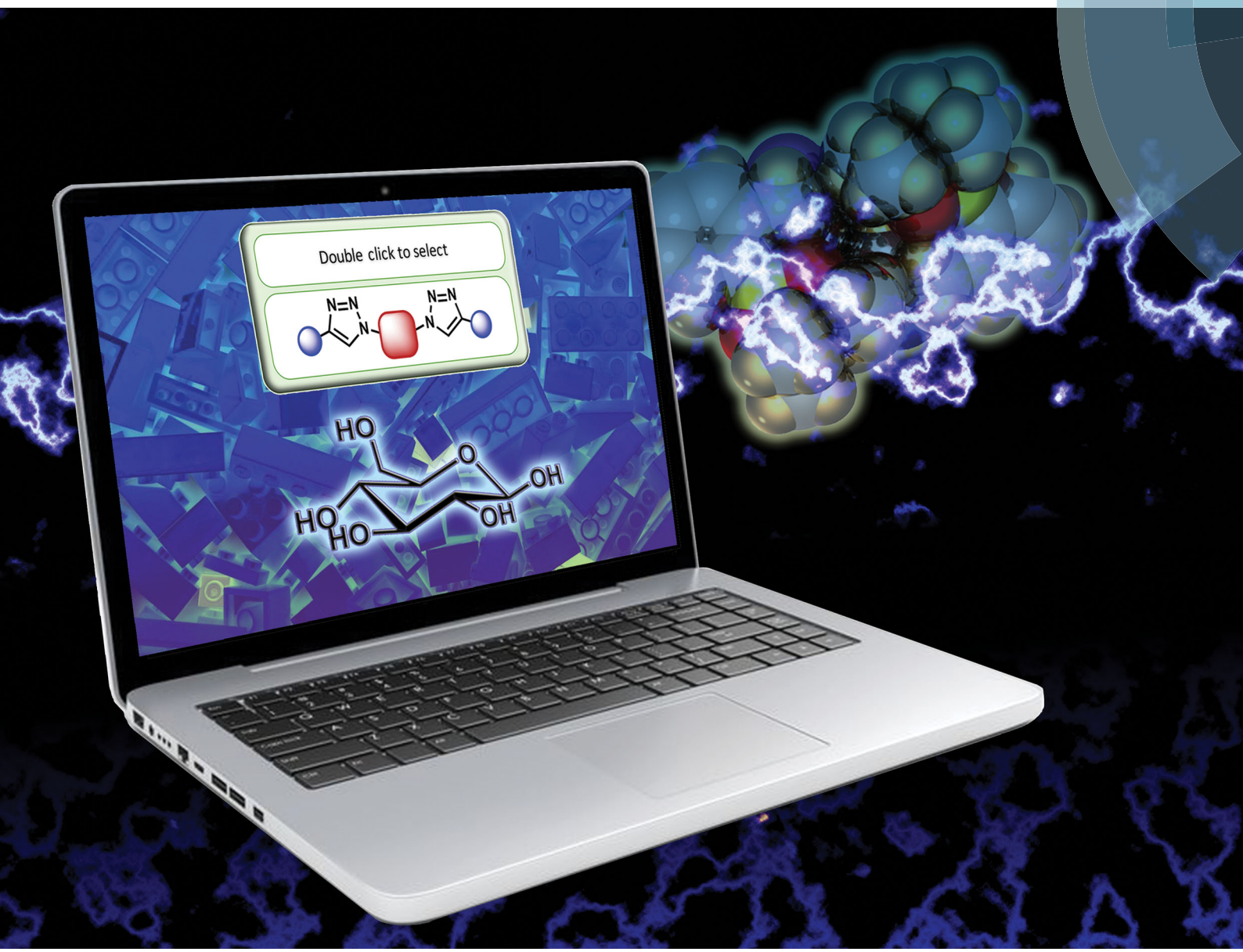
Volume 53 | Number 14 | 18 February 2017 | Pages 2189–2300

ChemComm

Chemical Communications

rsc.li/chemcomm



ISSN 1359-7345

ROYAL SOCIETY OF CHEMISTRY

COMMUNICATION

John S. Fossey *et al.*

Glucose selective bis-boronic acid *click-fluor*

ChemComm

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2017, **53**, 2218

Received 25th October 2016,
Accepted 23rd November 2016

DOI: 10.1039/c6cc08534b

www.rsc.org/chemcomm

Glucose selective bis-boronic acid *click-fluor*†‡

Wenlei Zhai,[a] Louise Male[b] and John S. Fossey*[a]

Four novel bis-boronic acid compounds were synthesised *via* copper catalysed azide–alkyne cycloaddition (CuAAC) reactions. Glucose selectivity was observed for a particular structural motif. Moreover, a new glucose selective fluorescent sensor was designed and synthesised as a result.

In the past few decades, synthetic probes have shown significant promise for real-time and accurate detection of biomolecules.[1] Much attention has been devoted to boronic acid derivatives for saccharide detection.[2] Although boronic acid-mediated saccharide sensing showed encouraging results, a lack of selectivity for higher order saccharides needs to be addressed, and remains challenging. Phenylboronic acid derivatives have greater affinity for fructose over glucose, under physiological conditions.[3] For the purpose of increasing the binding affinity to glucose, and other saccharides, a more sophisticated receptor structure is required.

James *et al.* were the first to report that two appropriately positioned boronic acids could modulate selectivity by two-point binding interactions with glucose (sensor **1**, Fig. 1).[4] Further studies conducted by James *et al.* demonstrated that design of the spacer unit between the borons, of a bis-boronic acid, was crucial for glucose recognition (sensor **2**, Fig. 1).[5] Drueckhammer *et al.* showed that the distance between two *p*-tolylboronic acids could be optimised, for glucose selectivity, through a computational study, and a rigid four-fused ring scaffold was produced (sensor **3**, Fig. 1).[6] Thus, appropriate positioning of boronic acids can give glucose selectivity. However, application of a universally simple methodology to selective saccharide receptor design remains a challenge.[7]

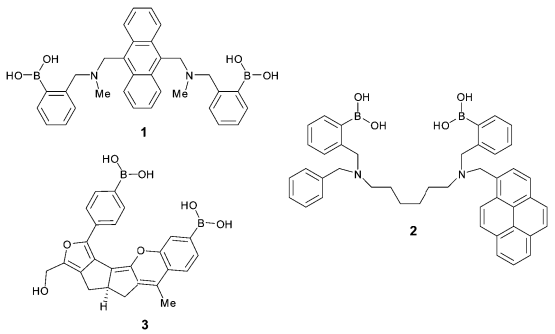


Fig. 1 Chemical structures of reported glucose-selective fluorescent sensors developed by Shinkai *et al.* (**1**),[4a] James *et al.* (**2**)[5a] and Drueckhammer *et al.* (**3**).[6]

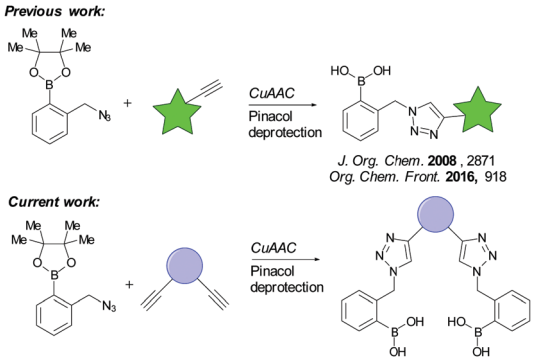
Copper catalysed azide–alkyne cycloaddition (CuAAC) reaction, often referred to as the "*click reaction*,"[8] has been employed to synthesise novel boronic acid derivatives for various applications. In order to explore the advantages of the CuAAC reaction in the area of saccharide sensing,[9] Scrafton *et al.* employed the CuAAC reaction for a five-step synthesis of a boronic acid-based sensor molecule (Scheme 1).[10] The term "*click-fluor*" was used to refer to this class of molecular sensors. Recently, the scope has been elaborated to include incorporation of fluorophores.[11] Although these studies demonstrated the possibility of rapid synthesis of boronic acids *via* the CuAAC reaction, the reported mono-boronic acids cannot serve as glucose-selective sensors due to their relatively weak glucose binding. In order to engineer selectivity, CuAAC may be employed to construct bis-boronic acids. Wang and co-workers reported two triazole-linked bis-α-amidoboronic acids.[12] Their result showed significantly enhanced binding affinity for oligosaccharides. Zhao *et al.* also designed and synthesised three bis-boronic acid sensors through triazole formation.[13] However, in their studies, the receptors preferentially recognise D-fructose over D-glucose.

In this report, three bis-boronic acid molecules were designed such that the CuAAC reaction may be used to rapidly construct a series. Their synthesis is studied and saccharide

[a] *School of Chemistry, University of Birmingham, Edgbaston, Birmingham, West Midlands, B15 2TT, UK. E-mail: J.s.fossey@bham.ac.uk*
[b] *X-Ray Crystallography Facility, School of Chemistry, University of Birmingham, Edgbaston, Birmingham, West Midlands, UK*

† Dedicated to the 60th Birthday of Tony Czarnik.

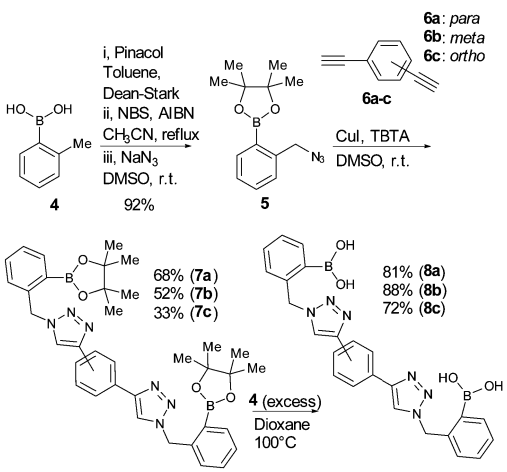
‡ Electronic supplementary information (ESI) available. CCDC 1510897–1510899. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c6cc08534b



Scheme 1 Synthesis of mono and bis-boronic acids as saccharide sensors *via* the CuAAC reaction.

binding evaluated, for the purpose of developing a novel multi-boronic acid "click" platform. As such, we built upon the knowledge that glucose selectivity may be achieved by correctly spacing two boronic acids in one molecule, to demonstrate "click-compatibility" for selectivity, and pave the way for future exploration in higher order saccharide sensor design.

Three regio-isomeric bis-boronic acids (**8a–c**), akin to the mono-boronic acid *click-fluors* already reported, were designed and their synthesis embarked upon (see Scheme 2). Following pinacol protection of commercially available *o*-tolylboronic acid, organic azide **5** was synthesised on a 10 gram scale, by bromination and displacement of bromide by azide, according to literature procedures (92% yield of compound **5**).[11] Three bis-alkynes were required; fortunately 1,3- and 1,4-diethynylbenzene are commercially available and were used as purchased. 1,2-Diethynylbenzene was readily synthesised from 1,2-dibromobenzene *via* a palladium-catalysed Sonogashira coupling and TMS removal following literature procedures.[14] Initially, the CuAAC reaction was conducted as per our previous reports, but poor yields of the target bis-boronic esters (**7a–c**), as a result of unwanted side reactions, plagued our experiments.[15] For example, during the synthesis of compound **7b**, oxidation and deborylation reactions occurred on one or both of the boronic



Scheme 2 Synthetic route of bis-boronic acid receptors **8a–c**.

esters (confirmed by mass spectrometry). Therefore, the conditions of the CuAAC reaction were further modified. More mild conditions, use of TBTA as a ligand for copper and lessening of catalyst loading helped improve the reaction outcomes and minimize (copper-catalysed) de-borylation.[16] Thus, the yields of these three key intermediates were improved from 32% to 68% (**7a**), 21% to 52% (**7b**), and 18% to 33% (**7c**), respectively. Next, pinacol was removed by addition of compound **4** under acidic conditions; note that this gives a by-product, pinacol protected-**4**, which may be (and was) used in further syntheses.[13a] Bis-boronic acids **8a–c** were obtained after trituration and flash chromatography in 72–88% isolated yield.

The bis-pinacol esters, **7a–c**, are crystalline solids, and crystals suitable for single crystal XRD structure determination were grown from mixtures of hexane and ethyl acetate. From the obtained structures presented in Fig. 2, the distance between

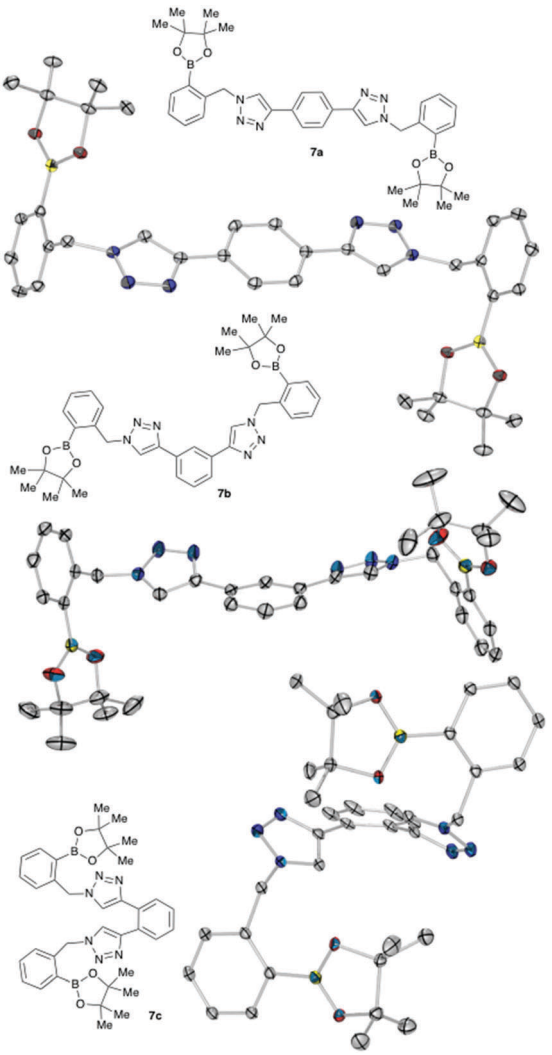


Fig. 2 Chemical and X-ray structures of key intermediates **7a–c** (ORTEP ellipsoids 30% probability rendered using Ortep III for Windows and PovRay). Hydrogen atoms have been omitted. In **7a** and **7c** the molecules are located on a symmetry element such that only half in each case are crystallographically unique. In **7c**, part of the boronic ester group is disordered over two positions, with only the major part being shown here.[17]

Table 1 Binding constants of compounds **8a–c** and fructose and glucose calculated according to ITC data

	8a	8b	8c
Binding site (fructose)	1.89 ± 0.280	0.48 ± 0.005	1.30 ± 0.046
Binding constant (M^{-1}) (fructose)	$1.90 \times 10^3 \pm 280$	$1.30 \times 10^5 \pm 1.10 \times 10^4$	$2.95 \times 10^3 \pm 183$
Binding site (glucose)	N/A	0.45 ± 0.081	1.33 ± 0.101
Binding constant (M^{-1}) (fructose)	N/A	$5.03 \times 10^3 \pm 479$	$6.19 \times 10^3 \pm 731$

the two boron atoms was measured as *para* **7a** B(1)···B(1)^{i} = 14.787 (3) Å; **7b** *meta* B(1)···B(2) = 14.101 (4) Å; and *ortho* **7c** B(1)···B(1)^{ii} = 7.619 (3) Å for each compound.[17] The distance in **7c** is obviously reduced compared to that in **7a**.

To determine the saccharide binding capability of the synthesised bis-boronic acids, isothermal titration calorimetry (ITC) was employed. ITC is an effective method to study the binding affinity between small molecules and large biomolecules like proteins and DNA.[18] Moreover, there are studies of using ITC to determine the binding strength between lectins and saccharide,[19] as well as boronic acid containing molecules with saccharide.[11,20] In our experiment, measurements were carried out in pH 8.21 PBS buffer with up to 20% DMSO, depending on the solubility of the tested compounds.

According to the ITC results, compound **8a** behaves like most mono-boronic acid derivatives, showing higher binding affinity for fructose than glucose. As shown in Table 1, the recorded binding constant between **8a** and fructose is $1.90 \times 10^3 \pm 280$ M^{-1}, which is similar to that of mono-boronic acid.[11]

However, attempts to determine the binding constant between **8a** and glucose failed due to such a weak interaction (see the ESI,‡ Fig. S2). Compared with compound **8a**, the glucose binding affinity of compound **8b** is improved. Surprisingly, the binding constant between **8b** and fructose is extremely high. Indeed, this superior fructose selectivity is noteworthy and further investigations are planned to better understand this observation. For both **8b** and **8c**, it takes at least 20 minutes to reach the equilibrium for each addition of glucose. On the other hand, each fructose addition equilibrates within 5 minutes under the same conditions. Perhaps, the glucose binding process requires the conformational change of analyte or receptor to facilitate the optimal interaction.

As presented in Fig. 3, compound **8c** showed good binding affinities to both fructose and glucose. The binding strength between **8c** and glucose is more than twice that of fructose. Therefore, the ITC data reveal that compound **8c** is a glucose selective receptor. The binding constants of compound **8a–c** with glucose increase across *meta*, *para* and *ortho* series respectively, which again demonstrates that the distance between the two boronic acid groups is critical for glucose recognition.

Encouraged by the positive result from the ITC experiment, we decided to combine this newly developed receptor architecture with a fluorophore, in order to construct a glucose-selective fluorescence sensor. Starting from commercially available 6,7-dihydroxycoumarin (**9**), bis-alkyne **12** was synthesised by converting the hydroxyl groups into triflates (**10**), followed by Sonogashira coupling and TMS deprotection using TBAF (Scheme 3).[21] The CuAAC reaction and pinacol deprotection were performed under the same conditions as earlier.

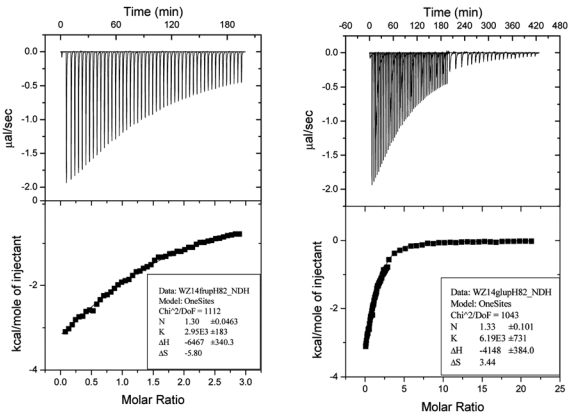
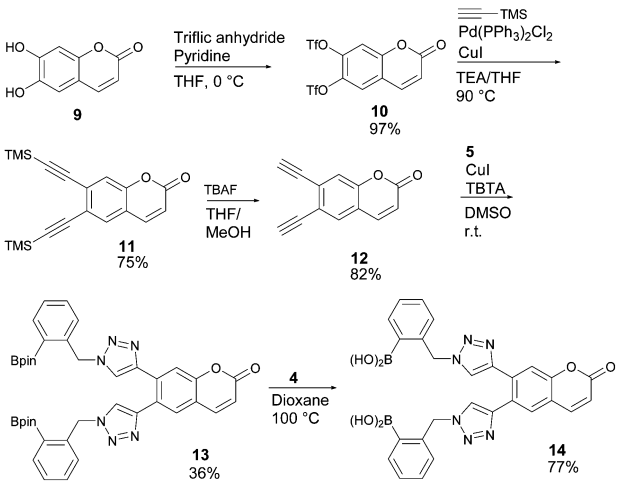


Fig. 3 ITC study of compound **8c** binding with fructose (left) and glucose (right) in pH 8.21 PBS buffer with 20% DMSO.



Scheme 3 Synthetic route of fluorescent bis-boronic acid compound **5**.

In Fig. 4, the fluorescence response of sensor **14** towards fructose and glucose is shown, respectively. It was observed that the fluorescence intensity was quite weak for sensor **14**, as the Raman scattering signal of the excitation source was also recorded on the spectrum. Upon addition of 5 mM fructose, the fluorescence signal was slightly enhanced (I/I_0 = 1.13, see Fig. 4a), which was similar to what we have observed with mono-boronic acid sensor binding with fructose in our previous study.[11]

However, the fluorescence intensity gradually decreased upon addition of 5 mM glucose (I/I_0 = 0.79, see Fig. 4b and c). Presumably, the different fluorescence responses towards fructose and glucose were caused by the differing binding modes.

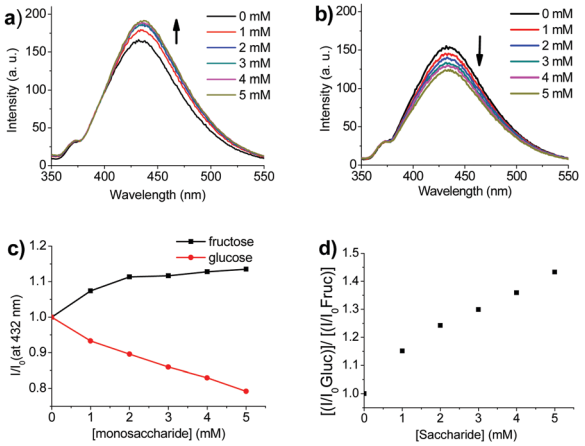


Fig. 4 (a) Fluorescence spectra of 1.33×10^{-6} M sensor **14** upon addition of fructose (0–5 mM) in pH 8.21 methanolic buffer; (b) fluorescence spectra of 1.33×10^{-6} M sensor **14** upon addition of glucose (0–5 mM) in pH 8.21 methanolic buffer; (c) plotting fluorescence responses of **14** against the concentrations of fructose and glucose; (d) plot of $[(I/I_0\ \text{Gluc})]/[(I/I_0\ \text{Fruc})]$ against the concentration of tested saccharide. Excitation wavelength: 330 nm; maximum emission: 432 nm.

Meanwhile, it took no less than 30 minutes for the fluorescence signal to be stabilised after adding glucose, which agrees with the slow binding process in our ITC studies.

Three regioisomeric bis-boronic acids were synthesised using the CuAAC reaction. The binding constants of the synthesised compounds with fructose and glucose were measured by ITC experiment, respectively. It was found that selectivity for glucose binding is modulated by the distance between the two boronic acid groups. To our delight, compound **8c** presented higher binding affinity towards glucose over fructose. Moreover, a fluorescent receptor, **14**, was synthesised showing divergent properties upon interaction with glucose *versus* fructose. It was shown that compound **14** can serve as an "on–off" fluorescence sensor for selective glucose detection. More studies are required to better understand the fluorescence modulation mechanism, which is an ongoing work in our laboratory. We showed the utility of combining different functional components using the CuAAC reaction to construct selective molecular receptors for more challenging targets.

WZ and JSF thank The Catalysis and Sensing for our Environment (CASE) group for networking opportunities.[22] China Scholarship Council (CSC) and the University of Birmingham are also thanked for providing full-fee studentship support to WZ. JSF thanks the University of Birmingham for support, the Royal Society for an Industrial Fellowship (6955) and the EPSRC for funding (EP/J003220/1).

Notes and references

1 A. P. de Silva, T. S. Moody and G. D. Wright, *Analyst*, 2009, **134**, 2385–2393.
2 (*a*) X. Sun and T. D. James, *Chem. Rev.*, 2015, **115**, 8001–8037; (*b*) W. Zhai, X. Sun, T. D. James and J. S. Fossey, *Chem. – Asian J.*, 2015, **10**, 1836–1848; (*c*) X. Sun, W. Zhai, J. S. Fossey and T. D. James, *Chem. Commun.*, 2016, **52**, 3456–3469.
3 (*a*) J. P. Lorand and J. O. Edwards, *J. Org. Chem.*, 1959, **24**, 769–774; (*b*) S. Guo, J. Chen, B.-Y. Cai, W.-W. Chen, Y.-F. Li, X. Sun, G.-R. Chen, X.-P. He and T. D. James, *Materials Chemistry Frontiers*, 2017, DOI: 10.1039/C6QM00158K.
4 (*a*) T. D. James, K. R. A. S. Sandanayake and S. Shinkai, *Angew. Chem., Int. Ed.*, 1994, **33**, 2207–2209; (*b*) T. D. James, K. R. A. S. Sandanayake, R. Iguchi and S. Shinkai, *J. Am. Chem. Soc.*, 1995, **117**, 8982–8987.
5 (*a*) S. Arimori, M. L. Bell, C. S. Oh, K. A. Frimat and T. D. James, *Chem. Commun.*, 2001, 1836–1837; (*b*) S. Arimori, M. L. Bell, C. S. Oh, K. A. Frimat and T. D. James, *J. Chem. Soc., Perkin Trans. 1*, 2002, 803–808.
6 W. Yang, H. He and D. G. Drueckhammer, *Angew. Chem., Int. Ed.*, 2001, **40**, 1714–1718.
7 J. S. Fossey, F. D'Hooge, J. M. H. van den Elsen, M. P. Pereira Morais, S. I. Pascu, S. D. Bull, F. Marken, A. T. A. Jenkins, Y.-B. Jiang and T. D. James, *Chem. Roc.*, 2012, **12**, 464–478.
8 H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004–2021.
9 C. Dai, Y. Cheng, J. Cui and B. Wang, *Molecules*, 2010, **15**, 5768–5781.
10 D. K. Scrafton, J. E. Taylor, M. F. Mahon, J. S. Fossey and T. D. James, *J. Org. Chem.*, 2008, **73**, 2871–2874.
11 W. Zhai, B. M. Chapin, A. Yoshizawa, H.-C. Wang, S. A. Hodge, T. D. James, E. V. Anslyn and J. S. Fossey, *Org. Chem. Front.*, 2016, **3**, 918–928.
12 S. Jin, C. Zhu, Y. Cheng, M. Li and B. Wang, *Bioorg. Med. Chem.*, 2010, **18**, 1449–1455.
13 (*a*) M. Shao and Y. Zhao, *Tetrahedron Lett.*, 2010, **51**, 2508–2511; (*b*) K. Mulla, P. Dongare, N. Zhou, G. Chen, D. W. Thompson and Y. Zhao, *Org. Biomol. Chem.*, 2011, **9**, 1332–1336.
14 Q. Zhou, P. J. Carroll and T. M. Swager, *J. Org. Chem.*, 1994, **59**, 1294–1301.
15 S. Jin, G. Choudhary, Y. Cheng, C. Dai, M. Li and B. Wang, *Chem. Commun.*, 2009, 5251–5253.
16 T. R. Chan, R. Hilgraf, K. B. Sharpless and V. V. Fokin, *Org. Lett.*, 2004, **6**, 2853–2855.
17 See ESI for atom labels and further details and corresponding CIF files. CCDC deposition numbers 1510897–1510899. (i) Symmetry code for the generation of equivalent atoms: $-x$, $1 - y$, $2 - z$; (ii) symmetry code for the generation of equivalent atoms: $-x$, y, $1/2 - z$.
18 M. W. Freyer and E. A. Lewis, *Methods in Cell Biology*, Academic Press, 2008, vol. 84, pp. 79–113.
19 T. K. Dam and C. F. Brewer, *Chem. Rev.*, 2002, **102**, 387–430.
20 (*a*) Ö. Torun, F. C. Dudak, D. Baş, U. Tamer and İ. H. Boyacı, *Sens. Actuators, B*, 2009, **140**, 597–602; (*b*) M. S. Melicher, J. Chu, A. S. Walker, S. J. Miller, R. H. G. Baxter and A. Schepartz, *Org. Lett.*, 2013, **15**, 5048–5051.
21 Z. Zhou and C. J. Fahrni, *J. Am. Chem. Soc.*, 2004, **126**, 8862–8863.
22 (*a*) D. T. Payne, J. S. Fossey and R. B. P. Elmes, *Supramol. Chem.*, 2016, **28**, 921–931; (*b*) J. S. Fossey and W. D. G. Brittain, *Org. Chem. Front.*, 2015, **2**, 101–105.