Supramolecular one-pot approach to fluorescent glycodendrimers†

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Homogeneous, fluorescent, sugar-functionalized metallic dendrimers that contain varying numbers and types of monosaccharides have been prepared using a self-assembly process and have been shown to be highly efficient lectin sensors in turbidity assays.

Dendrimers are hyper-branched polymers that emanate radially from a central core and are morphologically similar to biological macromolecules of well-defined three-dimensional architecture. The properties of dendrimers can be exploited for optical,[1a] biomedical,[1b,c] electrical[1a] as well as catalysis[1d] applications and may ultimately lead to new materials.[1] Dendrimers adorned with pharmaceutically active compounds,[2–5] carbohydrates,[2] photosensitizers[3] and redox units[4] have been reported. Glycodendrimers are attractive for potential biomedical applications using anti-viral[5a] and anti-adhesive[5b] properties. Applications as microbial toxin antagonists, anti-inflammatory and anti-cancer drugs[6] have been proposed. Glycodendrimers should contain a fluorescent marker or contrast agent for direct evaluation in biological assays. However, developing a facile synthetic route to these fluorescent probes has been a challenge. Copper(II) catalyzed Huisgen [2 + 3] cycloaddition[7] and template-based[8] dendrimer construction have been employed to construct glycodendrimers. More recently, a self-assembly process was used as an effective method for the formation of dendrimers.[9] The assembly of the dendron was controlled by electrostatic forces, hydrogen bonding, metal coordination and other non-covalent interactions. Using this process, metal complexes functionalized with carbohydrates have been reported; examples include Cu(II) Fe(II), Ru(II) Re(I) and Tc(I) complexes.[10] However, they are limited to 2–8 sugar substituted complexes and lack systematic methodology to tune the fluorescence, topology and physico-biological properties of the dendrimers.

We hereby present a hydroxyquinoline confined glycodendron to bind transition and lanthanide metal complexes by self-assembly to obtain high nuclear glycodendrimers. Self-assembly of the metal dendrimers was assessed by a variety of spectroscopic and other analytical means. Finally, we show that the interaction of specific high density metal glycodendrimers with Concavalin A (ConA) lectin results in the formation of colloidal aggregates.[11]

To synthesize metallic glycodendrimers, a versatile metal chelator was required to manipulate the carbohydrate density of sugars, such as mannose that specifically interact with ConA lectin. An amide derivative of 8-hydroxyquinoline, frequently employed as a ligand in coordination chemistry, was selected as metal chelator.[12,13] Fluorescent Zn(II),[12] lanthanide(III)[13] and Al(III)[13] ion complexes of 8-hydroxyquinoline derivatives have already been studied due to their non-bleaching fluorescence in the visible and NIR region.[12,13] With this information in hand, we prepared complexes **1–7** (Fig. 1) bearing carbohydrates. Mannose and galactose were selected for initial trials, as they are important for cell recognition and migration, as well as for bacterial attachment.[14]

Mannose, glucose or galactose-capped dendrons **12–14** (Scheme 1) were prepared starting from *N*-{tris[(2-cyanoethoxy)methyl]}methylamine **8**.[2] Following treatment of **8** with concentrated HCl in ethanol to yield tri-ester **9**, peptide coupling of **9** with Boc-β-alanine followed by 8-*O*-benzyl-quinoline-2-carboxylic acid[15] yielded tripod **10**. Ester hydrolysis of **10**, followed by coupling with pentafluorophenol, afforded activated ester **11** in 71% yield. Pentafluorophenol ester **11** was further

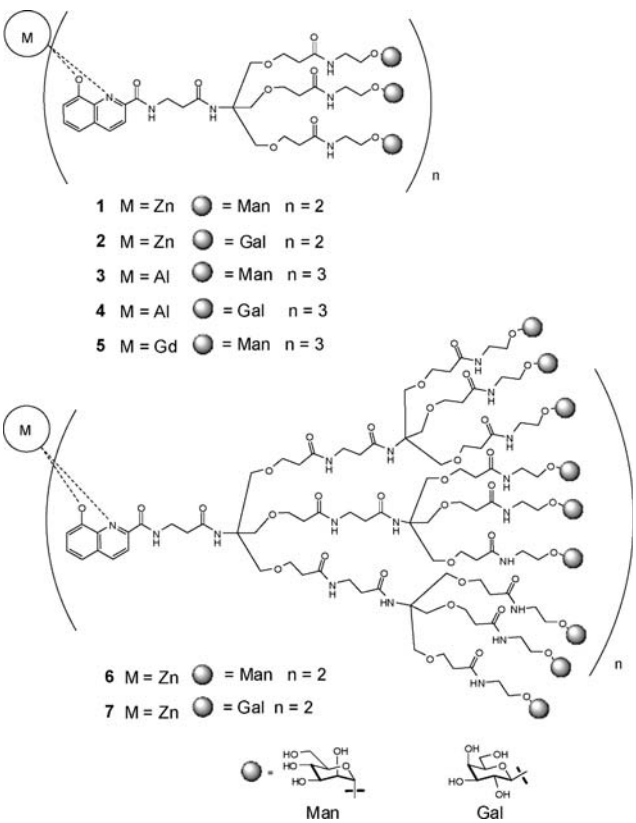

Fig. 1 Glycodendrimers produced by self assembly.

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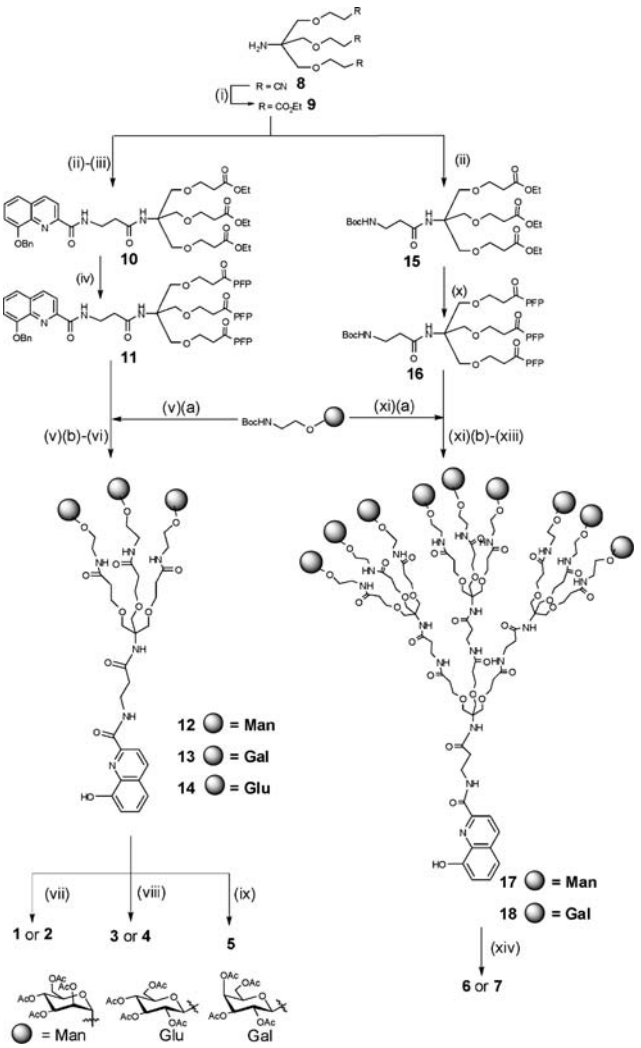

Scheme 1 Synthesis of dendrons **12–14** as well as **17–18** and formation of metal dendrimers **1–7**: (i) (a) conc. HCl, reflux, 4 h; (b) EtOH, reflux, 12 h, 51%; (ii) *N*-Boc-β-Ala, DIC, HOBT, DCM, 0 °C to rt, 12 h, 63%; (iii) DCM–TFA (3 : 1), rt, 1 h; then 8-*O*-benzyl-quinoline-2-carboxylic acid,[16] DIC, HOBT, DCM, rt, 12 h, 66%; (iv) (a) 1 N NaOH, EtOH, rt, 2 h; (b) pentafluorophenol, DIC, DCM, 0 °C to rt, 12 h, 71%; (v) (a) 2-(*tert*-butoxycarbonylamino)ethoxy-2,3,4,6-tetra-*O*-acetyl-β-D-galactose (**12**), glucose (**13**) or mannose (**14**),[1] DCM–TFA (3 : 1), 1 h, rt, yield = 58% (**12**), 53% (**13**), 61% (**14**)); (b) mixture from (a) was added to **11**, TEA, DCM, rt, 12 h; (vi) (a) NaOMe, MeOH, 2 h; (b) H_2, Pd/C, MeOH, 12 h, yield (over 2 steps) = 24% (**12**), 21% (**13**), 25% (**14**); (vii) $Zn(OAc)_2$, MeOH, reflux, 12 h, yield = 76% (**1**), 81% (**2**); (viii) $Al(OAc)_3$, MeOH, reflux, 12 h, yield = 75% (**3**), 75% (**4**); (ix) $GdCl_3{\cdot}6H_2O$, MeOH, reflux, 12 h, 75%; (x) (a) 1 N NaOH, EtOH, rt, 2 h; (b) pentafluorophenol, DIC, DCM, 0 °C to rt, 12 h, 47%; (xi) (a) 2-(*tert*-butoxycarbonylamino)ethoxy-2,3,4,6-tetra-*O*-acetyl-β-D-mannoside or galactoside,[1] DCM–TFA (3 : 1), 1 h, rt; (b) mixture from (a) added to **14**, TEA, rt, 12 h; (xii) (a) DCM–TFA (3 : 1), rt, 1 h; (b) **11**, DCM, TEA, rt, 12 h; (xiii) (a) NaOMe, MeOH, 2 h, rt; (b) H_2, Pd/C, rt, MeOH, 12 h, yield (over 3 steps) = 29 (**17**), 19% (**18**); (xiv) $Zn(OAc)_2$, MeOH, reflux, 12 h, yield = 82% (**6**), 80% (**7**).

reacted with peracetylated mannose, glucose or galactose containing an anomeric 2-aminoethoxy linker,[1] before treatment with base and hydrogenolysis yielding **12–14**. The metal dendrimers **1–5** were prepared by refluxing stoichiometric amounts of **12–14** with either $Zn(OAc)_2$, $Al(OAc)_3$ or $GdCl_3$ in methanol. The molecular weights of all complexes were determined by MALDI-ToF. Complexes **1** and **2** were further examined by NMR spectroscopy. Second generation dendrons **17** and **18** were prepared in analogy to the process employed for **12–14**. Complexes **6** and **7** were subsequently formed. Synthesis of Gd(III) and other lanthanide complexes of dendrons **17** and **18** resulted in the formation of polymetallic dendrimers.[16]

The photophysical properties of complexes **1**, **3** and **5** were investigated in methanol at room temperature (Fig. 2). Zinc complex **1** shows a maximum at 402 nm. This absorption corresponds to the ligand to metal charge transfer (LMCT) band of the complex, while the aluminium (**3**) and gadolinium complexes (**5**) showed LMCT bands at 388 nm and 392 nm, respectively. Bands at 355 nm (for **1**, **3** and **5**) correspond to the ligand centered (LC) excited state of the ligand (Fig. 2). λ_{max} for the fluorescence spectra of dendron **12** appears at 521 nm, while Zn(II) **1** and Al(III) **3** complexes showed strong fluorescence intensities at 532 nm and 528 nm, respectively (Fig. 3). The quantum yields of the Zn(II) **1** and Al(III) **3** complexes are approximately six to seven times higher than that of dendron **12**, due to excellent electron transfer between LUMO and HOMO of the complexes. The ligand to metal energy transfer (LMET) of the Gd(III) **5** complex was not observed as a result of the lowest excited states located at higher energy than the emitting state of the hydroxyquinoline ligand.[17]

After assessing the optical properties of complexes **1**, **3** and **5**, glycodendrimer–protein interactions were investigated. ConA served as model lectin since it selectively binds to α-mannopyranosides. When aqueous solutions of **1**, **6** or **7** were added to solution of ConA in Hepes buffer, only **6** showed an increase in turbidity of the mixture, indicative of binding (Fig. 4). As expected, complex **6** shows better binding than **1** due to a larger cluster and mannose density on the dendrimer surface and hence binding is seen with **6** and not **1**. In order to demonstrate that the turbidity increase observed is as a result of protein–carbohydrate interaction, a large excess of mannose was added to inhibit dendrimer–ConA binding. Indeed, the turbidity disappeared upon addition of mannose. Dendrimer **7**, bearing β-galactopyranoside, served as negative control and did not bind to ConA. Dendrimer–lectin interactions were also monitored by fluorescence measurements: upon addition of a solution of **6** to a buffered solution of ConA, fluorescence was slightly quenched, while complexes **1–5** and **7** failed to quench the signal. This could be interpreted as the simultaneous occurrence of various processes, such as agglutination of the fluorescent complex and

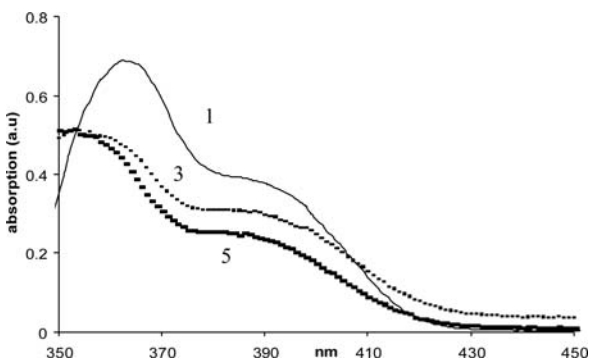

Fig. 2 UV-visible spectra of complexes **1** (solid line), **3** (dark dotted line) and **5** (dotted dashed line); 1.5 mM of (complexes **3** and **5**) and 1.9 mM of complex **1** in methanol.

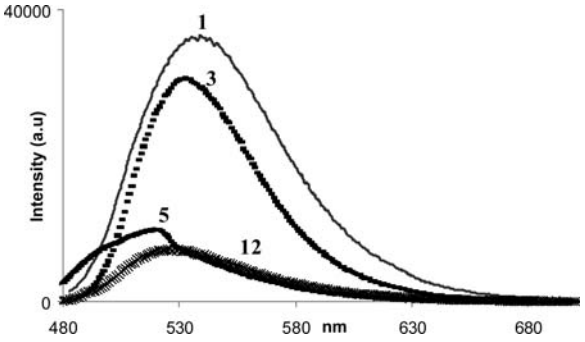

Fig. 3 Luminescence of **1** (solid line), **3** (dark dotted line), **5** (dark solid line); 1 mM in methanol, **12** (dark line with black feather)—1.5 mM in methanol, excitation λ_{max} = 400 nm.

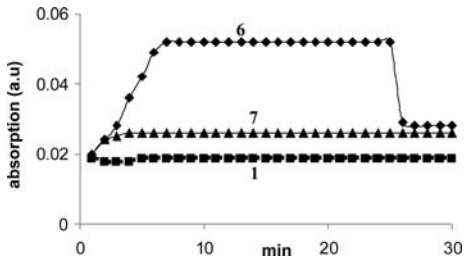

Fig. 4 Turbidity analysis: absorption change of compound **1** (■), **6** (◆) and **7** (▲) at 500 nm on addition of ConA (1 mg mL^{-1}). Mannose (100 mM) was added to **6** after 25 min.

photoinduced energy or electron transfer between the metal complex and Mn(II) in the ConA lectin (see ESI†).

In conclusion, hydroxyquinoline functionalised glycodendrons can be tuned to different homogenous fluorescent glycodendrimers, containing a defined number of sugars. We have shown that high sugar density was essential for lectin binding, as demonstrated by the interaction of metallo-glycodendrimer **6** with ConA compared to **1** which contains fewer mannose residues. Moreover, ConA binding to glycodendrimers was shown to be carbohydrate specific, as expected. This approach to glycodendrimers can be applied to the synthesis of non-bleaching fluorescent probes and active markers that may be easily incorporated into the dendrimer. The prospect of lanthanide-containing glycodendrimers (from dendrons **17**, **18**) will provide tunable fluorescent, MRI reagents, for imaging and treatment relying on multivalent interactions is currently under investigation.

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