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ortho-Substituted fluorescent aryl monoboronic acid displays physiological binding of D-glucose

J. S. Hansen [a,*], M. Ficker [a], J. F. Petersen [a], J. B. Christensen [a], T. Hoeg-Jensen [b]

[a] Department of Chemistry, Universitetsparken 5, DK-2100 Copenhagen, Denmark
[b] Novo Nordisk Park D6.1.142, DK-2760 Maaloev, Denmark

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ABSTRACT

An *ortho*-fluorinated phenylboronic acid incorporated into the BODIPY-fluorophore, sensor **1**, exhibits a significant response in emission intensity upon binding of D-glucose and D-fructose. This sensor displays a desired binding strength toward D-glucose at the physiological level, that is K_d values between 10 and 20 mM. This binding strength of sensor **1** has been shown to be independent of the employed buffer, that is, saline buffer, 50 mM phosphate buffer, and 50 mM phosphate buffer in 52.3 w/w% MeOH. The D-glucose binding strength and the fluorescence response of a BODIPY-based *ortho*-methylated phenylboronic acid, sensor **2**, show a significantly decreased D-fructose binding affinity. This sensor also exhibits buffer-dependent binding strength and fluorescence response upon binding of D-glucose and D-fructose.

Arylboronic acids are small and flexible molecules[1–6] in comparison to lectins[7] and artificial macrocycles designed to bind carbohydrates.[8–11]

The reactions of arylboronic acids with *cis*-1,2-diols or 1,3-diols in aqueous media generally afford cyclic esters rapidly and reversibly. This transformation is likely to alter the electronic properties of an arylboronic acid based sensor, since formation of the corresponding arylboronate is favored in the cyclic ester. An arylboronic acid based fluorescent sensor can potentially be used as a semi-invasive or non-invasive reporter for monitoring of D-glucose in diabetic patients.[12–15] The displacement constant, K_d, must have a value around 10–20 mM for binding of D-glucose in human blood, since blood glucose varies between 2 and 30 mM in diabetic patients.[16] The maximum sensitivity is achieved, when K_d is in the middle of the binding curve.

D-Glucose is the dominant monosaccharide present in human blood, that is, [D-glc] ≈5 mM,[17] in comparison to D-fructose. [D-frc] <0.5 mM, even after a fructose-rich meal.[18,19] Thus, selective recognition of D-glucose over other saccharides is important for accurate glucose readouts. D-Fructose generally exhibits a stronger binding toward aryl monoboronates due to the tridentate binding mode,[20] whereas D-glucose exhibits bidentate binding.[21] This stronger D-fructose binding leads to errors in D-glucose readouts. A substituent placed *ortho* to the boronic acid may likely decrease the binding of D-fructose. This has recently been shown by UV/Vis-titrations, using a colorimetric competitive assay, that is, Alizarin Red Sodium (ARS), and further rationalized by calculations at the B3LYP/6-31G(d) level with Gaussian.[22]

These findings have been employed in the engineering of *ortho*-substituted aryl monoboronic acids incorporated into the BODIPY fluorophore.

Sensor **1**, which has been synthesized similarly to the procedure described by DiCesare and Lakowicz,[23] contains an *o*-fluorine substituent. It exhibits excellent D-glucose binding in three types of buffers, with displacement constants, K_d, around 15–20 mM. D-Fructose binding of sensor **1** is not reduced significantly as expected, since the found D-fructose displacement constants are around 0.5–1.0 mM.[24] The tight D-fructose binding may be a consequence of a favorable hydrogen bond interaction between a hydroxyl group in D-fructose and the *o*-fluorine substituent in sensor **1**. Also the lower pK_a of sensor **1** compared to sensor **2** may contribute positively to the tighter D-fructose binding of sensor **1**.

Sensor **2** was synthesized according to a recently described procedure.[25] This sensor, which contains an *o*-methyl substituent, shows a significantly reduced D-fructose binding affinity, with K_d around 10 mM. This is a onefold reduction in D-fructose binding strength compared to that normally expected for binding aryl monoboronates.[24] An explanation of this phenomenon might be that sensor **2** is not capable of hydrogen bond formation as suggested for sensor **1**, and that the pK_a is significantly lower for sensor **1** compared to sensor **2**.

Sensors **1** and **2** are shown in Figure 1 (top), along with the unsubstituted sensor **3**, which has recently been evaluated.[25]

* Corresponding author. Tel.: +45 61337866.
E-mail address: jonhansen@chem.ku.dk (J.S. Hansen).

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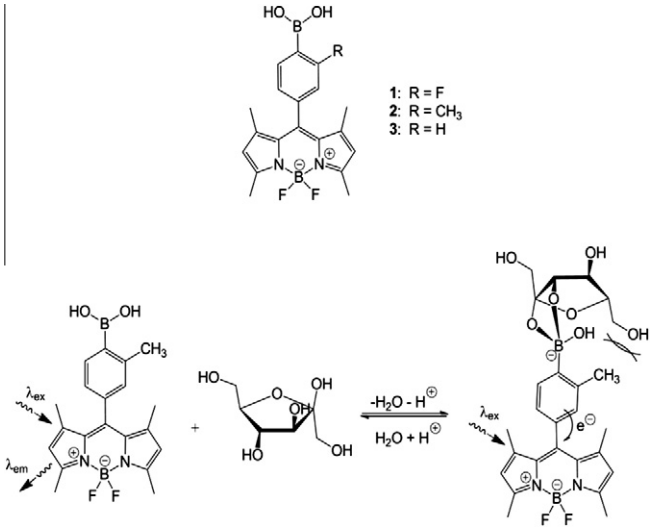

Figure 1. Top: *ortho*-substituted aryl monoboronic acids, **1** and **2**, incorporated into a BODIPY fluorophore, along with the unsubstituted analogue, **3**.[25] Bottom: proposed reductive quenching for the diminished tridentate D-fructose binding of sensor **2**, caused by the presence of an *ortho*-positioned methyl substituent.[26]

Research by the groups of Lakowicz and Shinkai revealed a similar binding efficiency for arylboronic acids and their corresponding ester analogues.[27,28] The similar binding efficiency is due to rapid ester exchange in boronic acid esters. Shinkai and Lakowicz have therefore used their boronic acids directly as the corresponding protected esters.

Based on the above, the pinacol ester analogues of boronic acids have been used throughout the binding studies herein reported, since these are easier to synthesize and purify. A control binding experiment with the neopentyl ester analogue of sensor **1** has been performed. The binding strength of this sensor matched the binding strength of sensor **1**. The neopentyl ester analogue of **3** has previously been tested by Lakowicz's group.[23]

The extinction coefficients of sensors **1** and **2** have been determined in methanol to be $\varepsilon_{1495\ nm}$ = 84,000 M^{-1} cm^{-1} and $\varepsilon_{2495\ nm}$ = 80,000 M^{-1} cm^{-1}, respectively. Such high extinction coefficients are as expected for BODIPY dyes.[29]

The impact of the buffer on the binding properties of sensor **1** is very similar in the three employed buffers, that is, saline buffer (10 mM phosphate, 2.7 mM KCl, and 137 mM NaCl), 50 mM phosphate buffer, and 52.3 w/w% methanolic and 50 mM phosphate buffer at physiological pH (7.4). This high assay robustness may be useful from a biological perspective. The excitation and emission response intensities are increased significantly upon raising the monosaccharide concentration. This phenomenon may be interpreted as oxidative quenching upon saccharide binding, similar to the proposed binding mechanism of sensor **3**.[25] The plot of the slight increase in emission for sensor **1** by addition of D-glucose is shown in Figure 2. The binding curves for binding D-glucose and D-fructose in saline buffer at pH 7.4 are shown in Figure 3.

Displacement constants, K_d, are listed in Table 1 for sensors **1** and **2**, where 1:1 D-glucose and D-fructose–arylmonoboronate complexes are anticipated. The displacement constants are calculated by plotting the emission intensity increase against the logarithm of the concentration, using a sigmoidal dose response (variable slope).

Sensor **1** does not exhibit the same increase in emission intensity as sensor **3**.[25] This lower emission response increase for **1** may be a consequence of the relatively high electron affinity of the *meso*-aryl substituent in sensor **1**, compared to the *meso*-aryl substituent in sensor **3**. This difference is caused by the electron- withdrawing effect of fluorine. The tight D-glucose binding of sensor **1** compared to sensor **3** may be due to the relatively lower pK_a of **1** (7.3) compared to sensor **3** (8.3–8.8).[23] The overall very similar increase in emission intensity of sensor **1** on addition of the two monosaccharides however, indicates a comparable binding mode in the boronate ester formation.

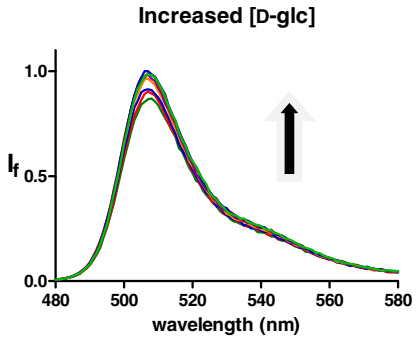

Figure 2. Increase in the emission intensity of sensor **1**, upon augmenting [D-glc] from 0 to 513 mM in a saline buffer at pH 7.4.

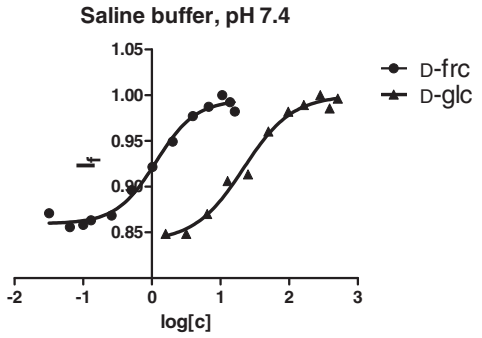

Figure 3. Curve fit using GraphPad Prism 5.0. The measured emission intensity is plotted against the logarithm of the concentration data (in mM), using a sigmoidal dose response (variable slope). Sensor **1** is used at 0.53 μM in the phosphate buffered saline solution at pH 7.4.

Table 1
Binding constants, K_d, in mM, determined from the change in emission intensities at pH 7.4

Sensor/buffer	K_d D-frc (*n*) σ	K_d D-glc (*n*) σ
1, Saline	1.0 (2) 0.3	17 (2) 3.9
1, 50 mM Phosphate	0.7 (2) 0.2	18 (2) 1.4
1, 52.3 w/w% MeOH/phos	0.8 (2) 0.1	16 (2) 3.3
2, Saline	9.2 (2) 1.3	<500 (2) —
2, 50 mM Phosphate	11.1 (2) 0.3	<500 (2) —
2, 52.3 w/w% MeOH/phos	7.1 (2) 0.1	97 (2) 0.1

The number of measurements (*n*) is shown in brackets, and the standard deviations (σ) are shown to the right.

Sensor **2** exhibits very weak binding toward D-glucose (K_d >500 mM). The tightest D-glucose binding is found in the 52.3 w/w% methanolic and 50 mM phosphate buffer with a K_d of 97 mM being observed. The overall low D-glucose binding affinity may be explained by the very high pK_a of sensor **2** (10.7). One would expect tighter D-glucose binding in the 50 mM phosphate buffer according to the pK_a argument, since sensor **2** is slightly more acidic in this buffer (pK_a of 10.4). The response upon D-glucose binding has been shown to be buffer dependent. A slight decrease in emission intensity is observed in the saline buffer and in the 50 mM phosphate buffer. Titration of sensor **2** with D-glucose in a 52.3 w/w% methanolic and 50 mM phosphate buffer, on the other hand, results in a slight increase in the emission intensity.

The reduced D-fructose binding affinity of sensor **2** in all three buffers is expected based on previous UV/Vis-titrations and calculations at the B3LYP/6-31G(d) level with Gaussian.[22] Titration of sensor **2** with D-fructose in saline buffer and phosphate buffer at pH 7.4, shows significantly decreased emission intensity on increasing the sugar concentration. The decreased excitation and emission intensities of sensor **2** are shown in Figure 4. The binding isotherm is shown in Figure 5. Titration of sensor **2** with D-fructose in 52.3 w/w% methanolic and 50 mM phosphate buffer, in contrast, results in increased emission intensity, see Figure 6. This is in good agreement with the observations from the titration of sensor **2** with D-glucose. This may suggest a reductive quenching in saline buffer and 50 mM phosphate buffer, while oxidative quenching might take place in 52.3 w/w% methanolic and 50 mM phosphate buffer.[26] The proposed oxidative quenching upon D-fructose binding is depicted in Figure 1 (bottom), while the sigmoidal curve for D-fructose binding of sensor **2** is shown in Figure 5. Additional excitation measurements mirror the found emission responses for sensors **1** and **2**, and the recorded excitation spectra of **1** and **2** match the absorption spectra.

The binding selectivities have been calculated for sensors **1** and **2** for the binding of D-glucose and D-fructose. The lowest K_d(D-glc)/K_d(D-frc) value for sensor **1** was found in saline buffer. The overall lowest K_d(D-glc)/K_d(D-frc) ratio was, however, found for sensor **2**. The selectivity ratios are listed in Table 2.

Determination of the pK_a values for the sensors **1** and **2** has been conducted in 50 mM phosphate buffer and in 52.3 w/w% methanolic and 50 mM phosphate buffer. The slight blue-shifting of the absorption and emission spectra upon boronate formation is exploited to calculate the respective acid–base equilibria. The titration curve for the determination of the pK_a of sensor **1** in 50 mM phosphate buffer is shown in Figure 7, and the found pK_a values are listed in Table 3.

Fluorometric evaluation of sensor **1** reveals excellent binding affinity toward D-glucose, with displacement constants, K_d, at the physiological level. K_d is found midway between the extremes of diabetic blood sugar value, 2–30 mM, that is, the probe should give good sensitivity over the relevant range.[16] The binding strength of sensor **1** must still be modulated toward weaker D-fructose binding. This can presumably be achieved by attachment of a larger electron-withdrawing substituent at the position *ortho*- to the boronic acid unit. Attachment of a CF_3-group at the *ortho*-position may result in the desired steric effect causing decreased D-fructose binding, along with a sufficiently low pK_a for optimal D-glucose binding. Alternatively attachment of an *ortho*-chlorine might also decrease D-fructose binding. However, this may increase the pK_a of the sensor, due to the decreased electron-withdrawing ability of this halogen. In order not to weaken the D-glucose binding, this latter change of sensor **1** will demand the attachment of electron-withdrawing substituents elsewhere in the dye to maintain a low pK_a. Reduced D-fructose selectivity is required in order to obtain accurate D-glucose readouts without any significant error.

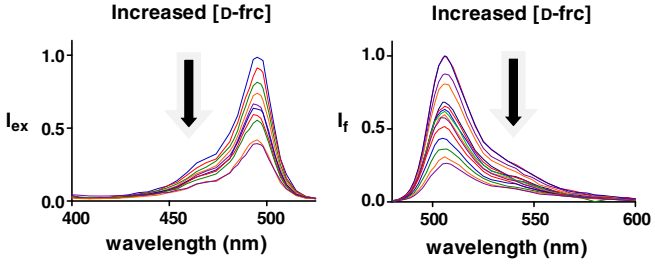

Figure 4. Significant decrease in the excitation and emission intensities of sensor **2** (left and right, respectively) upon addition of D-fructose from 0 to 2.0 M in a saline buffer.

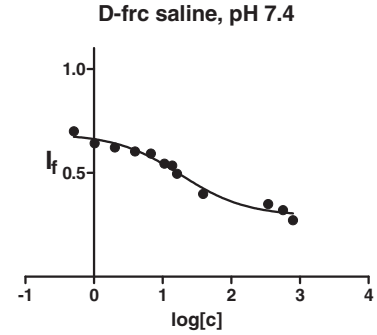

Figure 5. Decreased emission intensity of sensor **2** as a result of increasing [D-frc] in saline buffer at pH 7.4.

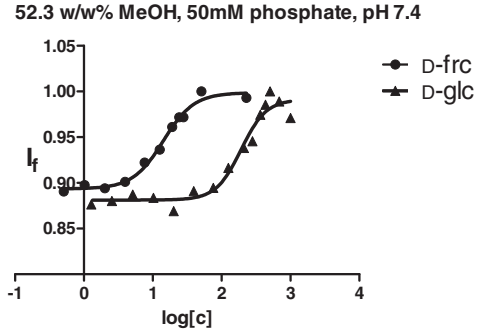

Figure 6. Increased emission intensity of sensor **2** as a result of increasing sugar concentration.

Table 2
K_d(D-glc)/K_d(D-frc) ratios for sensors **1** and **2** in the three employed buffers

Sensor/buffer	K_d (D-glc)/K_d(D-frc)
1, Saline	17
1, 50 mM Phosphate	25.7
1, 52.3 w/w%MeOH/phos	20
2, Saline	–
2, 50 mM Phosphate	–
2, 52.3 w/w%MeOH/phos	13.7

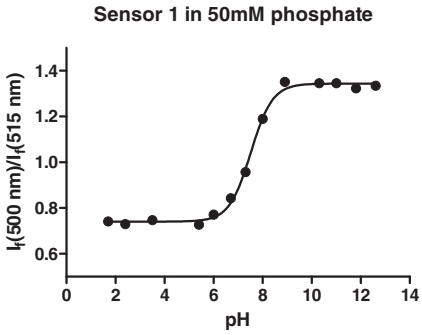

Figure 7. Titration curve for the pK_a determination of sensor **1**. The ratios of emission intensities at 500 and 515 nm are plotted against pH.

Sensor **2** exhibits significantly decreased D-fructose binding, but also poor D-glucose binding in all the three buffers. The poor D-glucose affinity of sensor **2** can be rationalized by the very high pK_a value. Decreasing the pK_a of the arylboronic acid by attachment of electron-withdrawing substituents elsewhere in the molecule can tune the binding strength of sensor **2** toward

Table 3
pK_a Values determined from the change in excitation and emission intensities

Sensor/buffer	pK_a (n) σ
1, 50 mM Phosphate	7.3 (2) 0.1
1, 52.3 w/w% MeOH/phos	7.9 (2) 0.1
2, 50 mM Phosphate	10.4 (2) 0.1
2, 52.3 w/w% MeOH/phos	10.7 (2) 0.5

The number of measurements (n) is shown in brackets, and the standard deviations (σ) are shown to the right.

binding of D-glucose at a physiological level. The high assay robustness of sensor **1** in the three employed buffers is promising in the engineering of a fluorescent sensor, which can be used for semi- or non-invasive D-glucose monitoring in human blood. The found assay robustness is quite different compared to the obtained results for the sensors **2** and **3**.[25] Here the employed buffer exhibits a much greater impact on the absorption and emission response, as well as the carbohydrate binding affinities and the D-glucose selectivity.

Superior selectivity toward D-glucose can ultimately be achieved by the use of fluorescent diboronate derivatives.[30–34] However, these usually contain a highly insoluble scaffold, which is required for appropriate spatial arrangement of the boronate moieties. Aryl diboronates are also generally more synthetically challenging than the engineering of aryl monoboronates.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.01. 101.

References and notes

1. Yang, W.; Yan, J.; Springsteen, G.; Deeter, S.; Wang, B. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1019–1022.
2. Cao, H.; Diaz, D. I.; DiCesare, N.; Lakowicz, J. R.; Heagy, M. D. *Org. Lett.* **2002**, *4*, 1503–1505.
3. Shinkai, S.; Takeuchi, M. *Trends Anal. Chem.* **1996**, *15*, 188–193.
4. Draffin, S. P.; Duggan, P. J.; Duggan, S. A. M.; Norrild, J. C. *Tetrahedron* **2003**, *59*, 9075–9082.
5. Hirata, O.; Kubo, Y.; Takeuchi, M.; Shinkai, S. *Tetrahedron* **2004**, *60*, 11211–11218.
6. Dowlut, M.; Hall, D. G. *J. Am. Chem. Soc.* **2006**, *128*, 4226–4227.
7. Goldstein, I. J.; Murphy, L. A.; Ebisu, S. *Pure Appl. Chem.* **1977**, *49*, 1095–1103.
8. Anderson, S.; Neidlein, U.; Gramlich, V.; Diederich, F. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1596–1600.
9. Barwell, N. P.; Crump, M. P.; Davis, A. P. *Angew. Chem., Int. Ed.* **2009**, *48*, 7673–7676.
10. Ke, C.; Destecroix, H.; Crump, M. P.; Davis, A. P. *Nat. Chem.* **2012**, *4*, 718–723.
11. Sookcharoenpinyo, B.; Klein, E.; Ferrand, Y.; Walker, D. B.; Brotherhood, P. R.; Ke, C.; Crump, M. P.; Davis, A. P. *Angew. Chem., Int. Ed.* **2012**, *51*, 4586–4590.
12. Hiraoka, M.; Firbank, M.; Essenpreis, M.; Cope, M.; Arridge, S. R.; van der Zee, P.; Delpy, D. T. *Phys. Med. Biol.* **1993**, *38*, 1859–1876.
13. Peyser, T.A.; Gamsey, S.; Romey, M.A.; Markle, D.R. US Patent 20100312483, 2010, *Chem. Abstr.* **2010**, *154*, 4538.
14. Hansen, J. S.; Christensen, J. B.; Petersen, J. F.; Hoeg-Jensen, T.; Norrild, J. C. *Sens. Actuators, B Chem.* **2012**, *161*, 45–79.
15. Huang, S.; Jia, M.; Xie, Y.; Wang, J.; Xu, W.; Fang, H. *Curr. Med. Chem.* **2012**, *19*, 2621–2637.
16. Kamata, K.; Morihiro, M.; Nishimura, T.; Eiki, J.-I.; Nagata, Y. *Structure* **2004**, *12*, 429–438.
17. Heise, H. M.; Bittner, A.; Koschinsky, T.; Gries, F. A. *Fresenius J. Anal. Chem.* **1997**, *359*, 83–87.
18. Donmoyer, C. M.; Eijofor, J.; Lacy, D. B.; Chen, S.-S.; McGuinness, O. P. *Am. J. Physiol. Endocrinol. Metab.* **2001**, *280*, E703–E711.
19. Arai, T.; Hashimoto, K.; Muzutani, H.; Kawabata, T.; Sako, T.; Washizu, T. *Vet. Res. Commun.* **1999**, *23*, 203–209.
20. Norrild, J. C.; Eggert, H. *J. Chem. Soc., Perkin Trans. 2* **1996**, 2583–2588.
21. Bielecki, M.; Eggert, H.; Norrild, J. C. *J. Chem. Soc., Perkin Trans. 2* **1999**, 449–455.
22. Hansen, J. S.; Christensen, J. B.; Solling, T. I.; Jakobsen, P.; Hoeg-Jensen, T. *Tetrahedron* **2011**, *67*, 1334–1340.
23. DiCesare, N.; Lakowicz, J. R. *Tetrahedron Lett.* **2001**, *42*, 9105–9108.
24. Van Duin, M.; Peters, J. A.; Kieboom, A. P. G.; Van Bekkum, H. *Tetrahedron* **1984**, *40*, 2901–2911.
25. Hansen, J. S.; Petersen, J. F.; Hoeg-Jensen, T.; Christensen, J. B. *Tetrahedron Lett.* **2012**, *53*, 5852–5855.
26. Loudet, A.; Burgess, K. *Chem. Rev.* **2007**, *107*, 4891–4932.
27. DiCesare, N.; Lakowicz, J. R. *J. Phys. Chem. A* **2001**, *105*, 6834–6840.
28. Mizuno, T.; Fukumatsu, T.; Takeuchi, M.; Shinkai, S. *J. Chem. Soc., Perkin Trans. 1* **2000**, 407–413.
29. Karolin, J.; Johansson, L. B. A.; Strandberg, L.; Ny, T. *J. Am. Chem. Soc.* **1994**, *116*, 7801–7806.
30. Yang, W.; He, H.; Drueckhammer, D. G. *Angew. Chem., Int. Ed.* **2001**, *40*, 1714–1718.
31. Karnati, V. R.; Gao, X.; Gao, S.; Yang, W.; Ni, W.; Sankar, S.; Wang, B. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3373–3377.
32. James, T. D.; Sandanayake, K. R. A. S.; Iguchi, R.; Shinkai, S. *J. Am. Chem. Soc.* **1995**, *117*, 8982–8987.
33. Suri, J. T.; Cordes, D. B.; Cappucio, F. E.; Wessling, R. A.; Singaram, B. *Langmuir* **2003**, *19*, 5145–5152.
34. Eggert, H.; Frederiksen, J.; Morin, C.; Norrild, J. C. *J. Org. Chem.* **1999**, *64*, 3846–3852.