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Multivalent ligand mimetics of LecA from *P. aeruginosa*: synthesis and NMR studies

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ABSTRACT

Molecular recognition of glycans plays an important role in glycomic and glycobiology studies. For example, pathogens have a number of different types of lectin for targeting host sugars. In bacteria, lectins exist sometimes as domains of bacterial toxins and exploit adhesion to glycoconjugates as a means of entering host cells. Herein, we describe the synthesis of three glycodendrons with the aim to dissect the fine structural details involved in the multivalent carbohydrate–protein interactions. LecA, from the pathogen *Pseudomonas aeruginosa*, has been used to characterize galactose dendrons interaction using one of the most widespread NMR technique for the elucidation of receptor-ligand binding in solution, the saturation transfer difference (STD) NMR. Furthermore, the effective hydrodynamic radius of each dendrimer recognized by LecA was estimated from the diffusion coefficients determined by pulsed-field-gradient stimulated echo (PFG-STE) NMR experiments.

1. Introduction

Interactions between carbohydrates and lectins mediate several biological processes such as cell–cell communication, molecular recognition for intracellular trafficking or interactions between pathogenic microorganisms and viruses and the surfaces of mammalian cells.[1,2] *Pseudomonas aeruginosa* (PA), like *Escherichia coli* and *Staphylococcus aureus*, is an opportunistic pathogen associated with chronic airway infections, especially in cystic fibrosis patients. PA synthesizes two lectins, LecA and LecB,[3] involved in the pathogenicity of this opportunistic bacterium.[4] LecA (or PA-IL) is a tetrameric cytotoxic galactose-specific soluble lectin consisting of four subunits of 121 amino acids (12.75 kDa).[3,4] Affinity between a single carbohydrate residue and a lectin is usually relatively low; to circumvent this issue, nature uses a cluster effect, through which multiple simultaneous weak interactions between carbohydrate ligands and their receptors reinforce one-another, yielding a higher affinity than the sum of the individual interactions.[5] Nowadays, the design of glycoclusters with high affinity and selectivity for lectins may rely on rational design.[6] Therefore, several multivalent unnatural glycoconjugates with various valencies and spatial arrangement of the ligands have been reported, and among them, some exhibited high affinities for LecA.[7]

In the search for new multivalent antagonists of LecA, we herein propose the synthesis of three glycoconjugates bearing, respectively, one, two and four β-galactose moieties (Fig. 1, **1**–**3**); their interactions with the protein and their hydrodynamic radius were analyzed by NMR studies.

Compounds **1**–**3** were obtained starting from zero, first and second generation heterobifunctional dendrons (**4**–**6**), respectively. The synthesis of dendrons **4**–**6** was performed using (Boc-aminooxy) acetic acid (**7**) and selected building blocks (**8**–**10**) through esterification and protection/deprotection steps, as outlined in Scheme 1.[8]

The introduction of the β-galactose moieties was performed reacting dendrons **4**–**6** with lactose (Scheme 2); a mixture of water and methanol, in the presence of acetic acid (0.15 M), was used as the solvent. These conditions allowed the reaction between the aminooxy functionalities and the reducing end of the disaccharide to give a hydrolitically stable oxime bond. In all cases, the reaction proceeded slowly, with low yields (from 10 to 22%), probably due to the unfavorable equilibrium between the hemiacetal and carbonyl forms of reducing carbohydrates and the increasing steric hindrance moving from mono- to tetra-functionalized glycoconjugates. Performing the reaction in different conditions (acetate buffer, pH = 4) as solvent, did not afford the desired products.

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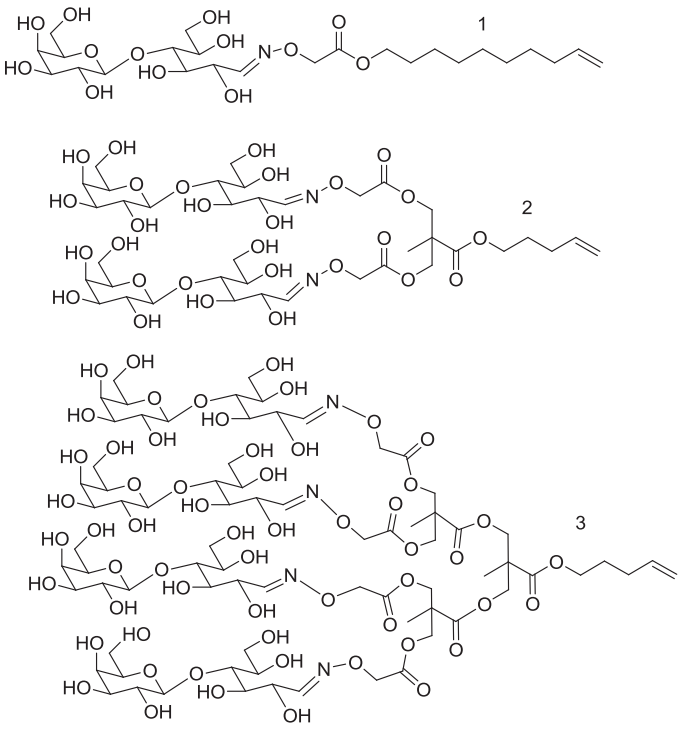

Fig. 1. Structures of multivalent LecA **1**–**3** antagonists.

1.1. STD NMR analysis

The interaction between the synthesized glycoconjugates and LecA lectin from *Pseudomonas aeruginosa* was investigated *via* NMR, by using STD (Saturation Transfer Difference) NMR.[9] This technique allows not only to deduce the existence of a physical protein-ligand binding in solution, but also to detect the regions in closer contact to the receptor, defining the binding epitope of the ligand.

Firstly, we have analyzed the interaction of the protein LecA and compound **1**; the absence of STD signals in the corresponding STD NMR spectrum suggested that the lectin was not able to recognize this glycoconjugate (data not shown).

Interesting results were instead obtained with compounds **2** and **3** in the presence of LecA (Figs. 2 and 3): a comparison of the STD enhancements and of their relative intensities, indeed, provided evidence of a similar mode of accommodation in the protein binding pocket of the two glycoconjugates, as also clearly inferred from a qualitative analysis of the STD NMR spectra. It is worth to note that the overlapping of several ligand resonances hampered a quantitative analysis of the STD effects. However, a qualitative study of the STD intensities suggested that in both cases, the interaction with the lectin preferentially involved the sugar region of the ligand. As shown in Figs. 2 and 3, the presence of STD signals ascribable to the galactose residues, both in the presence of the bi- and the tetrafunctionalized ligands (compound **2** and **3**), indicated that these moieties were pivotal for the recognition process.

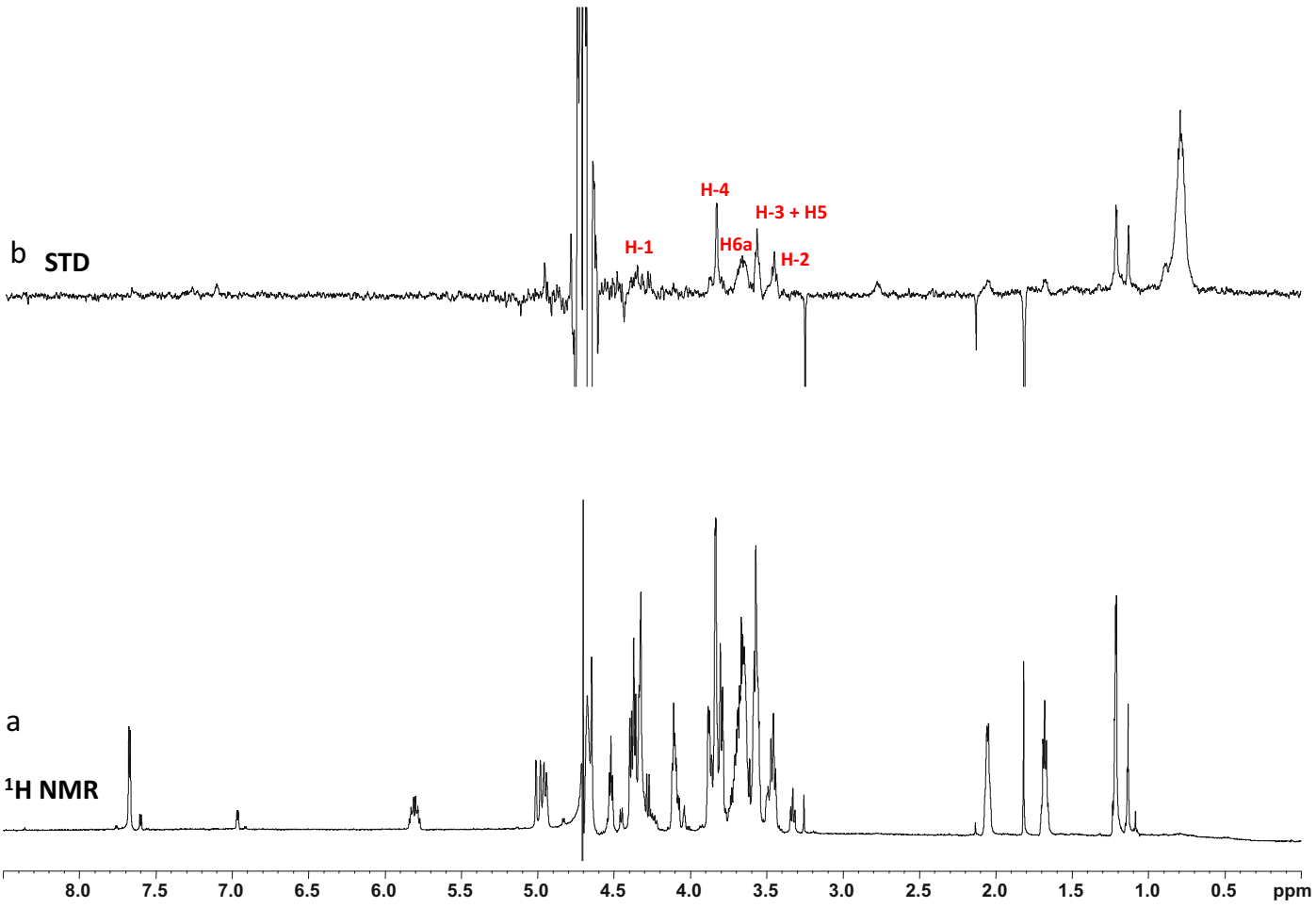

Fig. 2. a) ^{1}H NMR spectrum of compound **2** in the presence of the lectin LecA. b) STD NMR spectrum on the mixture ***LecA*: 2** 1:80.

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