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Synthesis of oligosaccharides to identify an immunologically active epitope against *Candida auris* infection†

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Candida auris (*C. auris*) is an emerging multidrug-resistant fungal pathogen that represents a significant public health challenge as it can spread rapidly and result in high mortality rates. The mannans on the *C. auris* cell surface are potent immunogens and attractive targets for developing a glycoconjugate vaccine. We synthesized the oligosaccharides resembling cell surface mannans of *C. auris* and printed them onto microarray slides that were used to screen plasma from mice infected with *C. auris*. IgM antibodies in mouse plasma recognize the β-1,2 linkage present in *C. auris* surface mannans. Disaccharide **19** emerged from glycan array screening as a lead for developing a vaccine against *C. auris*, as the majority of patient plasma samples showed antibodies against this glycan. The synthetic oligosaccharides can be used for the early detection of *C. auris* infections.

Introduction

Candida auris (*C. auris*) is a species of yeast that was first reported[1] in 2009 and is now on the World Health Organization (WHO) list as a "critical priority group" pathogen.[2] The fungus causes multidrug-resistant and potentially life-threatening infections that can result in sepsis, meningitis, and other serious conditions if left untreated. *C. auris* infections spread most commonly in healthcare settings from person to person through contact with contaminated surfaces or medical equipment.[3] *C. auris* infections are typically diagnosed by a combination of laboratory testing and medical history.[4] Treatment of *C. auris* infections is often difficult as resistance to anti-fungal drugs such as fluconazole, amphotericin, voriconazole and caspofungin is emerging.[5] Anti-fungal vaccines are urgently needed.

The outer membrane of the fungus contains a variety of proteins, including outer membrane proteins, lipoproteins, and glycoproteins. The polysaccharide-rich cell membrane is composed of chitin and β-glucans in the inner layer, and mannoprotein in the outer layer of cell wall.[6] *C. auris*

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Fig. 1 (A) Structure of *C. auris* mannans; (B) structure of synthetic mannans resembling the *C. auris* cell surface.
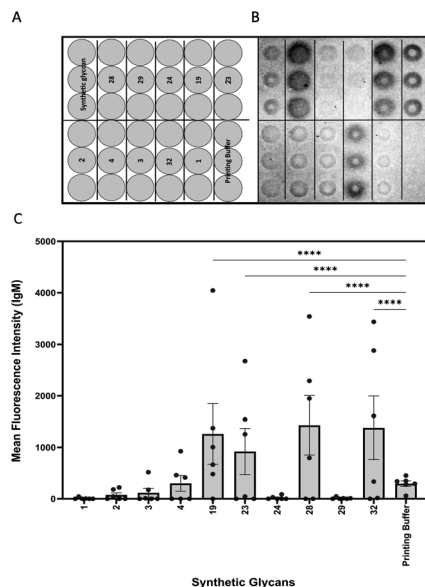
Fig. 2 Glycan microarray analysis of *Candida auris* mannans. (A) Printing pattern of glycan microarray. (B) Exemplary binding pattern of mice plasma to immobilized synthetic glycans. (C) Mean fluorescence intensity of IgM antibody binding to synthetic glycans. A plasma dilution of 1 : 50 was used. Values represent mean ± SEM. Differences were tested for significance using one-way ANOVA followed by Tukey's post hoc test with (****) $p < 0.0001$.

mannoproteins are composed of *O*- and *N*-linked mannans with α-1,2-, α-1,3-, α-1,6- and β-1,2-linked mannopyranose units as well as phosphodiester-linked mannopyranose residues (Fig. 1).[6–8] The cell membrane is important for protecting the fungus from environmental stress, providing a barrier to the entry of antimicrobial agents, and aiding in the adherence to surfaces. The cell membrane polysaccharides are recognized by pattern recognition receptors (PRRs) on the surface of the immune cell and in some cases may contribute to the pathogenicity of *C. auris* by allowing it to evade the host immune system.[9]

Isolated mixtures of *Candida* mannans and mannoproteins were explored as potential vaccine antigens in animal models of candidiasis two decades ago without follow-up.[10,11] Rather than using isolated mixtures of glycans, defined, synthetic antigens resembling cell surface mannans may constitute attractive vaccine candidates. As a first step in the vaccine design and development process, we report the synthesis of a series of conjugate-ready glycans related to the *C. auris* mannans (Fig. 1) that were used to screen plasma of infected mice for antibodies on glycan microarrays (Fig. 2) to identify a minimal epitope.[12]

Results and discussion

Glycan assembly is based on orthogonally protected building blocks. *H*-phosphonate building block **13** (Scheme 1), was
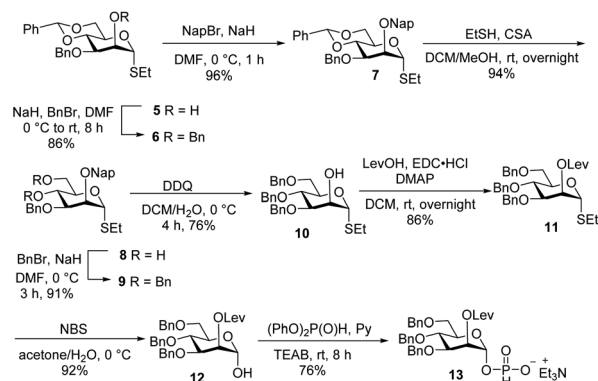
Scheme 1 Synthesis of *H*-phosphonate building block **13**.

prepared by the formation of a Nap ether at the C-2 hydroxyl of commercially available thioglycoside **5** to form **7** in 96% yield.[13] The benzylidene acetal in **7** was hydrolysed using ethanethiol and camphorsulfonic acid (CSA) to give dihydroxyl mannose thioglycoside **8**. Dibenzylation of **8** afforded fully protected thioglycoside **9** in 91% yield. Oxidative removal of naphthyl ether using DDQ afforded thioglycoside[14] **10** in 76% yield. Levulinoylation of the C2–OH in **10** using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl) and DMAP produced **11** in 86% yield.[15,16] Hydrolysis of thioglycoside **11** using *N*-bromosuccinimide (NBS) and subsequent treatment of hemiacetal[16] **11** with diphenyl phosphite provided *H*-phosphonate **13**.[17]

Union of D-thiomannoside[18] **15** and aminopentyl linker[19] **14** promoted by NIS/TfOH afforded desired α-linked glycoside **16** in 85% yield (Scheme 2). The linker is placed in anticipation of the conjugation to a carrier protein or a microarray surface. Benzoyl ester cleavage using sodium methoxide gave differentially protected monosaccharide **17**.[20] The synthesis of β-(1→2)-linked disaccharide mannan[21] **19** began with the activation of thioglycoside[22] **6** by addition of 1-benzene-sulfonyl piperidine (BSP), 2,4,6-tri-*tert*-butylpyrimidine (TTBP) and triflic anhydride (Tf₂O) at −60 °C followed by addition of acceptor **17** at −70 °C to get exclusively fully protected β-(1→2)-linked mannan **18**. The newly formed β-glycosidic bond was confirmed by coupled HSQC NMR. The global deprotection[23] of protected mannan **18** using Pd/C provided β-(1→2)-linked disaccharide **19** with 70% yield. Glycosylation of monosaccharide **17** with building block **15** using NIS/TfOH followed by cleavage of the benzoyl ester using sodium methoxide provided disaccharide **21**. Hydrogenation of disaccharide **21** using Pd/C yielded 64% of deprotected disaccharide **24**. For the synthesis of trisaccharide **23**, disaccharide acceptor **21** was glycosylated with building block **6** using BSP, TTBP, and triflic anhydride to afford β-(1→2)-linked trisaccharide **22** in 84% yield. Hydrogenation of trisaccharide **22** furnished deprotected trisaccharide **23** in 70% yield. Synthesis of tetrasaccharide **28** began with the union of disaccharide **21** with donor **15** using

Scheme 2 Synthesis of oligosaccharides mannans resembling the cell surface of *C. auris*.

NIS/TfOH, followed by debenzoylation using sodium methoxide to yield protected tetrasaccharide **26**. Trisaccharide **26** was glycosylated with thioglycoside donor **6** to produce protected tetrasaccharide **27** in 87% yield. Tetrasaccharide **27** was freed from all protective groups using Pd/C to yield tetrasaccharide **28**. Similarly, **26** was converted to trisaccharide **29** in 72% yield.

Oligosaccharides containing a phosphodiester linkage are essential for immunological studies aimed at identifying the minimal glycan epitope (Scheme 3). Glycosylation of the aminopentyl linker with **6** using BSP, TTBP and triflic anhydride provided exclusively β-linked, fully protected monosaccharide **30** in 84% yield (confirmed by coupled HSQC NMR). The benzylidene ring in monosaccharide **30** was opened using dichlorophenyl borane and TMSOTf to yield 79% of the 6-OH containing **31**. Coupling of *H*-phosphonate **13** and monosaccharide **31** using pivaloyl chloride as coupling reagent followed by iodine/H_2O assisted oxidation of the newly formed *H*-phosphonate diester, levulinoyl (Lev) ester cleavage using hydrazine acetate and hydrogenation provided phosphate diester disaccharide **1**. The stereochemistry of the newly formed linkage was confirmed by coupled HSQC NMR. For the synthesis of trisaccharide **2**, the benzylidene ring in **18** was opened to obtain disaccharide **33** in 86% yield. Coupling of *H*-phosphonate **13** with disaccharide **33**, followed by oxidation using iodine/H_2O and global

deprotection provided trisaccharide **2** containing a phosphodiester linkage in 27% yield over three steps. The synthesis of tetrasaccharide **3** started with benzylidene ring opening in trisaccharide **22** using dichlorophenyl borane and TMSOTf to obtain trisaccharide **34** in 92% yield. Reaction of *H*-phosphonate **13** with trisaccharide **34**, oxidation of the newly formed *H*-phosphonate diester followed by global deprotection provided tetrasaccharide **3**. Similarly, pentasaccharide **4**, containing a phosphodiester linkage, was synthesized from tetrasaccharide **27**.

Glycan microarrays are useful tools for screening plasma samples in order to identify minimal glycan epitopes.[24] Synthetic glycans **1–4**, **19**, **23**, **24**, **28**, **29** and **32** were immobilized in triplicates on glass slides to detect antibody binding to the glycans in plasma samples derived from mice three days after infection (Fig. 2).

IgM antibodies from infected mice specifically bound to structures **19**, **23**, **28** and **32**. All these synthetic glycans contain the β-(1→2)-linked mannan. Five of six animals produced antibodies to disaccharide **19** that emerged as a vaccine lead for incorporation into a glycoconjugate. No IgM antibodies were detected against glycans **1–4** possibly be due to shielding of the phosphodiester linker. Since antibodies against the synthetic glycans were detected already after three days of infection, oligosaccharides **19**, **23**, **28** and **32** serve for early detection of infection.

Scheme 3 Synthesis of oligosaccharides having phosphodiester linkage.

Conclusions

We synthesized a library of oligosaccharides resembling the cell surface mannans of *C. auris*. The challenging phosphodiester linkage was synthesized using *H*-phosphonate building block **13**. Glycan array analysis of plasma from mice infected with *C. auris* for three days were screened for antibodies to the synthetic glycans. Disaccharide **19** is recognized by IgM and appears to be a promising vaccine lead for further development of a glyco-conjugate vaccine candidate.

Data availability

Data supporting this article have been uploaded as ESI.†

Author contributions

P. H. S. conceived and supervised this project. R. K. S. synthe-sized all the target compounds. E. E. R. performed glycan microarray experiments. M. B. and M. G. N. provided plasma for glycan microarray experiments. R. K. S. and E. E. R. wrote the original draft of the manuscript which was edited by all authors.

Conflicts of interest

There are no conflicts to declare.

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