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3-[2-(Boronophenyl)benzoxazol-5-yl]alanine derivatives as fluorescent monosaccharide sensors

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ABSTRACT

Two 3-[2-(boronophenyl)benzoxazol-5-yl]alanine derivatives were synthesized and their potential application as fluorescent monosaccharide sensors was studied. It was found that both non-proteinogenic amino acids bound glucose and fructose at physiological pH, however, the latter much stronger. As a result they are selective sensors for fructose. Moreover, one of them (3-[2-(3-boronophenyl)benzoxazol-5-yl]alanine methyl ester) can be used to quickly distinguish, which monosaccharide is present in the solution because of the different character of fluorescence intensity changes (increase in the presence of fructose and decrease in the presence of glucose).

1. Introduction

The design of receptors for neutral molecules, such as monosaccharides, that exhibit both high affinity and high selectivity is recently at the center of interest of many research groups.[1–12]

The mechanism of monosaccharide detection by designed sensors is often based on hydrogen bond formation but only in aprotic solvents. In aqueous environment, the boronic acid is used. This functional group interacts strongly and reversibly with 1,2- or 1,3-diols forming five- or six-membered ring, which was first reported in 1959.[13] The result of this covalent binding with monosaccharides is the formation of a cyclic boronic ester leading to an increase in the Lewis acidity of the boron atom.[1,2,13–17] This ester forms faster in aqueous basic media where the boron atom is present in its tetrahedral anionic form.[13,17–20] However, the diol complexation process is affected by common buffer components, such as phosphate, which may diminish the observed stability constants.[17,21]

Based on the above-described mechanism, many saccharide sensing molecules have been designed and studied.[1–17,21–48] These compounds are used for saccharide detection in many analytical methods with applications in nanostructures[37,38] as well as optical methods.[1–12,17,21–37,40–50] The latter, based on fluorescence and absorption measurements, are especially promising in the selective detection of very low concentrations of the sensing molecule. These parameters are easy for the quantitative detection in comparison to other analytical methods.

In the search for new fluorescent monosaccharide sensors, we synthesized two non-proteinogenic amino acids based on the benzoxazol-5-ylalanine skeleton with a phenylboronic acid moiety and studied their interactions with monosaccharides.

2. Results and discussion

2.1. Synthesis

N-Boc-3-[2-(4-boronophenyl)benzoxazol-5-yl]alanine methyl ester (**1**) and *N*-Boc-3-[2-(3-boronophenyl)benzoxazol-5-yl]alanine methyl ester (**2**) were obtained from *N*-Boc-3-aminotyrosine methyl ester, via the intermediate Schiff base which underwent oxidative cyclization to the heterocyclic compound in the presence of lead tetraacetate in DMSO, according to the procedure published previously.[51,52] The removal of the Boc group was performed by means of acidic hydrolysis giving both studied compounds (**1a** and **2a**, Fig. 1).

2.2. Acid–base properties

2.2.1. Absorption measurements. Absorption spectra of both the studied compounds in water lay in the near ultraviolet upto about 340 nm and have poor vibrational structure. Their maxima are at about 307 nm (ε=18,000 $cm^{-1}M^{-1}$) and 302 nm (ε=13,900 $cm^{-1}M^{-1}$) for **1a** and **2a**, respectively. The position and

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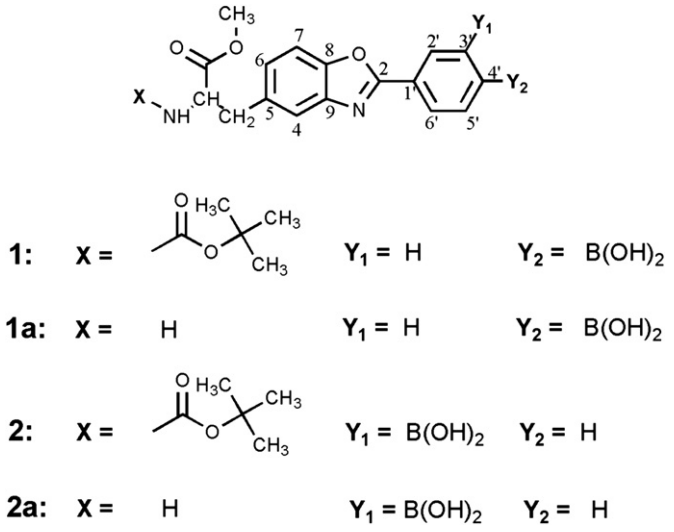

Fig. 1. Structures of synthesized compounds.

shape of these spectra do not change significantly with an increase in pH (Figs. 2 and 3). Only a slight increase of the absorbance is observed in the studied pH range (from 3.7 to 10.6). From these results, pK_a values in the ground state were determined using global analysis, which takes into account absorbance changes at different wavelengths. For compound **1a**, pK_a is equal to 8.05±0.9 whereas for **2a** – 8.09±0.16. These values are comparable to these determined for other monoboronic acid derivatives.[5,30,45,49]

In order to check the stability of these compounds at different pH, the measurements were repeated for all solutions after 24 and 48 h (Figs. 1S–6S). It was observed that the absorbance increases more with time, especially in the strongly alkaline environment (for pH >9.5, Figs. 1S and 4S). Moreover, it results in slight decline of pK_a value (7.86±0.20 and 8.05±0.22 for **1a** and **2a**, respectively, after 24 h and 7.68±0.21 and 7.89±0.22 for **1a** and **2a**, respectively, after 48 h, Table 1S). The observed changes are smaller in the case of compound **2a** than **1a** indicating on its higher stability.

The influence of pH on the position and shape of the absorption spectra of both compounds in the presence of 50 mM of fructose or glucose is similar as in the aforementioned case. However, pK_a values of compounds studied in the presence of fructose decrease significantly to the value of 5.89±0.11 for **1a** and 5.54±0.12 for **2a**. The influence of glucose is smaller as pK_a values are equal to 7.86±0.14 and 7.29±0.16 for **1a** and **2a**, respectively. Such changes are in agreement with those observed for other monoboronic acid esters with monosaccharides.[5,13,30,45,49]

The stability experiments reveal that for both compounds studied for pH >9 after 24 h or more, a new absorption band appears with a maximum at 340 nm, at the expense of parent compound absorption, regardless of the monosaccharide used. It is worth mentioning that for the same pH and monosaccharide concentration, the absorption spectra of **2a** changes to a lesser extent than those of **1a** (Figs. 2S, 3S, 5S and 6S). The observed new band may be a result of instability of the benzoxazole moiety in an alkaline environment leading to oxazole ring opening with the restoration of the Schiff base,[53] or the dissociation of boronic acid. However, the data presented in the literature concerning the stability of boronic acid derivatives in alkaline solution do not confirm this supposition.

2.2.2. Fluorescence measurements. Both studied compounds are fluorescent with emission maxima in water at about 360 nm and fluorescence quantum yields equal to 0.13 and 0.09 for **1a** and **2a**, respectively. In both cases the increase in pH causes a decrease in

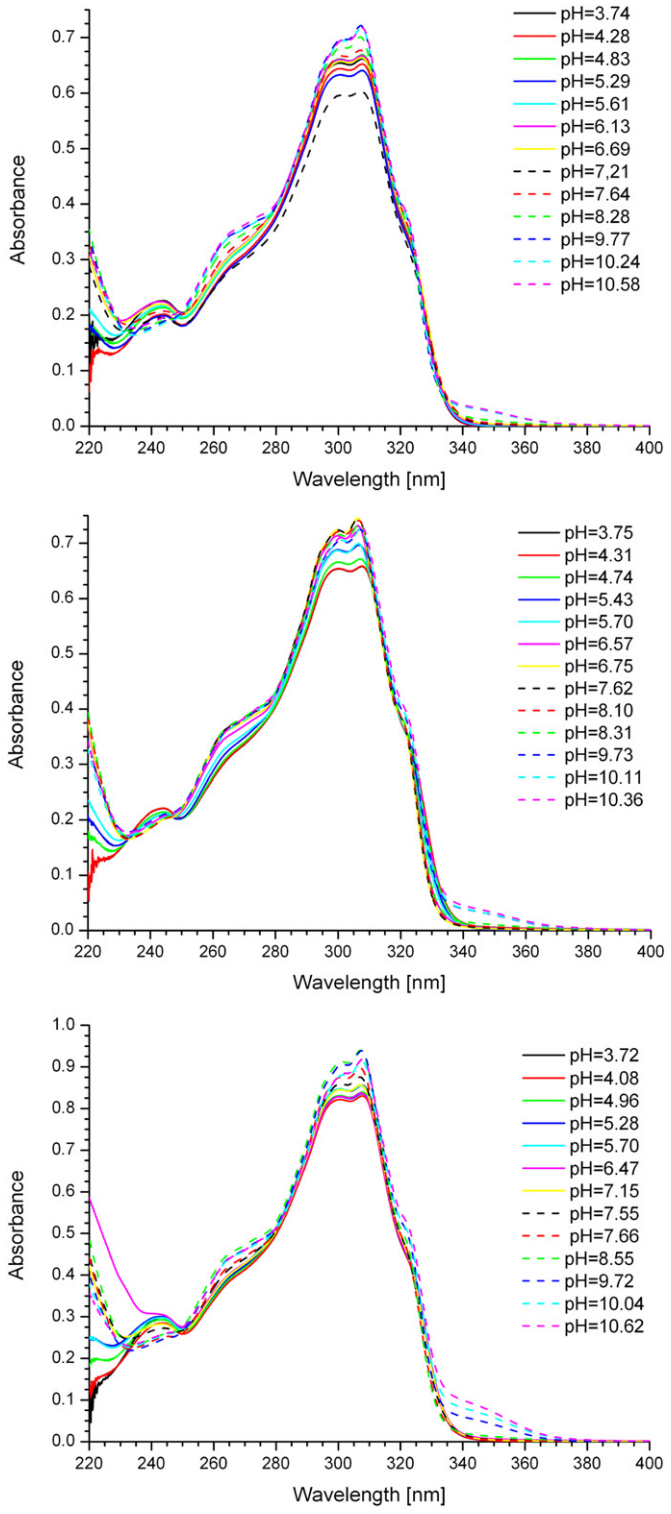

Fig. 2. Absorption spectra of **1a** in water solutions of different pH in the absence (at the top) and in the presence of 50 mM of fructose (in the middle) or 50 mM of glucose (at the bottom).

fluorescence intensity with a small hypsochromic shift of the spectrum as a result of the change in the electronic properties and the geometry at the boron atom (Figs. 4 and 5).[1,2,13,17] Moreover, for pH >9 a diffuse vibrational structure appears. The apparent pK_a values in the excited state, calculated from the titration curves, are equal to 7.32±0.03 for **1a** and 7.26±0.03 for **2a** (Table 1, Fig. 6). Thus, in the excited state both compounds are stronger acids than in the

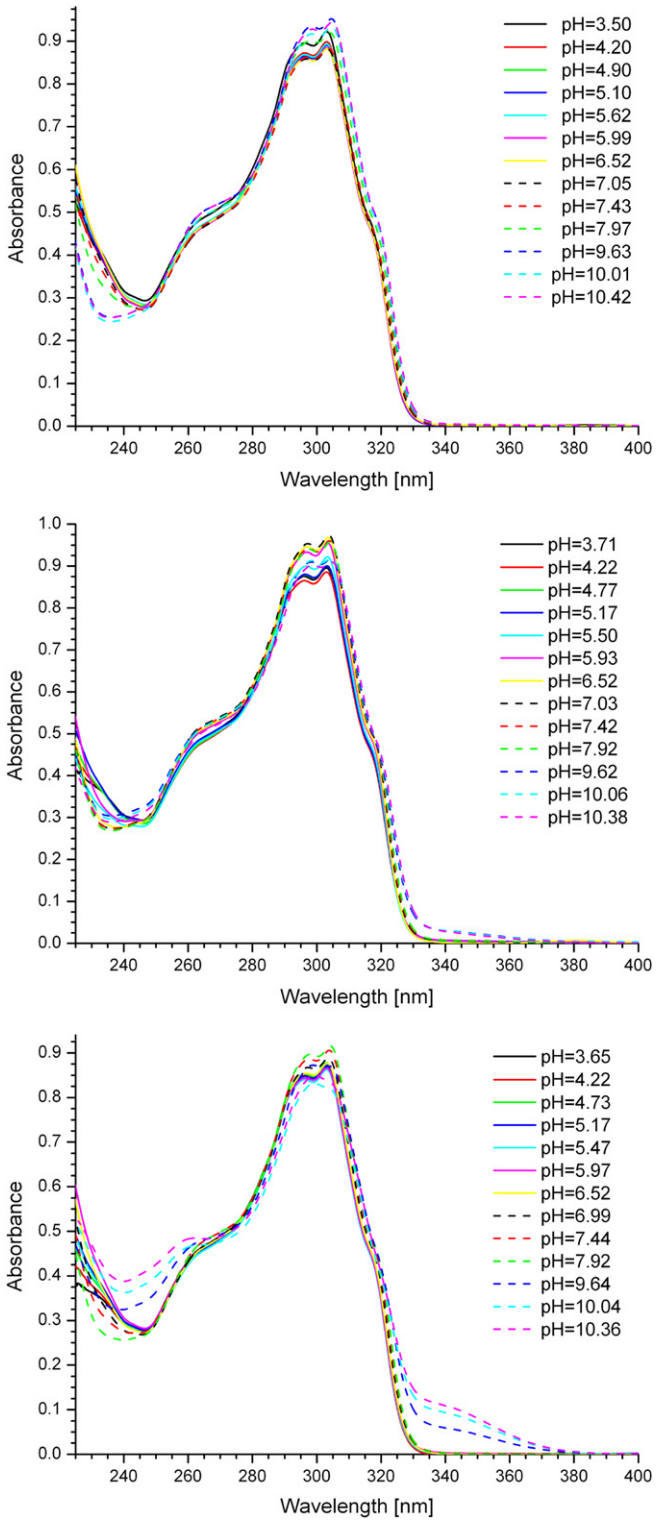

Fig. 3. Absorption spectra of **2a** in water solutions of different pH in the absence (at the top) and in the presence of 50 mM of fructose (in the middle) or 50 mM of glucose (at the bottom).

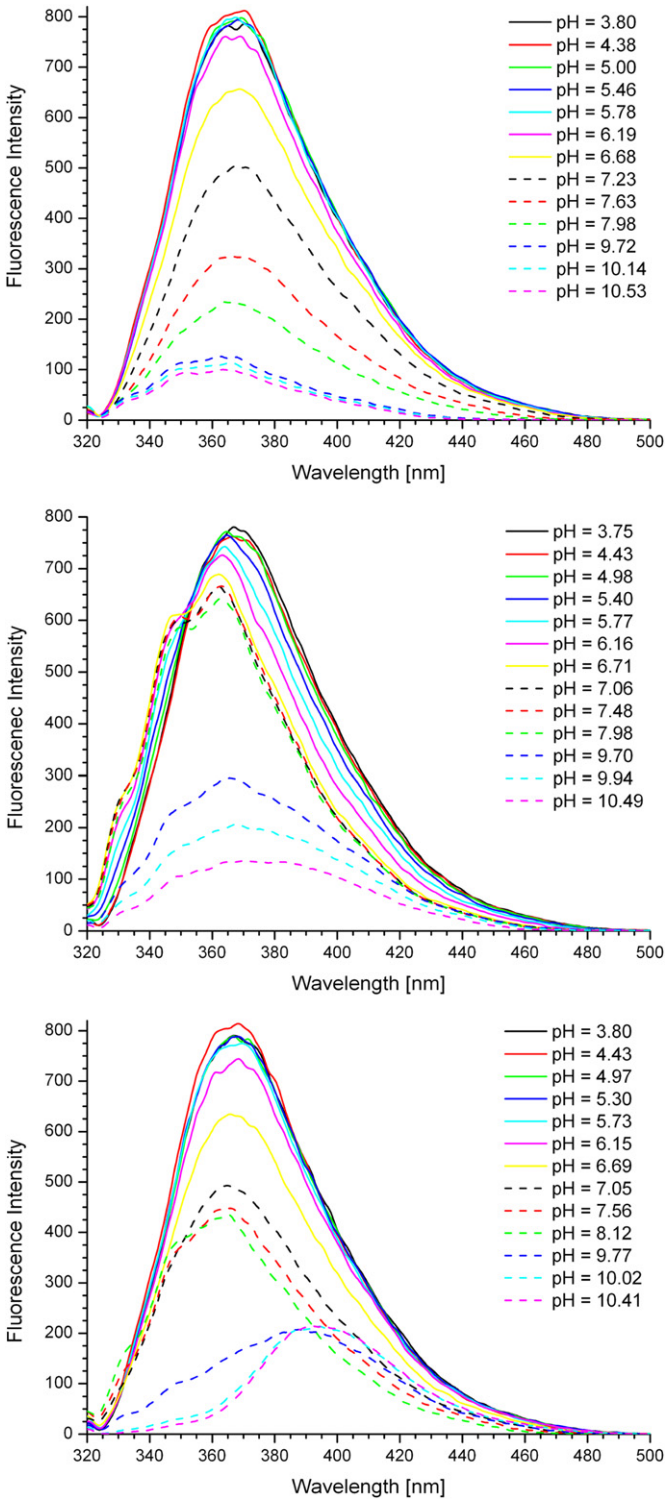

Fig. 4. Fluorescence spectra of **1a** in water solution of different pH in the absence (at the top) and in the presence of 50 mM of fructose (in the middle) or 50 mM of glucose (at the bottom).

ground state. Moreover, regardless of the position of boronic group in the phenyl ring, both derivatives show very similar acid–base properties.

The pH titration of both compounds in the presence of 50 mM of monosaccharide (fructose or glucose) reveals that increase of pH causes a decrease in fluorescence intensity. Moreover, in the case of **1a**, a simultaneous blue shift is observed. For solutions with pH >7, the diffuse vibrational structure of the emission band appears. It is more pronounced in the presence of fructose suggesting a stiffening of the compound structure probably as a result of interactions between the boronic group and the monosaccharide. The observed quenching effect of the fluorescence intensity in the presence of a monosaccharide is much smaller in comparison with pure

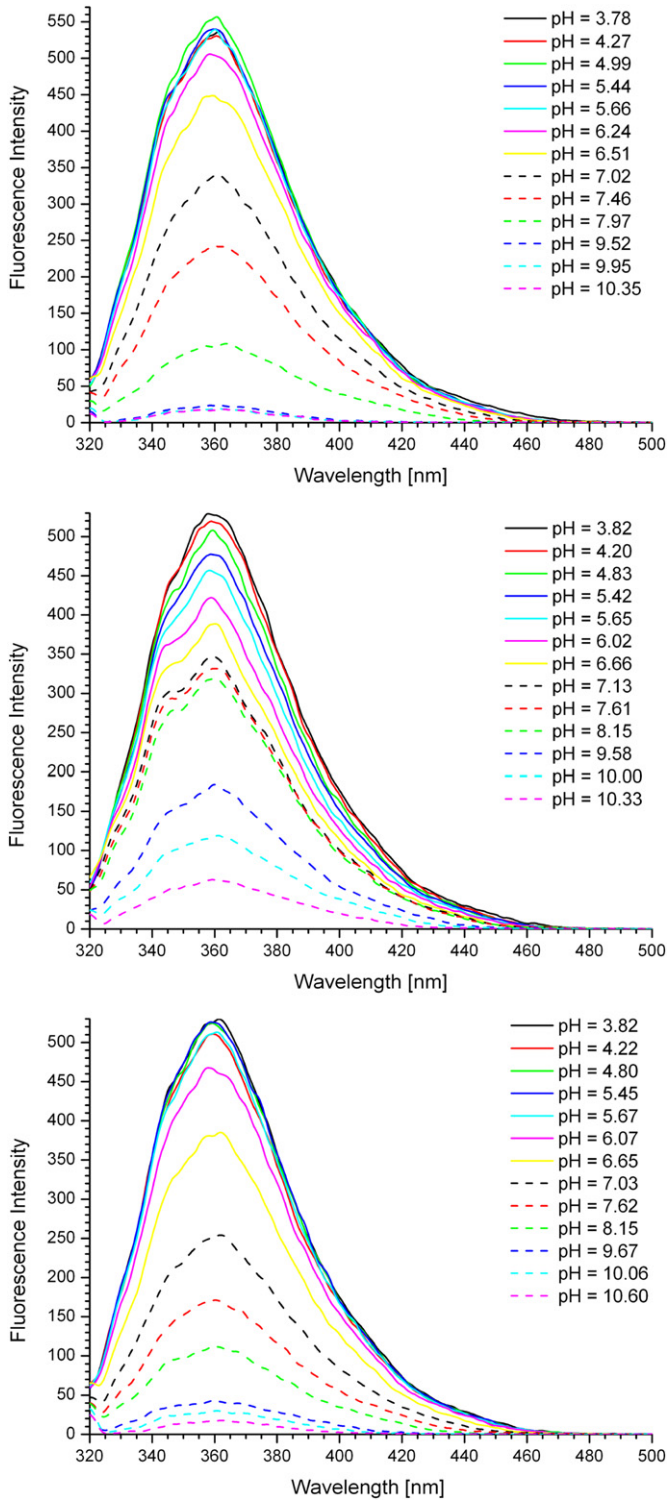

Fig. 5. Fluorescence spectra of **2a** in water solutions of different pH in the absence (at the top) and in the presence of 50 mM of fructose (in the middle) or 50 mM of glucose (at the bottom).

Table 1
The pK_a values of compounds studied in the ground and excited state (pK_a*) in the absence and presence of 50 mM of fructose or glucose determined using global analysis (pK_a) or single data file analysis (pK_a*)

	pK_a	R^2	pK_a*	r^2
1a	8.05±0.09	0.9997	7.32±0.03	0.9982
1a+50 mM of fructose	5.89±0.11	0.9680	6.51±0.11	0.9806
1a+50 mM of glucose	7.86±0.14	0.9217	6.74±0.09	0.9865
2a	8.07±0.16	0.9988	7.26±0.03	0.9976
2a+50 mM of fructose	5.54±0.12	0.9462	6.01±0.10	0.9838
2a+50 mM of glucose	7.29±0.16	0.9514	6.88±0.06	0.9943

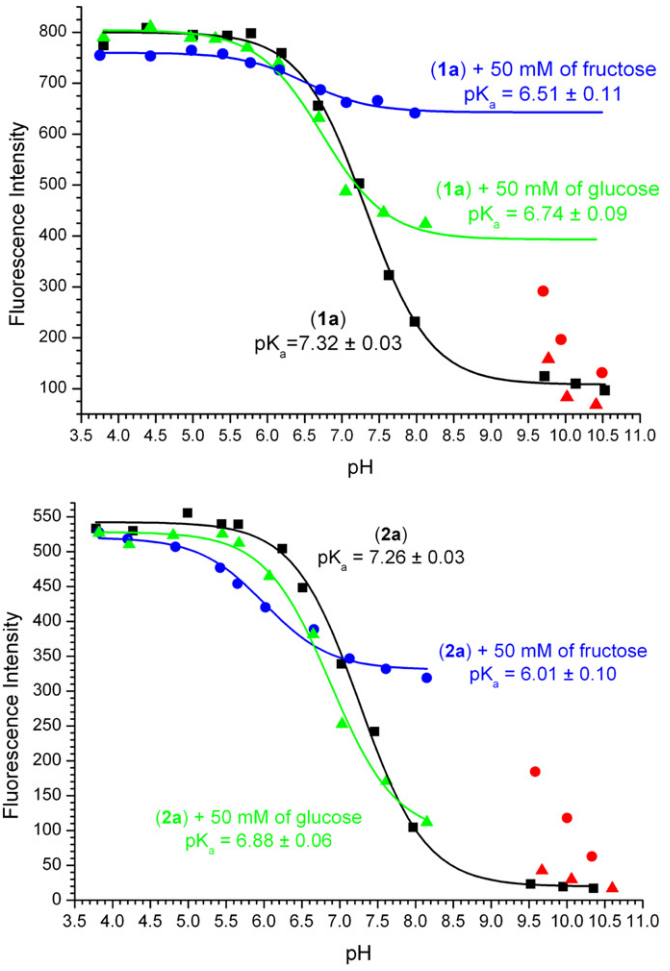

Fig. 6. The dependence of fluorescence intensity on pH for **1a** (at the top) and **2a** (at the bottom) in the absence (black points) and in the presence of 50 mM of fructose (blue points) or glucose (green points). The red points were disregarded in the pK_a calculations.

compounds (for **1a**: about 14% in the presence of fructose and 54% in the presence of glucose; for **2a**: about 60% in the presence of fructose and 20% in the presence of glucose). However, for solutions with pH >8, a violent reduction in fluorescence intensity is observed. For compound **1a** the reduction is about 40% of initial intensity in the presence of fructose and 25% in the presence of glucose, together with the red shift of the emission band (clearly visible in the spectra recorded after 24 h, Fig. 7S). It is probably a result of the benzoxazole ring opening and formation of a weak fluorescent Schiff base whose emission band maximum is about 400 nm. In the case of **2a**, in the presence of glucose the fluorescence intensity is totally quenched, whereas in the presence of fructose it is equal to about 90% of initial intensity (Fig. 8S).

The calculated pK_a values in the presence of monosaccharides are smaller than those for the pure compounds (Table 1, Fig. 6) indicating that both compounds studied are stronger acids in their presence, especially fructose. It is probably a result of interaction between the compound and monosaccharide resulting in formation of an electron-rich boronic anion.[13,45–48]

2.3. Interaction with monosaccharides

2.3.1. Absorption measurements. The presence of a monosaccharide in solution (pH=7.5, phosphate buffer) does not influence the shape and position of the absorption band of both compounds studied, however, an increase in monosaccharide concentration results in an increase in absorbance (Fig. 7, Figs. 9S–11S). The titration curves reveal a linear increase in absorbance for high monosaccharide concentration, which is connected with the light scattering by precipitated monosaccharide microcrystals (Fig. 7, Figs. 9S–11S). This effect was confirmed by an independent experiment—spectrophotometric titration of the buffer with monosaccharide (not shown). Thus, binding constant calculations were performed assuming the presence of two equilibrium constants, one denoting the actual binding constant with monosaccharide, while the other was added to include the impact of scattered light and has no physical meaning. However, such an approach (two equilibrium constants) significantly improves the quality of the fit.

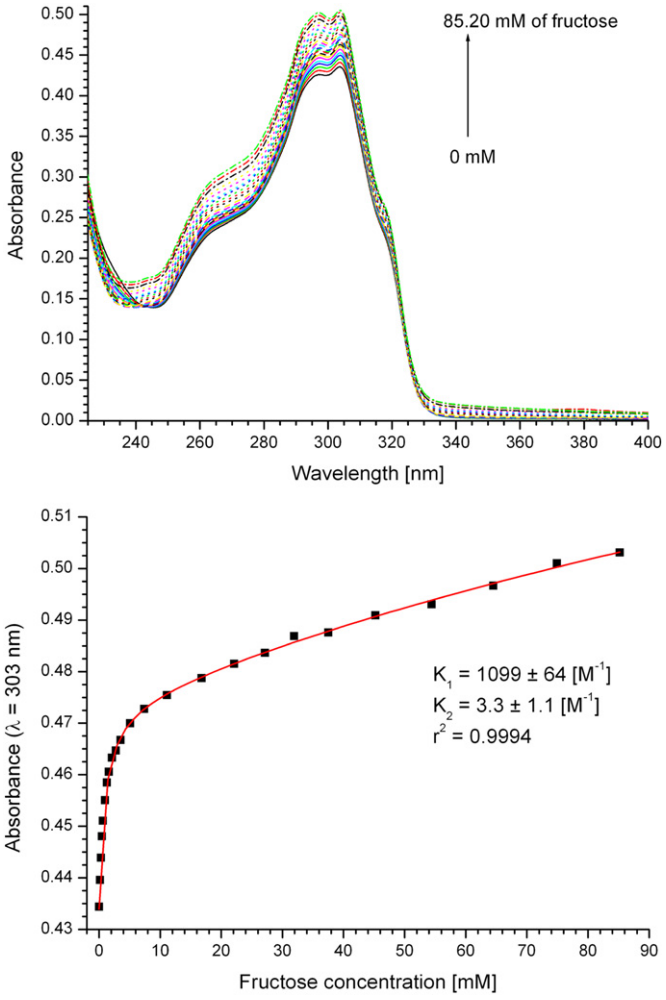

Fig. 7. Absorption spectra of **2a** in phosphate buffer (pH=7.5) in the presence of increasing concentrations of fructose (at the top) and the obtained binding curve (at the bottom).

The obtained binding constants (Table 2) indicate that fructose is more strongly bound by both compounds than glucose. Moreover, compound **1a** interacts sligthly stronger with both monosaccharides. Such effects of monosaccharides binding is characteristic for monoboronic acid derivatives,[1,2,13,30,35,37,47] however, the obtained binding constants for these compounds are higher than those reported by

Table 2
The binding constants values of compounds studied with fructose and glucose in phosphate buffer (pH=7.5)

	1a		**2a**	
	Fructose	Glucose	Fructose	Glucose
K [M^{-1}] (absorption)	895±71	24.9±0.8	847±34	14.2±0.1
R^2	0.9942	0.9988	0.9993	0.9964
K [M^{-1}] (fluorescence)	1419±19	27.7±0.8	1135±26	17.0±0.3
R^2	0.9999	0.9995	0.9995	0.9999

Wang et al. for dibenzofuran-4-boronic acid (514 M^{-1} for fructose and 0.6 M^{-1} for glucose),[30] 6-(*N*,*N*-dimethylamino)-naphthalene-2-boronic acid (120 M^{-1} and 2.4 M^{-1} for fructose and glucose, respectively),[35] or 5-(*N*,*N*-dimethylamino)-naphthalene-1-boronic acid (311 M^{-1} and 3.6 M^{-1} for fructose and glucose, respectively),[37] but comparable with those obtained for some of stilbene derivatives of boronic acid, published by Lakowicz et al.[45]

2.3.2. Fluorescence measurements. For both compounds, an increase in the monosaccharide concentration results in a small blue shift of the emission band, as well as appearance of its diffuse vibrational structure (Figs. 8–10). Moreover, the significant changes of fluorescence intensity are observed but their character depends on the monosaccharide used.

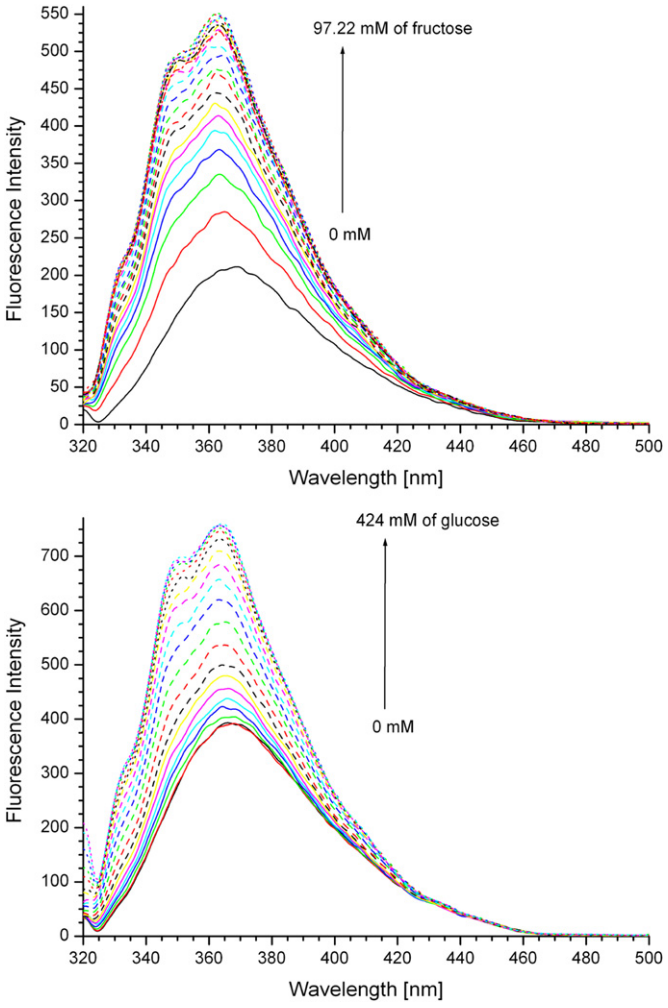

Fig. 8. Fluorescence spectra of **1a** in phosphate buffer (pH=7.5) in the presence of increasing concentrations of fructose (at the top) or glucose (at the bottom).

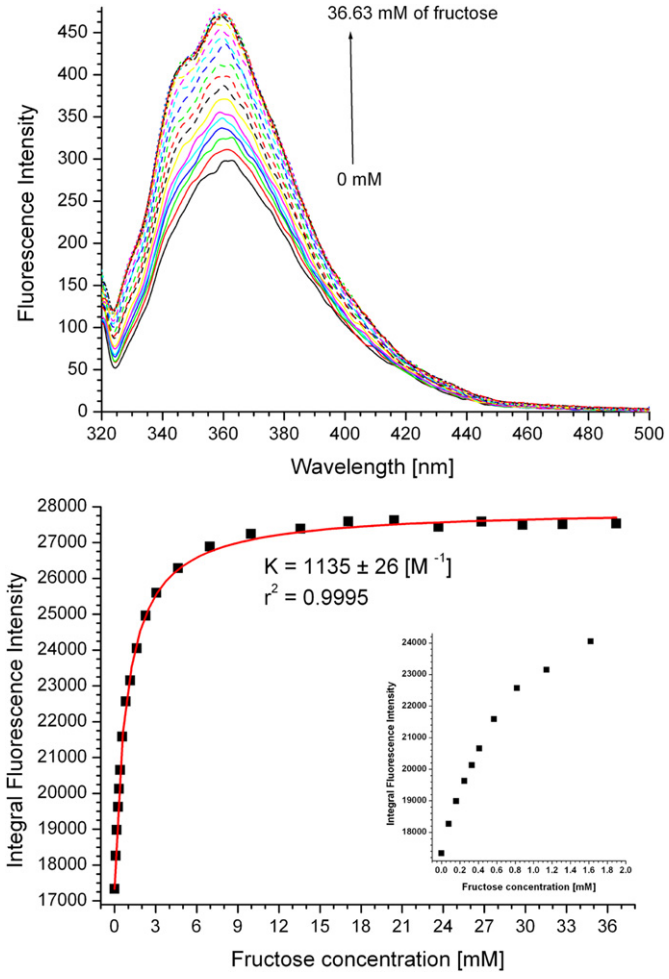

Fig. 9. Fluorescence spectra of **2a** in phosphate buffer (pH=7.5) in the presence of increasing concentrations of fructose (at the top) and obtained binding curve (at the bottom).

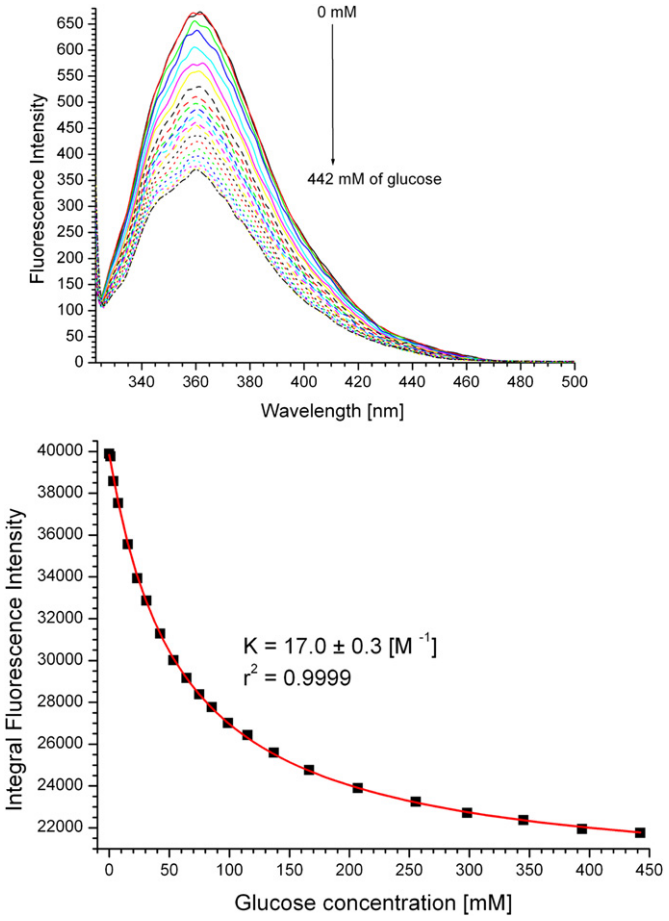

Fig. 10. Fluorescence spectra of **2a** in phosphate buffer (pH=7.5) in the presence of increasing concentrations of glucose (at the top) and obtained binding curve (at the bottom).

The presence of fructose in both cases results in an increase in the fluorescence intensity (Figs. 8 and 9, and Fig. 12S) but the bigger changes are observed for compound **1a** (about 2.4- and 1.5-fold fluorescence enhancement for **1a** and **2a**, respectively, Fig. 11). Also, the calculated binding constant value is higher for **1a** than **2a** indicating a stronger interaction of this compound with fructose. Moreover, for both compounds, the obtained binding constants in the excited state are significantly higher than those in the ground state obtained from the absorption measurements (Table 2).

The presence of glucose in the solution causes an increase in the fluorescence intensity of **1a** (about 1.9-fold, Figs. 8 and 11) whereas the fluorescence intensity of **2a** is quenched (by about 50%, Figs. 8 and 10). Such behavior of isomers may be a result of the different structure of the anionic ester of boronic acid with glucose. For compound **2a**, the structure of the complex formed probably allows glucose hydroxyl groups to interact with the heteroatom(s) of the oxazole moiety through the hydrogen bonds resulting in fluorescence quenching. The calculated binding constants of glucose in the excited state are small and comparable to these obtained from the absorption titration (Table 2).

The observed stronger interaction of both compounds with fructose is consistent with the literature: for all simple monoboronic acids the binding constants increase as follows: D-fructose>D-galactose>D-glucose.[1,5,13,15–17,26,54] Also, greater fluorescence enhancement on the addition of fructose than glucose (Fig. 11) has been observed for other saccharide sensors.[1,22,23,25,49,50] It is worth

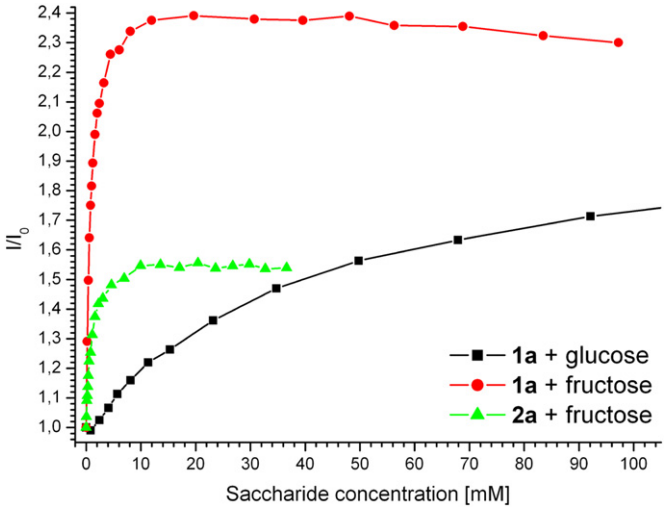

Fig. 11. Relative fluorescence intensity (I/I_0) versus saccharide (fructose or glucose) concentration profile of **1a** or **2a** in phosphate buffer (pH=7.5).

mentioning that both compounds enabled the determination of small fructose concentrations as the maximal changes in their fluorescence intensity are observed up to about 10 mM of fructose (Fig. 11). Compound **1a** saturates with glucose at concentrations higher than 100 mM (Fig. 11).

The interaction of benzoxazolylalanine derivatives with monosaccharides was also studied by means of time-resolved fluorescence spectroscopy. It was found that the fluorescence intensity decays of both pure compounds in phosphate buffer at pH=7.5 are heterogeneous (Table 3). This heterogeneity of the fluorescence intensity decay decreases in the presence of fructose, especially in the case of **1a** (the fluorescence intensity decay becomes monoexponential), in contrast to the presence of glucose. These data also indicate that the binding of monosaccharide causes the shortening of the decay times, however, these are minor changes only. Thus, these compounds cannot be used as lifetime sensitive monosaccharide sensors.

Table 3
The fluorescence lifetimes (τ), pre-exponential factors (α) and values of χ^2_R parameter for **1a** and **2a** in the phosphate buffer (pH=7.5) in the absence and presence of fructose or glucose

Compound	τ_i [ns]	α_i	χ^2_R
1a (c=15 μM)	1.41	1.00	7.02
	1.57	0.30	1.02
	0.28	0.70	
1a (c=15 μM)+fructose (c=50 mM)	1.16	1.00	1.15
1a (c=15 μM)+glucose (c=50 mM)	1.14	1.00	4.21
	1.36	0.38	1.09
	0.58	0.62	
2a (c=15 μM)	1.54	1.00	3.55
	1.69	0.54	0.94
	0.51	0.46	
2a (c=9 μM)+fructose (c=37 mM)	1.31	1.00	1.76
	1.39	0.67	0.99
	0.51	0.33	
2a (c=5 μM)+glucose (c=442 mM)	1.06	1.00	45.71
	1.56	0.14	1.12
	0.42	0.86	

3. Conclusion

The studies of interactions of two 3-[2-(borophenyl)benzoxazol-5-yl]alanine derivatives with monosaccharides revealed that these non-proteinogenic amino acids may be used as efficient fluorescent fructose sensors in water. Despite some limitations, such as low stability in alkaline environment as well as absorption in the UV range, both of them show, at physiological pH, significant fluorescence intensity changes after binding fructose, which is very strong (high binding constant value). Moreover, small value of glucose binding constant makes them selective sensors for fructose in the presence of glucose. Additionally, because of the different character of fluorescence intensity changes, compound **2a** can be used to quickly distinguish, which monosaccharide (glucose or fructose) is present in the solution. Due to the presence of an amino acid moiety these compounds may be used in biological applications as monosaccharide sensor residues in peptides.

4. Experimental

4.1. Synthesis

4.1.1. General. *N*-Boc-3-nitro-L-tyrosine methyl ester was prepared according to literature procedure.[55,56] 4-Formylphenylboronic acid and 3-formylphenylboronic acid were purchased from Lancaster whereas lead tetraacetate from Sigma–Aldrich and used as received. TLC was carried out on Merck silica gel plates (Kieselgel 60 F_{254}). The spots were revealed using a UV lamp (254 nm, 366 nm). All solvent ratios are in volume parts. The purification was carried out by means of column chromatography (Merck, Silica gel 60, 0.040–0.063 mm) and semi-preparative RP-HPLC (Kromasil column, C-8, 5 μm, 250 mm long, ID=20 mm or Supelcosil™ SPLC-ABZ column, C-18, 5 μm, 250 mm long, ID=10 mm). The purity of the obtained compounds was checked by means of analytical RP-HPLC (Kromasil column, C-8, 5 μm, 250 mm long, ID=4.6 mm) with detection at 223 nm. Melting points (mp) were determined in capillary tubes using Gallenkamp Griffin MPA-350.MB2.5 apparatus and are uncorrected. Specific rotation ($[\alpha]_D^{20}$) was determined on a Perkin Elmer 343 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Varian, Mercury-400 BB spectrometer (400 and 100 MHz, respectively) in DMSO-d_6. Infrared spectra were recorded on a Bruker IFS-66 instrument. Mass spectra were recorded on a Bruker Biflex III instrument (MALDI-TOF). Elemental analysis were taken on a Carlo Erba CHNS–O EA1108 instrument.

*4.1.2. N-Boc-3-[2-(4-boronophenyl)benzoxazol-5-yl]alanine methyl ester (**1**) and N-Boc-3-[2-(3-boronophenyl)benzoxazol-5-yl]alanine methyl ester (**2**).* Both compounds were obtained according to the procedure published previously.[51,52]

A mixture of *N*-Boc-3-nitro-L-tyrosine methyl ester (0.69 g, 2.04 mmol) and 5% palladium on active carbon in MeOH (20 mL) was stirred under a hydrogen atmosphere at rt for about 90 min. The catalyst was filtered off and the solvent evaporated in vacuo to give a brownish oily product, which was dissolved in absolute EtOH (3 mL) and mixed with a solution of appropriate aldehyde (4-formyphenylboronic acid or 3-formylphenylboronic acid, 0.31 g, 2.06 mmol) in absolute EtOH (7 mL). The mixture was stirred at rt overnight (TLC monitoring). After this time the solvent was removed by evaporation and the obtained Schiff base (brown oil, R_f=0.57 (AcOEt) for (**1**), R_f=0.76 (AcOEt/petroleum ether 2:1) for (**2**)) was dissolved in DMSO (10 mL) and lead tetraacetate (1.36 g, 3.07 mmol) was added. The mixture was stirred at rt for an hour (TLC monitoring) and then dissolved in ethyl acetate (AcOEt, 30 mL) and washed in turn with a saturated aqueous solution of NaCl (3×). The combined organic layers were dried over anhydrous $MgSO_4$ and evaporated in vacuo giving the product, which was isolated by means of column chromatography ((**1**)—AcOEt/petroleum ether 2:1; (**2**)—AcOEt). The crude product was recrystallized from AcOEt/petroleum ether giving compound (**1**) as a yellowish solid (0.52 g, 1.18 mmol, 58%) or compound (**2**) as a brown solid (0.26 g, 0.59 mmol, 29%). The purity of the obtained compound was checked by means of TLC ((**1**): R_f=0.61 (AcOEt/petroleum ether 2:1); (**2**): R_f=0.64 (AcOEt)) and analytical RP-HPLC (t_R=52.5 min (**1**), t_R=52.6 min (**2**), the mobile phase was a gradient running from 0% to 100% of B over 60 min (A=0.01% water solution of trifluoroacetic acid (TFA), B=80% of acetonitrile in A)).

Compound (**1**): [found: C, 59.62; H, 6.06; N, 6.03. $C_{22}H_{25}BN_2O_7$ requires C, 60.02; H, 5.72; N, 6.36%]; mp 276–278 °C (decomposition); $[\alpha]_D^{20}$ +24.6 (*c* 0.013, MeOH); ν_{max} (KBr) 3612.1, 3425.4, 3342.2, 3071.4, 2981.5, 2927.5, 1738.6, 1686.4, 1571.4, 1548.5, 1503.6–1407.0, 1372.7–1163.4, 1057.9, 926.0–709.8 cm^{-1}; δ_H (400 MHz, DMSO-d_6) 1.30 (9H, s, $(CH_3)_3$), 2.96–3.18 (2H, m, $C^{\beta}H_2$), 3.63 (3H, s, OCH_3), 4.22–4.28 (1H, m, $C^{\alpha}H$), 7.30–7.33 (2H, m, C^6H, NH), 7.70 (2H, d, *J* 8.01 Hz, C^4H, C^7H), 8.00 (2H, dd, *J* 2.00, 6.61 Hz, $C^{2'}H$, $C^{6'}H$), 8.15 (2H, dd, *J* 2.00, 6.41 Hz, $C^{3'}H$, $C^{5'}H$), 8.21 (2H, s, $B(OH)_2$); δ_C (100 MHz, DMSO-d_6) 28.02 $(CH_3)_3$, 36.30 C^{β}, 51.75 OCH_3, 55.44 C^{α}, 78.25 $C^{t\text{-Bu}}$, 110.36 C^7, 120.27 C^4, 126.04 C^6, 126.74 $C^{2'}$, $C^{6'}$, 127.57 $C^{3'}$, $C^{5'}$, 134.50 $C^{1'}$, $C^{4'}$, 134.75 C^5, 141.25 C^9, 149.06 C^8, 155.37 NHCO, 162.57 C^2, 172.44 CO; *m*/*z* (MALDI): MH^+, found 441. $C_{22}H_{25}BN_2O_7$ requires 440.

Compound (**2**): [found: C, 59.95; H, 5.85; N, 6.02. $C_{22}H_{25}BN_2O_7$ requires C, 60.02; H, 5.72; N, 6.36%]; mp 178–180 °C (decomposition); $[\alpha]_D^{20}$ +22.8 (*c* 0.016, MeOH); ν_{max} (KBr) 3462.9, 3348.1, 2971.1, 1737.3, 1683.2, 1548.8, 1519.9, 1475.3, 1438.4, 1366.6–1166.9, 1066.6, 1018.4, 971.4–699.1 cm^{-1}; δ_H (400 MHz, DMSO-d_6) 1.30 (9H, s, $(CH_3)_3$), 2.97–3.18 (2H, m, $C^{\beta}H_2$), 3.64 (3H, s, OCH_3), 4.22–4.28 (1H, m, $C^{\alpha}H$), 7.31 (1H, dd, *J* 2.00, 8.21 Hz, C^6H), 7.34 (1H, s, NH), 7.58 (1H, t, *J* 8.01, 7.61 Hz, $C^{5'}H$), 7.69 (1H, d, *J* 2.00 Hz, C^7H), 7.71 (1H, s, C^4H), 8.02 (1H, dt, *J* 1.20, 7.61 Hz, $C^{4'}H$),

8.22 (1H, dt, *J* 1.60, 8.01 Hz, $C^{6'}H$), 8.29 (2H, s, $B(OH)_2$), 8.65 (1H, s, $C^{2'}H$); δ_C (100 MHz, DMSO-d_6) 28.02 (CH_3)$_3$, 36.29 C^{β}, 51.74 OCH_3, 55.44 C^{α}, 78.23 $C^{t\text{-Bu}}$, 110.29 C^7, 120.15 C^4, 125.60 C^6, 126.53 $C^{1'}$, 128.30 $C^{2'}$, $C^{5'}$, 128.71 $C^{6'}$, 132.94 $C^{4'}$, 134.41 $C^{3'}$, 137.44 C^5, 141.56 C^9, 149.06 C^8, 155.34 NHCO, 162.81 C^2, 172.44 CO; *m*/*z* (MALDI): MH^+, found 441. $C_{22}H_{25}BN_2O_7$ requires 440.

*4.1.3. 3-[2-(4-Boronophenyl)benzoxazol-5-yl]alanine methyl ester (**1a**) and 3-[2-(3-boronophenyl)benzoxazol-5-yl]alanine methyl ester (**2a**).* To magnetically stirred solution of (**1**) (50 mg, 0.11 mmol) or (**2**) (100 mg, 0.23 mmol) in CH_2Cl_2 (2 mL) trifluoroacetic acid (2 mL) was added. After 1 h solvent was evaporated in vacuo. The oily residue was twice dissolved in toluene and evaporated in vacuo to remove traces of TFA. The crude products were purified by means of semi-preparative RP-HPLC ((**1a**)—Kromasil column, mobile phase—gradient running from 0% to 60% of B over 120 min, detection at 223 nm; (**2a**)—1. Kromasil column, mobile phase—gradient running from 0% to 70% of B over 120 min, detection at 223 nm, 2. Supelcosil column, mobile phase—gradient running from 20% to 60% of B over 120 min, detection at 304 nm, A=0.01% water solution of TFA, B=80% of acetonitrile in A). Compound (**1a**) was obtained as grey solid (40 mg, 0.09 mmol, 86%) whereas compound (**2a**) as a beige solid (22.5 mg, 0.05 mmol, 22%).

Compound (**1a**): t_R=27.8 (gradient running from 0% to 100%B over 60 min); mp 290–293 °C (decomposition); *m*/*z* (MALDI): MH^+, found 341. $C_{17}H_{17}BN_2O_5$ requires 340.

Compound (**2a**): t_R=31.0 (gradient running from 0% to 100%B over 60 min); mp >360 °C (decomposition); *m*/*z* (MALDI): MH^+, found 341. $C_{17}H_{17}BN_2O_5$ requires 340.

4.2. UV–vis and fluorescence measurements

4.2.1. General. Monosaccharide used, D-(+)-glucose and D-(−)-fructose, were purchased from Sigma–Aldrich.

Absorption spectra were measured on a Perkin–Elmer Lambda 40P spectrophotometer at 25 °C, stabilized by a P-1 Peltier system with magnetic stirrer. Fluorescence spectra were measured on a Perkin–Elmer LS-50B spectrofluorometer with magnetic stirrer in the cuvette holder (Electronic Stripper Model 300). The temperature of the solution was maintained using Julabo F26-MP refrigerated circulator. Quantum yields were calculated using 2-aminopyridine in 0.05 M H_2SO_4 (QY=0.605) as a reference and were corrected for different refractive indices of solvents.[41] In all fluorometric measurements, the optical density of the solution does not exceed 0.1.

The fluorescence lifetimes were measured with a time-correlated single-photon counting apparatus Edinburgh CD-900. The excitation source was a NanoLed N15 (λ_{ex}=277 nm) from IBH. The half-width of the response function of the apparatus, measured using a Ludox solution as a scatter, was about 1.0 ns. The emission wavelengths were isolated using a monochromator (about 12 nm spectral band-width). Fluorescence decay data were fitted by the iterative convolution to the sum of exponents according to equation:

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

where α_i is the pre-exponential factor obtained from the fluorescence intensity decay analysis and τ_i the decay time of the *i*-th component, using software supported by the manufacturer. The adequacy of the exponential decay fitting was judged by visual inspection of the plots of weighted residuals as well as by the statistical parameter χ^2_R and shape of the autocorrelation function of the weighted residuals and serial variance ratio (*SVR*).[57]

Titration curves against pH were measured in buffer solutions: acetate buffer for pH 3.50–5.78, phosphate buffer for pH 5.90–8.56 and carbonate buffer for pH 9.46–10.60. The pH of the solution was measured using Hanna Instruments HI 9321 pH-meter with a combined glass electrode.

All calculations were performed using Origin v. 6.1 software.

4.2.2. pH titrations. Three series of thirteen samples of the same concentration of each compound were prepared in appropriate buffer solution (pH from 3.50 to 10.60). Two of them contained additionally 50 mM of monosaccharide (glucose or fructose). For absorption measurements the concentration of compound was about 44 μM for **1a** and 56 μM for **2a** whereas for fluorescence measurements the concentration of both compounds was equal to 15 μM. The spectra of each series were measured just after preparation and additionally after about 24 and 48 h.

Titration curves were fitted and pK_a values (pK_a=−logK_a) were obtained using the equation:

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

where: I_{acid} and I_{base} are the absorbance or fluorescence intensity limits in the acid and basic region, respectively. The single data file and global analysis (simultaneous analysis of all data files with pK_a as common parameter) were used to determine pK_a values.

In the case of fluorometric titrations the measured fluorescence intensity of each sample was corrected for differences in absorbance according to the equation:

$$I_p = I \times \left(1 - 10^{-A_{wz}}\right) \Big/ \left(1 - 10^{-A_{pr}}\right)$$

where: I is measured fluorescence intensity of the sample, I_p—fluorescence intensity corrected for the sample absorbance, A_{wz} and A_{pr} are absorbances of the standard and the sample at the excitation wavelength, respectively.

4.2.3. Monosaccharide binding experiments. In the monosaccharide titration experiments a solution of compound in phosphate buffer pH=7.5 was treated with increasing amounts of a solution of monosaccharide (glucose or fructose) containing a fluorophore at the same concentration as in the cuvette. After each addition, using a Hamilton microsyringe with micrometer screw, the solution was stirred for a few minutes to reach equilibrium and the UV–vis or fluorescence spectrum was subsequently recorded. All titrations were performed twice.

Titration curves against monosaccharide were fitted, and binding constants (K_D) were obtained from global analysis of data obtained from two independent titrations using the equation:

$$I = \frac{(I_0 + I_1 K_D[C])}{1 + K_D[C]} + \frac{(I_0 + I_2 K_S[C])}{1 + K_S[C]}$$

where: I denotes absorbance or integral fluorescence intensity and I_0, I_1 and I_2 are the initial (no monosaccharide), intermediate and final (plateau) intensity on the titration curve. The second part of the equation is added to take into account the effect of light scattering in absorption measurements and K_S has no physical meaning. This part of the equation was omitted in fluorescence data analysis.

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Supplementary data

Spectrophotometric and spectrofluorometric pH titrations of compounds studied measured after 24 and 48 h as well as

appropriate pK_a values in the ground and excited state. Absorption spectra of **1a** and **2a** in phosphate buffer (pH=7.5) in the presence of increasing concentrations of fructose or glucose. The monosaccharide binding curves obtained from absorption and fluorescence measurements together with the fits determined from single data file analysis. Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2012.08.085.

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