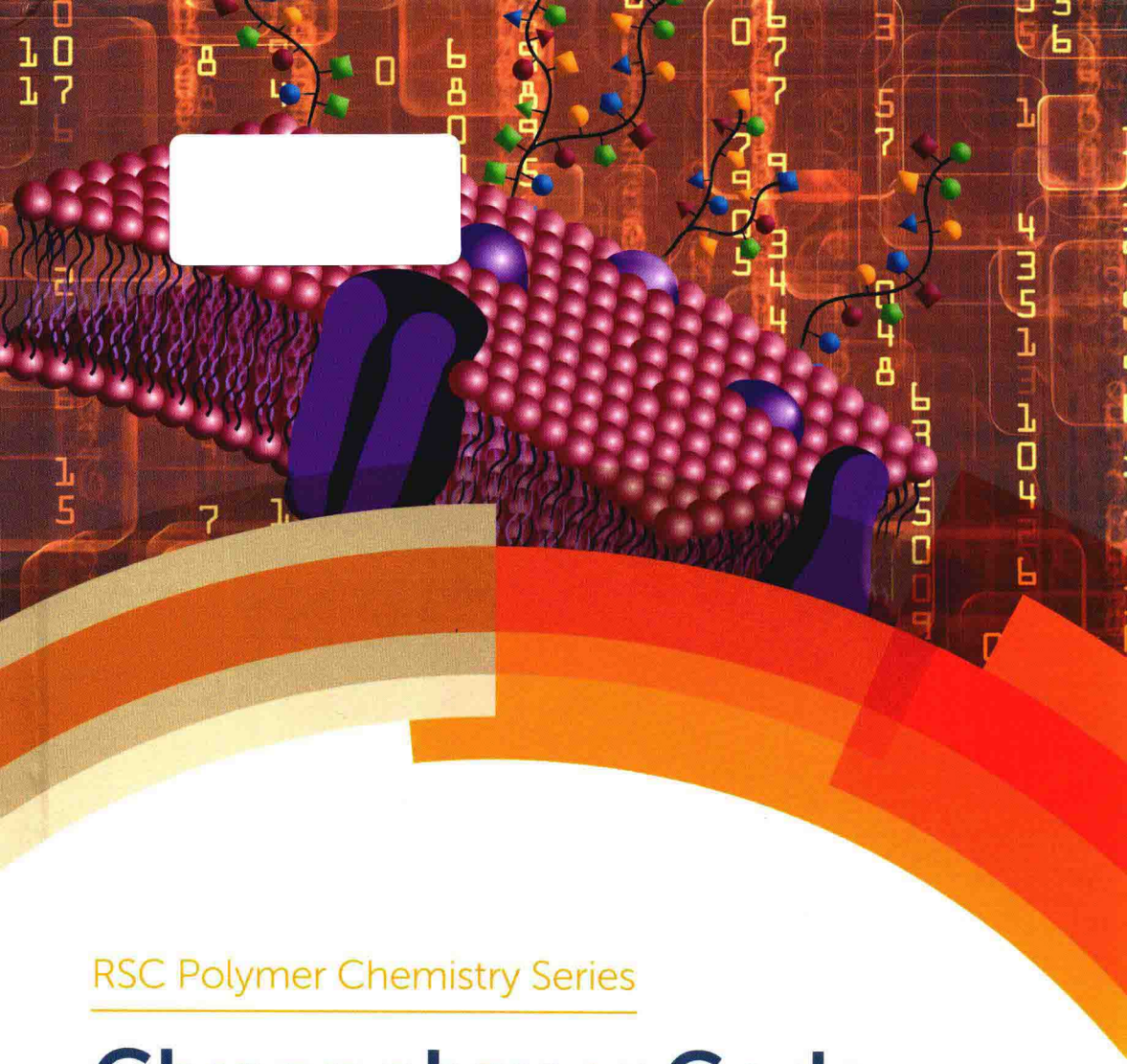

RSC Polymer Chemistry Series

Glycopolymer Code

Synthesis of Glycopolymers and their Applications

Edited by C. Remzi Becer and Laura Hartmann

ROYAL SOCIETY OF CHEMISTRY

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Synthesis of Glycopolymers and Their Applications

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Preface

Sugars have long been recognized for their role in biology and medicine and their synthesis and modifications are a constant challenge for organic chemists. However, in the world of polymer chemistry and material sciences, sugars have often been neglected. While peptides and oligonucleotides – the other two biomacromolecules – have inspired tremendous efforts in creating biohybrid or bioinspired polymers and materials, the sugars have been mainly used as a renewable resource for starting materials not taking into account their biological properties. Sugars mediate a number of biological events such as inflammation and infection *via* their interaction with protein receptors. However, the interaction of a single sugar ligand is very weak and Nature uses the so-called glycocluster or multivalency effect to have several sugar ligands interact simultaneously and create a strong binding event. Based on this concept, polymer chemists have synthesized glycopolymers presenting a large number of sugar ligands along a polymeric backbone, thus creating high-affinity ligands. Although the concept is fairly simple, the covalent attachment of sugar ligands to a polymeric backbone, the synthesis can be challenging, including different strategies for the conjugation of sugar ligands and the variation of the polymer chains. Tremendous progress has been made in recent years in synthesizing glycopolymers and glycomaterials. The ability of sugars to introduce biofunctionality into synthetic materials has been recognized and shown for a number of applications ranging from drug design to biosensors. However, the field is still in its infancy and many of the synthesized glycopolymers and -materials have not yet been studied for their potential properties and applications.

The chapters in this book are concerned with different classes of glycopolymers and glycomaterials and specifically focus on the different synthetic strategies that have been developed over the last few years. In the first chapter,

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Lindhorst focuses on the lectin structures that are the lock of Glycopolymer Code. We believe that it is crucial to understand the locking mechanism first in order to attempt to crack the code. The following chapter is concerned with the preparation of glycopolymers, where Ting and Stenzel provide an extremely detailed account of state-of-the-art synthesis techniques for glycopolymers. In the third chapter, Krannig and Schlaad provide insights into glycopolypeptides. This chapter is followed by Dondoni and Marra's excellent introduction to glycocalixarenes and their molecular recognition. In Chapter 5, Voit and her colleagues focus on the dendritic architectures of synthetic glycan structures and their use in brain disease therapy. In Chapter 6, Chen and her colleagues discuss the glycomaterials that grow larger as we look at the self-assembly of glycopolymers and their formation of vesicles and hydrogels. In the last three chapters, the focus is concentrated on the applications of glycopolymers. In Chapter 7, Narain and his colleagues discuss glyconanoparticles and their biomedical applications. Following on, Chapter 8, by Fernández-García and Muñoz-Bonilla, then outlines the great potential for such hybrid glycomaterials in various biomedical applications. In the final chapter, Miura and Seto discuss recent literature examples on the use of glycopolymers in biosensing applications. We believe that all these various applications provide insights into Glycopolymer Code that require a more systematic approach, similar to glycomics, in order to crack Nature's sugar code and create the Glycopolymer Code.

Overall, we hope that this book will inspire interested research students and academics alike and support their education, teaching and research and thus further promote the field of glycopolymers and glycomaterials.

We are very grateful to all authors of the chapters in this book who kindly agreed to support this project and we would like to thank them for their enthusiasm and excellent work.

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CHAPTER 1

Small Molecule Ligands for Bacterial Lectins: Letters of an Antiadhesive Glycopolymer Code

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1.1 Introduction

This chapter discusses how glycopolymers might function in the context of microbial adhesion. This is an important topic as attachment of viruses and bacteria to surfaces is a global problem and for host organisms it has fundamental implications for their vitality. This was considered when the human microbiome project was launched in 2008. Consequently, the human microbiome project is dedicated to research into how changes of microbial colonization influence human health and disease.[1]

It has turned out that microbial colonization of the body is largely associated with the glycoconjugate decoration of the host cells, named the 'glycocalyx.' The glycocalyx of a cell is an extracellular compartment comprising a huge variety of different glycoconjugates. Strikingly, it forms an anchoring

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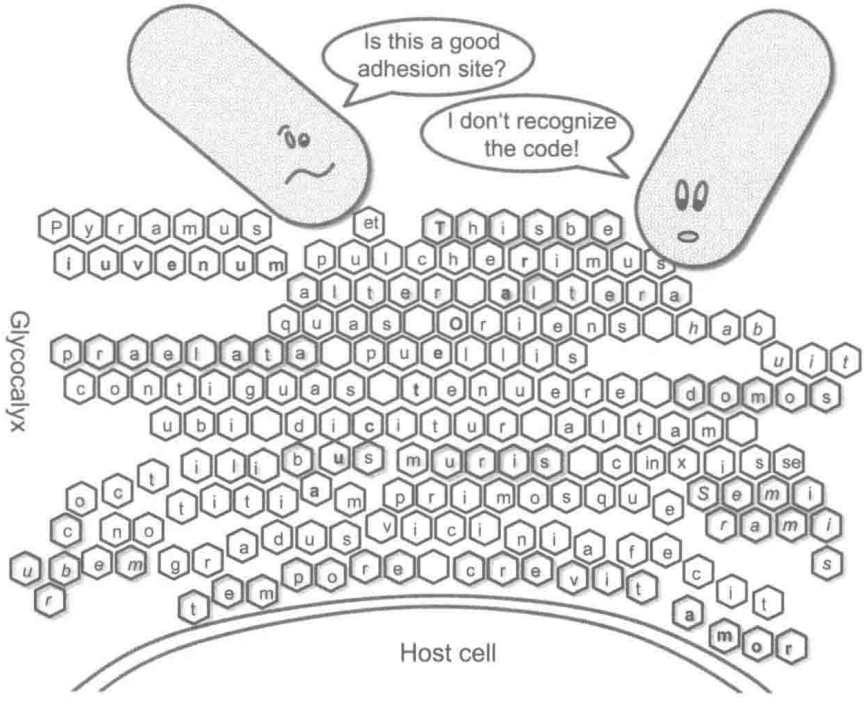

Figure 1.1 'Deciphering the glycocode.'[4] Cartoon to exemplify that carbohydrate-specific adhesion of, *e.g.*, a bacterial cell to the glycocalyx of a host cell might be looked at as reading a code.

platform for invading microbes. It has been asked how carbohydrate recognition has evolved among microbes,[2] how it is regulated and how it develops during the lifetime of an organism, in other words, how binding to cell surface carbohydrates is being 'spelled' (Figure 1.1). It has been suggested that the oligo- and polysaccharide structures that are expressed on cell surfaces function in the sense of a 'glycocode,'[3] thus paralleling the biology of carbohydrates with the alphabet of a language, in order to decipher its meaning.[4] Of course, it is sensible to consider the diversity of carbohydrate structures as a biologically meaningful concert corresponding to the whole of molecular interactions. Glycopolymers can be regarded as a means to interrogate a putative carbohydrate alphabet and, moreover, as a powerful tool to prevent microbial colonization of surfaces.

1.2 Lectin-Mediated Bacterial Adhesion

To colonize cell surfaces of the host, bacteria, for example, have to accomplish a process of adhesion in order to withstand natural defence mechanisms and mechanical shear stress. Stable adhesion can lead to the formation of bacterial biofilms, which is accompanied by vital advantages for the microbial colonies[5] but disadvantages for the host. Finally, adhesion apparently is a prerequisite for bacterial infections that constitute a major global health

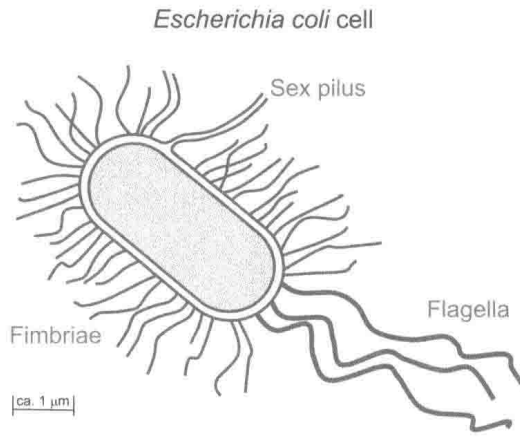

Figure 1.2 A majority of bacterial cells, such as *E. coli*, are equipped with three types of hair-like protein appendages, named pili, fimbriae and flagella. Fimbriae serve as adhesive organelles, mediating adhesion to the glycocalyx of host cells. *E. coli* cells are covered with several hundred copies of fimbriae of different carbohydrate specificity.

problem, in particular in developing countries. Bacterial infections are especially dangerous for newborns and young children,[6] with the most common serious neonatal infections involving bacteremia, meningitis and respiratory tract infections. Key pathogens in these infections are *Escherichia coli*, *Klebsiella* sp., *Staphylococcus aureus* and *Streptococcus pyogenes*.[7]

One important mechanism of bacterial adhesion is based on molecular interactions between cell surface carbohydrates of the host and specialized carbohydrate-specific bacterial proteins called adhesins or lectins. Lectins were first described at the end of the 19th century,[8] when it was shown that plant lectins have the ability to agglutinate erythrocytes blood group specifically. As we know today, this is a result of a multivalent carbohydrate–lectin interaction. In 1954, Boyd and Shapleigh proposed the term lectin 'for these and other antibody-like substances' with blood group-specific agglutination properties.[9] In the 1990s, Lis and Sharon[10] suggested that 'lectin' should be used as a general name for all proteins of non-immune origin that possess the ability to agglutinate erythrocytes and other cell types. Early classification of lectins relied on their carbohydrate specificity. However, today lectins are grouped on the basis of their structural features and especially the relatedness of their carbohydrate binding sites, which are often called 'carbohydrate recognition domains,' or CRDs.[11–13]

It is common knowledge today that lectins are ubiquitously spread in Nature, comprising many different functions in different organisms.[14] Also, many bacteria, in particular those of the Enterobacteriaceae family, have the ability to agglutinate erythrocytes by their own lectins. This haemagglutination activity of bacteria is almost always associated with the presence of multiple filamentous protein appendages projecting from the surface of the bacteria.[15] These are called fimbriae (from the Latin word for 'thread') and also, less correctly, pili (from the Latin word for 'hair') (Figure 1.2). Whereas

pili are involved in gene transfer between bacteria ('sex pili') and flagellae have the role of sensory organelles used for moving, fimbriae serve as adhesive organelles. Fimbriae contain lectin subunits, which mediate carbohydrate-specific adhesion to cell surfaces (and also cell agglutination). Thus, bacteria utilize the sugar decoration of cells – the glycocalyx – to colonize the cell surface, wherever cells are in contact with the outside environment, as for example in the case of epithelial cells.

1.3 Carbohydrate Specificity of Type 1 Fimbriae

Type 1 fimbriae are particularly efficient adhesion tools of bacteria to mediate the colonization of various biotic and abiotic surfaces. They are uniformly distributed on the bacterial cell surface with their length varying between 0.1 and 2 μm and a width of ~7 nm. Since the 1970s, numerous studies have been carried out to elucidate the carbohydrate specificities of bacterial adhesion, in particular of type 1 fimbriae-mediated adhesion of *E. coli*.[6] A key finding of this research was that the type 1 fimbrial lectin, called FimH, requires α-D-mannose and α-D-mannosides for binding. The other anomer, namely β-mannosides, cannot be complexed within the carbohydrate binding site. This knowledge suggested that type 1 fimbriated bacteria can adhere to tissues expressing glycoproteins of the high-mannose type, exposing multiple terminal α-D-mannosyl units.[16] For example, urinary tract infections are caused by uropathogenic *E. coli* (UPEC). Type 1 fimbriae are present in at least 90% of all known UPEC strains, where they are important pathogenicity factors.[6,15] Today, it is known that bacterial adhesion to the surface of urothelial cells is mediated by FimH binding to oligomannoside residues of the glycoprotein uroplakin Ia. This interaction is a prerequisite for bacterial invasion.[17] Consequently, much effort has been invested in the development of potent inhibitors of type 1 fimbriae-mediated bacterial adhesion in order to prevent bacterial adhesion to mucosa and thus treat bacterial infection in an approach that has been called antiadhesion therapy.[18,19]

In this context, a second feature of type 1 fimbriae-mediated bacterial adhesion that was discovered already quite early is important.[16] It was found that α-D-mannosides with an aromatic aglycone moiety exhibit an improved affinity to the bacterial lectin and an enhanced potency as inhibitors of type 1 fimbriae-mediated bacterial adhesion to surfaces. Today, this finding is well understood based on the X-ray studies of the structure of the type 1 fimbrial lectin FimH that have been published since 1999.[20–23] Structural biology has shown that the entrance of the carbohydrate binding site of FimH is flanked by two tyrosine residues, Y48 and Y137, which make π–π interactions with an aromatic aglycone of an α-D-mannoside ligand that is complexed within the cavity of the FimH carbohydrate binding site (Figure 1.3).

This and other structural features of the bacterial lectin FimH have been described elsewhere[6,24–26] and are not further detailed in this account. Similarly, the biosynthesis of type 1 fimbriae has been elucidated and reviewed.[27,28] Briefly, the fimbrial appendage is assembled in the outer membrane of

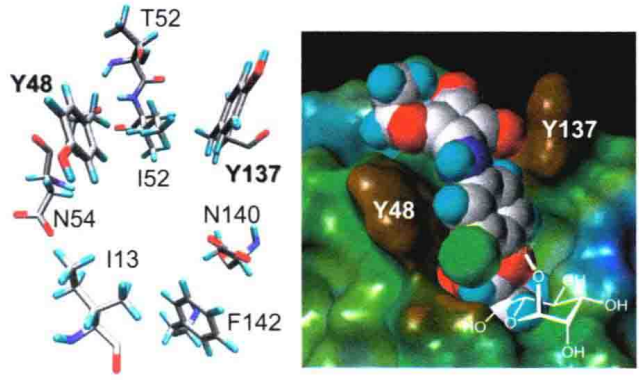

Figure 1.3 Left: spatial orientation of the amino acid residues at the entrance of the carbohydrate binding site of the bacterial lectin FimH as revealed by crystallography. The tyrosine residues Y48 and Y137 form a so-called 'tyrosine gate' that mediates the comparatively high affinity of mannosides with an aromatic aglycone by formation of π–π interactions. Right: the FimH carbohydrate binding site depicted as a Connolly surface, complexed with the mannoside **2** (*cf.* Figure 1.5). Mannoside **2** is shown as a CPK model. The large chloro substituent of **2** pointing towards the observer fills a depression at the ridge of the carbohydrate binding site, thereby improving affinity. The ring structure in white explains how α-D-mannoside ligands are located within the FimH cleft, the α-glycosidic bond sticking out of the binding site.

Gram-negative bacteria in a process called the chaperone–usher pathway. To be able to judge the potential value of especially glycopolymers as inhibitors of type 1 fimbriae-mediated bacterial adhesion, it is important to know that FimH is a two-domain protein, terminating every type 1 fimbrial rod (Figure 1.4). The so-called pilin domain of FimH, $FimH_P$, is required to anchor the protein at the fimbrial tip, comprising also the subunits FimF and FimG. The lectin domain $FimH_L$, on the other hand, accommodates the α-D-mannoside-specific carbohydrate-binding site. Both FimH domains are interconnected by a hinge region, permitting allosteric regulation of the carbohydrate binding site (see below).[29,30]

1.4 Tailor-Made FimH Antagonists

Based on the information obtained in structural biology studies, molecular modelling was employed to design tailor-made ligands of FimH and FimH antagonists. Synthesis and testing of these non-natural α-D-mannosides have only recently led to a revival of the idea of an antiadhesion therapy against microbial infection.[31] In the 1990s, Lindhorst *et al.* introduced the idea of using multivalent α-D-mannoside clusters to inhibit effectively type 1 fimbriae-mediated bacterial adhesion in a potential therapeutic context.[32] Later, molecular docking studies led to promising new monovalent mannosides as potent FimH antagonists.[33] Many groups have added various