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Binding and stabilisation effects of glycodendritic compounds with peanut agglutinin

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ABSTRACT

A number of new polyhydroxy-dendritic structures have been constructed from a few basic modules. The cores were derived from *N-tert*(butyloxycarbonyl)tris[(propargyloxy)methyl]aminomethane, *N,N′*-bis-1,3-(tris-(propargyloxymethyl)methyl)-5-(hydroxymethyl)isophthalamide, and *N,N′,N″*-tris-1,3,5-(tris-(propargyloxymethyl)methyl)-1,3,5-benzene tricarboxamide while the terminal groups were derived from β-azido-galactose and β-azido-lactose leading to six new glycodendrimeric compounds with up to 63 hydroxyl groups on the periphery. The binding ability of the new compounds to peanut agglutinin (PNA), a galactose recognizing lectin from *Arachis hypogaea*, was investigated by nano-Isothermal Titration Calorimetry and nano-Differential Scanning Calorimetry. We found that the compounds had stronger stabilising effect on the macromolecules compared to the corresponding sugars. The interaction between lectin and the glycodendrimeric unit is entropically driven with only a low enthalpic contribution. A trend was found with increasing number of carbohydrates that is strongly influenced by the steric constraints of the ligands. Our results indicate the significance of multivalency and size control in the successful design of lectin inhibitors.

1. Introduction

Carbohydrates play a vital role in many biological processes [1–3], and their interactions with specific carbohydrate-binding proteins (lectins) are prevalent in living organisms owing to their involvement in intercellular recognition events, such as cell adhesion and migration, cell differentiation and apoptosis, infection by viral, parasitic and bacterial pathogens, glycoprotein synthesis and in blood protein level regulation [4–10]. The sugar specificity of these proteins has made them an active subject of investigation relating to their potential application as diagnostic markers as well as for the development of carbohydrate-based therapeutics [11–13]. Although plant lectins were the first to be identified, their role is still not completely established but they are implicated in plant defence mechanisms and plant symbiosis [14,15]. Their study is also medically relevant since, for example, there are indications of their role in cell proliferation [16,17] as well as of their potential in cancer treatment [18–24], while, in other cases, their possible allergenic effects are of interest, such as those associated with peanut agglutinin (PNA) [25].

Monosaccharide-lectin binding affinity is often weak (in the millimolar to micromolar range) but this affinity may be enhanced by exploiting the so-called "cluster glycoside effect" where there are multivalent interactions between lectin and ligands containing numerous copies of the same carbohydrate group [26–32]. As a consequence, in the quest for potential lectin ligands, the concept of multivalent carbohydrate analogues with enhanced binding affinity has led to the synthesis of many glycoconjugate compounds [11,33–36]. Lectins are usually aggregated into oligomeric structures with the tetrameric one being the most commonly observed, but, depending on the pH, this can dissociate into dimers or monomers [37,38], and these oligomeric structures open up the possibility of multivalent binding to multiple lectins or to multiple sites on one lectin [27,39,40]. The substrate binding pocket is usually found on the surface of each monomer and this

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arrangement can provide suitable conditions for the multivalent binding of macromolecules (polymers, dendrimers) when agglutination or precipitation are the biochemical aims. Due to the current interest in the development of potential pharmaceuticals based on carbohydrate-lectin interactions, there has been much recent research activity concerning molecular scaffolds with a multivalent carbohydrate periphery such as glyconanoparticles, glycopolymers and glycodendrimers [41–44]. Glycodendrimers have been known for almost two decades [45–48] and display a number of attractive features due to their monodispersity and regular branching pattern, as well as the possibility of manipulating their chemical composition so as to control their physicochemical properties. As a result, glycodendrimers are foreseen to have a wide range of potential biomedical applications, for example as anti-adhesion drugs [49,50], as drug delivery systems [51,52], as functional antigens and anti-tumour vaccines [53], and as conjugates to gold nanoparticles for the monitoring of recognition events [54–57].

The possibility of designing dendritic structures with specific properties has provided major challenges for the development of synthetic procedures that give products with acceptable yields and purity. However, in spite of extensive activity in this area, very few dendrimer-based products have reached the stage of commercial development, and one reason for this is that the synthesis of dendrimers is often a time-consuming process which translates into a high cost of the final product. For efficient dendrimer synthesis, efficient reactions are a prerequisite, and one of the most useful types of reaction for this purpose is that covered by the term "Click Chemistry" introduced by Sharpless [58]. The first example of a convergent synthesis of dendritic macromolecules based on click chemistry appeared in 2004 [59], and this approach has since been widely used for the preparation of dendrimers [60–64]. We are interested in the design and synthesis of new dendritic molecules with possible biomedical applications *e.g.* in formulations of Liposomal-Locked in-Dendrimers (LLD) nanoparticles as drug delivery systems [65,66], and in this context we have recently employed click chemistry routes involving divergent and convergent synthetic steps using a small number of modular building blocks which offer the possibility of building a small library of new dendritic compounds with multiple hydroxyl groups on the periphery of the molecule.

As part of these studies, investigations have been initiated into the interactions of lactose and β-D-galactose terminated low generation dendrimeric compounds with lectins. Although the selectivity of these sugar-recognizing proteins is well described [4,67–71], there are still unanswered questions regarding the cluster glycoside effect or non-specific binding. Recently, a crystallographic investigation has been reported of the interactions with human galectin-7 of certain of the new glycodendrimeric compounds synthesised by us [72], and here we report on parallel calorimetric studies with the galactose specific plant lectin peanut agglutinin (PNA) derived from *Arachis hypogaea*. These studies were performed using nano-ITC and nano-DSC which are high precision techniques for the measurement of protein–ligand interactions [73–77], and allow for a thermodynamic approach to the description and quantification of the binding aspects [78–80], while the information they provide serves to complement that recently reported from related studies with PNA using enzyme-linked lectin assays, dynamic light scattering and atomic force microscopy [32].

2. Results and discussion

2.1. Synthesis of materials

The syntheses of the dendritic structures are based on the attachment of large building blocks which can be coupled efficiently to the appropriate core using click chemistry protocols

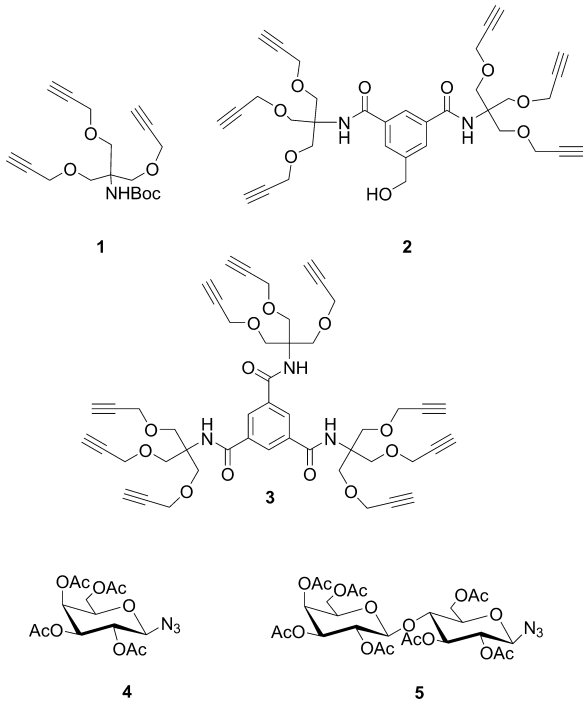

Fig. 1. Structures of building blocks. The three core modules (**1–3**) and the two peripheral modules (**4, 5**).

in such a way as to facilitate chromatographic separation of the desired mono-disperse compounds [81–84]. For the particular study reported here, two series of molecules were prepared. Each series consists of the same terminal unit coupled to three different propargylated cores, **1**, **2** and **3** (Fig. 1). Thus, in the first series, peracetylated 1-β-azido-D-galactose, **4**, was attached to three cores of increasing size while the second series consists of the same cores terminated by reaction with peracetylated 1-β-azido-lactose, **5**, thus giving glycodendrons and glycodendrimers containing from 12 to 63 peripheral hydroxyl groups. The propargyl building blocks **1**–**3** were synthesised as outlined in Scheme 1, using tris(hydroxymethyl)aminomethane (TRIS) as starting material. Copper-catalysed coupling of β-azido galactoside **4** with **1**–**3** gave the desired compounds **7**, **9** and **11** in 67, 77 and 60% yields, respectively. Subsequent deprotection with MeONa in MeOH and, in the case of the Boc protected compounds with 3 M HCl/dioxane or TFA/DCM, provided **8b**, **10** and **12** in almost quantitative yields (Scheme 2). In a similar fashion, clicking the β-azido lactose **5** to **1**, **2** and **3** gave the desired products in isolated yields ranging from 55 to 98% (Scheme 3) and deprotection led to compounds **14b**, **16** and **18**.

2.2. Calorimetric studies

Fig. 2 displays the ITC results of the free sugar–protein binding interaction. In order to assess the thermodynamic details of the binding interaction, the best fit on the plots was performed according to thermodynamic models. The simplest traditional thermodynamic model, *i.e.* single site with fixed $n = 1$ stoichiometry was sufficient to give a good fit (taking into account the experimental errors) and the relevant thermodynamic parameters are reported in Table 1. We observe a significant enthalpic contribution (indicating a well defined binding cavity) but also a negative entropic contribution resulting in binding constants of an order of 10^3.

These results are in substantial agreement with similar studies reported in the literature in spite of the suggestion by some authors of a more complex process in the case of the PNA – β-D-galactose