Electronic Supplementary Information

Self-assembly of a "double dynamic covalent" amphiphile with a glucose-responsive imine bond

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1. General

All commercial reagents and solvents were used as received. Turbidity measurements were performed on a Varian Cary 300 UV-Vis spectrophotometer. Fluorescence spectra (λ_{ex} = 500 nm, λ_{em} = 623 nm) of Nile red were obtained on a Hitachi F-4500 or a Varian Cary Eclipse fluorescence spectrophotometer. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on Bruker AV400 or AV500 NMR spectrometers. Dynamic light scattering (DLS) measurements were performed on a Malvern Zetasizer Nano ZS. Transmission electron microscope (TEM, unstained) images were taken on a JEOL JEM-1400 transmission electron microscope. All sample preparation and spectroscopic measurements were carried out at ambient temperature of 298 K. Liquid chromatography–mass spectrometry (LC-MS) was performed using a Waters (Manchester, UK) TQD mass spectrometer equipped with a triple quadrupole analyser.

2. Experimental

A stock solution of 50 mM 4-formylphenylboronic acid (4FBA) was prepared in 100 mM sodium carbonate buffer of pH 10.5, with 2 eqv of NaOH (i.e. 100 mM) added (to ionize the boronic acid and neutralize octylamine-HCl for the self-assembly study). A stock solution of 500 mM octylamine-HCl (C8AM-HCl) was prepared in water. Stock solutions of D-glucose (1 M), D-fructose (1 M) and D-galactose (0.5 M) were prepared in water and diluted with buffer prior to use. For ^{1}H NMR determination of imine formation, the solutions were prepared in D_2O.

In the self-assembly study, stock solutions of a saccharide (if used), C8AM-HCl, 4FBA-2NaOH were added to 100 mM sodium carbonate buffer of pH 10.5 to obtain the final solution with desired component concentrations. For NMR and DLS studies, the solution was incubated for 30 min and then subject to the measurements. For the fluorescence experiments, the solution (2 mL) was incubated for 30 min, treated with Nile red (10 μL of 1 mM methanol solution), and then immediately subject to fluorescence measurements. For TEM imaging, the solution was incubated for 30 min, pipetted onto a copper grid, dried under vacuum for 30 min and subject to TEM imaging.

3. ESI-MS spectra

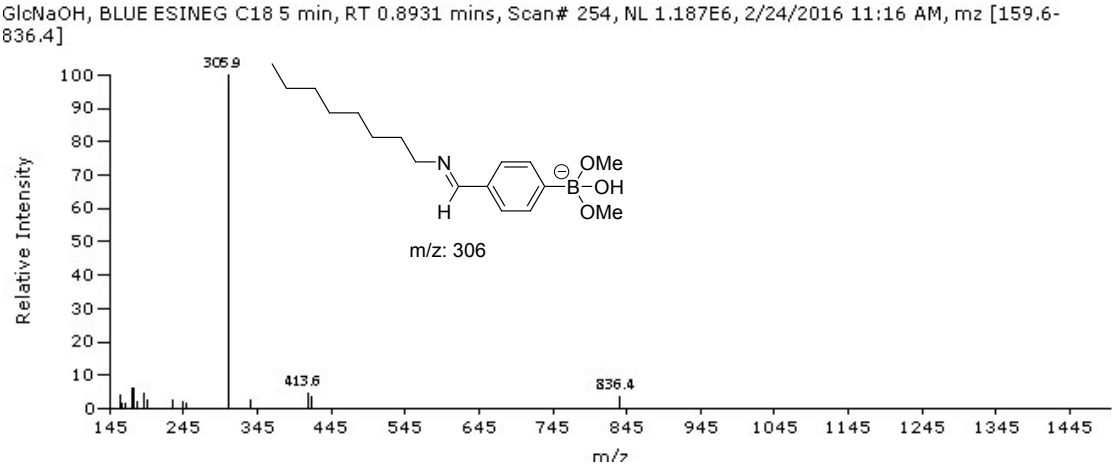

Fig. S1 ESI-MS spectrum of a methanol solution of 4FBA (sodium salt), C8AM, and glucose, diluted from a concentrated methanol solution of 4FBA (sodium salt, 20 mM), C8AM (20 mM) and glucose (10 mM).

4. Fluorescence spectra

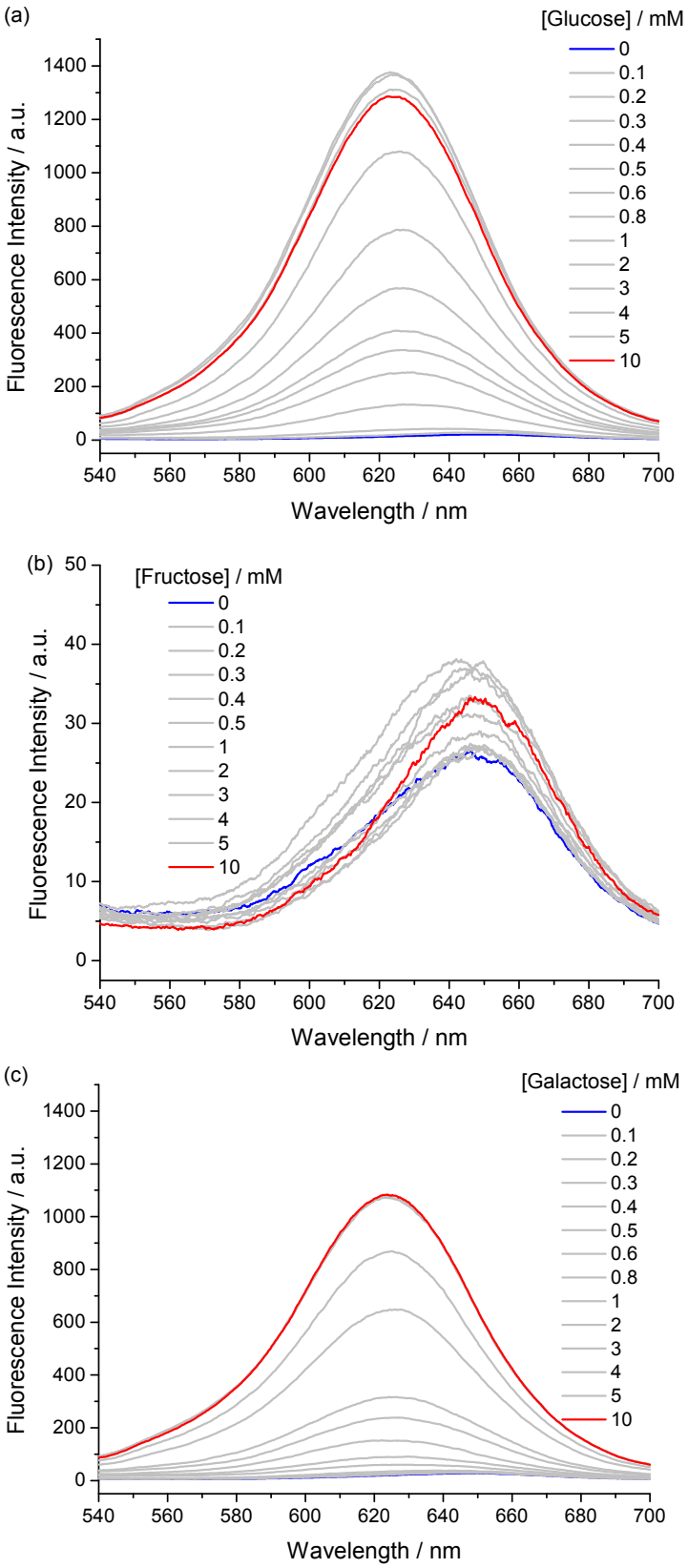

Fig. S2 Fluorescence spectra of Nile red (5 μM) added to a mixture of 4FBA (3 mM), C8AM (3 mM) and increasing concentration of D-glucose (a), D-fructose (b) and D-galactose (c) in pH 10.5 100 mM $NaHCO_3$–Na_2CO_3 buffer. 4FBA, C8AM and the saccharide component (if used) were mixed in the buffer for 30 min before the addition of Nile red.

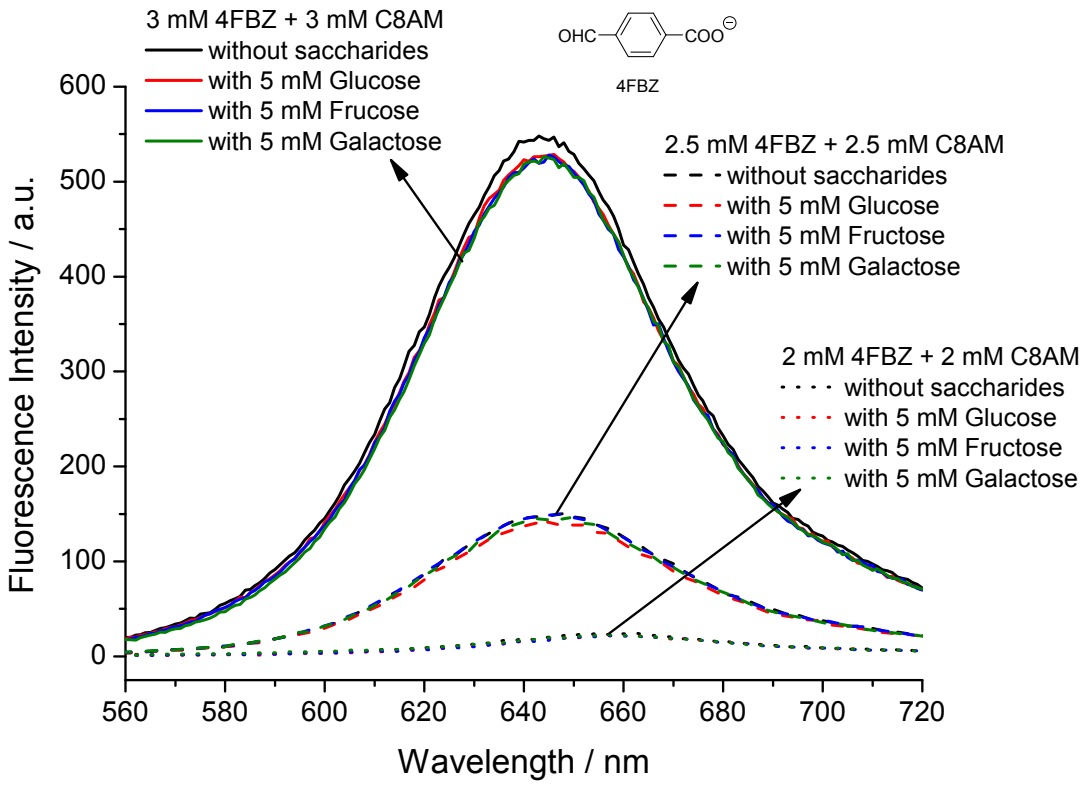

Fig. S3 Fluorescence spectra of Nile red (5 μM) added to a mixture of 4-formylbenzoate (4FBZ, 2 mM, 2.5 mM or 3 mM) and C8AM (2 mM, 2.5 mM or 3 mM) in the absence and presence of saccharides (5 mM) in pH 10.5 100 mM $NaHCO_3$–Na_2CO_3 buffer. 4FBZ, C8AM and the saccharide component (if used) were mixed in the buffer for 30 min before the addition of Nile red. Inset shows the structure of 4FBZ.

5. NMR evidence of imine and boronate ester formation

To confirm the ability of 4FBA to form an imine with C8AM and bind saccharides via boronate ester linkages, we conducted NMR binding studies. 4FBA (10 mM) was mixed with a large excess of C8AM (100 mM), in the presence of the non-ionic detergent octaethylene glycol monododecyl ether (C12E8, 200 mM, to solubilize all components and prevent vesicle formation). ^{1}H NMR spectrum in the absence of saccharides (Fig. S4 black) showed that the boronic acid existed predominantly as the imine. The presence of saccharides led to appearance of new signals in the ^{1}H NMR spectra of the imine, confirming saccharide binding to the imine. We attempted to perform ^{11}B NMR on those micelle samples; however, the ^{11}B NMR signals of the imine broadened to baseline, likely due to restricted molecule motion as a result of the imine aligning with the C12E8 surfactant molecules in micelles. Therefore ^{11}B NMR saccharide binding study was only performed for 4FBA (Fig. S5).

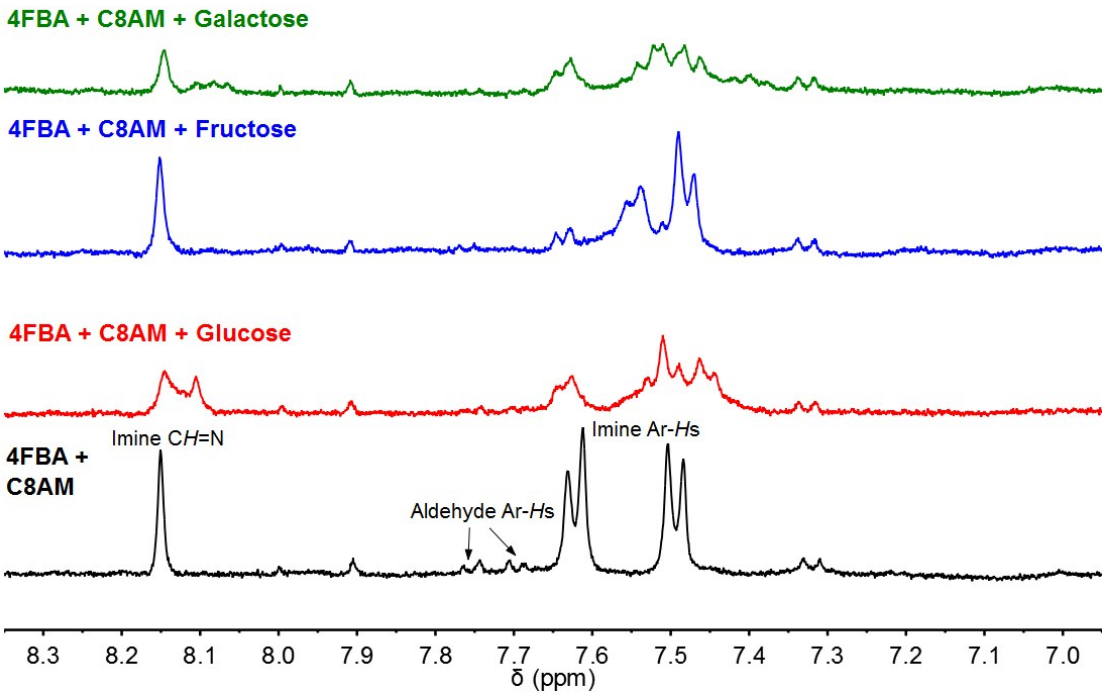

Fig. S4 ^{1}H NMR spectra (400 MHz) of a mixture of 4FBA (10 mM, sodium salt used), C8AM (100 mM) and C12E8 (200 mM) in the absence and presence of saccharides (10 mM) in 9:1 H_2O/D_2O. The imine product was solubilized in C12E8 micelles.

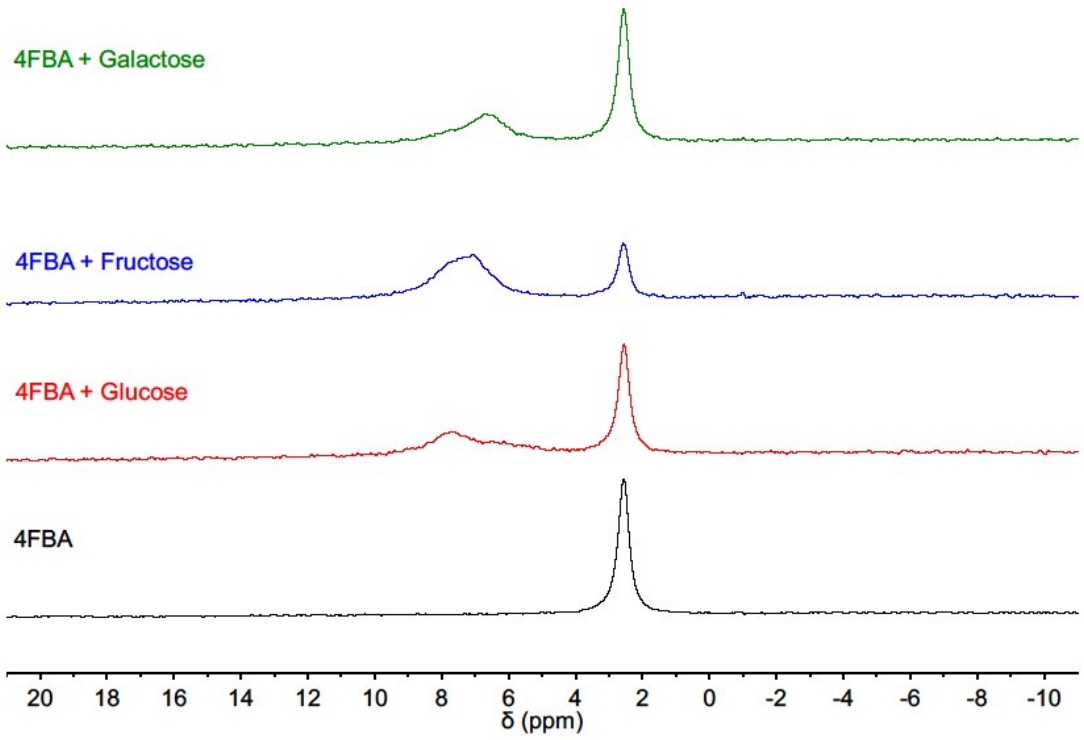

Fig. S5 ^{11}B NMR spectra (160 MHz, without decoupling, referenced to $BF_3{\cdot}Et_2O$ in $CDCl_3$) of 4FBA (10 mM) in the absence and presence of saccharides (10 mM) in 9:1 H_2O/D_2O buffered at pH 10.5 with 100 mM $NaHCO_3$–Na_2CO_3.

6. NMR determination of imine formation

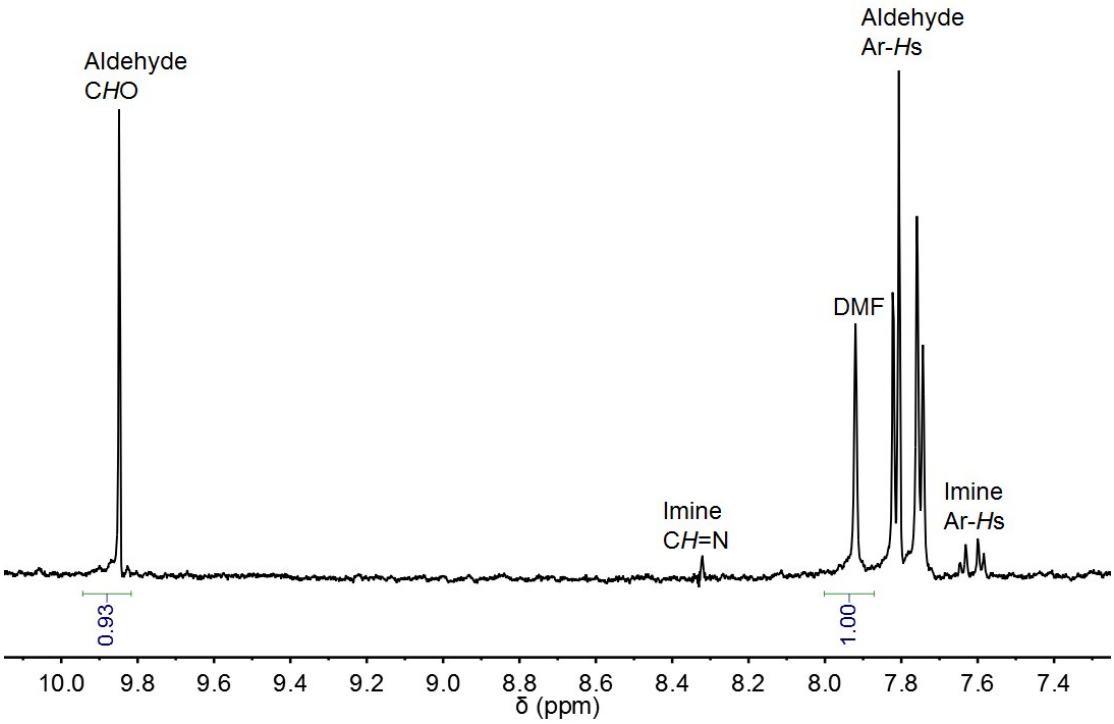

Fig. S6 ^{1}H NMR (500 MHz) spectrum of a mixture of 4FBA (3 mM) and C8AM (3 mM) in D_2O buffered at pD 10.5 with 100 mM $NaHCO_3$–Na_2CO_3. DMF (3 mM) was added as an internal reference for determination of imine formation.

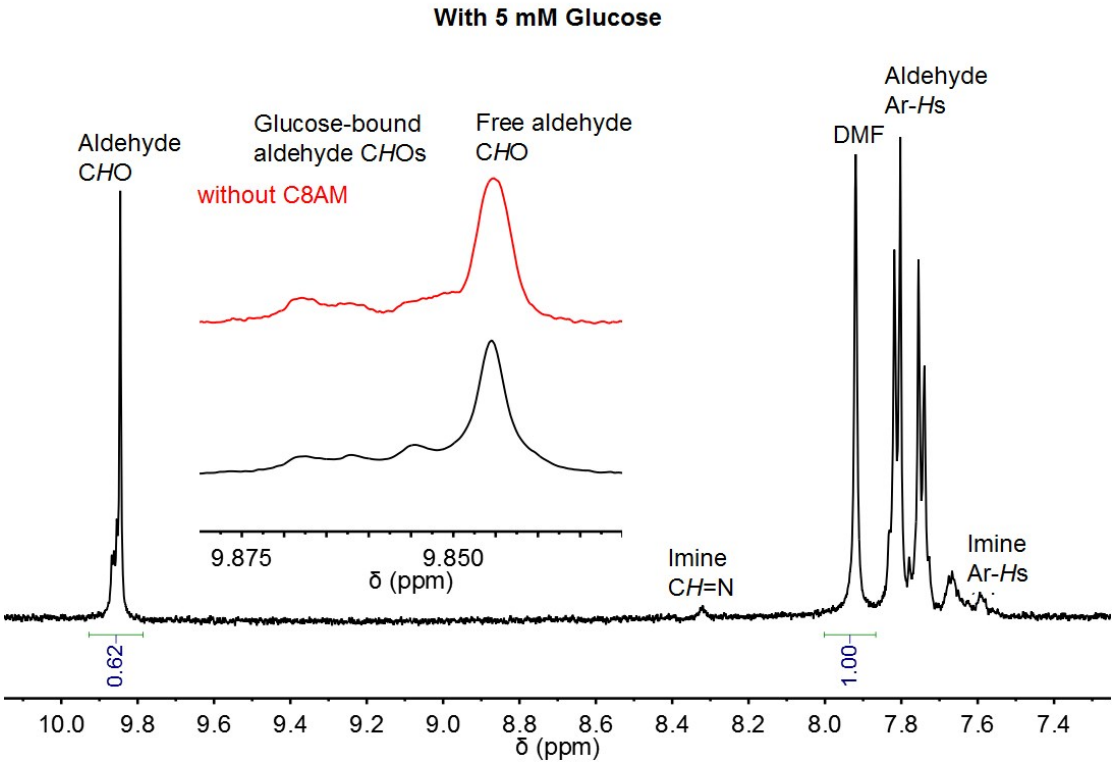

Fig. S7 ^{1}H NMR (500 MHz) spectrum of a mixture of 4FBA (3 mM), C8AM (3 mM) and glucose (5 mM) in D_2O buffered at pD 10.5 with 100 mM $NaHCO_3$–Na_2CO_3. DMF (3 mM) was added as an internal reference for determination of imine formation. Note that multiple sets of signals were observed in the aldehyde CHO region and the aromatic regions of aldehydes and imines. This is due to the coexistence of the free aldehyde, glucose-bound aldehydes, free imine, and glucose-bound imines. The aldehyde region was zoomed and compared with the control sample without the amine component C8AM (red spectrum). Similar multiple signals were observed without C8AM, indicating that the multiple signals are due to coexistence of the free aldehyde and glucose-bound aldehydes (a mixture of complexes[1]).

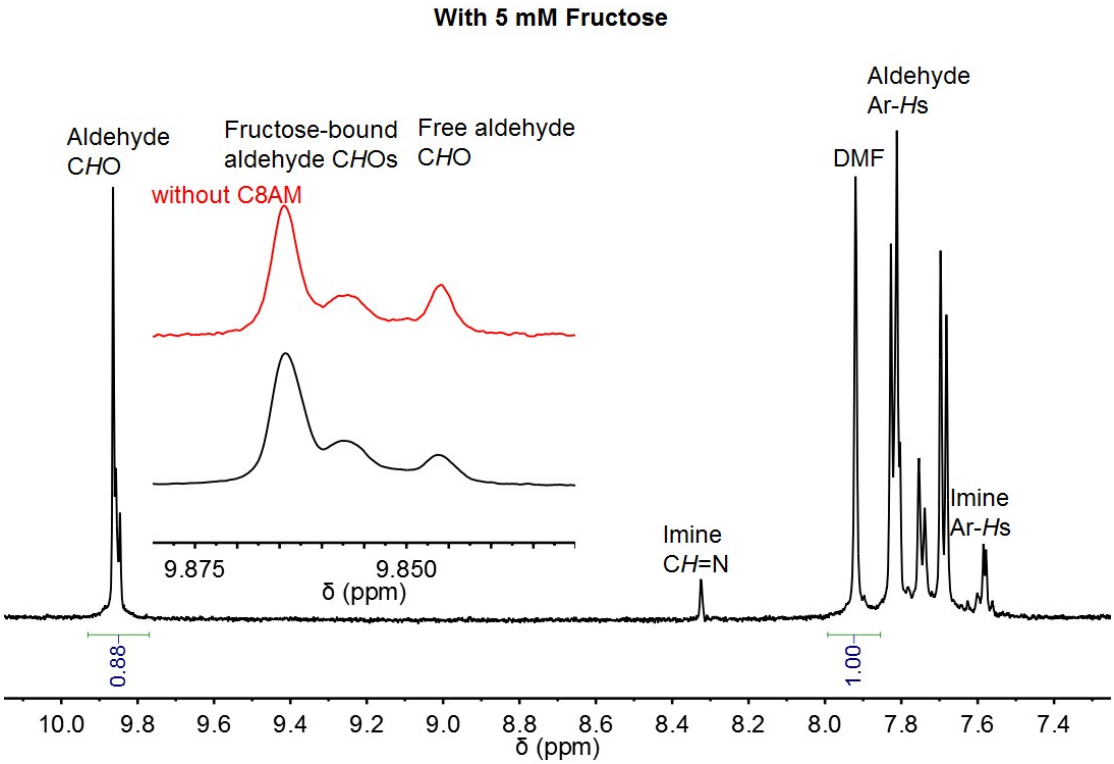

Fig. S8 ^{1}H NMR (500 MHz) spectra of a mixture of 4FBA (3 mM), C8AM (3 mM) and fructose (5 mM) in D_2O buffered at pD 10.5 with 100 mM $NaHCO_3$–Na_2CO_3. DMF (3 mM) was added as an internal reference for determination of imine formation. Note that multiple sets of signals were observed in the aldehyde CHO region and the aromatic regions of aldehydes and imines. This is due to the coexistence of the free aldehyde, fructose-bound aldehydes, free imine, and fructose-bound imines. The aldehyde region was zoomed and compared with the control sample without the amine component C8AM. Similar multiple signals were observed without C8AM, indicating that the multiple signals are due to coexistence of the free aldehyde and fructose-bound aldehydes (a mixture of complexes[2]). Note that comparison of Fig. S6 and S7 reveals that in the bulk solution most of 4FBA was bound to fructose whereas glucose binding only occurred to a low extent. This is consistent with the much higher affinity of monoboronic acid for fructose than for glucose.[3]

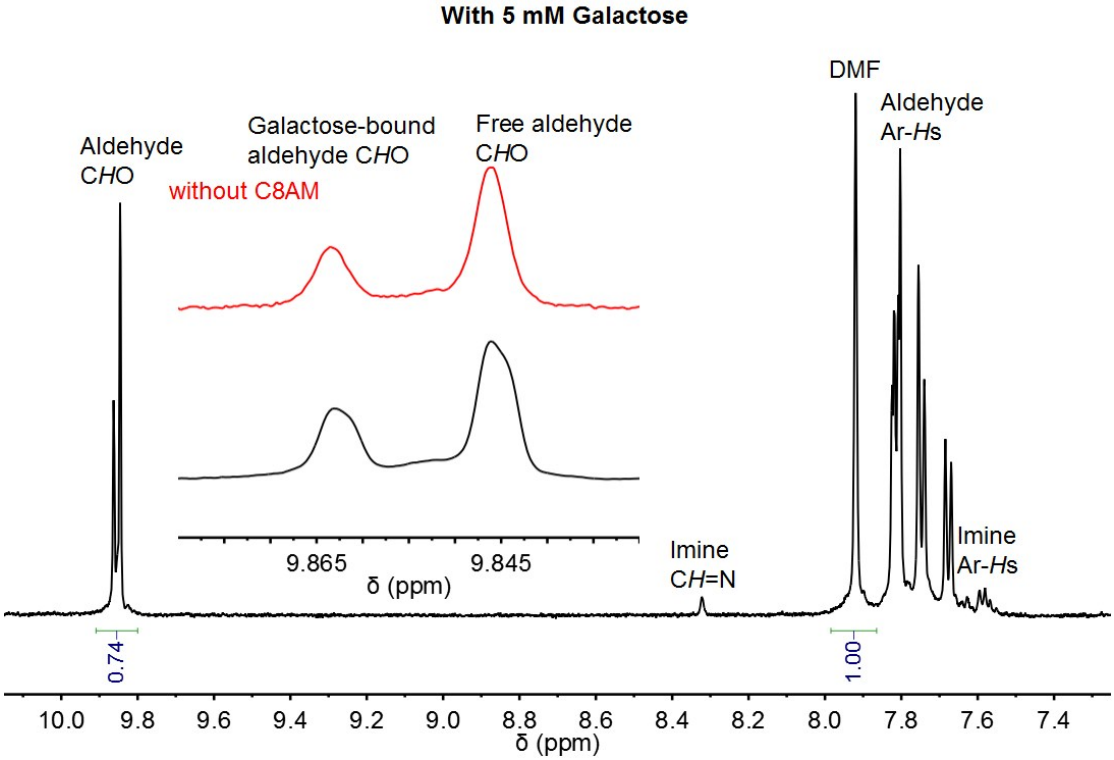

Fig. S9 ^{1}H NMR (500 MHz) spectra of a mixture of 4FBA (3 mM), C8AM (3 mM) and galactose (5 mM) in D_2O buffered at pD 10.5 with 100 mM $NaHCO_3$–Na_2CO_3. DMF (3 mM) was added as an internal reference for determination of imine formation. Note that multiple signals were observed in the aldehyde CHO region and the aromatic regions of aldehydes and imines. This is due to the coexistence of the free aldehyde, galactose-bound aldehydes, free imine, and galactose-bound imines. The aldehyde region was zoomed and compared with the control sample without the amine component C8AM. Similar double signals were observed without C8AM, indicating that the multiple signals are due to coexistence of the free aldehyde and the galactose-bound aldehydes.

7. TEM images

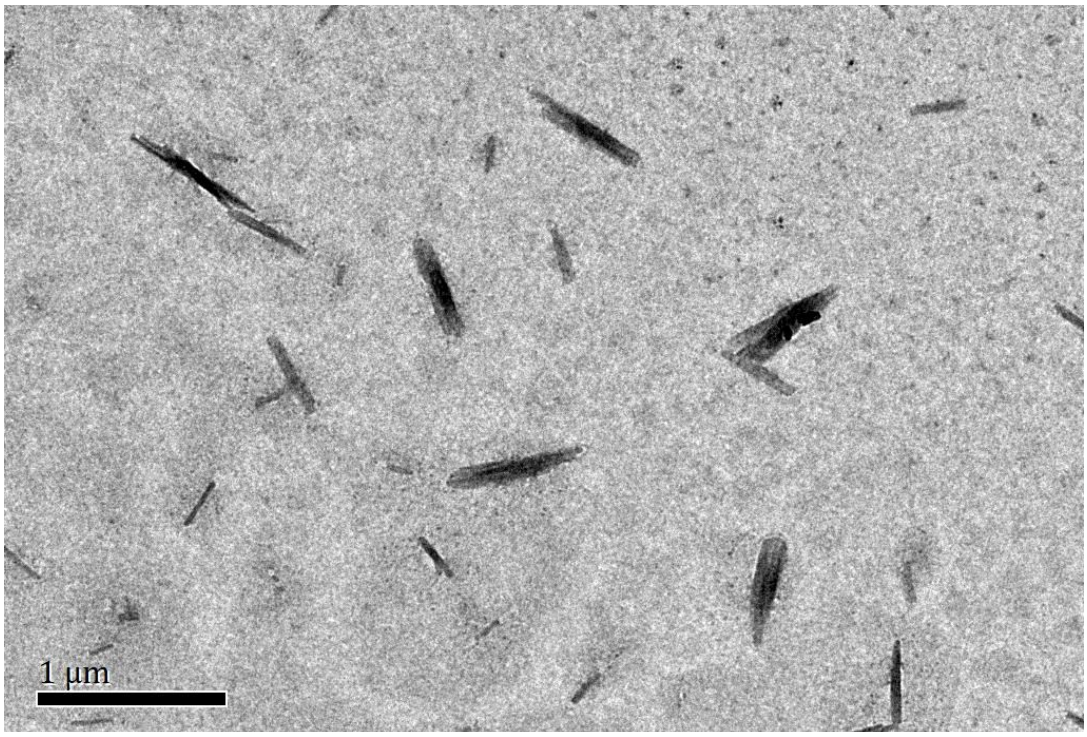

Fig. S10 Transmission electron microscope (TEM) images of dried samples of a solution of 4FBA (3 mM) and C8AM (3 mM) in 100 mM $NaHCO_3$–Na_2CO_3 buffer of pH 10.5.

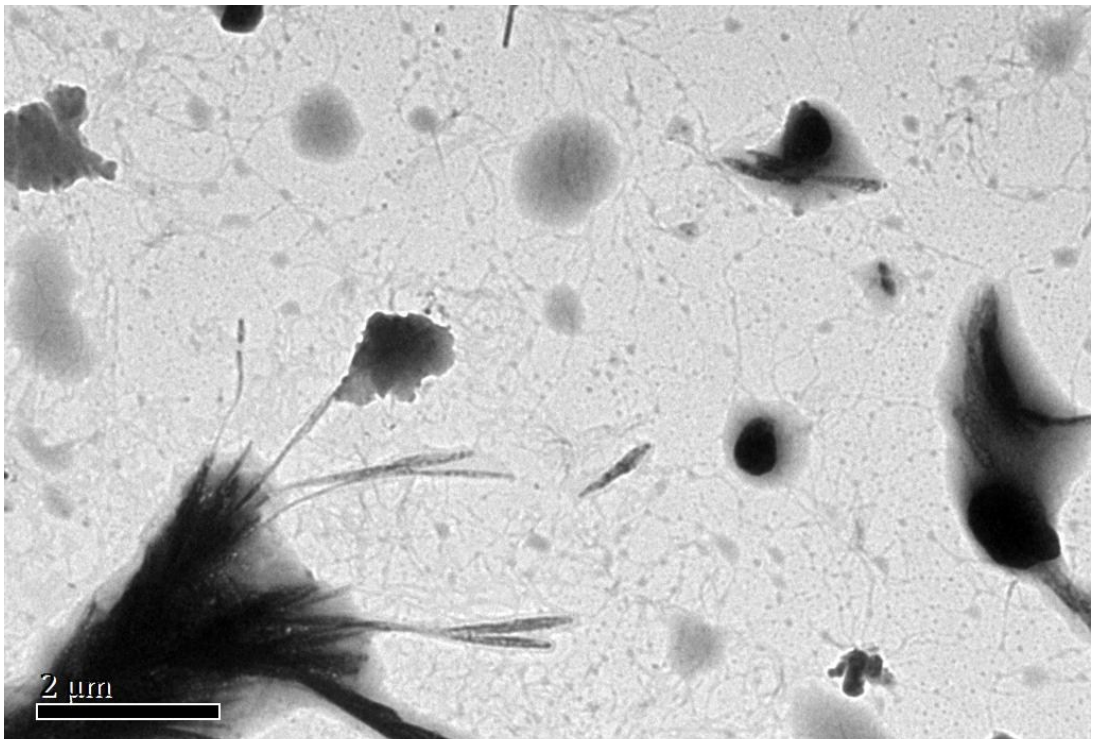

Fig. S11 Transmission electron microscope (TEM) images of dried samples of a solution of 4FBA (3 mM), C8AM (3 mM), and fructose (5 mM) in 100 mM $NaHCO_3$–Na_2CO_3 buffer of pH 10.5.

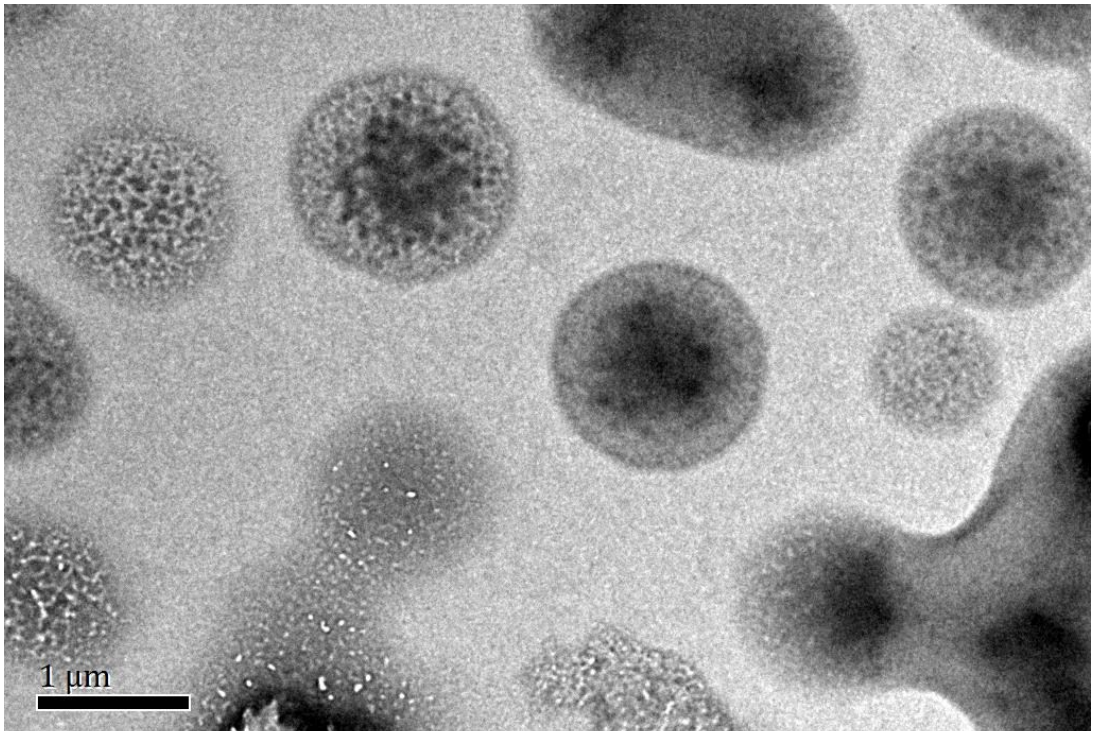

Fig. S12 Transmission electron microscope (TEM) images of dried samples of a solution of 4FBA (3 mM), C8AM (3 mM), and galactose (5 mM) in 100 mM $NaHCO_3$–Na_2CO_3 buffer of pH 10.5.

8. DLS data

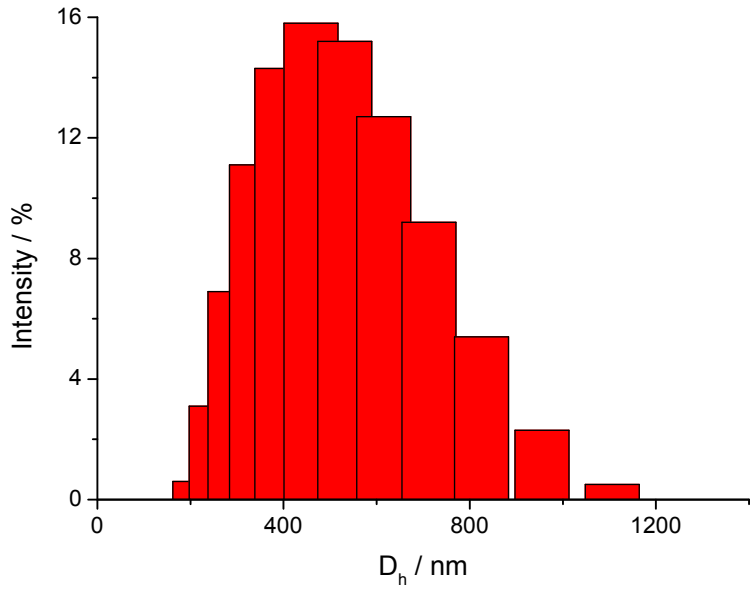

Fig. S13 Size distribution of aggregates formed by 4FBA (3 mM), C8AM (3 mM), and galactose (5 mM) in 100 mM $NaHCO_3$–Na_2CO_3 buffer of pH 10.5, measured by dynamic light scattering. A zeta potential of -29.5 mV was determined for this sample. The light scattering intensity of the control samples without saccharide (count rate 16.0 kcps) and with 5 mM fructose (count rate 5.8 kcps) was too low for DLS measurements, consistent with the conclusion that no or negligible amphiphile aggregation occurred.

9. Fluorescence response to glucose in the presence of saccharide interferents

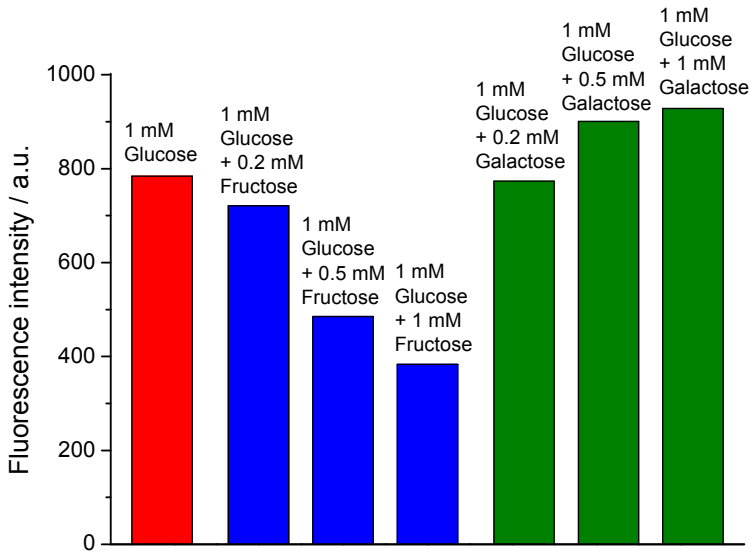

Fig. S14 Fluorescence intensity of Nile red (5 μM) added to the assembly formed by 4FBA (3 mM), C8AM (3 mM) and glucose (1 mM) in the absence and presence of saccharide interferents in pH 10.5 100 mM $NaHCO_3$–Na_2CO_3 buffer.

10. References

1 J. C. Norrild and H. Eggert, *J. Am. Chem. Soc.*, 1995, **117**, 1479-1484.

2 J. C. Norrild and H. Eggert, *J. Chem. Soc., Perkin Trans. 2*, 1996, 2583-2588.

3 (a) N. J. Gutierrez-Moreno, F. Medrano and A. K. Yatsimirsky, *Org, Biomol. Chem.*, 2012, **10**, 6960-6972; (b) J. P. Lorand and J. O. Edwards, *J. Org. Chem.*, 1959, **24**, 769-774.