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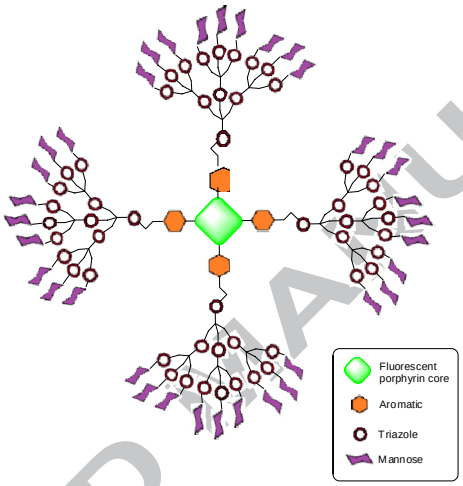

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Use of 'click chemistry' for the synthesis of carbohydrate-porphyrin dendrimers and their multivalent approach towards lectin sensing

Rituparna Das,*[a] Balaram Mukhopadhyay*[a]

[a] *Department of Chemical Sciences, Indian Institute of Science Education and Research (IISER) Kolkata, Mohanpur, Nadia 741246, India*
E-mail: ritu_iiser@yahoo.com (RD), sugarnet73@hotmail.com (BM)

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ABSTRACT

Multivalent carbohydrate dendrimers having 12 and 36 α-D-mannose units on the periphery of a porphyrin-cored dendritic scaffold have been synthesized using "click chemistry". Synthesized dendrimers were characterized by ^{1}H and ^{13}C NMR, UV, IR and MALDI-TOF MS. The fluorescence behavior of the glycodendrimers was examined and they were used successfully for the detection of mannose binding lectin (MBL) Concanavalin A.

Introduction

Lectins are the proteins that interact with carbohydrates with high specificity and play an integral role in establishing various cell-cell interactions.[1] These non-covalent interactions act as a mediator in a range of biological events like cell growth, cell agglutination, interaction of bacteria/viruses and cell signaling.[2] Naturally, lectins responsible for definite recognition are often found in aggregates offering multiple binding sites to the carbohydrates. This multivalency or technically "the glycoside cluster" effect[3] poses as a perfect solution to the low binding affinities provided by monovalent interactions or that predicted from the sum of constitutive interactions. This enhanced binding of lectins with carbohydrates is at present an attractive area of research by virtue of its pivotal role in diverse biological processes like promoting inflammatory response, functioning of the immune system in malignancy, metastasis and apoptosis. Proper and significant elucidation of the key characteristics of these interactions may facilitate the development of various therapeutic strategies. The concept of natural multivalency has been explored by various research groups and several multivalent ligands have been synthesized to modulate the biological systems.[4] However, glycodendrimers go a long way in achieving the multivalent platform in the cellular processes. Many glycodendrimers have been reported in order to initiate lectin recognition[5] by virtue of their inherent optical, photophysical and electronic properties.

Metallated porphyrins are natural scaffolds that constitute various biologically relevant macromolecules like haemoglobin, myoglobin and most of the cytochromes. Porphyrin glycoconjugates are gaining much interest as they open up the scope of exploring the photochemical behavior of the core scaffold. In addition to their high photostability, the rigidity offered by the porphyrin architecture also aids in fine tuning the carbohydrate epitopes directly grafted on the scaffolds and thus exhibiting strong potential to act as selective inhibitors capable of discriminating between lectins of similar sequence. Porphyrins are capable of generating singlet oxygen upon shining light of specific wavelength which is lethal to the tumor cells. The multivalent approach with the porphyrin dendrimeric glycoconjugates thus plays a crucial role in photodynamic therapy (PDT) of tumors.[6] Although porphyrin glycodendrimers have been synthesized[7] and used as various photosensitizers,[8] their capability to act as fluorescent markers[9] is less explored. Considering these potential applications, in this paper we report the synthesis of novel tetra-substituted mannosyl moiety appended dendrimeric metalloporphyrins (**Figure 1**).

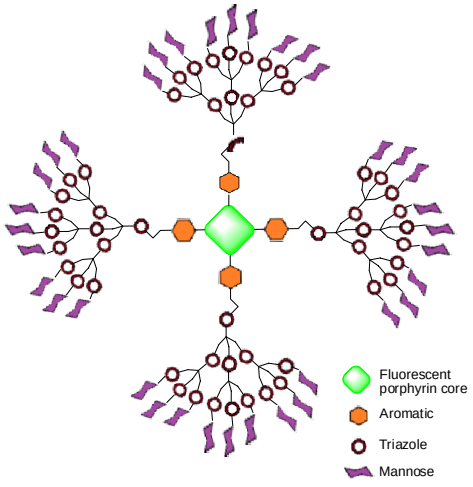
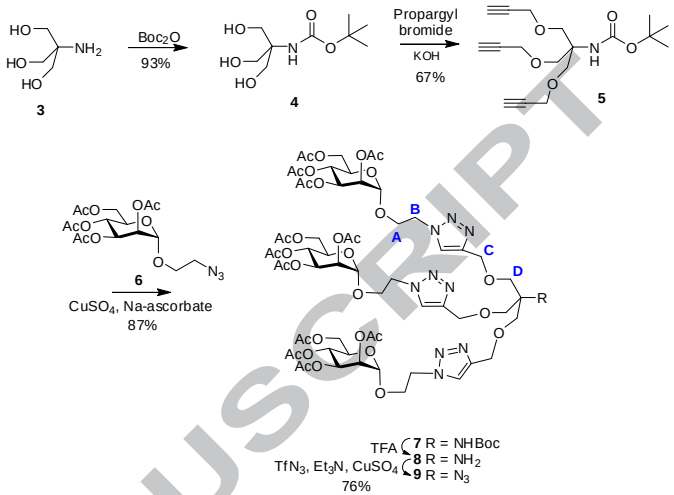


Figure 1. Representative structure of the proposed mannose conjugated porphyrin-dendrimer

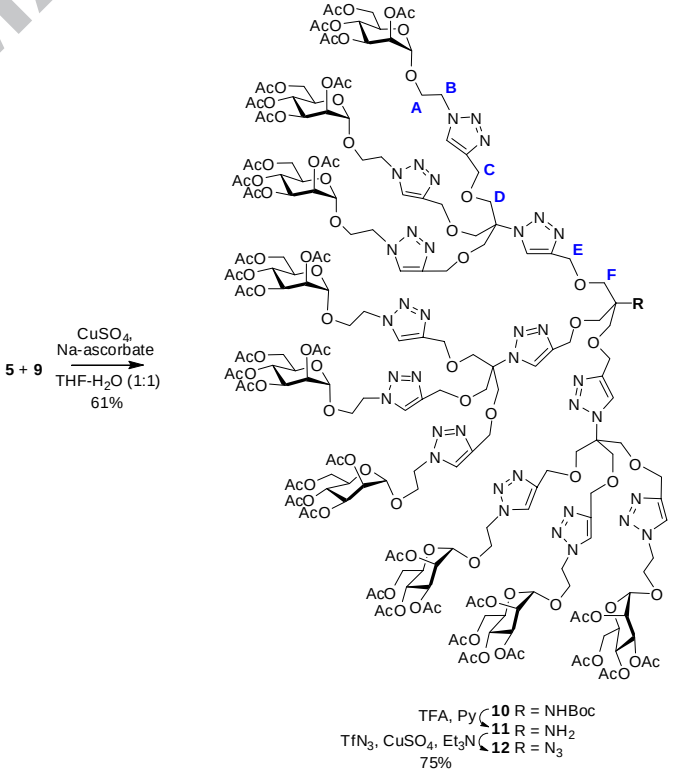
Concanavalin A[10] (Con A, *Canavalia ensiformis*), a mannose binding lectin (MBL) has been chosen as the protein model to explore the capability of the synthesized carbohydrate dendrimers as potential fluorescent sensors and the studies have revealed promising insight to the specific and efficient binding affinities of such carbohydrate dendrimers over natural porphyrin biomolecules due to multivalent ConA-mannose interaction.

To explore the influence of the multivalency on carbohydrate lectin interaction, we aimed at the synthesis of first and second generation mannose conjugated zinc(II)porphyrin dendrimers by the use of mild and environment friendly, regioselective Cu(I) catalyzed 'click reaction'. Tris(hydroxymethyl)aminomethyl group was implemented as the branching platform and the potential linker for the carbohydrate appendages connected to the porphyrin architecture. The flexible linker was strategically employed to introduce relatively high degrees of freedom and adaptability to chelate with the binding sites of the protein with the optimized affinity enhancements. For the effective synthesis of the 1st and 2nd generation carbohydrate dendrimers, a divergent approach was chosen as the better route wherein an 'inside-out' strategy was employed. The porphyrin functionalized zinc(II)porphyrin core was synthesized followed by the insertion of the separately constructed azide functionalized mannose conjugated dendrimeric building blocks. Starting with the synthesis of the carbohydrate dendrimeric wedges, we started with the commercially available *tris*-(hydroxymethyl)aminomethane (**3**), commonly known as TRIS. The use of tris hydrochloride was avoided as it demanded an additional step of neutralizing the compound. The free amine of **3** was initially protected with *tert*-butyloxycarbonyl (Boc) functionality using di-*tert*-butyl dicarbonate[11] to give the *N*-Boc protected derivative **4** in 93% yield. The hydroxyl groups were then propargylated to furnish the tris-propargyl derivative **5** in 67% isolated yield. Alkylation with propargyl bromide in the presence of KOH provided the best possible yield compared to alkylation in the presence of NaH at -55 ºC which gave yield of 45%. Derivative **5** was then coupled with known 2-azidoethyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside[12] (**6**) using $CuSO_4$ and sodium L-ascorbate to afford the 1st generation mannosylated dendron **7** in 87% yield. The formation of the desired dendron was assured from the characteristic triazole peak at δ 7.66 respective to the anomeric proton at δ 4.76 and complete disappearance of the acetylenic signals (δ 2.42), in the ^{1}H NMR spectrum. In the ^{13}C NMR spectrum, peaks at δ 145.1 and 123.7 for the triazole and 97.4 for the anomeric-α-carbon further supported the formation of the desired product. Next, TFA mediated selective deprotection of the *N*-Boc followed by reaction with freshly prepared TfN_3 in the presence of Et_3N and $CuSO_4$[13] gave the desired azide-functionalized dendron **9** in 76% yield over two steps (**Scheme 1**). The disappearance of the strong singlet at δ 1.35 in ^{1}H NMR spectrum for the *tert*-butyloxy group and corresponding carbonyl peak at δ 154.7 in ^{13}C NMR confirmed the complete removal of the Boc functionality.

The second generation glycodendrons were prepared with the synthesized azido-functionalized first generation dendritic wedges (**9**) by clicking them on Boc protected tris-propargyl derivative **5** using $CuSO_4$ in the presence of Na-ascorbate. The formation of the 2nd generation glycodendrons (**10**) was similarly confirmed by two sets of triazole signals at δ 7.82 and δ 7.68 in 1:3 ratio with the disappearance of the acetylenic signals (*δ* 2.42), and the corresponding mannoside anomeric signal at δ 4.79 in the ^{1}H spectrum. Usual TFA-catalyzed deprotection of the NHBoc functionality was employed followed by reaction with the freshly prepared TfN_3 in the presence of triethylamine and $CuSO_4$ to afford the desired azide-functionalized 2nd generation glycodendron **12** in 75% yield over two steps (**Scheme 2**).



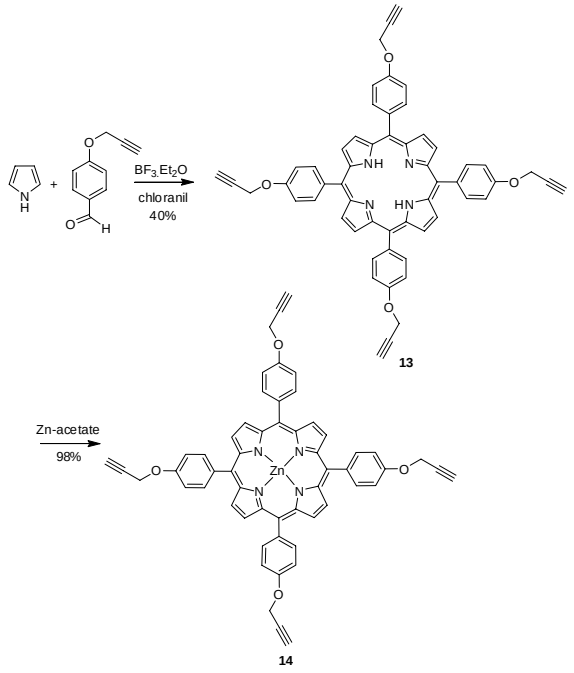
Scheme 1. Synthesis of the first generation azide-functionalized glycodendron **9**



Scheme 2. Preparation of the second generation azide-functionalized dendrimeric wedge **12**

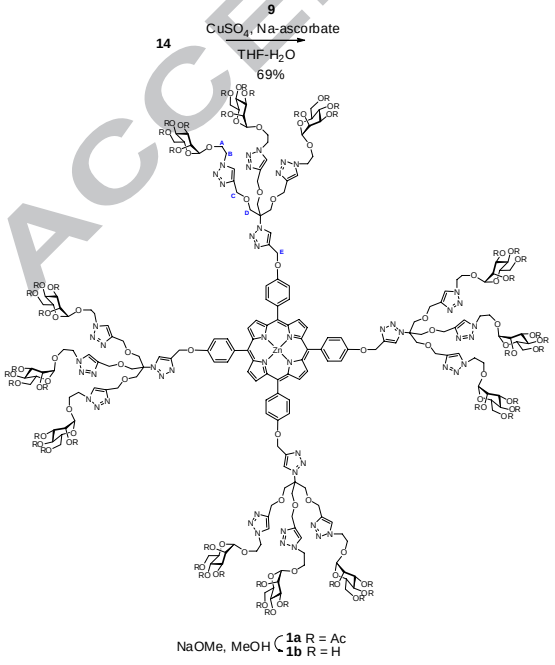
The synthesis of tetra-substituted porphyrins has always been challenging for the synthetic chemists because of its low yield. Based on the known protocols and methodologies, the propargyl functionalized porphyrin derivative **13** was synthesized in a modest 40% yield employing Lindsey type condensation[14] between 4-*O*-propargyl benzaldehyde and pyrrole. It was followed by metalation using Zn-acetate in refluxing $CHCl_3$-

MeOH mixture to give propargyl functionalized Zn-porphyrin derivative (**14**) in 98% yield (**Scheme 3**). The introduction of zinc was confirmed by the disappearance of the characteristic N*H* signal at δ -2.76 in the ^{1}H NMR spectrum.



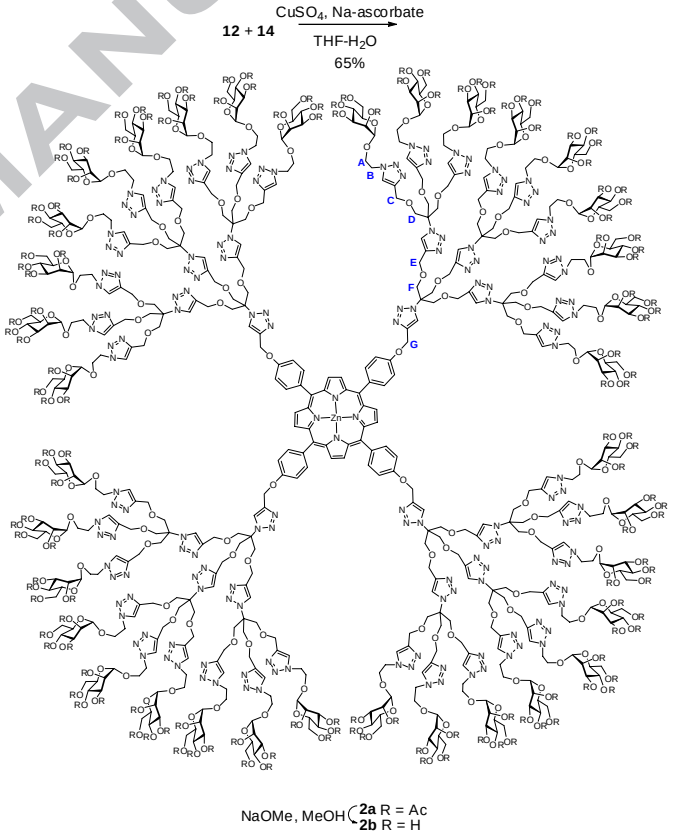
Scheme 3. Synthesis of the propargyl derivative of Zn-porphyrin core **14**

It is worth noting that the pre-metalation of the free porphyrin is essential prior to the Cu-catalyzed "click reaction" to prevent insertion of Cu in the porphyrin core. The choice of zinc as the insertion metal was triggered by the fact that it was often inert to insertion by other heavy metals at room temperature. The size affinity of the heavy metals towards porphyrin core is in the order Zn(II)~Cu(II)>>Mg(II).[15] Moreover the introduction of heavy metals in dyes promoting inter-system crossing has also been of high interest.[16]



Scheme 4. Synthesis of the Zn-porphyrin dendrimer **1b**

The propargyl-functionalized Zn-porphyrin core (**14**) was coupled with the glycodendrons **9** and **12** separately employing 'click chemistry' to afford the 1st generation and 2nd generation Zn-porphyrin glycodendrimers **1a** and **2a** respectively (**Schemes 4** and **5**). After chromatographic purification, compound **1a** and **2a** were isolated in 69% and 65% yields indicating that the influence of the sterically crowded glycodendrons is insignificant for the coupling reaction. The symmetrically tetra-substituted porphyrin carbohydrate dendrimers were fully characterized by ^{1}H and ^{13}C NMR, IR and MALDI-TOF MS. Complete absence of the characteristic azide peak in the IR spectra confirmed the purity of the final glycodendrimers (**Figure S1** and **S2**). In the ^{1}H NMR of **1a**, peaks at δ 8.15 and δ 7.68 for the formed triazoles in 4:12 ratio and the anomeric signal at δ 4.78 confirmed the formation of the symmetrical glycodendrimer. ^{13}C NMR showed the new anomeric signal at δ 97.5 which confirmed the formation of the α-mannosides. Similarly, for compound **2a**, ^{1}H NMR showed signals at δ 8.10 , δ 7.68 and δ 7.50 in 9:36:4 ratio in its ^{1}H spectrum and the new anomeric signal at δ 97.5 in ^{13}C spectrum.

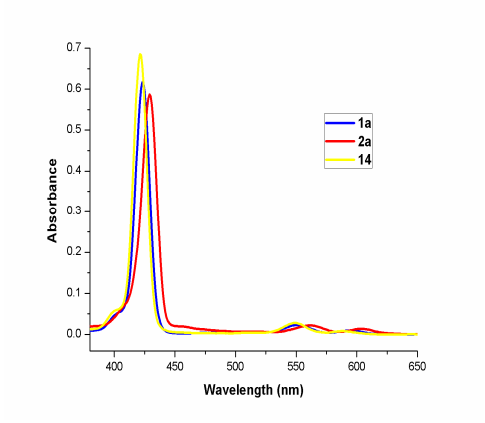


Scheme 5. Synthesis of the Zn-porphyrin dendrimer **2b**

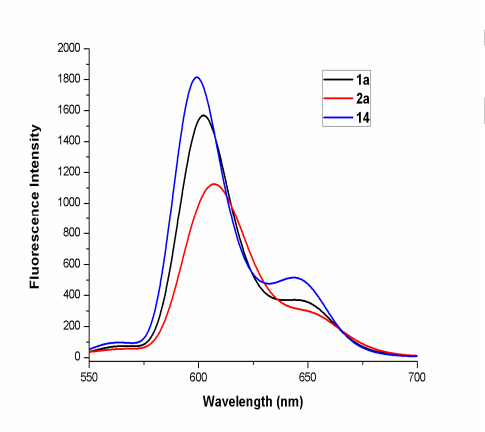
The acetate groups of the per-*O*-acetylated dendrimers **1a** and **2a** were quantitatively removed by using NaOMe in MeOH by implementing Zémplen de-*O*-acetylation method to afford the water soluble dendrimers **1b** and **2b** (**Schemes 4** and **5**). The compositions of the de-*O*-acetylated derivatives were confirmed by MALDI-TOF MS only. The extensive use of 'click chemistry' for the entire synthetic route marks the easy applicability of the said process.

The effect of the dendritic environment on the metalloporphyrin behavior was examined by the absorption and emission studies on the protected dendrimers **1a** and **2a**. The visible purple colour of the carbohydrate porphyrin dendrimers is

due to the absorption(s) within the porphyrin ligand involving the excitation of electrons from π to π* porphyrin ring orbitals. The UV-Vis spectra of **1a** and **2a** in dichloromethane showed typical metalloporphyrin behavior having Soret or B bands in the 423-429 nm range representing strong transition to the second excited state ($S_0 \rightarrow S_2$) and Q-bands in the 549-562 nm and 592-603 nm ranges representing weak transitions to the first excited state ($S_0 \rightarrow S_1$). When compared with the UV-Vis spectrum of the Zn-porphyrin derivative **14**, slight red shifts from **14** to **1a** and **1a** to **1b** were observed (**Figure 2a**). Next, the fluorescence behavior of the acetylated dendrimers **1a** and **2a** were studied and compared with that of the Zn-prophyrin derivative **14**. No significant difference was observed in the emission behavior of the dendrimers due to the dendritic environment (**Figure 2b**).



2a



2b

Figure 2. a. UV-Vis spectra of **1a**, **2a** and **14** in CH_2Cl_2, **b**. emission spectra of **1a**, **2a** and **14** in CH_2Cl_2

The conjugation of the carbohydrates onto the hydrophobic porphyrin core renders the required water-solubility to make it available in the biological media. The UV-Vis spectra for the de-*O*-acetylated dendrimers **1b** and **2b** were recorded in Tris buffer (pH 7.4) at room temperature. Prominent Soret and Q-bands indicated good solubility of the deprotected dendrimers in water due to effective shielding of the hydrophobic porphyrin and aromatic core by the hydrophilic sugar units in the periphery. However, significant broadening of the Soret band suggests significant aggregation arising from hydrophobic interactions in water (**Figure S3**, **ESI**).

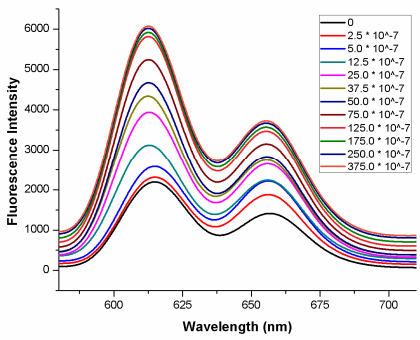


Figure 3. Increase of fluorescence upon binding of ConA with dendrimer **1b**

Once the carbohydrate dendrimers were characterized satisfactorily, we focused our attention to see the potential of the carbohydrate functionalized Zn-porphyrin dendrimers for the detection of lectins. The dendrimers were purposefully decorated with mannose so that they can be utilized for the interaction experiments with the mannose-binding lectin Concanavalin A (ConA). The tetrameric ConA has four carbohydrate binding domains and thus, it can form aggregated networks when exposed to multivalent mannose conjugates. The tetrasubstituted 1st and 2nd generation carbohydrate dendrimers portray an ideal aggregation platform to the four equivalent and diverged carbohydrate binding sites of the lectin. The fluorescence intensity of water soluble Zn-porphyrin dendrimers remains moderate in the buffer solution having high dielectric constant. However, upon interaction with the lectins when the aggregates are formed, it creates a hydrophobic microenvironment around the dendrimer resulting considerable enhancement of the fluorescence intensity. ConA which exists as a tetramer at pH>7 can ideally bind with mannose derivatives on all four wedges of our synthesized molecules by virtue of their tetrasubstituted nature, Therefore, the extent of binding to the lectin can be titrated by measuring the increase of fluorescence intensity. Based on this 0.5×10^{-6} molar solutions of dendrimer **1b** and **2b** in TRIS buffer (0.1M, pH 7.4) containing 0.1 mM $CaCl_2$ and 0.1 mM $MnCl_2$ were separately mixed with varied concentrations of ConA in the same buffer and the fluorescence was measured. The concentration of the ConA solution was gradually increased until the fluorescence intensity enhancement became constant suggesting maximum binding of ConA with the first and second generation dendrimers (**Figure 3** and **4** respectively).

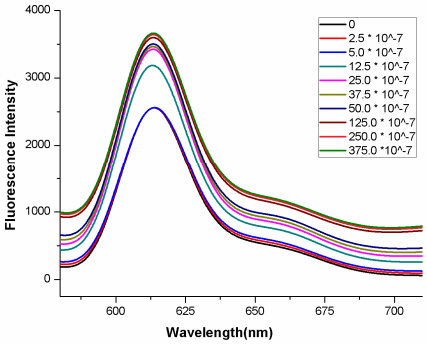


Figure 4. Increase of fluorescence upon binding of ConA with dendrimer **2b**

By comparing the 1st and 2nd generation dendrimers (**Figure 5**), it can be seen that greater aggregation is achieved with the first generation dendrimer **1b** with an overall change in emission of 3800 a.u. from dispersed to fully aggregated condition upon

addition of ConA, as compared to 1000 a.u. obtained with the second generation dendrimer **2b**. **1b** display a dynamic linear range from 0-350×10^{-7} M concentration of ConA compared to that of only 0-50×10^{-7} M for **2b**. It is clear that the first generation dendrimer **1b** provides superior aggregation with ConA, displaying a greater change in emission along a larger linear range. The quicker saturation of the second generation dendrimer **2b** may be explained by the much higher peripheral carbohydrate density that sterically hinders the possibility of interaction with incoming ConA. The obtained observations are also in sync with the various studies done in the past to state that higher number of epitopes doesn't necessary indicate enhanced binding potency towards proteins.[17] The titration data of the fluorescence intensity against the concentration of ConA displayed non-linearity indicating some degree of cooperativity in the binding process. Therefore, the non-linear equation (**Equation S1**, **ESI**) was used to fit the titration data to incorporate the factor for the cooperative behavior. The binding constant $\log K_b$ with 1st generation dendrimer **1b** was found to be 5.46 with the value of n=1.30. The same with the 2nd generation dendrimer **2b** found to be 5.91 with the value of n=1.82 (**Figure S4** and **S5**). Further, to judge the specificity of the mannose modified carbohydrate dendrimer towards the MBL ConA, first generation dendrimer similar to that of **1b** was synthesized with galactose in the periphery (compound **F**, **Scheme S1**, **ESI**). When it was subjected to titration with ConA, no enhancement of fluorescence was observed suggesting no binding with ConA (**Figure 5**). Thus, the specific nature of the carbohydrate-lectin interaction is established.

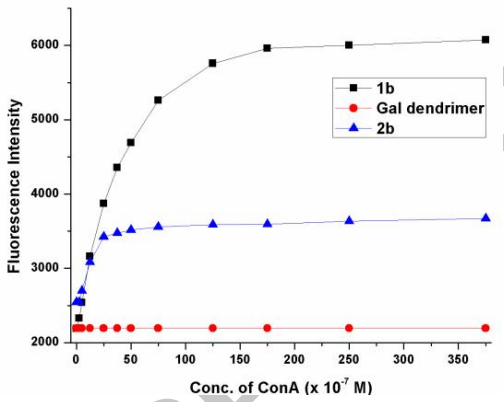


Figure 5. Comparison of the interaction of ConA with dendrimers **1b**, **2b** and the galactose modified dendrimer

Thus, in conclusion, we have developed the synthetic strategy for the preparation of porphyrin carbohydrate dendrimer with peripheral mannose ligands using the versatile 'click reaction' and established their application as sensors for the detection of lectins. The lack of significant previous reports employing porphyrin dendrimers for lectin sensing marks the novelty of our reported work. The first generation dendrimer displayed greater aggregation with the lectin ConA compared to that of the second generation. This is clearly showing that the extent of carbohydrate-lectin binding depends not only on the number of peripheral carbohydrate ligands but also the ligand density that dictates the number of available carbohydrates for effective binding. This particular application of carbohydrate appended porphyrin carbohydrate dendrimers for lectin-sensing may provide the required opening for the further use of porphyrin based dendrimers as lectin sensors.

Acknowledgments

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Supplementary Material

Experimental details and copies of the ^{1}H ^{13}C and selected COSY and HSQC NMR spectra.

Highlights

- Rational synthesis of 1st and 2nd generation Zn-porphyrin glycodendrimer
- Use of water soluble dendrimers for lectin sensing through fluorescence
- Insight of the 1st vs 2nd generation efficiency of binding with lectin