

Supramolecular Chemistry

Monosaccharides as Versatile Units for Water-Soluble Supramolecular Polymers

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Abstract: We introduce monosaccharides as versatile watersoluble units to compatibilise supramolecular polymers based on the benzene-1,3,5-tricarboxamide (BTA) moiety with water. A library of monosaccharide-based BTAs is evaluated, varying the length of the alkyl chain (hexyl, octyl, decyl and dodecyl) separating the BTA and saccharide units, as well as the saccharide units (α -glucose, β -glucose, α -mannose and α -galactose). In all cases, the monosaccharides impart excellent water compatibility. The length of the alkyl chain is the determining factor to obtain either long, one-dimensional supramolecular polymers (dodecyl spacer), small aggregates (decyl spacer) or molecularly dissolved (octyl and

hexyl) BTAs in water. For the BTAs comprising a dodecyl spacer, our results suggest that a cooperative self-assembly process is operative and that the introduction of different monosaccharides does not significantly change the self-assembly behaviour. Finally, we investigate the potential of post-assembly functionalisation of the formed supramolecular polymers by taking advantage of dynamic covalent bond formation between the monosaccharides and benzox-aboroles. We observe that the supramolecular polymers readily react with a fluorescent benzoxaborole derivative permitting imaging of these dynamic complexes by confocal fluorescence microscopy.

Introduction

Saccharide-based amphiphiles, in nature typically found in membranes as structural or recognition units,^[1,2] have become an important class within the fields of soft matter and materials science because of their excellent water-solubility, biocompatibility and the potential for saccharide recognition. Numerous saccharide-based amphiphiles have been synthesised and extensively studied, both in bulk and in aqueous solution.^[3-7] Alkyl glycosides are an appealing subclass because of their straightforward molecular structure.^[8] Changes in the relative size of the alkyl- or saccharide part influence the aggregation in line with the theory of Israelachvili,^[9] and so does changing the spatial orientation of the saccharide head group by modifying the anomeric centre.^[10,11] Introducing different epimers also influences the aggregation behaviour, which is attributed to differences in hydrogen bonding of the alcohol units.^[12-14]

The natural role of saccharides as recognition units through selective binding by lectins provides a strategy for the development of responsive systems or post-assembly functionalisation.^[15-21] Furthermore, saccharides are well known to form

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dynamic covalent bonds with boronic acids.^[22-24] Researchers have relied on this dynamic covalent bond formation for the development of synthetic lectin mimics,^[25,26] and dynamic systems with applications in sensing,^[25,27] responsive materials,^[28-34] drug delivery,^[35-38] and separation techniques.^[39] Benzoxaborole is of particular interest, because it forms dynamic covalent bonds with the pyranose form of monosaccharides.^[34,40-44]

In our previous work, we showed that benzene-1,3,5tricarboxamide (BTA) derivatives connected to water-soluble tetraethylene glycol units through a dodecyl spacer form responsive supramolecular polymers in water (Figure 1, compound 5).^[45] The mechanism of exchange of monomers between different supramolecular polymers was studied in detail by super resolution fluorescence microscopy,^[46] and a detailed computational study showed the importance of the formation of a local hydrophobic domain and suggested stabilisation through intermolecular hydrogen bond formation.^[47] Recently, the presence of intermolecular hydrogen bonds stabilising the supramolecular polymers in water was experimentally verified by infrared spectroscopy.^[48]

Here, we explore the application of monosaccharides as versatile water-solubilising groups to create BTA-based water-soluble supramolecular polymers. Two important aspects of the molecular design are evaluated with respect to the self-assembly behaviour. First, the role of the spacer length is studied by introducing a dodecyl, decyl, octyl or hexyl spacer connected to β -D-glucopyranoside (Figure 1, compounds **1a**-**d**). In a second series the aliphatic spacer is kept constant and either β -D-glucopyranoside, α -D-glu

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Figure 1. Chemical structure of BTAs 1–5.

side, or α -D-mannopyranoside is introduced. This allows us to assess the effect of changing the anomeric centre (1 a vs. 2) and the effect of changing between different epimers (1 a vs. 3 and 4) on the self-assembly behaviour. The monosaccharide is connected to the BTA moiety by a Cu^I-catalysed azide-alkyne cycloaddition because of ease of synthesis and functional group tolerance. The self-assembly of the monosaccharidebased BTAs is studied by ¹H NMR spectroscopy, UV/Vis, and fluorescence spectroscopy, as well as by cryogenic transmission electron microscopy (cryoTEM) and small angle X-ray scattering (SAXS). Our results show that a dodecyl spacer is required to form supramolecular polymers, and these polymers can be targeted by benzoxaborole through dynamic covalent bond formation. Interestingly, the introduction of different monosaccharides does not seem to significantly influence the self-assembly behaviour of the BTAs in water.

Results and Discussion

Synthesis of compounds 1-4

The synthetic route towards β -D-glucopyranoside-based BTAs **1 a-d** is shown in Scheme 1. The α, ω -dibromoalkanes of required length were converted into *N*-(ω -azidoalkyl)phthalimides **6a-d** in a statistic one-pot procedure. The Gabriel synthesis was completed by treating **6a-d** with hydrazine monohydrate affording the ω -azidoalkyl- α -amines **7a-d** in overall yields of 30–40%. Subsequently, treatment of trimesoyl chloride with three equivalents of **7a-d** provided azide-functionalised benzene-1,3,5-tricarboxamides **8a-d** in yields of 51–88%. The acetyl-protected glucose functionalised BTAs **9a-d** were produced in high yields by Cu¹-catalysed cycloaddition of **8a-d** with 2-propynyl-tetra-*O*-acetyl- β -D-glucopyranoside. Saponification of **9a-d** with sodium hydroxide resulted in the desired glucose-functionalised BTAs **1a-d** in near quantitative yields. Compounds **1a-d** were obtained as white solids and were



Scheme 1. Synthesis of BTAs 1 a-d.

fully characterised by ¹H NMR-, ¹³C NMR- and IR spectroscopy and MALDI-TOF-MS (see the Supporting Information).

The synthesis of BTA derivatives **2–4** was achieved by coupling **8a** to the corresponding alkyne-functionalised monosaccharide by Cu¹-catalysed azide–alkyne cycloaddition (Scheme 2). Workup by reversed phase column chromatography (which was also applied to **1a**) effectively removed traces of salts. BTAs **2–4** were obtained as white solids and were fully characterised by ¹H NMR-, ¹³C NMR-, IR spectroscopy and MALDI-TOF MS (see the Supporting Information).



Scheme 2. Synthesis of BTAs 2-4.

Exploring the aqueous self-assembly behaviour of 1 a-d

The aggregation behaviour of 1 a-d in D₂O was first assessed by ¹H NMR spectroscopy at room temperature ($c=1 \times 10^{-3}$ M) (Figure 2). All samples were heated to 90 °C during sample preparation and allowed to equilibrate overnight at room temperature, which promotes H–D exchange of the amides and alcohols. The sharpness of the resonances differs significantly in the NMR spectra of the different compounds. Whereas the spectrum of 1 c, d shows sharp signals, only one of the aromatic signals is visible in the spectrum of 1 b, and all remaining signals are relatively broad. In the spectrum of 1 a, all signals corresponding to the BTA have disappeared and only the solvent peaks remain visible. These observations suggest that 1 dand 1 c do not form aggregates, whereas the absence of



Figure 2. ¹H NMR spectra of **1a–d** ($c=1 \times 10^{-3}$ M) in D₂O at 20 °C. The peaks at δ = 7.9 and 8.1 ppm were assigned by 1D ROESY NMR spectroscopy to the triazole and aromatic core of the BTA, respectively (the Supporting Information, Figure S1).

a signal for the aromatic core and the broader remaining signals suggest that **1b** does form aggregates. In the spectrum of **1a** all signals are lost, indicative of the formation of large aggregates.

To study the aggregation behaviour of **1 a**–**d** in more detail, UV/Vis spectroscopy measurements were performed. Samples were prepared by injection of a concentrated solution in methanol (5 μ L, $c=5 \times 10^{-3}$ M) into water (2.5 mL, $c=1 \times 10^{-5}$ M) and spectra were recorded at 20 °C after equilibration of the samples overnight (Figure 3 A). The UV spectra of BTAs **1 c** and **1 d** perfectly overlap and show an absorption maximum at 207 nm. The UV spectrum of **1 b** shows a maximum at 208 nm, and that of **1 a** shows a maximum at 211 nm with a shoulder around 225 nm.

The previously studied BTAs comprising aliphatic side chains show a C_3 -symmetrical, helical arrangement of the hydrogen bonds stabilising the aggregates in alkane solvents and the



Figure 3. A) UV/Vis spectra of 1 a-d ($c \approx 1 \times 10^{-5} \text{ M}$) at 20 °C in water; the UV spectra of 1 c and 1 d perfectly overlap (small deviations in absorption intensities are attributed to small variations in the sample concentrations); B) Fluorescence emission spectra of Nile Red in aqueous solutions of 1 a and 1 b and in water at 20 °C ($c_{\text{BTA}} = 1 \times 10^{-5} \text{ M}$, $c_{\text{NR}} = 5 \times 10^{-6} \text{ M}$, $\lambda_{\text{ex}} = 550 \text{ nm}$).

angle between the amide and central benzene group is typically between 35 and 45°.^[49] This arrangement of the hydrogen bonds is reflected by an absorption maximum at 193 nm in the UV spectrum. In contrast, when these aliphatic BTAs are molecularly dissolved, the absorption maximum shifts to 208 nm.^[49,50] As a result, the single absorption maximum at 207 nm in the case of 1b and 1c, and 208 nm for 1d suggests that these BTAs are molecularly dissolved in water. For 1 a, the absorption maximum at 211 nm with a shoulder at around 225 nm is very similar to the UV/Vis spectrum we previously observed for aggregates of 5 in water.^[45] Recent IR measurements unambiguously show that aggregates formed by 5 are stabilised by intermolecular hydrogen bonds in water.^[48] In analogy, we believe that also the aggregates formed by 1 a are stabilised by hydrogen bonds. Interestingly, the redshift in the UV spectra observed for both 5 and 1a in water indicates that the packing of the BTAs in the aggregated state is different from those observed for the aliphatic BTA derivatives. We speculate that either the angle of the amide group with respect to the central benzene ring is different or that there is an offset between BTAs within the aggregate resulting in a J-type aggregate. Such offsets have been reported in several crystal structures for BTA derivatives.^[51] Although in both cases a redshift in the UV spectrum is expected, the details of the hydrogen-bond arrangement in aggregates of 1a and 5 are not yet fully understood.

To assess the formation of a hydrophobic domain upon selfassembly, experiments with the solvatochromic dye Nile Red were performed (Figure 3B). Both the fluorescence emission wavelength and intensity of Nile Red depend on the polarity of its environment.^[52] In our experiments, the emission intensity and λ_{\max} are directly related to the presence and polarity of a hydrophobic pocket. Samples were prepared by injection of a solution of Nile Red in methanol (5 μ L, $c = 2.5 \times 10^{-3}$ M) into water (2.5 mL), followed by the desired BTA (5 μ L, $c=1\times$ 10^{-2} M, final concentrations: $c_{NR} = 5 \times 10^{-6}$ M, $c_{BTA} = 1 \times 10^{-5}$ M). Solutions of 1c and 1d showed no change in fluorescence intensity or λ_{max} compared to water, which indicated that no hydrophobic domains were formed (data not shown). The emission intensity of Nile Red in a solution of 1a increased two orders of magnitude and λ_{max} shifted to 625 nm (Figure 3 B). In the solution of 1 b, the intensity increase and the blueshift of λ_{max} to 633 nm was much smaller (Figure 3B).

The results from the Nile Red assay, UV absorption and ¹H NMR experiments show that **1c** and **1d** do not aggregate in water at these concentrations. The UV spectrum of **1b** ($c = 1 \times 10^{-5}$ M) does not suggest assembly of the BTAs, whereas the Nile Red study indicates the formation of domains that are slightly more hydrophobic than water. Furthermore, in the ¹H NMR experiments, measured at two orders of magnitude higher concentration, compound **1b** does form small aggregates. These results may be explained by the amphiphilic nature of the molecules driving the formation of micelles, whereas the UV spectrum indicates that within these micellar aggregates no significant dipole interactions between the BTA cores occur. In contrast, all techniques indicated assembly of **1a** at 20 °C.

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Figure 4. A) CryoTEM image of **1 a** in water ($c=6.7 \times 10^{-4}$ M); scale bar is 0.5 µm. Inset: zoom, scale bar is 100 nm; B) SAXS profiles of **1 a** in aqueous solution ($c=3.2 \times 10^{-3}$ M) measured at 20 °C (\Box); fitted with the Schurtenberger–Pedersen form factor for wormlike, self-avoiding chains (—). $L_{\rm k} = (6.6 \pm 0.2)$ nm, $r_{\rm cs} = (3.4 \pm 0.1)$ nm and $L_{\rm c} > 70$ nm (beyond the experimentally accessible range).

To determine the dimensions of the assemblies formed by **1a** in water, cryoTEM ($c = 6.7 \times 10^{-4}$ m, Figure 4A) and smallangle X-ray scattering (SAXS) measurements ($c = 3.2 \times 10^{-3}$ M, Figure 4B) were carried out. The cryoTEM experiments revealed fibres of high aspect ratio with lengths of several micrometres and a diameter of approximately 5 nm. The fibres did not appear to form bundles; but this was difficult to assess due to the high fibre density in the sample. The SAXS profile was characteristic for one-dimensional fibres with lengths beyond the experimentally accessible *q*-range. Data extraction from the profile using a wormlike chain model^[53] yielded a contour length beyond the limit of 70 nm. The Kuhn length (L_k) , a measure for the stiffness of supramolecular polymers, was estimated at (6.6 \pm 0.2) nm, and the cross-sectional radius (r_{cs}) was estimated to be (3.4 ± 0.1) nm at 20 °C. These values are in good agreement with the cryoTEM images, and suggest that even at high concentrations these fibres do not tend to form bundles.

The effect of temperature on the self-assembly behaviour of 1 a and 1 b

A major advantage of using saccharides as water-compatibilising units is that, unlike tetraethyleneglycol, which we used previously,[45] they do not show a lower critical solution temperature (LCST). Therefore, we can investigate the temperature-dependent behaviour of the supramolecular polymers formed by 1a and the aggregates formed by 1b with the aim to elucidate their stability and the mechanism of their self-assembly process. Variable-temperature ¹H NMR spectroscopic measurements were performed using pyrazine as an internal standard with a sharp signal at $\delta = 8.6$ ppm ($c = 1 \times 10^{-3}$ M, Figure 5). The ¹H NMR spectrum of **1**a shows no signals other than water at $\delta =$ 1.8 ppm up to 60 °C. At 70 °C, a signal for the triazole at $\delta =$ 7.9 ppm, and broad signals corresponding to the aliphatic spacer and the saccharide end group appear. At 80 °C, a broad peak at $\delta =$ 7.6 ppm emerges, corresponding to the aromatic core, which shifts to $\delta = 7.8$ ppm at 90 °C. At 90 °C, also a broad peak at $\delta = 3.2$ ppm appears, which is assigned to the --CH2- next to the amide by 2D COSY NMR spectroscopy.



Figure 5. Variable temperature 1H NMR spectra of $1\,a$ (top) and $1\,b$ (bottom) in D2O ($c\!=\!1\!\times\!10^{-3}\,{}_{M}$) using water presaturation for improved signal-tonoise.

At 25 °C, the spectrum of **1b** displays broad signals for the aliphatic spacer and saccharide end group. Only one signal appears in the aromatic region at $\delta = 7.9$ ppm, corresponding to the triazole resonance. At 40 °C, a broad signal appears at $\delta = 8.1$ ppm, corresponding to the aromatic core of the BTA, and a signal at $\delta = 3.2$ ppm emerges which is assigned to the – CH₂– next to the amide by 2D-COSY NMR spectroscopy. Interestingly, the shift of the aromatic core in **1a** is $\delta = 7.8$ ppm at 90 °C, whereas in **1b** it is already $\delta = 8.1$ ppm at 40 °C. This suggests π - π stacking of the cores of **1a**, even at 90 °C, and indicates a stronger interaction of the BTA cores in the aggregates of **1a**. The abrupt appearance of signals for **1a** compared to the gradual sharpening of signals for **1b** indicates a higher temperature stability of the supramolecular polymers formed by **1a**.

The normalised integral of the triazole signal plotted as function of temperature clearly shows the different temperature response of the aggregates of **1a** and **1b** in water (Figure 6). For **1b** a plateau is reached at 50°C. Based on the



Figure 6. Normalised integral of the triazole ¹H NMR signal as function of temperature for **1 a** and **1 b** ($c=1 \times 10^{-3}$ M) taken from spectra in Figure 5.

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line broadening persisting up to 90 °C, we hypothesise that above 50 °C **1b** is present in little clusters of only a few molecules. Taken that the integral of the triazole signal reaches its maximum value at 50 °C, we estimate that at 25 °C the fraction of **1b** in aggregates too large to be detected by ¹H NMR spectroscopy is roughly $\varphi = 0.5$. For **1a**, the normalised integral of the triazole signal sharply increases above 60 °C. Based on the shape of the curve, it seems that a plateau is not yet reached even at 90 °C. For **1a**, we estimate $\varphi = 0.5$ is reached between 65 and 70 °C. The sharpness of the transition in **1a** suggests a cooperative supramolecular polymerisation process.^[54]

More insight into the nature of the supramolecular polymerisation processes of **1a** and **1b** was gathered by variabletemperature UV measurements. The UV spectra of **1a** and, to a much lesser extent, of **1b** change upon increasing the temperature (Figure 7 and Figure 8), whereas the UV spectra of



Figure 7. UV/Vis spectra of **1 b** ($c=1 \times 10^{-5}$ M) in water measured between 0 and 90 °C with 10 °C intervals.



Figure 8. UV/Vis spectrum of **1 a** in water ($c = 1 \times 10^{-5}$ M) measured at temperatures between 20 and 90 °C displaying two different transitions. A) 20 to 62 °C: B) 64 to 90 °C.

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1 c and **1 d** show no temperature dependency (the Supporting Information, Figure S2).

By heating a solution of **1b** from 20 to 90 °C, the UV absorption gradually shifts from 208 to 207 nm accompanied by a small increase in intensity. The UV spectrum of **1a** shows a more complex temperature-dependent behaviour. At 20 °C, the UV spectrum of **1a** shows a maximum and a shoulder at 211 and 225 nm, respectively. Increasing the temperature to 62 °C (Figure 8A) induces a decrease in intensity of the absorption and a shift to 208 nm. Concurrently, a shoulder at 195 nm emerges, which is separated by an isosbestic point. By increasing the temperature to 90 °C (Figure 8B), the shoulder at 195 nm diminishes and the absorption maximum at 208 nm further increases, again through an isosbestic point. The temperature behaviour is completely reversible and upon cooling to 20 °C the initial spectrum is obtained again.

The transition between 20 and 62 °C has a non-linear temperature dependency (see below) and appears to be a transition from one aggregated state to another, indicated by the upcoming band at 195 nm and the presence of an isosbestic point. Previously, the opposite transition has been observed after injection of 5 from methanol into water.^[45] Also, an absorption maximum at 194 nm has previously been attributed to BTA self-assembly into columnar helical stacks stabilised by threefold hydrogen bonding in organic solvents,^[49] and to helical BTA assembly in water.^[45] The absorption band at 208 nm may indicate that a part of the BTAs is in an unordered conformation, however, the 1,2,3-triazole unit also has an absorption maximum at 210 nm in water,^[55] which may contribute to the overall intensity at this wavelength. Taking the results from the ¹H NMR experiment into account, the observed transition between 20 and 60 $^\circ\text{C}$ seems to be accompanied by a reduction in the size of the aggregates. The intensity increase at 208 nm indicates partial disruption of the aggregates at high temperatures.

The differences in the temperature-dependent behaviour of the assemblies of **1a** and **1b** were further assessed by probing the hydrophobic domains with Nile Red at various temperatures. In both cases, an initial shift towards shorter wavelengths was observed at low temperatures, which is attributed to the temperature dependency of the Nile Red emission. The same shift was reproduced in variable temperature measurements of Nile Red both in ethanol and acetonitrile (the Supporting Information, Figure S3). By heating a solution of 1a to 70 °C the Nile Red emission shows a sharp decrease and shift to longer wavelengths (Figure 9A), in agreement with the variable temperature UV measurements. In solutions of 1b only a gradual intensity decrease and shift of λ_{max} is observed (Figure 9B). Interestingly, at 80 $^{\circ}\text{C}$ λ_{max} is more blueshifted for 1a than for 1b. This indicates that at 80 °C hydrophobic domains are still present in case of 1a, which further supports that the sharp transition is one between different aggregation types.





Figure 9. A) Nile Red fluorescence emission in an aqueous solution of **1 a** $(c_{BTA}=5\times10^{-5} \text{ m}, c_{NR}=5\times10^{-6} \text{ m})$ measured at temperatures between 10 and 80 °C (10 °C intervals) displaying an abrupt shift between 60 and 70 °C; B) Nile Red fluorescence emission in an aqueous solution of **1 b** $(c_{BTA}=5\times10^{-5} \text{ m}, c_{NR}=5\times10^{-6} \text{ m})$ measured at temperatures between 10 and 80 °C (10 °C intervals) displaying a gradual temperature-dependent trend in the Nile Red emission.

The role of the monosaccharides in the self-assembly process

After assessing the importance of an adequately long hydrophobic spacer, the role of the configuration of the monosaccharides in the BTA self-assembly was explored. To that purpose, enantiomerically pure **2–4**, all comprising a dodecyl spacer, were prepared in addition to **1a** and studied with UV and circular dichroism (CD) measurements at room temperature ($c = 1 \times 10^{-5}$ M). Like L-proline,^[56] monosaccharides may induce a helical preference in the BTA-based supramolecular polymers. Although the UV spectra were indicating that all derivatives were self-assembled (see below), no CD effect was observed in any of the samples, indicating that no helical bias was induced by the monosaccharides.

CryoTEM measurements were performed to visualise the aggregates formed by **2–4** in aqueous solution and to assess their shape and dimensions (Figure 10). Similar to **1a**, both **3** and **4** form long, fibrillar aggregates of micrometres in length and a diameter of approximately 5 nm at a concentration of 3.4×10^{-4} M (Figure 10). For **2** no fibres could be observed, possibly due to poor-quality ice formation during the vitrification process. However, based on our results (see below) we expect **2** to form supramolecular polymers similar to **1a**, **3** and **4**. Interestingly, the supramolecular polymers of **3** display a periodic variation in diameter and intensity, as can be seen at a higher magnification (Figure 10, left inset). A similar feature was observed in the cryoTEM images of **5** and is tentatively attributed to a non-circular cross-section of the supramolecular polymers.^[45] The higher magnification image of **4** (Figure 10,



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Figure 10. CryoTEM images of **3** and **4** in water ($c = 3.4 \times 10^{-4}$ M); scale bar is 0.5 µm. Inset: zoom, scale bar is 100 nm.

right inset) does not clearly show the same feature, possibly due to poorer contrast or focus. Both **3** and **4** show some bundling, but this appears to be a result of the relatively high concentration rather than a general effect.

Temperature stability of assemblies of 1 a and 2-4

The influence of the configuration of the monosaccharides on the self-assembly behaviour was further explored by variabletemperature UV spectroscopy. The UV spectra of **2**–**4** in water $(c=1\times10^{-5} \text{ m})$ were recorded at temperatures between 20 and 90 °C and compared to those obtained for **1a** (Figure 8). Samples were prepared by addition of water to the solid material and upon gentle heating the solid material dissolved readily, resulting in a clear solution.

At 20°C, compounds 2-4 show the same typical UV spectrum as 1a and the same transitions occur when increasing the temperature to 90 °C (the Supporting Information, Figure S4). Interestingly, the changes and transitions in the UV spectra of 1 a and 2-4 occur at almost identical temperatures. Only the shape and intensity change of the shoulder at 195 nm varies slightly between 1a-4. Possibly, this is due to the solvent cut-off of water, which is 190 nm at 20°C and shifts with temperature, making this part of the spectrum sensitive to small deviations of the background correction. Nevertheless, in all cases the same trend is observed. Interestingly, for 2, a transition in the absorption spectrum is observed directly after sample preparation and over the course of 105 min (the Supporting Information, Figure S4 inset). The absorption maximum below 200 nm diminishes while simultaneously a maximum and shoulder at 211 and 225 nm, respectively, emerge similar to what was reported for 5 previously.^[45] We expect that this process may occur for all BTA derivatives 1 a and 2-4. It appears that heating the solution to 60°C induces the reversed transition.

To visualise the trends more clearly, the normalised absorption intensity of 1a and 2-4 at 225 nm is plotted as function of temperature (Figure 11). BTA derivatives 1a and 2-4 all display a very similar temperature profile. A gradual decrease of the intensity is observed going from 90 to $60 \,^\circ$ C, followed by an apparent cooperative intensity increase between 60 and $20 \,^\circ$ C. The final absorption intensity is reached fastest by 1a and 4, whereas 2 deviates slightly. The gradual decrease

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Figure 11. Normalised UV/Vis absorption of aqueous solutions of 1 a–4 ($c = 1 \times 10^{-5}$ M) at 225 nm plotted as a function of temperature.

during cooling from 90 to 60 °C corresponds to the formation of small aggregates according to the ¹H NMR experiments. Below 60 °C the aggregation into long supramolecular polymers seems highly cooperative. Possibly, the small aggregates formed at high temperature act as nucleus for elongation. The minor difference between the temperatures at which the elongation occurs for **1a** and **2–4** is surprising because a strong relation between the introduction of different anomers or epimers and the critical micelle concentration (CMC), solubility, and aggregate shape has been observed in alkyl glycosides.^[10–14]

Targeting the saccharide units through dynamic covalent bond formation with benzoxaborole

As a versatile strategy for post-assembly functionalisation, the potential for selectively targeting the saccharides in **1 a** and **2**–**4** by dynamic covalent bond formation with benzoxaborole was assessed.^[40,41] Fluorescein-labelled benzoxaborole **10**



Figure 12. Fluorescein-labelled benzoxaborole 10. (Figure 12, $c = 5 \times 10^{-7}$ M) was added to pre-equilibrated solutions of **1a**, **3** and **4** (3×10^{-5} M) and the binding was qualitatively assessed by fluorescence microscopy (Figure 13).

The snapshots from imaging the solutions (see the Supporting Information for the movie files) show fluorescent fibrillar objects, although with some differences between **1a**, **3** and **4**. The sample of



Figure 13. Fluorescence microscopy images of **1 a**, **3**, and **4** ($c=3 \times 10^{-5}$ M) in the presence of fluorescein-labelled benzoxaborole **10** ($c=5 \times 10^{-7}$ M) showing fibrillar objects in all cases. Scale bar is 10 µm.

1 a shows a mixture of fibres longer than 10 μ m and short fibres. For **3**, mostly long fibres of around 10 μ m long are observed. Instead, short fibres are present in the solution of **4**, but some fibres reach up to 10 μ m in length. To rule out nonspecific or hydrophobic interactions, fluorescein-labelled benzoxaborole **10** was added to a pre-equilibrated solution of **5** in water. In this solution, no aggregates could be visualised with fluorescent microscopy. This strongly suggests selective dynamic covalent-bond formation to occur between the benzoxaborole and the monosaccharides in **1 a**, **3** and **4**.

Although cryoTEM measurements showed that 1 a, 3 and 4 form fibres of similar dimensions of several micrometres in length, the fluorescence microscopy measurements suggest that the average length of the fibres varies. Possibly, the length scale of the cryoTEM is too small to detect these differences. However, the observed differences may also result from complexation of benzoxaborole with the supramolecular polymers, which may influence the self-assembly and exert a different effect on the different monosaccharides. Importantly, these measurements show the proof of principle of a post-assembly dynamic step to functionalise, label, or target the supramolecular polymers, and preparation of multivalent benzoxaborole derivatives may lead to higher affinity. Furthermore, in a multicomponent system, this approach may allow for the development of selective cross-linkers, or recruiters to gain spatiotemporal control on the population distribution over the length of the supramolecular polymer.^[57]

Conclusion

A series of BTA derivatives functionalised with monosaccharide end groups has been synthesised and studied. A dodecyl spacer separating the BTA core from the saccharide end group is required for the formation of supramolecular polymers of high aspect ratio. The introduction of different monosaccharides has little effect on the self-assembly behaviour. When increasing the temperature, the supramolecular polymers are disrupted at almost identical temperature. This is in sharp contrast to alkyl glycosides, which show a strong dependency of the solubility and CMC on the configuration of the saccharide, and indicates these BTAs do not behave simply as saccharidebased amphiphiles. The formation of long supramolecular polymers appears to be a cooperative process. However, different species are present at different temperature regimes. Possibly, the small aggregates present at high temperature act as nucleus for elongation, although this needs to be studied in more detail. Finally, we show that the monosaccharides in the supramolecular polymers can be selectively targeted through dynamic covalent bond formation. This strategy may provide a powerful method to functionalise or label the supramolecular polymers in a post-assembly stage.

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