give **4** as a white powder (45%): ^{1}H NMR (300 MHz, $CDOD_3$): $\delta = 7.92$ (d, $J = 8.1$, 1H), 7.78 (d, $J = 3.6$, 1H), 7.64 (m, 3H), 7.50 (d, $J = 6.6$, 1H), 4.87 (s, 2H), 3.30 (s, 3H), 1.73 ppm (s, 3H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 173.7$, 141.9, 137.6, 132.1, 130.7, 130.1, 128.1, 127.2, 126.4, 123.7 ppm; HR ESI-MS: m/z calcd for $C_{13}H_{15}BNO_3$: 244.1145 $[M+H]^+$; found: 244.1146.

Procedures for the binding studies: Distinct solutions of the sensors and the sugars (various concentrations) were prepared in 0.1 M phosphate buffer at pH 7.40. Then, a sensor solution (2 mL) was mixed with a sugar solution (2 mL). After stirring for 20 min, the mixture was transferred into a 1-cm quartz cuvette and the fluorescence intensity was recorded immediately.

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[14] CCDC-279133 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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