Mannose-functionalized PAMAM dendrimers : their synthesis, characterization and use in refining the model of protein-carbohydrate interactions
by Eric Kevin Woller

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry
Montana State University

Abstract:
The interaction between proteins and carbohydrates is an important first step for many biological processes. For example, viral and bacterial infections, antibody-antigen interactions, fertilization and other cell-cell interactions all rely on protein-carbohydrate interactions as a first step in recognition. It is known, however, that the affinity between a lectin and a single sugar is quite weak. The increase in binding affinity in most biological systems is a result of what is often generically referred to as multivalent binding.

A number of carbohydrate-containing synthetic organic molecules have been synthesized to study multivalent protein carbohydrate interactions. Examples include small molecules that typically contain fewer than 10 sugars, medium sized molecules that contain between 10 and 100 sugars, and large polymeric systems that can contain thousands of sugars.

While most carbohydrate-containing systems have provided valuable insight into the mechanism of multivalent interactions, no class of carbohydrate containing system existed prior to this work in which the number of sugars and the size of the ligand could be varied independently. Such a system should provide specific information on the optimal geometric arrangement of ligands toward various receptors.

We have homogeneously and heterogeneously functionalized polyamidoamine (PAMAM) dendrimers up to generation six with mannose using a thiourea linkage by the reaction of an isothiocyanate on the carbohydrate with the terminal amines on the dendrimer. The result is water-soluble carbohydrate-containing dendritic molecules. Methods to characterize these dendritic macromolecules by nuclear magnetic resonance spectroscopy and matrix-assisted laser desorption ionization time-of-flight mass spectrometry were developed.

The effectiveness of the dendrimers as ligands for the lectin Concanavalin A has been demonstrated by means of a hemagglutination assay, a precipitation assay and a turbidity assay. Results indicate that the size of the dendrimer has a great effect, with the larger dendrimers being most active with Concanavalin A. In addition, the degree of functionalization has a profound effect, with the optimum degree of functionalization for binding being below full functionalization. Results also suggest mannose-functionalized PAMAM dendrimers effectively recruit and cluster lectins.

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APPROVAL

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Eric Kevin Woller

This dissertation has been read by each member of the dissertation committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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To Denice

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ABSTRACT

The interaction between proteins and carbohydrates is an important first step for many biological processes. For example, viral and bacterial infections, antibody-antigen interactions, fertilization and other cell-cell interactions all rely on protein-carbohydrate interactions as a first step in recognition. It is known, however, that the affinity between a lectin and a single sugar is quite weak. The increase in binding affinity in most biological systems is a result of what is often generically referred to as multivalent binding.

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CHAPTER 1

INTRODUCTION, BACKGROUND AND RATIONALE

Protein-Carbohydrate Interactions

The interaction between proteins and carbohydrates is an important first step for many biological processes. For example, viral and bacterial infections, antibody-antigen interactions, fertilization and other cell-cell interactions all rely on protein-carbohydrate interactions as a first step in recognition (Figure 1.1).[1-8]

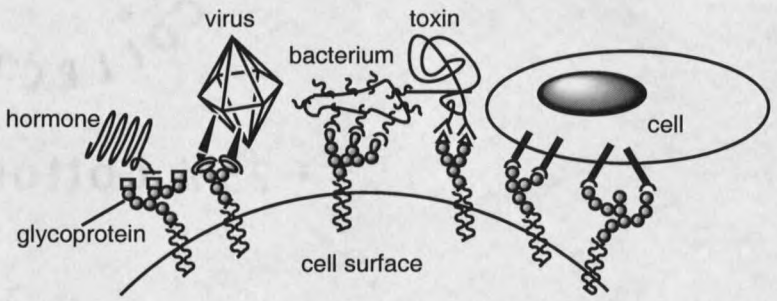

Figure 1.1 Various biological interactions that rely on protein-carbohydrate interactions. Adapted from reference 4.

A well studied case is the infection of human cells by influenza virus.[2, 9-21] In the first step of infection, hemagglutinin on the surface of the virus interacts with sialic acid (a sugar that is present on many glycoproteins) located on the surface of a bronchial epithelial cell. Once the interaction has occurred, barring an adequate immune response, viral infection will proceed.

It is known, however, that the affinity between a carbohydrate receptor, such as a lectin, and a single sugar is quite weak. In the case of binding between hemagglutinin and monomeric α-sialosides, the association constant has been determined to be about 10^3 M^{-1}.[17] However, the interaction between influenza virus and the cell surface is strong enough to lead to an eventual infection.

Other studies of the affinity of lectins for saccharide units have also shown that, typically, a single sugar is only weakly bound.[22, 23] The increase in binding affinity in most biological systems is apparently a result of what is often generically referred to as multivalent binding.

Multivalent Interactions and Glycoside Clustering

Yuan C. Lee at Johns Hopkins University performed pioneering work on the study of multivalent interactions. Lee and co-workers found tremendous enhancements in affinity between small clusters of galactose residues and mammalian hepatic carbohydrate receptors.[24] They found that the binding affinity for monovalent, divalent and trivalent ligands (Figure 1.2) increased roughly from 1 to 1000 then to 1 million, respectively! To describe this general observation, Lee first coined the term "glycoside cluster effect." The glycoside cluster effect is defined by Lee as "affinity enhancement achieved by multivalent ligands over

monovalent ones that is greater than would be expected from a simple effect of concentration increase."[25]

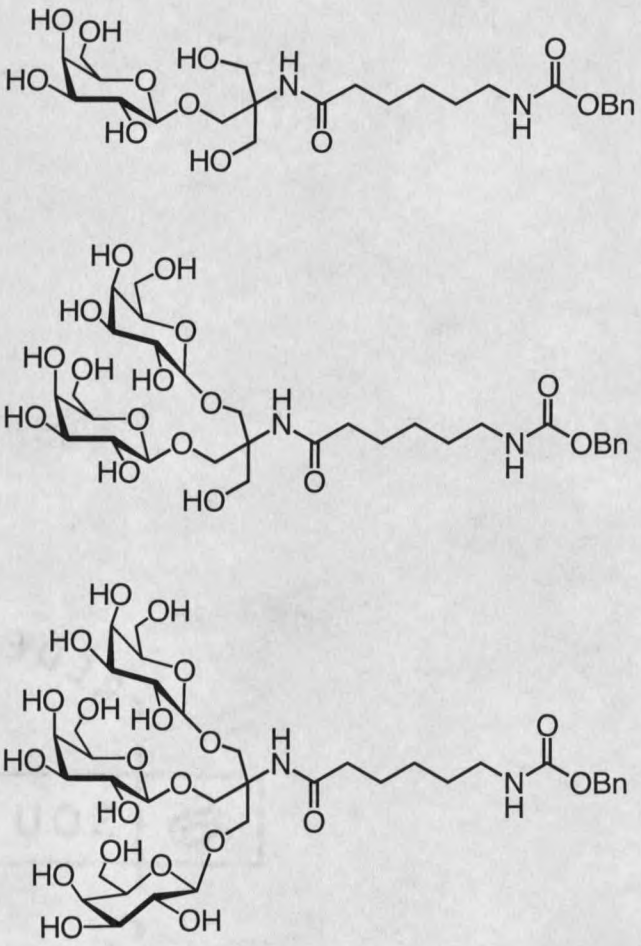

Figure 1.2 Mono-, di-, and tri-valent galactose ligands designed and used by Lee and co-workers to observe the *glycoside cluster effect*.

The term "multivalent interaction" refers to a more specific interaction, that being when a ligand presents multiple epitopes (carbohydrate residues) and binds to two or more distant binding sites on a receptor (protein). What is remarkable is that multivalent interactions are often stronger than the sum of