Facile synthesis of size dependent Ru(II)–carbohydrate dendrimers *via* click chemistry†

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Received (in Cambridge, UK) 1st December 2009, Accepted 5th February 2010
First published as an Advance Article on the web 24th February 2010
DOI: 10.1039/b925113h

A facile and flexible approach for the preparation of Ru(II) complexes containing different carbohydrates based on the Cu(II)-catalyzed Huisgen-[3 + 2] cycloaddition is described.

Lectins are carbohydrate binding proteins involved in cell–cell interactions that are the basis of a variety of biological processes, such as cell adhesion and migration, phagocytosis, cell differentiation and apoptosis.[1] Moreover, carbohydrates that selectively bind lectins have been used for imaging and delivery.[2] The interest in lectin–carbohydrate interactions has increased considerably. Monomeric binding affinities for carbohydrate–lectin interactions are typically in the milli- to micromolar range. Multivalent presentation of carbohydrates, as is the case on the cell surface, overcomes the problem and can be mimicked using glycodendrimers.[3] A host of glycodendrimers has been prepared[4–6] and explored in different applications.[3] The facile, Cu(II)-assisted Huisgen-[3 + 2] cycloaddition has been applied to attach carbohydrates to PMMAM dendrimers,[4b] nanoparticles[5] and dendrimers formed *via* a self-assembly process[6] in order to construct glycodendrimers. Glycodendrimers designed for biological assays should ideally contain means to optically, electrochemically or gravimetrically detect them in biological systems. We previously described Ru(II)–glycodendrimers as strong optical and electrochemical probes.[7] A general synthetic protocol to rapidly and effectively synthesize structurally diverse Ru(II)–glycodendrimer complexes remained elusive.

Here, we report the design and synthesis of different generations of Ru(II)–glycodendrimers (**1–8**) bearing varying numbers of carbohydrates (Fig. 1). Increased carbohydrate density around the $Ru(bipy)_3$ core strongly influences the photophysical and colloidal aggregation properties of the dendrimers compared to organic fluorescent glycodendrimers (Table 1).

Ru(II)–alkynes are readily accessible in high yields using inexpensive starting materials and are an ideal basis for the rapid synthesis of Ru(II)–glycodendrimers *via* "click chemistry". Carbohydrate dendrimers containing an azido-linker and Ru(II)–acetylene complexes are prepared separately. Subsequent Huisgen-[3 + 2] cycloaddition, followed by the removal of protecting groups on carbohydrate moieties, will provide access to the desired complexes in a straightforward, modular fashion. Tripodal sugar-azides **14** and **15** were prepared from tris(hydroxylmethyl)aminomethane **9** (Scheme 1). Coupling of **9** with acrylonitrile, followed by treatment with HCl in ethanol and amide bond formation with 5-bromo valeric acid, yielded **12**. Nucleophilic substitution of bromide by azide, saponification and esterification with pentafluorophenol afforded **13** in 86% yield. The same procedure was utilized to obtain activated ester **13a**. Pentafluorophenol ester **13** was further reacted with 2-aminoethoxy per-acetylated mannose and mannose-tripod to obtain first and second generation azide mannose dendrons **14** and **15** respectively (Scheme 1). Tripod **17** was prepared by mixing active ester **13a** with propargyl amine.

Ru(II)–alkyne derivatives **21** and **24** were readily prepared from 4,4′-dimethyl bipyridine **18** (Scheme 2). Oxidation with chromic acid and coupling with **17** after Boc-cleavage in the presence of triethyl amine followed by complexation with *cis*-$Ru(bipy)_2Cl_2$ yielded complex **21**. Similarly, **24** was synthesized by selenium dioxide oxidation of **18** to the monocarboxylic acid derivative followed by coupling with propargyl amine and complexation with $RuCl_3$. Finally, **21** or **24** and fluorescein alkyne were coupled with stoichiometric amounts of dendrons **14** and **15** *via* the [3 + 2] cycloaddition reaction. Deacetylation yielded dendrimers **1–8**. When a similar method was applied to the synthesis of a Ru(II) complex substituted with 54 sugars, a mixture of 27 and 36 mannose substituted Ru(II) dendrimers was obtained. Steric effects are likely responsible for the failure to obtain the fully glycosylated dendrimer. These results indicate that the Ru(II)-complex is a facile template to obtain varying carbohydrate densities when compared to the fluorescein probe. The optical properties of Ru(II)-complexes **1–8** were investigated at room temperature. Complexes **1–7** showed a maximum emission at 643–648 nm. The quantum yield of **7** is almost twice that of complexes **1–6**, thus demonstrating size dependent optical dendrimer properties. The quantum yield of **8** is almost comparable to the fluorescein dye.

After assessing the optical properties, lectin–carbohydrate interactions between *galanthus nivilis agglutinin* (GNA), concanavalin A (ConA) lectin and α-manno- and β-glucopyranosides were investigated.[8] GNA and ConA have twelve

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† Electronic supplementary information (ESI) available: Full experimental details for synthesis of metal-dendrimers and its optical and turbidity studies. See DOI: 10.1039/b925113h

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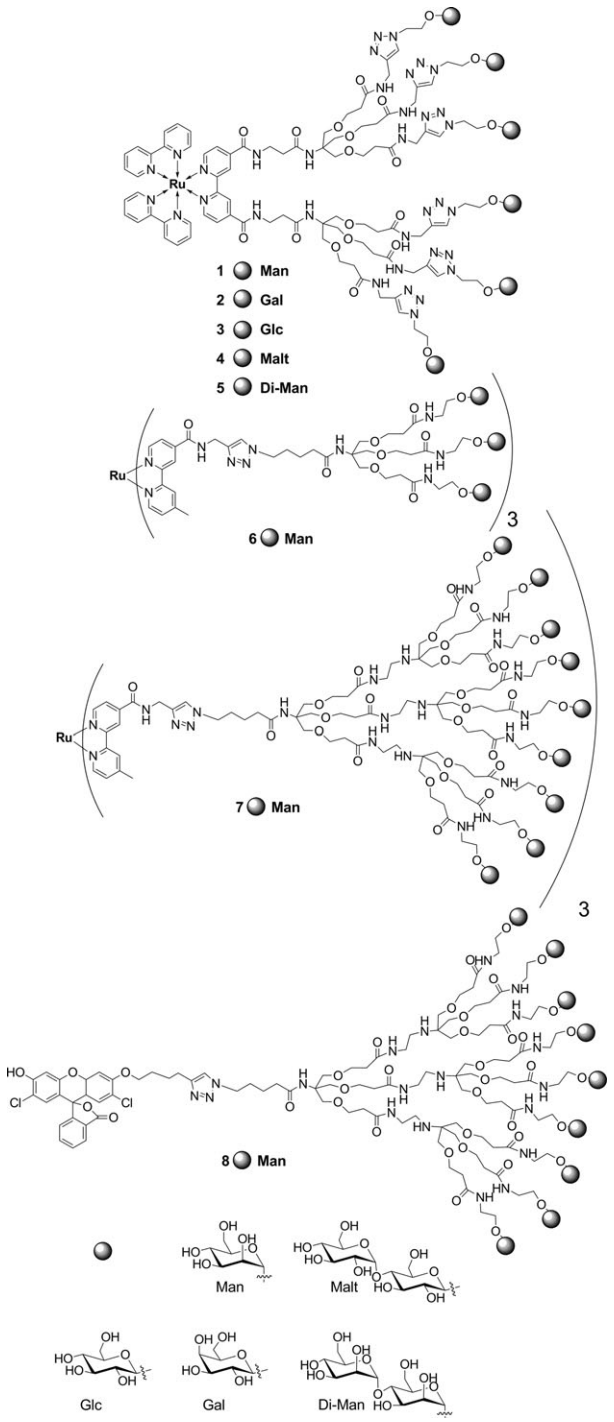
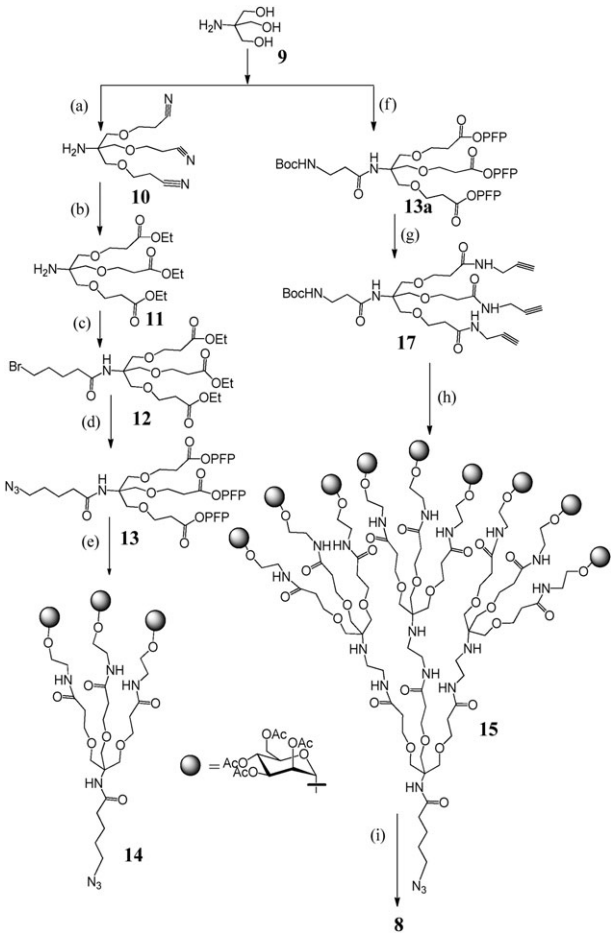


Fig. 1 Structures of Ru(II)-complexes **1–8**.

Table 1 Photophysical properties of dendrimers **1**, **6**, **7** and **8**. Dendrimer size was calculated for the minimum energy conformation of the glycodendrimers using MM2 Chem 3D structure and Mercury programs

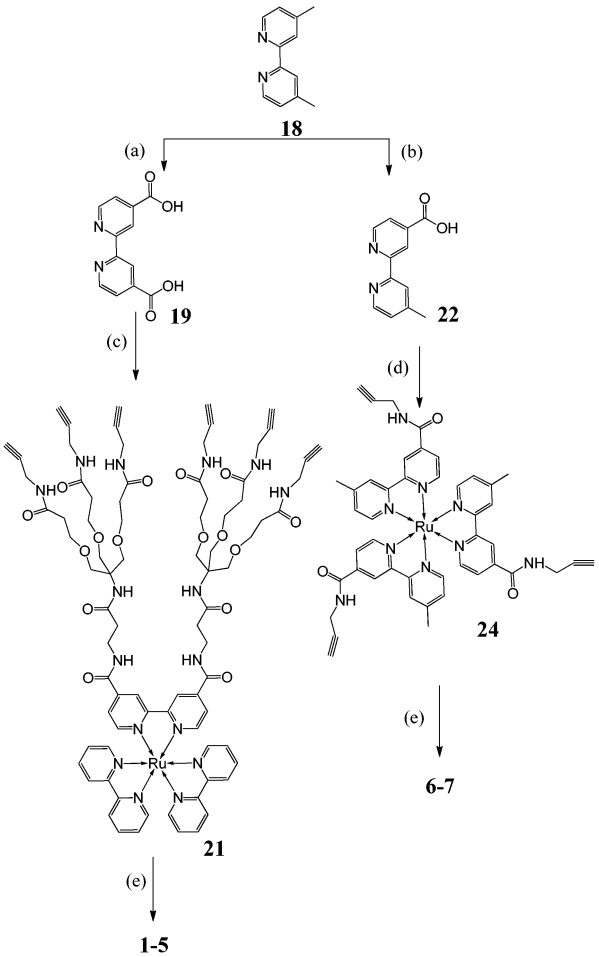
Compound	λ_{max}/nm	QY (Φ)	Size/Å
1	645	0.061–0.066	~12–19
6	648	0.098	~36–56
7	648	0.125	~68–86
8	548	0.058	~13–21



Scheme 1 Synthesis of sugar-tripod azides and fluorescein dendrimer **8**. (a) Acrylonitrile/NaOH (40%); (b) conc HCl/EtOH, 51%; (c) 5-bromo-valeric acid/DIC/HOBT/DCM, 69%; (d) NaN_3/DMF, 72%; NaOH/MeOH; pentafluorophenol/DIC/HOBT/DCM, 86%; (e) 2-(*tert*-butoxycarbonyl amino)ethoxy-2,3,4,6-tetra-*O*-acetyl-α-D-mannoside/DCM/TEA, 71%. (f) Acrylonitrile/NaOH (40%), conc HCl/EtOH, 51%; *N*-Boc-β-Ala/DIC/HOBT/DCM, 63%; pentafluorophenol/DIC/DCM, 71%; (g) propargyl amine/TEA/DCM, 77%; (h) tripod-mannose[7b]/DCM/TEA, 47%; (i) $CuSO_4$/ascorbic acid/THF : H_2O (1 : 1), 12 h, 84%; NaOMe/MeOH, 61%.

and four binding sites for mannose respectively.[9] In HEPES buffer binding of complexes **1–8** correlates with turbidity and increased density of the mannose and glucose sugars (Fig. 2). As expected, complex **2** containing β-galactose did not result in turbidity, whereas **7** resulted in highly turbid solutions. All other complexes showed weak turbidity. Similarly, ConA showed strong turbidity with complex **7** and no aggregation with any other complex. In order to demonstrate that the turbidity is due to specific carbohydrate–protein interactions, excess mannose was added to inhibit dendrimer–GNA binding.

In conclusion, we have developed an efficient synthetic route to non-bleaching, high quantum yield fluorescent Ru(II)–carbohydrate dendrimers using the copper catalyzed Huisgen-[3 + 2] cycloaddition. Lectin binding affinity and optical properties of the metallo-glycodendrimers can be readily tuned by changing the number of carbohydrate moieties. Specific carbohydrate–protein interactions of these



Scheme 2 Synthesis of Ru(II)-cores **21** and **24** as well as formation of metal glycodendrimers **1–7**. (a) CrO_3/conc H_2SO_4, 98%; (b) SeO_2/conc H_2SO_4, 98%; (c) comp **17**/TEA/DCM, 12 h, 69%; *cis*-Ru(bipy)$_2$Cl$_2$/EtOH, 12 h, 51%; (d) propargyl amine/DCM/TEA, 76%; $RuCl_3$/EtOH/AcOH, 12 h, 39%; (e) $CuSO_4$/ascorbic acid/THF : H_2O (1 : 1) 12 h, 84–86%; NaOMe/MeOH, 76%.

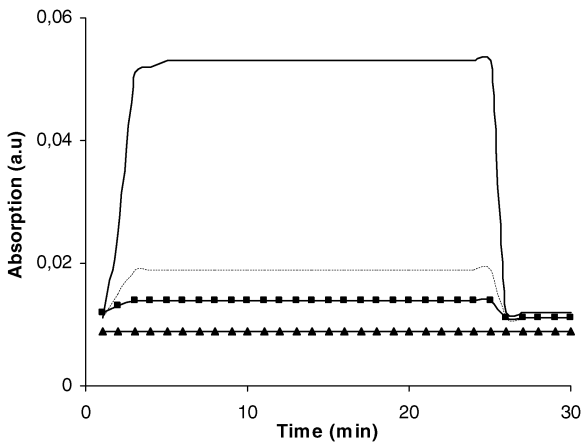


Fig. 2 Turbidity assay: absorption change of complexes **1**, **3** and **4** (■), **2** (▲), **5**, **6** and **8** (dotted line) and **7** (straight line) at 530 nm upon addition of GNA (1 mg mL^{-1}). Mannose (100 mM) was added to all complexes after 25 min.

dendrimers are of potential use for further applications in imaging as well as bio-sensing processes.

We thank the Max-Planck Society and the DAAD (fellowship to Y.H.T.) for financial support.

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