Synthesis of biotinylated pentasaccharide structurally related to a fragment of glucomannan from Candida utilis*

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The polysaccharide mannans are the main surface antigen of the cell wall of Candida fungi, playing an important role in the pathogenesis of diseases caused by these mycopathogens. Mannan has a complex, comb-like structure and includes a variety of structural units, with their combination varying depending on the Candida species and strain. Glucomannan, a polysaccharide from Candida utilis, contains terminal α-D-glucose residues attached to oligomannoside side chains. This paper describes the first synthesis of a pentasaccharide structurally related to C. utilis glucomannan fragment, which is an α-(1→2)-linked tetramannoside terminated at the non-reducing end by an α-D-glucopyranosyl residue. The pentasaccharide was obtained as a 3-aminopropyl glycoside, which made it possible to synthesize also its biotinylated derivative, suitable for various glycobiological studies. The most complicated step in the pentasaccharide synthesis was stereoselective 1,2-cis-glycosylation to attach the α-D-glucopyranosyl residue. This was accomplished using a glucosyl donor specially developed in our laboratory, the protecting groups of which provide the necessary α-stereoselectivity. The target biotinylated pentasaccharide thus obtained will be used in the future as a model antigen for the detection of immunodeterminant epitopes of Candida mannans.

Key words: Candida utilis, glucomannan, antigen, oligosaccharide, 1,2-cis-glycosylation.

The yeast-like fungus Candida is normally a part of natural microflora of humans; however, as a result of certain pathological processes, this microorganism can induce severe and difficult-to-cure surface (located on skin and mucous membranes) and invasive candidiasis.1,2 The invasive candidiasis is the most severe disease caused by Candida spp., which appears when the fungal cells penetrate into the bloodstream and spread from the focus of infection throughout the body.2 The invasive candidiasis affects approximately 750,000 people annually all over the world and has a high lethality of up to 30—55%.3 In Russia, more than 11,000 cases of this disease are recorded every year.4 In 2020, due to the spread of the new coronavirus infection SARS-CoV-2, cases of COVID-19-associated invasive candidiasis started to be recorded and systematized.5 Currently, the development of acute respiratory distress syndrome and stay in the intensive care units are regarded as the main risk factors for the development of invasive candidiasis associated with COVID-19.5

Currently, about 17 Candida species capable of causing the disease in humans are known, with more than 90% of all systemic candidiasis being caused by C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, and C. krusei. However, in recent years, increased morbidity and mortality due to rare Candida species have been observed. There are serious concerns about infectious agents such as C. auris, C. utilis, C. lipolytica, C. catenulata, and so on, which cause stubborn infections, especially in hospitalized patients with significant comorbid conditions.6,7

Mannan is the main surface polysaccharide component of the Candida cell wall.8 This polysaccharide plays a key role as an antigen in the recognition of this pathogen by the immune system.8,9 Normally, the interaction of immunologically active mannan fragments with pathogen-associated molecular pattern recognition receptors (DC-SIGN, Dectin-2, CD206, etc.) results in activation of immune cells and pathogen neutralization.8,10 In addition, mannan is a convenient diagnostic marker for detection of invasive or another candidiasis in patients without specific symptoms.10

The most well-known commercial enzyme-linked immunosorbent assay used to determine mannan circulating in the blood is PLATELIA™ Candida Ag PLUS (Bio-Rad Laboratories, France).11,12 This assay kit uses a specific monoclonal antibody, which recognizes the oligo-α-
(1→2)-D-mannoside sequence terminated by one or two β-(1→2)-linked D-mannoside units, as shown in our recent study using a series of synthetic models.\textsuperscript{14}

From the chemical standpoint, mannan is the carbohydrate part of the mannoprotein with a comb-like structure. Mannan structure is formed by the α-(1→6)-linked backbone, in which some mannose residues bear relatively short linear and branched oligomannoside side chains attached via α-(1→2)-bonds.\textsuperscript{8,15} They can be composed of α-(1→2)-, α-(1→3)-, α-(1→6)-, and β-(1→2)-linked mannose residues and significantly differ in the size, chemical structure, and antigen properties (Fig. 1, a).\textsuperscript{8} The mannan structure varies depending on the Candida species and strain. For example, mannan from \textit{C. utilis} contains a unique structural unit: mannose side chains terminated by α-D-glucosyl residue (Fig. 1, b).\textsuperscript{16} Due to the presence of this residue, this polysaccharide is called glucomannan.

Natural polysaccharides are too complex and heterogeneous to be used for analysis of immunobiological properties of mannans. Furthermore, the result of biotechnological growth of fungal mass and subsequent isolation of polysaccharides is poorly controllable and poorly reproducible.\textsuperscript{17} Therefore, synthetic oligosaccharides related to the key fragments of this polysaccharide are an indispensable tool for many interdisciplinary studies of natural polysaccharide antigens.\textsuperscript{18,19} Oligosaccharides form the basis for the development of vaccines and diagnostic kits and the search for immunodeterminant fragments of fungal cell wall polysaccharides\textsuperscript{14,20–25} and are valuable models for the structural studies of natural fungal polysaccharides.\textsuperscript{19,26–29}

In order to elucidate the immunological role of the glucomannan chains terminated by the α-D-glucosyl residue, we prepared spacered pentasaccharide \textit{I} consist-
The key steps of the synthesis of spacered pentasaccharide 1 include the stereoselective formation of four glycosidic bonds. In the case of mannosyl moieties, the formation of \( \alpha \)-glycosidic bond is a relatively simple task. The stereoselective formation of the 1,2-\( \alpha \)-mannosidic bond can easily be attained by using the stereocontrolling effect of the protecting group at O(2). However, \( \alpha \)-glucosylation requires thorough selection of protecting groups and reaction conditions.\(^{30-32}\) As the starting compound, we took trimannoside 3 (Scheme 1), prepared previously in our laboratory.\(^{33}\) Glycosylation of this derivative by trichloroacetimidate mannosyl donor 4 afforded tetramannosyl acceptor 6. The \(^1\)H NMR spectrum of derivative 6 does not show a signal for the acetyl group, which attests to the presence of a free hydroxy group at C(2D).

The selective glucosylation of compound 6 was accomplished by using glucosyl donor 7, synthesized recently in our laboratory, at the final step of assembly of the penta-saccharide chain.\(^{35}\) The use of compound 7 containing stereocontrolling protecting groups at O(3) (levulinoyl (Lev) group) and O(6) (\( \text{tert} \)-butyldiphenylsilyl (TBDPS) group) allows the stereospecific construction of the 1,2-\( \beta \)-glucosidic bond. As expected, the reaction of glucosyl donor 7 with tetramannosyl acceptor 6 in the presence of \( \text{tert} \)-butyldimethylsilyl triflate (TBSOTf) resulted in the stereospecific formation of the \( \alpha \)-(1\( \rightarrow \)2)-glucosidic bond, thus giving protected pentasaccharide 8 in 87% yield. The \( \alpha \)-configuration of the glucosidic bond was confirmed by the characteristic value of the spin—spin coupling constant \(^{3}J_{H(1),H(2)} = 3.5\) Hz between the H(1) and H(2) protons of the glucose residue.

Treatment of compound 8 with hydrazine hydrate to remove the levulinoyl protection and the subsequent removal of \( \text{tert} \)-butyldiphenylsilyl group on treatment with tetrabutyllummonium fluoride resulted in partially deprotected pentasaccharide 9. The benzyl and N-trifluoroacetyl deprotection of derivative 9 via successive palladium-catalyzed hydrogenolysis and alkaline treatment gave the target pentasaccharide 1. The \( \alpha \)-configuration of the four mannosyl residues in oligosaccharide 1 unambiguously followed from the spin—spin coupling constants between the H(1) protons and the C(1) atoms: \(^{1}J_{C(1),H(1)} = 174\) Hz; the \( \alpha \)-glucosidic bond configuration was confirmed (as in the case of protected derivative 8) by the characteristic spin—spin coupling constant \(^{3}J_{H(1),H(2)} = 3.5\) Hz. Biotinylation of compound 1 by treatment with activated ester of biotin derivative 36 in the presence of triethylamine gave the desired product 2 (see Scheme 1). The NMR spectra of the products (see Experimental) were fully in line with their structures.

The synthetic moieties of polysaccharides that are poorly accessible from natural sources are popular models for various glycobiological studies, which stimulates search for methods of the targeted chemical synthesis of these products.\(^{37}\) Compounds 1 and 2 obtained in this study will be used, together with other oligosaccharides related to Candida mannans, as model antigens to determine the immunological roles of certain structural fragments of cell wall polysaccharides of this group of pathogens and also as models for the calculation of spectral (NMR) effects of glycosylation in the branched oligosaccharide fragment shown in Fig. 1, b. The results of these studies will be published elsewhere.
Experimental

All reactions were carried out in solvents purified by standard procedures. The glycosylation was conducted in anhydrous solvent. The molecular sieves were activated before the reaction at 180 °C for 2 h in an oil pump vacuum. Thin layer chromatography was carried out on Kieselgel 60 F254 silica gel plates (Merck); the spots of compounds were visualized in the UV light or by spraying the plates with an orcinol solution (180 mg of orcinol in a mixture of 85 mL of water, 10 mL of orthophosphoric acid, and 5 mL of ethanol) followed by heating at ~150 °C. Column chromatography was carried out on Silica gel 60 (40—63 μm, Merck), and gel chromatography of free oligosaccharides was performed on a column with the TSK HW-40(S) gel (1.5×90 cm) in 0.1 M acetic acid; the eluate was analyzed using a Knauer K-2401 flow refractometer. Optical rotation was measured on a JASCO P-2000 polarimeter at 18—22 °C in the indicated solvents. NMR spectra were recorded at 25 °C on Bruker Avance 600, Bruker AM 300, and Bruker AV 400 instruments. The spectra of the protected derivatives were measured in CDCl3; the spectra of unprotected oligosaccharides were recorded in D2O; acetone was used as the internal standard (δH 2.225, δC 31.45). The signals were assigned using COSY and HSQC 2D NMR correlation procedures. In the description of the NMR spectra, the monosaccharide residues are designated by letters A, B, C, D, and E (see Fig. 2), starting from the reducing end of the oligosaccharide.

High-resolution electrospray ionization (ESI) mass spectra were measured on a Bruker microOTOF II instrument.

Reagents, conditions, and yields: a. TMSOTf, CH2Cl2, 0 °C; b. MeONa, CH2Cl2—MeOH, 20 °C, 83% (over two steps); c. TBSOTf, CH2Cl2, molecular sieves AW300, −35 °C, 87%; d. NH2NH2, pyridine—AcOH, 20 °C; e. TBAF, THF, 60 °C, 76% (over two steps); f. H2, Pd(OH)2/C, EtOAc—MeOH, 20 °C; g. NaOH, MeOH—H2O, 20 °C, 50% (over two steps); h. Et3N, DMF, 20 °C, 75%.

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Scheme 1

![Scheme 1](image-url)
(m, 60 H, 12 Ph). 13C NMR (150 MHz, CDCl3), δ: 28.0 (OCH2CH2CH2N), 37.9 (OCH2CH2CH2N), 65.7 (OCH2CH2CH2N), 68.5 (C(2D)), 68.8, 69.4, 69.5, and 70.0 (C(6A,B,C,D)), 71.6, 71.7, 71.8, 71.9, 72.0, 72.1, 73.1, 73.2, 73.3, 74.3, 74.7, 74.8, 74.9, 75.0, 75.1, 75.7, and 76.1 (C(2A,B,C,D)), 79.8, 79.3, 79.7, and 80.0 (C(1A,B,C,D), C(3A,B,C,D)), 99.0 (C(1A)), 100.9(2) and 101.4 (C(1B,C,D)), 127.3, 127.4, 127.6, 127.7, 128.1, 128.3, 128.4, 128.5, 129.6, 129.7, 133.2, 133.6, 135.9, 137.9, 138.1, 138.2, 138.3, 138.4, 138.5, 138.6 (12 Ph). MS, found: m/z 1922.8164 [M + Na]+; calculated for C174H172F3NNaO34S: 1922.8152.

3-Trifluoroacetamidopropyl 2,4-di-O-benzyl-6-O-tert-butylphenethyl-3-O-leuvinolyl-α-α-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-α-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-α-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-α-mannopyranoside (8). tert-Butyl dimethyl sulfoxide (3.4 mL, 0.015 mol, 18 mol%) was added at -35 °C to a stirred mixture of N-phenyltrifluoracetamide imidate 75S (70 mg, 0.081 mmol, 1.9 equiv.), monohydroxy saccharide 3,28.3 (MeCOCH2CH2CO), 29.9 (MeCOCH2CH2CO), 26.9 (MeCOCH2CH2CO), 28.1 (OCH2CH2CH2N), 74.3, 74.7, 74.8, 74.9, 75.0, 75.1, 75.7, and 76.1 (C(2 A,B,C,D), C(3 A,B,C,D)), 97.4 (C(1 A)), 100.6 (2) and 101.6 (C(1 B,C,D)), 127.3, 127.4, 127.6, 127.7, 128.1, 128.3, 128.4, 128.5, 129.6, 129.7, 133.2, 133.6, 135.9, 137.9, 138.1, 138.2, 138.3, 138.4, 138.5, 138.6 (12 Ph). MS, found: m/z 1922.8164 [M + Na]+; calculated for C174H172F3NNaO34S: 1922.8152.

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This paper does not contain descriptions of studies on animals or humans.

The authors declare no competing interests.

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