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**P-19**

# Synthesis of Rhamnose-containing Oligosaccharides, and their Interaction with a Horseshoe Crab Plasma Lectin (HPL)

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## 1. Introduction

Appearance of pathogenic bacterial strains which are resistant to currently available antibiotics is a serious problem worldwide. These antibiotic-resistant organisms may cause life-threatening infection that is highly difficult to manage even with combinations of available methods. To solve this problem one possible approach may be the use of lectins. Lectins are carbohydrate-binding proteins which have high specificity for properly arranged sugar moieties. Lectins can recognize carbohydrates on the bacterial cell-wall, and this specific recognition may provide an alternative way for the detection and inhibition of pathogens.[1] A soluble and functional recombinant horseshoe crab plasma lectin (rHPL) possessing specific L-rhamnose-binding capability has recently been expressed in an *Escherichia coli* system.[2] It was shown that rHPL selectively bound to pathogens isolated from clinical samples, such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Streptococcus pneumoniae* serotypes. The binding was established to be a specific molecular interaction between rHPL and rhamnose moiety of pathogen-associated molecular patterns on the bacterial surface. Besides that, rHPL is capable to inhibit the growth of *P. aeruginosa* PAO1 in a concentration-dependent manner.[1,2] The results show that a specific protein-glycan interaction between rHPL and rhamnosyl moieties may facilitate the development of new diagnostic and therapeutic strategies for the treatment of microbial pathogens-associated diseases.

Therefore, our research group designed and performed the synthesis of several different rhamnose-containing derivatives as potential ligands of rHPL.[3-5]

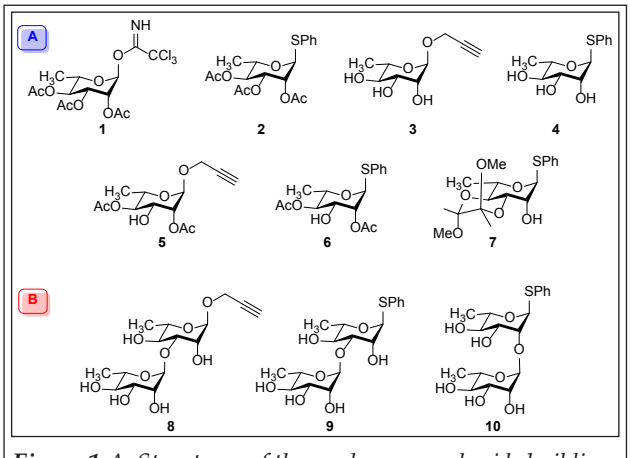


***Figure 1*** *A: Structures of the used monosaccharide building blocks B: The synthesized rhamnobiosides*

## 2. Results

Preliminary results suggested that rHPL could bind to rhamnose-containing components in the bacterial biofilm and interrupt biofilm development.

In order to produce oligosaccharides for further lectin binding studies the α-(1,2)- (**10**) and α-(1,3)-rhamnobioside derivatives (**8**, **9**) were synthesized by our research group.

The rhamnobiosides were synthesized by two different methods, namely open and conventional glycosylations. In most cases, stereo- and regioselective glycosylation reactions are performed by using properly protected glycosyl acceptors and donors. In contrast, in the case of open glycosylation an unprotected acceptor is used which may shorten the reaction pathway if sufficient selectivity can be achieved between the hydroxyl groups of the acceptor. For the production of the planned dirhamnosides two kinds of donor, one imidate (**1**) and one thioglycoside (**2**) and two kinds of acceptor, unprotected (**3** and **4**) and selectively protected ones (**5-7**), were applied. In each cases we

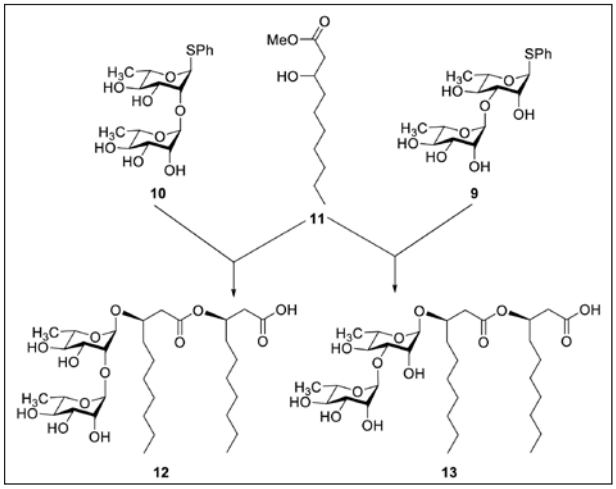


***Figure 2*** *The structure of the building blocks of the dirhamno-dilipids and the planned dirhamno-dilipids*

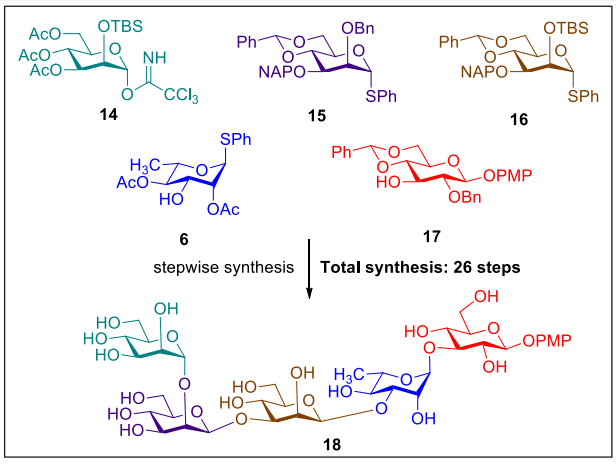


***Figure 3*** *Structure of the monosaccharide building blocks and the synthesized pentasaccharide*

successfully prepared the needed disaccharides (**8**, **9**) and (**10**) in moderate to high yields.[4]

We synthesized further derivatives in which the dirhamnosides were coupled with 3-hydroxydecanoic acid methyl ester (**11**). These are the so-called dirhamno-dilipids (**12**, **13**) which can be found in the bacterial biofilm layer and are also known to inhibit the biofilm formation of the bacterium through an interaction with the rHPL lectin.[6] For the syntheses of these derivatives the earlier prepared dirhamnosides (**9**) and (**10**) were used.

The pentasaccharide part of the psl exopolysaccharide of *Pseudomonas aeruginosa* consisting of three mannose units (**14**, **15**, **16**) one glucose (**17**) and one rhamnose (**6**) was also prepared, because it has also high affinity to bind to the rHPL lectin. First, the monosaccharide building blocks were prepared in properly protected forms. After the synthesis of the monosaccharide units the coupling reactions were performed in a linear synthesis method. As a result, we have successfully synthesized the desired pentasaccharide in a new and efficient pathway in 26 steps. Biological assays were performed after the preparation of the oligosaccharides. These results will be presented in detail.

## 3. Conclusions

We successfully synthesized several monosaccharide building blocks in properly protected form for the oligosaccharide synthesis. Three rhamnobiosides, two dirhamno-dilipids and the pentasaccharide part of the *Pseudomonas aeruginosa* psl exopolysaccharide were also synthesized in good yields and high efficiency. The prepared molecules then were subjected to ELISA assays in which the ability of the synthesized oligosaccharides to inhibit rHPL-*Pseudomonas aeruginosa* biofilm binding was measured. The results showed that the prepared rhamnobiosides (**8**, **9**, **10**) are good inhibitors and the (1-3)-linked disaccharides (**8**, **9**) are better, than the (1-2)-linked counterpart (**10**). Psl pentasaccharide (**18**) showed the strongest inhibitory effect among all synthesized oligosaccharide molecules and the dirhamno-lipids (**12**, **13**) were poor inhibitors.

## 4. Acknowledgements

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