JOC Notes

"Click-fluors": Modular Fluorescent Saccharide Sensors Based on a 1,2,3-Triazole Ring

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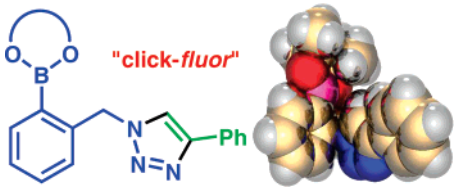

A carbohydrate sensing "*click-fluor*" is reported which displays a nontypical binding preference with sample saccharides. That a fluorescent sensor is generated as a result of triazole formation (*click reaction*), rather than simply bringing the sensor and reporter units together via a triazole linkage, and the potential applicability of the wide range of easily accessible acetylene units means a simple strategy for the development of modular fluorescent sensor arrays for rapid screening of target saccharides will be available.

Saccharides are ubiquitous in nature as building blocks for processes ranging from the production of metabolic energy through to tissue recognition.[1] Despite quantitative analysis and detection of saccharide-containing biomolecules being of paramount importance, reliable and accurate sensors are not widely available.

Many synthetic receptors developed for neutral guests have relied on noncovalent interactions, such as hydrogen bonding, for recognition. It is the case, however, that in aqueous systems neutral guests may become heavily solvated. While biological systems have the capacity to expel water from their binding pockets and sequester analytes wholly while exploiting noncovalent interactions, synthetic monomeric receptors have not yet been designed where hydrogen bonding has been able to compete with bulk water for low concentrations of saccharides.[2,3] The capacity of boronic acid receptors to function effectively in water is reflected by the number of published saccharide sensory systems designed around them. Additionally, boronic acids are known to bind saccharides via covalent interactions in aqueous media.[4−6]

The most common interaction is with *cis*-1,2- or -1,3-diols of saccharides to form five- or six-membered rings, respectively. Formation of this cyclic ester leads to an increase in the Lewis acidity of a central boron atom and it is this property that has been exploited to create saccharide sensing molecules. Typically, boronic acids have either been directly or indirectly (via a linker) connected to fluorophores and the change in boron Lewis acidity following subsequent complexation to a saccharide triggers a change in observed fluorescence.[5,6]

The synthesis of boronic acid containing fluorescent sensors involves either the direct attachment of a boronic acid to a fluorophore, or the attachment of a boronic acid to an amine to create boronic acid derivatives such as ortho, meta, and para sensors **1a**−**c** (Figure 1).[7]

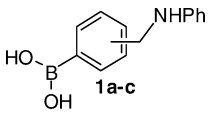

FIGURE 1. Previously reported amino-sensors **1a** *ortho*, **1b** *meta*, and **1c** *para*.

We are particularly interested in developing modular approaches since they offer the possibility of rapidly constructing sensor molecule libraries. Surprisingly, the Huisgen 1,3-dipolar cycloaddition,[8] to the best of our knowledge, has never been employed in the construction of boronic acid sensor molecules even though its importance in fluorescent sensor development has been proposed.[9] *Click chemistry* has the potential to rapidly create fluorescent saccharide sensors from boronic acid azides and any fluorophore bearing a terminal alkyne.

The so-called "*click reaction*"[10] forms an aromatic 1,2,3-triazole ring following the addition of an azide to a terminal

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(11) We have used the phrase "*click-fluor*" to describe the generation of a fluorophore from non-fluorescent constituent parts via a so called "click reaction". In this case, the triazole ring forms an integral part of the fluorophore; i.e., a new property is imparted on a molecule conceived by a "*click reaction*".

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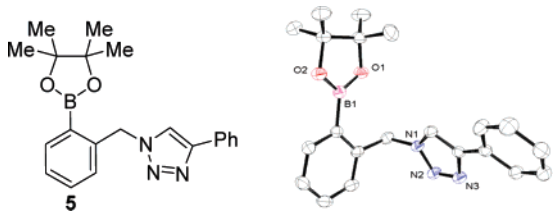
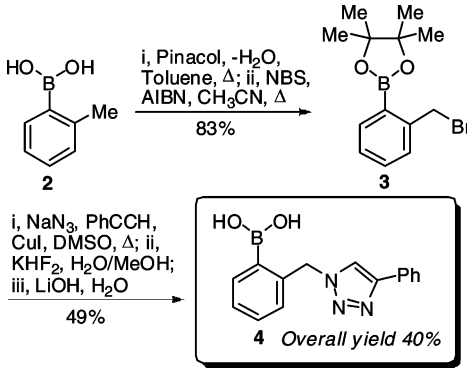


FIGURE 2. Chemical and X-ray structure of intermediate **5**.

SCHEME 1. Five Step Synthesis of "*click-fluor*" 4



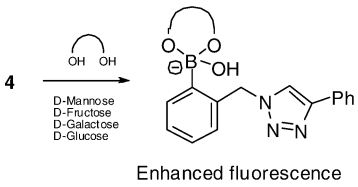
alkyne has the potential to create a fluorescent sensor from nonfluorescent constituent parts.[11]

Fluorescent sensor **4** was prepared as outlined in Scheme 1 in 40% yield over five steps. Commercial boronic acid **2** was pinacol protected and brominated to give boronate ester **3**. Subsequently, a copper(I)-catalyzed azide−alkyne [3 + 2] cycloaddition developed by Ham was employed,[12] enabling the synthesis of 1,2,3-triazole ring as predominantly the 1,4-regioisomer. At this point during the synthesis, X-ray quality crystals of intermediate **5** were obtained (Figure 2).[13] The target sensor **4** was then obtained via a two-step deprotection of the pinacol ester.[14]

The fluorescence titrations of **4** (1.67×10^{-6} mol dm^{-3}) with different saccharides were carried out in a pH 8.21 buffer (52.1 wt % methanol in water with KCl, 0.01000 mol dm^{-3}; KH_2PO_4, 0.002752 mol dm^{-3}; Na_2HPO_4, 0.002757 mol dm^{-3}).[15] The fluorescence spectra of **4** in the presence of D-fructose (0−2.2 mol dm^{-3}) are shown in Scheme 2 and Figure 3 (upper); the analogous spectra for the addition of D-glucose, D-galactose, and D-mannose are available as Supporting Information.

The stability constants (K) of fluorescence sensor **4** with D-fructose, D-glucose, D-galactose, and D-mannose were calculated by fitting the emission intensity at 430 nm (λ_{ex} 276 nm) vs concentration of saccharides (Figure 3, lower). The stability constants calculated from these titrations are given in Table 1.[16,17]

SCHEME 2. Binding of Different Saccharides with Sensor 4 in Aqueous Solution



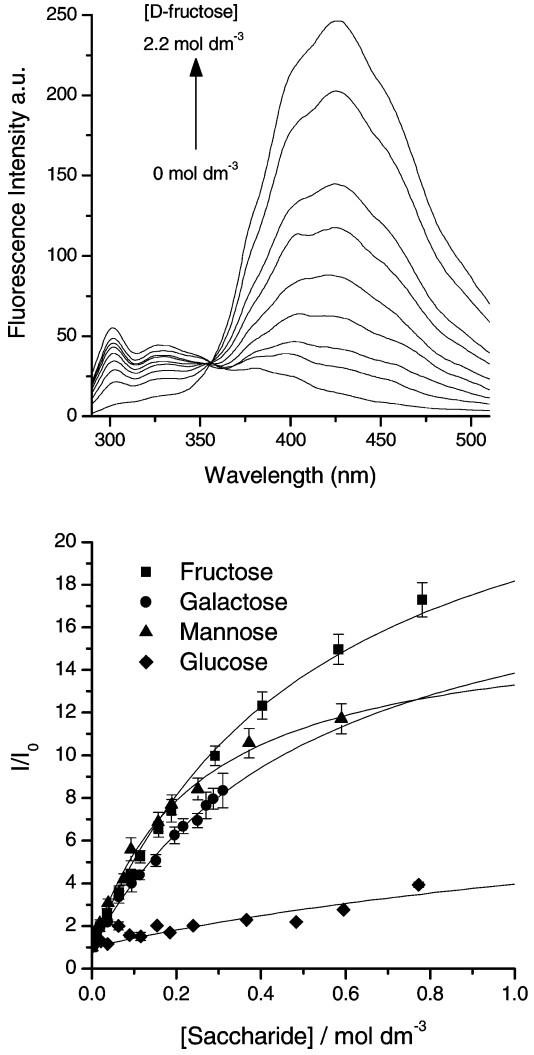


FIGURE 3. Fluorescence spectra of **4** in the presence of increasing concentrations of D-fructose, with the peak at 300 nm decreasing in intensity and the peak at 430 nm increasing in intensity, resulting in an isoemissive point at 360 nm (upper) and binding curves for ■ D-fructose; ◆ D-glucose; ● D-galactose, ▲ D-mannose (lower).

The fluorescence enhancements (I/I_0) obtained for **4** on the addition of D-fructose, D-galactose, and D-mannose are 27-, 20-, and 16-fold, respectively (Figure 3, lower, see the Supporting Information). We believe that these large fluorescence enhancements can be attributed to fluorescence recovery of the 1,2,3-triazole fluorophore. Similar fluorescence responses were

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(13) Crystal Data for **5**: $C_{21}H_{24}BN_3O$, M = 361.24, monoclinic, $P2_1/n$, a = 8.7850(1) Å, b = 11.7050(2) Å, c = 18.7420(3) Å, b = 90.368(1)°, U = 1927.17(5) Å^3, Z = 4, D_c = 1.245 g cm^{-3}, m = 0.08 mm^{-1}, F(000) = 768. Crystal size 0.35 × 0.2 × 0.2 mm, unique reflections 32826 [R(int) = 0.0474], observed [$I > 2s(I)$] 3497. R1, wR2 (obsd data) = 0.0565, 0.1013. R1, wR2 (all data) = 0.0403, 0.0924. Max peak/hole = 0.260, −0.205 e Å^{-3}. Bond lengths (Å), angles (deg) and torsions (deg): N(1)−N(2) 1.3467-(13), N(2)−N(3) 1.3165(14), B(1)−O(1) 1.3664(16), N(1)−C(7) 1.4681-(15), B(1)−C(1) 1.5653(17); N(1)−N(2)−N(3) 107.12(9), O(1)−B(1)−C(1) 126.09(11); C(1)−C(6)−C(7)−N(1) 98.90, O(1)−B(1)−C(1)−C(6) 6.83.

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TABLE 1. Stability Constant *K* (coefficient of Determination; r^2) for the Binding of Sensors 1a−c and 4 with Monosaccharides (52.1 wt % Methanol in Water, pH 8.21, 22 °C)

saccharide	*K* (mol^{-1} dm^3) (r^2) **1a**	*K* (mol^{-1} dm^3) (r^2) **1b**	*K* (mol^{-1} dm^3) (r^2) **1c**	*K* (mol^{-1} dm^3) (r^2) **4**
D-fructose	79.2 ± 1.7 (0.99)	212.1 ± 6.9 (0.99)	128.6 ± 2.6 (0.99)	1.9 ± 0.1 (0.99)
D-glucose	6.4 ± 0.4 (0.99)	8.7 ± 1 (0.99)	6.7 ± 0.5 (0.99)	-
D-galactose	14.2 ± 0.6 (0.99)	26.6 ± 1.3 (0.99)	17.7 ± 0.3 (0.99)	1.8 ± 0.3 (0.99)
D-mannose	7.9 ± 0.3 (0.99)	13.9 ± 1.4 (0.99))	16.2 ± 0.8 (0.99)	4.1 ± 0.4 (0.99)

observed for sensors **1a**−**c**.[7] In the absence of saccharides the normal fluorescence of the LE (locally excited) state of the 1,2,3-triazole donor of sensor **4** is quenched by energy transfer to the neutral phenylboronic acid acceptor, weakly Lewis acidic boron center. When saccharides are added, a negatively charged boronate anion is formed due to the enhancement of the Lewis acidity of the boron center on saccharide binding. Under these conditions, energy transfer from the 1,2,3-triazole donor becomes unfavorable and fluorescence is recovered. Interestingly the stability order for sensors **1a**−**c** with fructose > galactose > mannose > glucose was the "inherent stability order" observed for all simple boronic acids.[4−6,18] However, the unprecedented stability order with sensor **4** is mannose > fructose > galactose > glucose. We attribute the deviation in selectivity to the available hydrogen bond acceptor sites on the 1,2,3-triazole ring of **4** influencing the selectivity among the saccharides (such additional selectivity is not observed with sensors **1a**−**c**).

We have reported a simple "click-fluor" we believe that this strategy will make it possible to develop fluorescent modular sensor arrays for rapid screening of target saccharides.[19] The two most attractive aspects of "click-fluor" are that a fluorophore is generated upon triazole formation and the wide availability of acetylene units. Exploitation of this approach to prepare multiple receptor sensors is currently underway in our laboratories.

Experimental Section

2-(2-(Bromomethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane 3. Part i: Toluene-2-boronic acid **1** (2.11 g, 15.5mmol) and pinacol (2.20 g, 18.6mmol) were mixed in toluene (200 mL). A Dean−Stark head was fitted, and the reaction mixture was heated under reflux for 2 h. The mixture was allowed to cool to room temperature and then was washed with water (3 × 100 mL), condensed under reduced pressure, and dichloromethane (100 mL) was added. This was washed with water (2 × 100 mL), dried over sodium sulfate, and filtered, and the solvents removed under reduced pressure to yield 4,4,5,5-tetramethyl-2-*o*-tolyl-1,3,2-dioxaborolane as a colorless oil (3.23 g, 96%); ν (film)/cm^{-1} 2979, 1602, 1490, 1438, 1381, 1347, 1312, 1273, 1214, 1146; δ_H (300 MHz, $CDCl_3$) 1.21 (12H, s), 2.42 (3H, s), 6.99−7.22 (3H, m), 7.14−7.22 (1H, m); δ_B (96 MHz, $CDCl_3$) 32.57; δ_C PENDENT[20] (75 MHz, $CDCl_3$) 22.7 (+), 25.4 (+), 83.8 (−), 125.2 (+), 130.2 (+), 131.3 (+), 136.4 (+), 145.3 (−); *m*/*z* (ES+) 241.1370 ([M+Na]$^+$, $C_{13}H_{19}BO_2Na$ requires 241.1376). **Part ii:** 4,4,5,5-Tetramethyl-2-o-tolyl-1,3,2-dioxaborolane (3.20 g, 14.7mmol), *N*-bromosuccinimide (3.92 g, 22.0mmol), azobisisobutylonitrile (0.03 g, 1 mol %), and acetonitrile (100 mL) were refluxed at 90 °C for 2 h under nitrogen and then allowed to cool to room temperature. The mixture was condensed under reduced pressure until a solid began to form. The solid was filtered, and the filtrate was condensed further. The crude product was then purified using flash chromatography on silica gel (with petroleum ether as eluent) to yield compound **3** as a white solid (3.74 g, 86%): R_f (6:1, petrol/EtOAc) 0.86; mp 78−80 °C; ν ($CDCl_3$)/cm^{-1} 2976, 1599, 1444, 1386, 1350, 1324, 1269, 1144, 1102; δ_H (300 MHz, $CDCl_3$) 1.36 (12H, s), 4.90 (2H, s), 7.22−7.43 (3H, m), 7.77−7.83 (1H, m); δ_B (96 MHz, $CDCl_3$) 31.32; δ_C PENDENT (75 MHz, $CDCl_3$) 24.9 (+), 33.96 (−), 83.9 (−), 127.6 (+), 130.1 (+), 131.3 (+), 136.4 (+), 144.3 (−); *m*/*z* (ES+) 319.0475 ([M + Na]$^+$, $C_{13}H_{18}BBrO_2Na$ requires 319.0481).

2-((4-Phenyl-1*H*-1,2,3-triazol-1-yl)methyl)phenylboronic Acid 4. Part i: Compound **3** (1.04 g, 3.53mmol) and sodium azide (0.27 g, 4.12mmol) were dissolved in dimethyl sulfoxide (10 mL) and were stirred at room temperature for 10 min. Ethynylbenzene (0.30 g, 2.94 mmol) and copper(I) iodide (0.17 g, 0.88 mmol) were added, and the reaction mixture was heated at 80 °C for 4 h. The system was then heated at 60−70 °C, and the solvents were removed under high vacuum. Dry dichloromethane (200 mL) was added, and the resulting suspension was filtered through Celite in order to remove the inorganic salts. The filtrate was concentrated under reduced pressure, and the product was obtained by purification using flash chromatography on silica gel (petroleum ether/ethyl acetate eluent, 6:1). The appropriate fractions were combined, and the solvents were removed under vacuum to yield 4-phenyl-1-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)-1*H*-1,2,3-triazole (**5**) as a cream solid (0.86 g, 81%): R_f (4:1, petrol/EtOAc) 0.35; mp 99−101 °C; ν ($CDCl_3$)/cm^{-1} 2978, 1601, 1572, 1484, 1466, 1443, 1382, 1372, 1349, 1322, 1271, 1224, 1144; δ_H (300 MHz, $CDCl_3$) 1.37 (12H, s), 5.91 (2H, s), 7.24−7.48 (6H, m), 7.77 (1H, s), 7.77−7.82 (2H, m), 7.92−7.97 (1H, m); δ_B (96 MHz, $CDCl_3$) 31.79; δ_C PENDENT (75 MHz, $CDCl_3$) 24.9 (+), 53.4 (−), 84.2 (−), 119.9 (+), 125.6 (+), 125.7 (+), 127.9 (+), 128.0 (+), 128.8 (+), 129.1 (+), 129.2 (+), 130.8 (−), 131.9 (+), 134.8 (−), 136.7 (+), 140.9 (−), 147.6 (−); *m*/*z* (ES+) 384.1845 ([M + Na]$^+$, $C_{21}H_{24}BN_3O_2Na$ requires 384.1859). **Part ii:** 4-Phenyl-1-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)-1*H*-1,2,3-triazole (0.36 g, 1.0 mmol) in methanol (7 mL) was added to aqueous potassium hydrogen difluoride (3 mL, 4.5 M, 13.5mmol) within a plastic beaker. The resulting white slurry was stirred at room temperature for 15 min, concentrated in vacuo, and then dissolved in hot acetone. The mixture was filtered, the filtrate concentrated in vacuo and the residue recrystallized from a minimal amount of hot acetone and diethyl ether in order to afford the corresponding potassium trifluoroborate salt, potassium trifluoro(2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)phenyl)borate, as a yellow solid (0.27 g, 78%): mp 242−245 °C; ν (KBr)/cm^{-1} 3435, 3134, 2362, 1610, 1466, 1444, 1431, 1364, 1273, 1234, 1207; δ_H (300 MHz, CD_3OD) 5.79 (2H, s), 7.02−7.46 (6H, m), 7.61−7.80 (3H, m), 8.15 (1H, s); δ_B (96 MHz, CD_3OD) 4.84; δ_C PENDENT (75 MHz, CD_3OD) 55.2 (+), 122.5 (−), 125.7 (+), 126.8 (−), 128.0 (−), 128.1 (−), 129.1 (−), 129.3 (−), 129.5 (−), 130.0 (−), 132.0 (+), 133.89 (−), 133.93 (−), 139.1 (+), 148.8 (+); *m*/*z* (ES-) 302.1075 ([M − K], $C_{15}H_{12}N_3BF_3$ requires 302.1082). **Part iii:** Potassium trifluoro(2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)phenyl)borate (100 mg, 0.29 mmol) and lithium hydroxide (24 mg, 1.00 mmol) were added to a solution of water (5 mL) and acetonitrile (10 mL) and the solution was then stirred

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(20) The PENDANT technique results in primary and tertiary carbon atoms havid a different phase to secondary and quaternary carbon atoms. The phase is represented in the following manner: (+) positive phase; (−) negative phase. Homer, J.; Perry, M. C. *J. Chem. Soc., Chem. Commun.* **1994**, 373−374.

at room temperature for 24 h. The solution was then acidified with saturated ammonium chloride (8 mL) and hydrochloric acid solution (2 mL, 1 M) and then extracted with ethyl acetate (3 × 20 mL). The combined organic extracts were washed with hydrochloric acid solution (2 × 20 mL, 0.5 M), dried over sodium sulfate, and filtered, and the solvents were removed under reduced pressure to afford the title compound **3** as a white solid (0.062 g, 77%): ν ($CDCl_3$)/cm^{-1} 3580, 3141, 1600, 1448, 1397, 1280, 1253; δ_H (300 MHz, $CDCl_3$) 5.88 (2H, s), 7.00−7.85 (9H, m), 7.92−8.20 (1H, m); δ_B (96 MHz, CD_3OD) 31.00; δ_C PENDENT (75 MHz, CD_3OD) 55.8 (−), 122.7 (+), 127.1 (+), 129.3 (+), 129.8 (+), 130.4 (+), 130.6 (+), 131.0 (+), 131.6 (+), 132.0 (−), 134.0 (+), 136.1 (+), 140.0 (−), 149.5 (−); *m/z* (ES-) 302.1069 ([M − H + Na], $C_{15}H_{14}BN_3O_2$-Na requires 302.1077).

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Supporting Information Available: General procedures, fluorescence data, and tables of XRD data and X-ray data (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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