Conference paper

Vadim B. Krylov, Lucia Paulovičová, Ema Paulovičová, Yury E. Tsvetkov and Nikolay E. Nifantiev*

Recent advances in the synthesis of fungal antigenic oligosaccharides

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Abstract: The driving force for the constant improvement and development of new synthetic methodologies in carbohydrate chemistry is a growing demand for biologically important oligosaccharide ligands and neoglycoconjugates thereof for numerous biochemical investigations such as cell-*to*-pathogen interactions, immune response, cell adhesion, etc. Here we report our syntheses of the spacer-armed antigenic oligosaccharides related to three groups of the polysaccharides of the fungal cell-wall including α- and β-mannan, α- and β-glucan and galactomannan chains, which include new rationally designed synthetic blocks, efficient solutions for the stereoselective construction of glycoside bonds, and novel strategy for preparation of furanoside-containing oligosaccharides based on recently discovered pyranoside-*into*-furanoside (PIF) rearrangement.

Keywords: fungal antigens; fungal cell wall; ICS-28; oligosaccharide; pyranoside-*into*-furanoside rearrangement.

Introduction

Natural oligosaccharides and glycoconjugates play a crucial role acting as lectin receptors in different processes determining cell life cycle, cell-*to*-pathogen interactions and infection, cell adhesion and others. This makes spacer-armed oligosaccharide ligands and neoglycoconjugates thereof (i.e. molecular probes in which an oligosaccharide is attached via a spacer to a label or carrier) indispensable tools for the research of the processes which are determined by carbohydrate-lectin interactions to investigate and assess the structural features responsible for specific recognition of carbohydrate ligands in such processes, define ligand-receptor binding topology, and understand the biological functions and mechanisms of action of the corresponding natural glycoconjugates [1, 2].

Recent studies of the mechanisms of cell recognition have revealed a key role of various natural glycoconjugates. Among them, the carbohydrate antigens of fungal and bacterial pathogens are remained to be investigated. Their study is often complicated or even impossible because of very low availability of oligosaccharide of these groups from natural objects that additionally stimulates the development of efficient

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***Corresponding author: Nikolay E. Nifantiev,** Laboratory of Glycoconjugate Chemistry, N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky Prospect 47, 119991 Moscow, Russia, e-mail: nen@ioc.ac.ru; nifantiev@gmail.com

Vadim B. Krylov and Yury E. Tsvetkov: Laboratory of Glycoconjugate Chemistry, N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky Prospect 47, 119991 Moscow, Russia

Lucia Paulovičová and Ema Paulovičová: Department of Immunochemistry of Glycoconjugates, Center for Glycomics, Institute of Chemistry, Slovakia Slovak Academy of Sciences, Dubravská cesta 9, 84538 Bratislava, Slovakia

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chemical schemes for their preparation in practical amounts. In this communication, we provide an overview of the schemes which were recently developed by us for the syntheses of linear and branched oligosaccharides related to the polysaccharides of fungal cell wall including α- and β-mannans, α- and β-glucans and galactomannan. These developments include new rationally designed synthetic blocks, efficient solutions for the stereoselective construction of glycoside bonds, and novel strategy for preparation of furanoside containing oligosaccharides based on recently discovered pyranoside-*into*-furanoside (PIF) rearrangement.

Synthesis of linear and branched oligosaccharides structurally related to the mannan of the *Candida* cell-wall

The yeast *Candida albicans* is an opportunistic pathogenic microorganism. It is a component of the normal microflora of the majority of healthy individuals, but is able to cause severe infections in immunocompromised patients such as HIV patients and those undergoing immunosuppressive therapy or prolonged treatment with antibiotics [3, 4]. The cell surface of *C. albicans* first comes into contact with host cells, plays the main role in the adhesion of the pathogen, intercellular communication, and carries important antigenic determinants of the fungus [5, 6]. The main surface antigen of *C. albicans* is mannan which represents the carbohydrate part of the cell wall mannoprotein. This polysaccharide has a comb-like structure with an α-(1→6)-linked main chain, to which relatively short oligomannoside side chains built of α-(1→2)-, α-(1→3)-, α-(1→6)- and β-(1→2)-linked mannose units are attached via α-(1→2)-linkages. These side chains are responsible for the antigenic specificity of *Candida* species. The generalized structure of the mannan and structures of epitopes corresponding to the various antigenic factors [7] of *Candida* are shown in Fig. 1.

We synthesized in the past decade a wide range of oligosaccharides corresponding to the antigenic factors 1, 4, 6, 9 and 34. The synthesis of the linear α-(1→2)-tetramannoside **7** related to the antigenic factor 1 is depicted in Fig. 2 [8]. It was based on the stepwise chain elongation by one monosaccharide unit. Thiomannoside **1** [9] was used as the glycosyl donor. On the one hand, the presence of a 2-*O*-acetyl group in **1** was sought to ensure α-stereoselectivity of mannosylation due to the anchimeric participation. On the other hand, this group could be easily removed to produce a glycosyl acceptor for the next glycosylation. Two initial cycles of mannosylation and deacetylation proceeded smoothly and provided corresponding glycosyl acceptors **2** and **3** in good yields. However, when thioglycoside **1** was coupled with **3**, the predominant formation of the product of β-mannosylation (42 % vs. 28 % of the α-product) was unexpectedly observed. Application of 2-*O*-benzoylated donor **4** instead of **1** strongly enhanced α-stereoselectivity of mannosylation and provided the α-linked trisaccharide in 64 % yield. Final glycosylation of trisaccharide acceptor **5** with

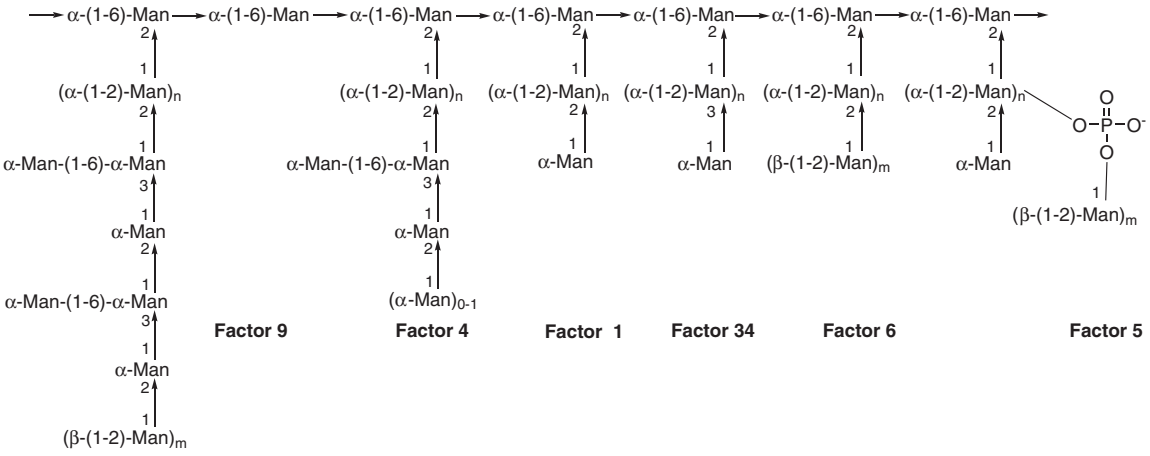

Fig. 1: Structure of the mannan of the *Candida* cell wall.

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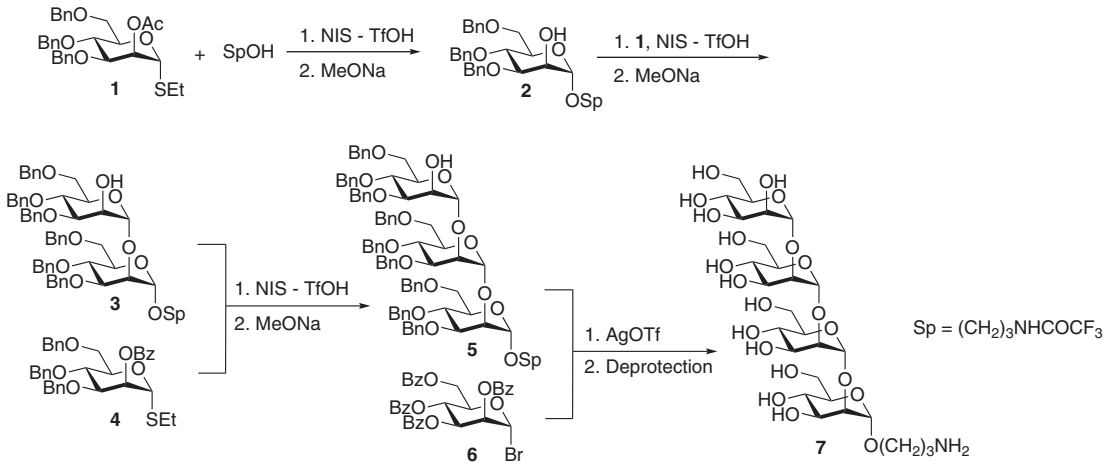

Fig. 2: Synthesis of the linear α-(1→2)-tetramannoside related to the antigenic factor 1.

mannosyl bromide **6** gave the expected tetrasaccharide that was subjected to deprotection to afford free tetrasaccaride **7**.

Unlike the synthesis of **7**, other oligomannosides described in this section were prepared using a convergent blockwise approach. Thus, [2+2] glycosylation was applied for the assembly of a tetrasaccharide **10** with the terminal α-(1→3)-linked mannose unit corresponding to the antigenic factor 34 (Fig. 3) [8]. Used α-(1→3)-linked disaccharide donor block **9** was obtained by AgOTf-promoted glycosylation of thiomannoside **8** with mannosyl bromide **6**. Subsequent coupling of **9** with dimannoside **3** and removal of the protecting groups from the resulting tetrasaccharide provided the target product **10**.

Linear heptasaccharide **21** built of alternating α-(1→2)- and α-(1→3)-mannose residues with a terminal β-(1→2)-mannose unit represented the antigenic factor 9 devoid of α-(1→6)-linked side mannose residues. The latter was synthesized using [3+4] glycosylation in the final step of the oligosaccharide chain assembly (Fig. 4) [10].

At first, the donor and acceptor blocks **15** and **19** were prepared. β-Mannosylation of allyl glycoside **12** [11] with thioglycoside **11** [12] under Crich's conditions [13] afforded β-disaccharide **13** in 69 % yield. Replacement of the 4,6-*O*-benzylidene group by benzoates and transformation of the allyl glycoside into trichloroacetimidate provided glycosyl donor **14** that was coupled with acceptor **8** to furnish trisaccharide donor block **15**. To prepare acceptor block **19**, acceptor **8** was glycosylated with mannosyl chloride **16** [14] with the formation of disaccharide **17**. The acetyl group in **17** was replaced by a chloroacetyl one to give donor **18** as the removal of the acetyl group by acidic methanolysis from the corresponding tetrasaccharide obtained by glycosylation of **3** with **17** was sluggish and produced acceptor **19** in a low yield. Condensation of **18** with **3** followed by removal of the chloroacetyl group from the glycosylation product with thiourea provided necessary acceptor **19**. Final glycosylation of **19** with **15** afforded heptasaccharide **20**; subsequent deprotection of **20** gave free oligomannoside **21**.

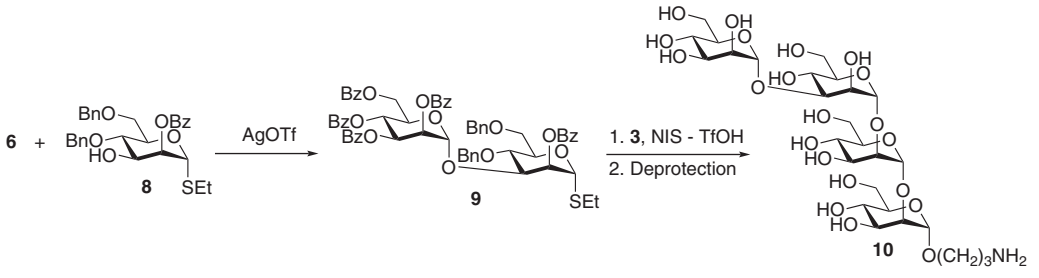

Fig. 3: Synthesis of the linear tetramannoside related to the antigenic factor 34.

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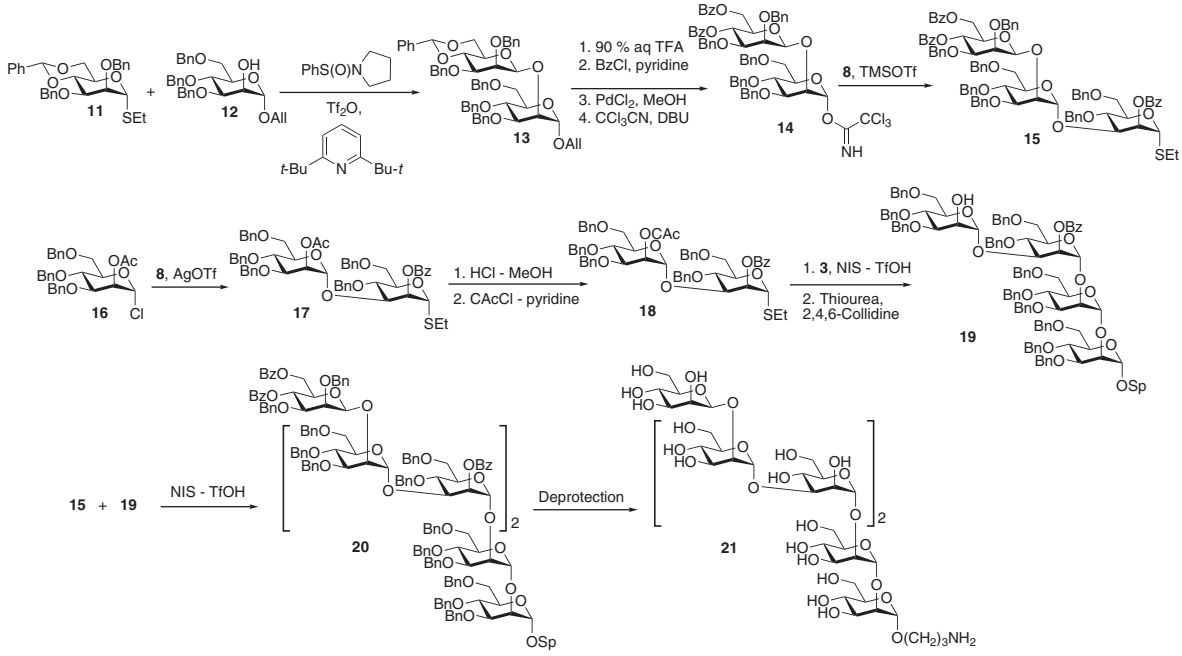

Fig. 4: Synthesis of the linear heptamannoside related to the antigenic factor 9.

3,6-Branched oligomannosides related to the antigenic factor 4 were also synthesized with the use of the blockwise approach. Pentasaccharide **25** was obtained by [3+2] glycosylation (Fig. 5) [15]. Branched trisaccharide glycosyl donor **23** was prepared by the Bredereck glycosylation [16] of 3,6-dityryl ether **22** with mannosyl bromide **6** in the presence of AgOTf. Further coupling of **23** with disaccharide acceptor **3** afforded pentasaccharide **24** that was subjected to deprotection with the formation of free product **25.**

Branched hexasaccharide **32** was assembled from disaccharide blocks **3**, **27** and **31** (Fig. 6) [15]. Monosaccharide precursor **26** of the mannose residue in the branching point contained different protecting groups at O-3 and O-6, thus providing an opportunity to attach different glycosyl substituents to these positions. The Bredereck glycosylation of **26** with mannosyl bromide **6** afforded disaccharide donor **27**. Its coupling with acceptor **3** followed by acidic removal of the silyl protection produced tetrasaccharide acceptor **28**. The third disaccharide block **31** was obtained in 60 % yield by glycosylation of thiomannoside **30** [17] with trichloroacetimidate **29** [18]. It is noteworthy that AgOTf-promoted glycosylation of **30** with bromide **6** provided disaccharide **31** in a much lower yield (~20 %) due to the predominant transfer of the EtS group from acceptor **30** to donor **6**. Since donor **31** contained a nonparticipating glycosyl substituent at O-2 of the glycosylating mannose residue, its reaction with **28** was not stereospecific and afforded the necessary α-hexasaccharide in a moderate yield (44 %) along with the corresponding β-anomer (14 %). Removal of protecting groups from the α-product gave hexasaccharide **32.**

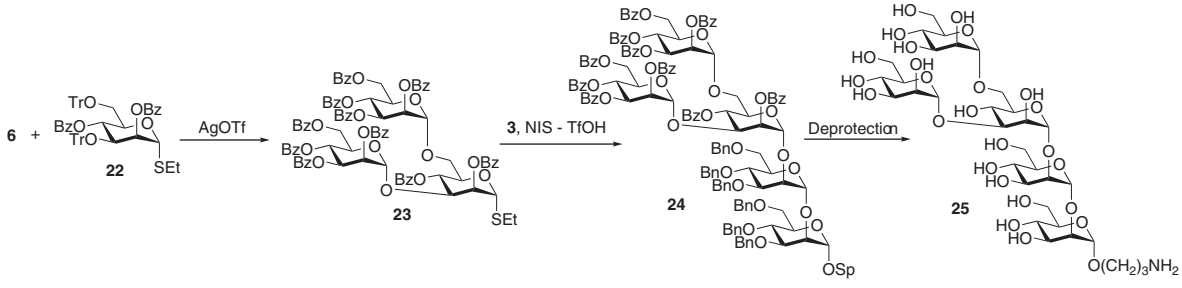

Fig. 5: Synthesis of the branched pentamannoside related to the antigenic factor 4.

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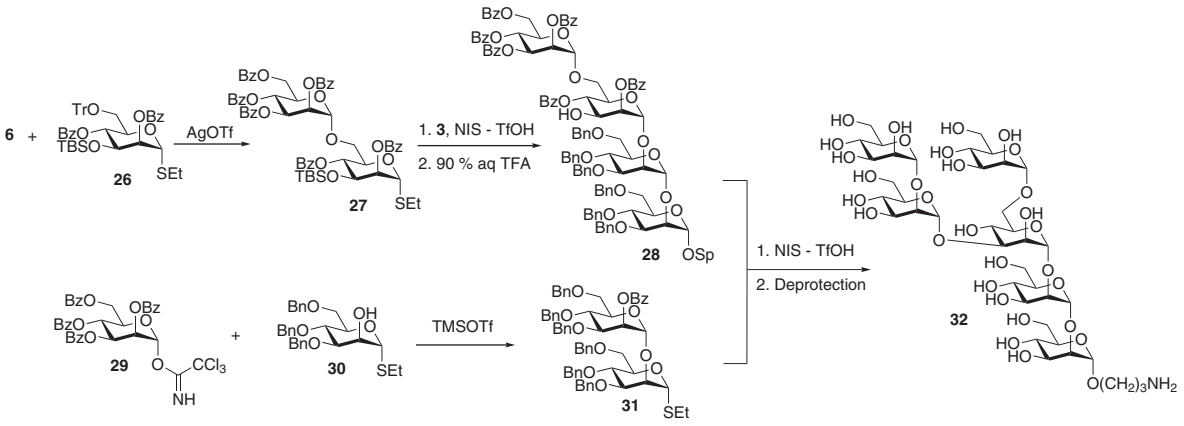

Fig. 6: Synthesis of the branched hexamannoside related to the antigenic factor 4.

We have also synthesized a series of oligosaccharides, in which β-(1 → 2)-mannoside blocks comprising 1–4 monosaccharide residues are attached via a glycoside bond to O-2 of the terminal unit of an α-(1 → 2)-oligomannoside chain. This type of β-(1 → 2)-oligomannosides is referred to as acid-stable mannan and corresponds to the antigenic factor 6. β-Mannosylation is one of the most difficult cases of glycosylation reactions [19]. Two general approaches to β-mannosylation are known to date. The first one is indirect and based on initial β-glucosylation followed by inversion of the configuration at C-2 in the glucose residue [20]. The second one is based on direct β-mannosylation with conformationally rigid 4,6-*O*-benzylidene-protected mannosyl donors [21]. In contrast to the first approach that allows the chain elongation to be performed by only one monosaccharide unit, more efficient blockwise assembly of (1 → 2)-oligomannoside chains [22, 23] is possible in the case of direct β-mannosylation. There is also a single example [24] of the preparation of β-(1 → 2)-oligomannosides using the intramolecular aglycon delivery (for a review see [25]).

Our synthesis was based on direct β-mannosylation with donor blocks containing up to four mannose residues for the assembly of the target oligosaccharides. One of our aims was to ascertain, whether the donor blocks larger than the reported dimannosides [22, 23] can successfully be applied to the introduction of long β-(1 → 2)-oligomannoside sequences into an oligosaccharide chain. The synthesis of the oligomannoside donors is outlined in Fig. 7 [26, 27]. Disaccharide thioglycoside **33** was used as the starting material. Its oxidation with *m*CPBA led to sulfoxide **34**, and removal of the PMP group provided disaccharide acceptor **35**.

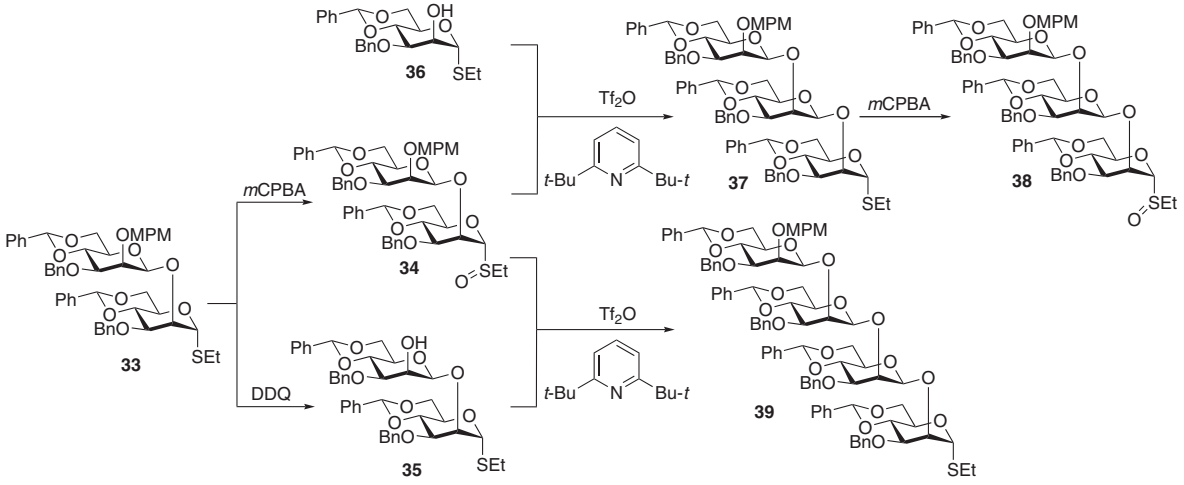

Fig. 7: Synthesis of oligomannosyl donors for the assembly of β-(1 → 2)-oligomannosides.

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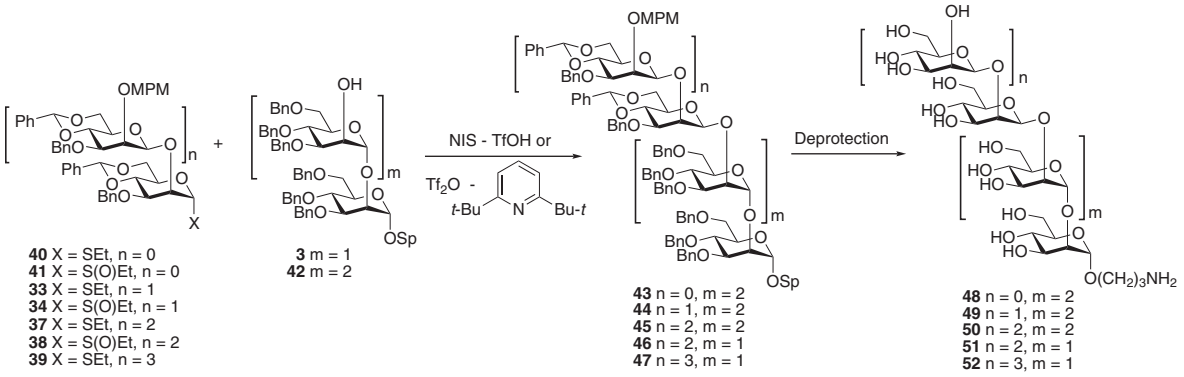

Fig. 8: Synthesis of oligomannosides related to the antigenic factor 6.

β-Mannosylation of acceptors **36** [28] and **35** with sulfoxide **34** under preactivation conditions [21] produced tri- and tetramannoside donors **37** and **39**, respectively. Oxidation of **37** gave sulfoxide **38**.

The set of available β-(1→2)-oligomannoside donors **33**, **34**, and **37–39** together with known monosaccharide donors **40** and **41** [29] allowed the synthesis of oligomannosides comprising from one to four β-mannosyl units (Fig. 8). Besides, having three pairs of thioglycoside and sulfoxide donors, we could study the influence of the donor type and activation conditions on the efficiency of β-mannosylation. Published data concerning these issues are contradictory [19, 21]. Within the pair of the monosaccharide donors, NIS–TfOH-promoted mannosylation of acceptor **42** with thioglycoside **40** without preactivation [21] provided a higher yield (37 %) of tetrasaccharide **43** than that with sulfoxide **41** under preactivation conditions (17 %). A similar result was obtained in the case of dimannoside donors: glycosylation of **42** with thioglycoside **33** without preactivation gave 65 % of pentasaccharide **44**, while sulfoxide **34** with preactivation provided a lower yield of **44** (37 %). On the contrary, only glycosylation of **42** with trisaccharide sulfoxide **38** under preactivation conditions gave hexasacchride **45** in a modest yield (27 %), while no formation of **45** was observed upon glycosylation with thioglycoside **37**. Finally, NIS–TfOH-promoted glycosylation of acceptor **3** with tri- and tetrasacchride thioglycosides **37** and **39** afforded oligosaccharides **46** and **47** in yields of 41 and 53 %, respectively.

Two conclusions may be drawn from these results: first, the donor blocks larger than dimannosides can be applied for the blockwise synthesis of oligosaccharides comprising long β-(1→2)-oligomannoside sequences; second, the efficiency of β-mannosylation with mono- and oligomannoside donors seems to depend more on the structure of the reaction partners than on the applied activation protocol (i.e. with or without preactivation of the donor). Removal of the protecting groups from compounds **43–47** furnished free oligomannosides **48–52**.

Some of the oligomannosides described in this section were conjugated to BSA [8, 10, 15] using a squarate procedure [30]. Immunomodulatory properties of these glycoconjugates have extensively been studied [31–35] as well.

Synthesis of linear and branched oligosaccharides structurally related to fungal β-glucan

β-(1→3)-Glucan is the major structural polysaccharide of the fungal cell wall, constituting approximately 50–60 % of the wall by dry weight [36]. The presence of this highly conserved glycopolymer in different pathogenic fungal species makes it a rational target for vaccine development. Conjugates of either natural β-(1→3)-glucan, laminarin [37], or linear synthetic β-(1→3)-oligoglucosides [38, 39] with carrier proteins were shown to be immunogenic and protective in mice against infections induced by *Candida albicans* and

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Aspergillus fumigatus. This opens a very good prospect to develop an efficient vaccine against both these dangerous pathogens because of the presence of β-(1 → 3)-glucan as the component of their cell wall. Important biological properties of β-(1 → 3)-glucans stimulated considerable activity in the chemical synthesis of β-(1 → 3)-oligoglucosides that was summarized by us in a recent review [40]. The most of published syntheses of β-(1 → 3)-oligoglucosides were based on application of glycosyl acceptors protected with 4,6-*O*-benzylidene and 2-*O*-acyl groups. However, the use of regioselective 3-*O*-glycosylation of 4,6-*O*-benzylidene-protected glycosyl acceptors with a free 2-OH group [41] seems to be more convenient. In our synthesis, we employed regioselective 3-*O*-glycosylation of 4,6-*O*-benzylidene-protected 2,3-diol glycosyl acceptors by a disaccharide donor block for the chain elongation. The synthesis of the donor block and a trisaccharide acceptor is presented in Fig. 9.

Glycosylation of 2,3-diol **54** [42] with trichloroacetimidate **53** [43] displayed good regioselectivity providing the corresponding β-(1 → 3)-disaccharide in 55 % yield along with its β-(1 → 2)-linked isomer (8 %), which were easily separated by silica gel column chromatography. Subsequent conventional benzoylation of free 2-OH gave disaccharide thioglycoside **55**. Similar regioselective glycosylation of spacer-armed 2,3-diol **56** [44] with **55** followed by benzoylation of the remaining 2-OH group resulted in the formation of trisaccharide **57**. Selective removal of the acetyl groups in the presence of benzoates was achieved by brief treatment of **57** with a large excess (50 equiv.) of hydrazine hydrate; as a result, acceptor **58** was obtained in 79 % yield. However, for the further chain elongation, we decided to use a glycosyl donor containing chloroacetyl groups as temporary protections of 2,3-diol to ensure its more reliable and efficient liberation for the next glycosylation step. To this aim, the acetyl groups in **55** were removed with hydrazine hydrate and the diol formed was chloroacetylated to furnish donor **59**. The assembly of a series of β-(1 → 3)-oligoglucosides comprising the odd number of monosaccharide units from donor **59** and acceptor **58** is presented in Fig. 10.

NIS–TfOH-promoted glycosylation of **58** with **59** was completely regioselective and produced β-(1 → 3)-linked pentasaccharide **60** in 91 % yield. The hydroxyl group in the former acceptor glucose residue was acetylated, and then the chloroacetyl groups were selectively removed to provide a new acceptor. Its glycosylation with **59** afforded heptasaccharide **61**. Iteration of acetylation, dechloroacetylation and glycosylation afforded in sequence nonasaccharide **62**, undecasaccharide **63** and tridecasaccharide **64**. Transformation of the protected oligomers into free 3-aminopropyl glycosides **65**–**70** included successive acidic removal of the benzylidene groups, basic deacylation and hydrogenolysis of the *N*-benzyloxycarbonyl group.

We have also synthesized two branched hexaglucosides in which a single β-(1 → 6)-glucose residue is attached either to the first or to the central glucose unit of a linear β-(1 → 3)-pentaglucoside core. The synthesis was based on the initial assembly of a linear β-(1 → 3)-pentaglucoside that bore a selectively removable 4,6-*O*-(*p*-methoxybenzylidene) group in the glucose residue subsequently forming the branching point (Figs. 11 and 12) [45]. Many of the mono- and oligosaccharide synthetic block used in the above synthesis of linear oligoglucosides were also employed for the preparation of the branched oligosaccharides.

Selective TMSOTf-promoted glycosylation of diol **56** with trichloroacetimidate **53** afforded disaccharide **71** that was subjected to benzoylation and selective deacetylation to give disaccharide diol **72**. The glucose block with the 4,6-*O*-(*p*-methoxybenzylidene) group was introduced by glycosylation of diol **72** with imidate

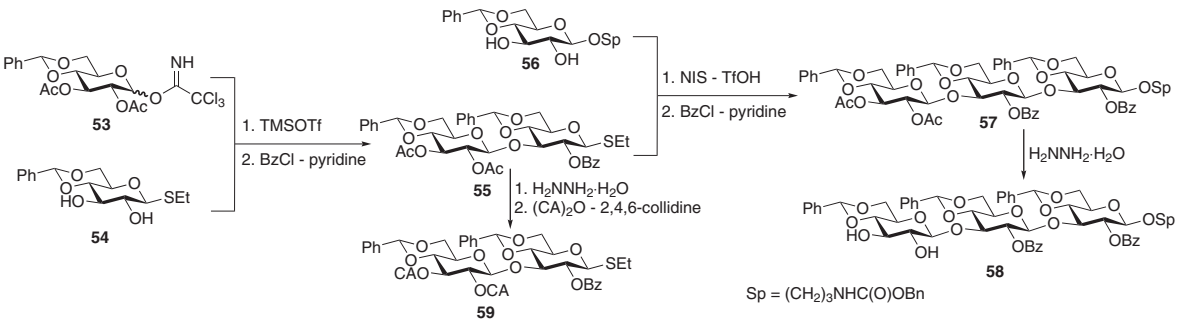

Fig. 9: Synthesis of the disaccharide glycosyl donor and the trisaccharide glycosyl acceptor.

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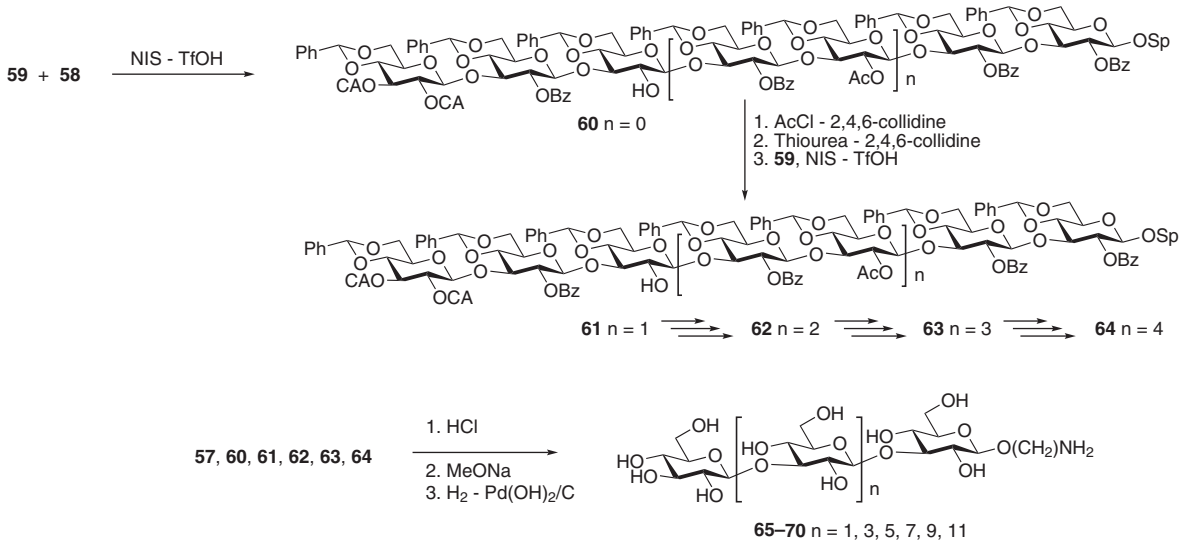

Fig. 10: Assembly of linear β-(1 → 3)-oligoglucosides.

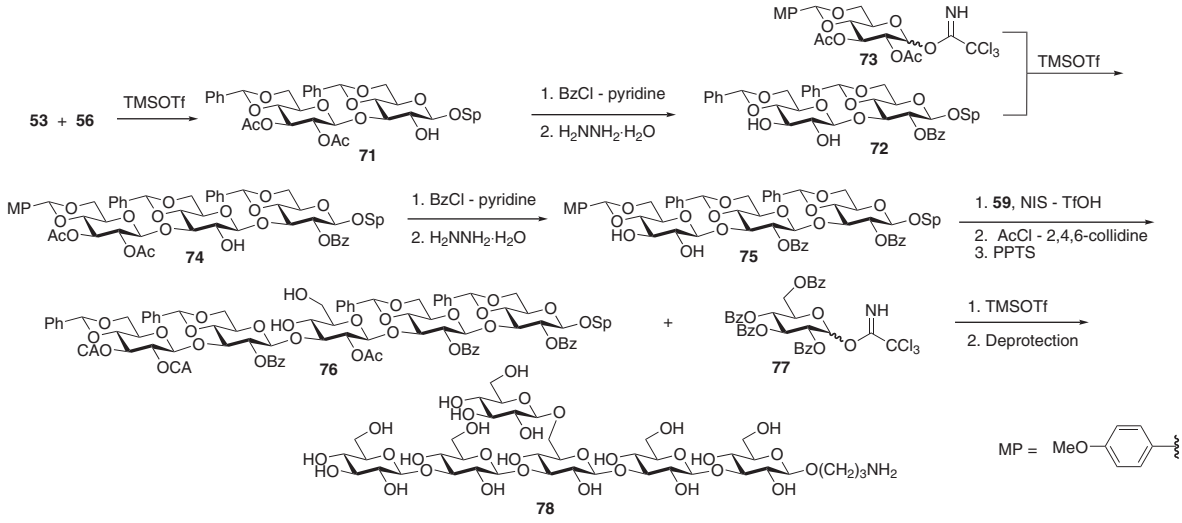

Fig. 11: Synthesis of the hexaglucoside with branching in the central glucose unit of the linear core.

73 resulting in the formation of trisaccharide **74.** Subsequent benzoylation and deacetylation of **74** provided trisaccharide diol **75.** Its glycosylation with thioglycoside **59**, acetylation of free 2-OH in the pentasaccharide formed and selective hydrolysis of *p*-methoxybenzylidene acetal produced pentasaccharide acceptor **76** for the final attachment of the side glucose unite. Selective glycosylation of the primary hydroxyl group in **76** with imidate **77** afforded a hexasaccharide (65 %) that was deprotected as described above for the linear analogs to provide target free oligosaccharide **78.**

The second branched hexasaccharide was prepared in a similar way (Fig. 12) [45]. 4,6-*O*-(*p*-Methoxybenzylidene)-protected acceptor **79** representing the glucose unit at the reducing end of the chain was twice elongated using disaccharide thioglycosides **59** and **55**. First glycosylation with **59** afforded trisaccharide **80** that was converted into acceptor **81**, and the second glycosylation with **55** produced pentasaccharide **82**. Selective removal of the *p*-methoxybenzylidene protection from **82** gave triol **83**. Glycosylation of **83** with trichloroacetimidate **84** proceeded regioselectively at the primary OH group and provided a hexasacchride in 70 % yield; its subsequent deprotection resulted in the formation of target product **85.**

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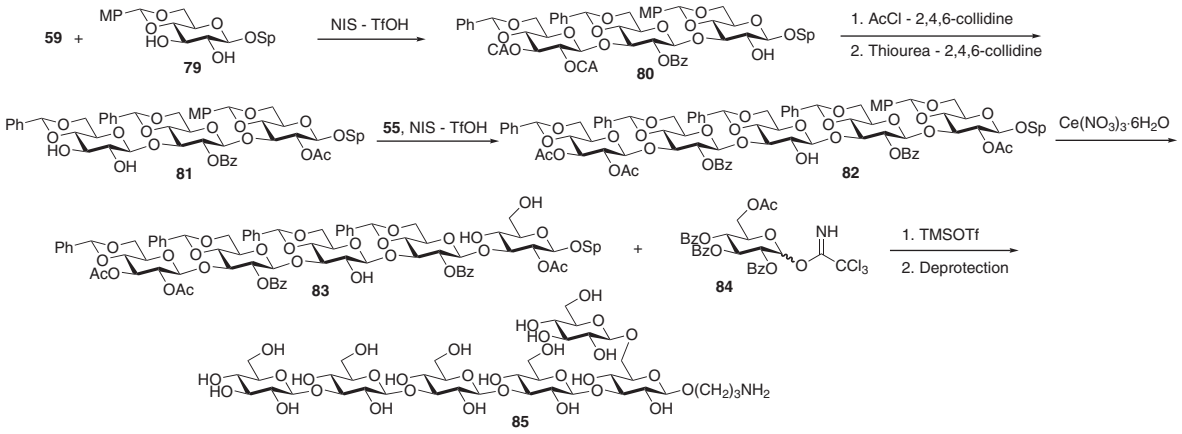

Fig. 12: Synthesis of the hexaglucoside with branching in the first glucose unit of the linear core.

Linear nonaglucoside **68** and branched hexaglucoside **78** were conjugated to BSA via a squarate linker. The conjugates were studied in respect to the development of an antifungal vaccine [34, 46]. Protective effect of active immunization with the conjugate of linear nonaglucoside **78** has been revealed in vivo in the experimental hematogenously disseminated *C. albicans* infection [46].

Synthesis of linear oligosaccharides structurally related to α-glucan of *Aspergillus fumigatus* cell wall

Aspergillus fumigatus is a very common air-borne mold causing severe and usually fatal invasive aspergillosis infection in immunosuppressed hospital patients. At risk are mainly patients in onco-hematology units and those undergoing intensive immunosuppressive therapy after receiving organ transplants. α-(1→3)-Glucan is the major polysaccharide of *A. fumigatus* cell wall and constitutes 19 and 40 % of the conidial and mycelial cell wall polysaccharides, respectively. It contributes to the virulence of diverse fungal pathogens and is involved in the aggregation of germinating conidia and biofilm formation. Moreover, it has been shown in experimental murine aspergillosis models that α-(1→3)-glucan has a prominent immunological role conferring a long-term survival [47]. The study of functions of this polysaccharide is complicated by its insolubility in water, therefore, synthetic soluble α-(1→3)-glucooligosaccharides of strictly defined structure and their conjugates with protein carriers and labels would provide tools for better control of antibody production with better definition of the epitope recognized and help in the identification of the human receptor for this polysaccharide.

The prerequisite for efficient preparation of sufficiently long α-(1→3)-oligoglucosides (Fig. 13) is high α-stereoselectivity at each glycosylation step. We planned to achieve it using the remote anchimeric assistance of acyl groups [48, 49]. Model investigations showed that glucosyl *N*-phenyltrifluoroacetimidates bearing two participating acyl groups at O-3 and O-6 or a single acyl group at O-6 only provided high efficiency and α-stereoselectivity of glucosylation [50]. The ability of the 6-*O*-acylated glucosyl donors to highly stereoselective α-glucosylation was of particular importance, since it enabled application of α-(1→3)-linked disaccharide and potentially higher oligosaccharide donor blocks, containing a 6-*O*-acyl group in the glycosylating glucose unit, for the chain elongation without loss of α-stereoselectivity.

The successful use of this finding for the synthesis of the α-(1→3)-pentaglucoside is presented in Fig. 13 [51]. Selectively removable *p*-methoxyphenyl and levulinoyl groups serving for temporary protection of 3-OH and the anomeric position, respectively provided the simple and efficient interconversion of donor and acceptor blocks. Glycosyl acceptor **89** representing the reducing end of the chain was synthesized from 4,6-*O*-benzylidene derivative **86** by successive removal of the anomeric MP group, conversion of the

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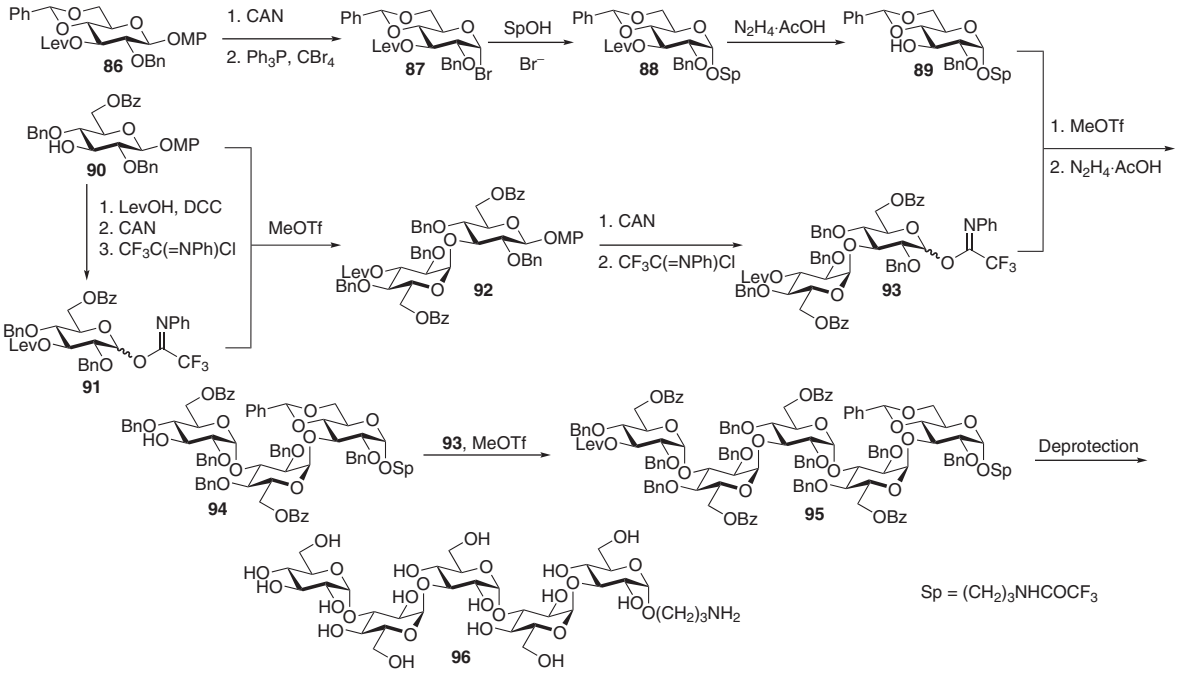

Fig. 13: Synthesis of α-(1→3)-pentaglucoside.

hemiacetal formed into glycosyl bromide **87**, halide-catalyzed α-glucosylation of 3-trifluoroacetamidopropanol and removal of the levulinoyl group from glycoside **88**. The donor and acceptor blocks for the chain elongation were prepared from the single precursor **90**. Acylation of 3-OH, removal of the anomeric MP group and acylation of the anomeric hydroxyl with $CF_3C(=NPh)Cl$ afforded monosaccharide glucosyl donor **91**. Its MeOTf-promoted coupling with acceptor **90** smoothly gave α-disaccharide **92** (90 %) that was converted in two steps into disaccharide donor **93** in total yield of 70 %. Glycosylation of acceptor **89** with **93** followed by removal of the levulinoyl group afforded trisaccharide acceptor **94**. In the final step, **94** was coupled with imidate **93** to provide α-pentasaccharide in 68 % yield. Deprotection of **95** included successive removal of the Lev group with hydrazine acetate, removal of the benzyl and benzylidene groups by catalytic hydrogenolysis and basic splitting of O- and N-acyl groups and gave free α-(1→3)-pentaglucoside **96**. This compound was conjugated to BSA via a squarate linker, and the conjugate obtained was used for induction of antibodies in mice. These polyclonal antibodies were shown to be efficient for immunolabeling α-(1→3)-glucan-positive morphotypes of *A. fumigatus* [51].

Synthesis of linear oligosaccharides structurally related to *Aspergillus* galactomannan

Galactomannan is a specific carbohydrate antigen of *A. fumigatus* [52]. Diagnosis of invasive aspergillosis infections commonly employs detection of this polysaccharide antigen circulating in the patient serum [52]. Moreover, galactomannan is considered as a promising base for development of approaches to the aspergillosis treatment using vaccines or therapeutic antibodies. With regard to the importance of *A. fumigatus* galactomannan, we initiated the synthesis of related antigenic oligosaccharides. Galactomannan represents a structurally diverse heteropolysaccharide build up from poly-D-mannose backbone with β-(1→5)-linked oligogalactofuranoside side chains attached to some of the mannose units via (1→6)- and/or (1→3)-bonds [53]. We performed (Fig. 14) the synthesis of two galactomannan-related heterosaccharides **109** and **112** containing both (1→6)- and (1→3)-linkages between galactose and mannose moieties [54].

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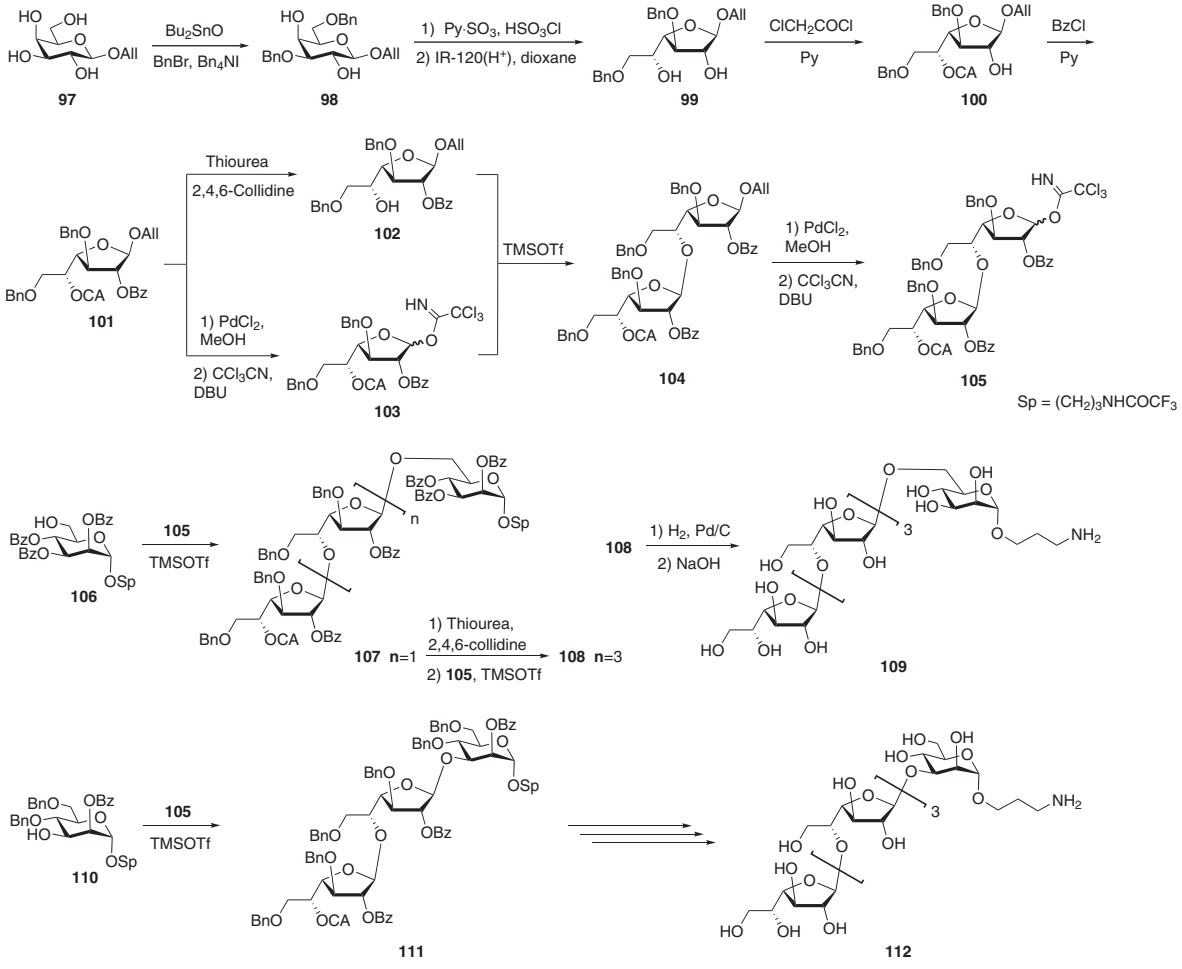

Fig. 14: Synthesis of pentasaccharides **109** and **112** related to the galactomannan from *A. fumigatus*.

The synthesis was carried out with the use of the recently discovered PIF-rearrangement [55–58] of selectively O-substituted galactopyranoside **98** which was prepared by regioselective 3,6-di-O-benzylation of allyl galactoside **97** via the organotin intermediate. PIF-rearrangement of pyranoside **98** into sulfated furanoside occurred upon treatment with the Py·SO_3 complex and chlorosulfonic acid; subsequent solvolytic *O*-desulfation in dioxane in the presence of Amberlite IR-120 (H^+) afforded furanoside **99**. The reactivity of the 5-OH and 2-OH groups in diol **99** was rather different. Thus, chloroacetylation of **99** in the presence of pyridine in DCM at low temperatures smoothly produced 5-O-acylated product **100**, which was then benzoylated to give orthogonally protected precursor **101**. A part of compound **101** was transformed into glycosyl-acceptor **102** by dechloroacetylation with thiourea and another part of **101** was deallylated and converted into trichloroacetimidate to give glycosyl donor **103**. The coupling of **102** and **103** in the presence of TMSOTf gave exclusively β-linked disaccharide **104**. Anomeric O-deallylation and subsequent trichloroacetimidoylation of disaccharide **104** gave desired disaccharide donors **105**.

Spacer-armed mannoside **106** was glycosylated with disaccharide donor **105** to give trisaccharide **107** with newly formed β-(1→6)-bond. O-Dechloroacetylation and subsequent glycosylation by trichloroacetimidate **105** gave pentasaccharide **108**. Its full deprotection yielded target spacer-armed pentasaccharide **109**. For the synthesis of isomeric pentasaccharide **112** comprising the β-(1→3)-bond between galactoside and mannoside residues, spacer-armed mannoside acceptor **110** was glycosylated with disaccharide donor **105**. Further chain elongation followed by complete O-deblocking gave desired pentasaccharide **112**.

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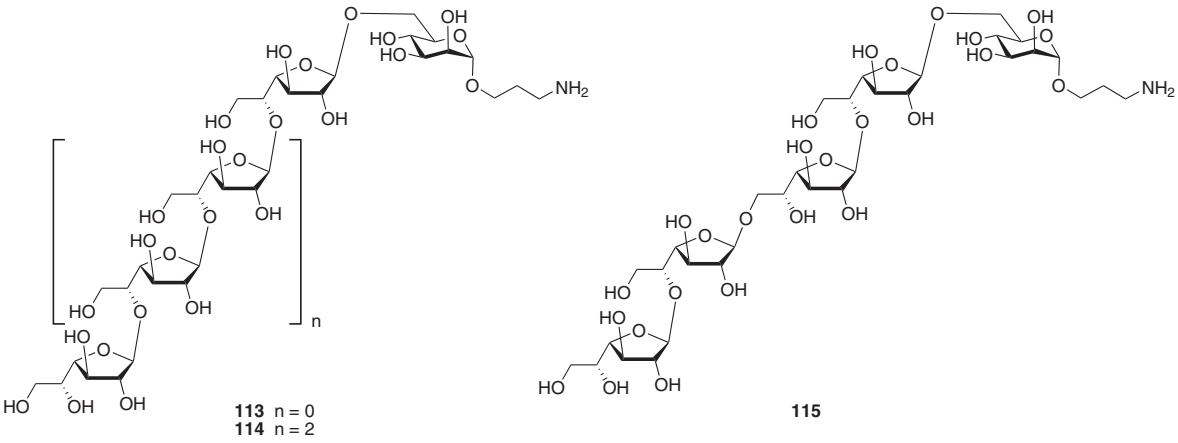

Fig. 15: Synthesized oligosaccharides **113–115** related to the galactomannan from *A. fumigatus*.

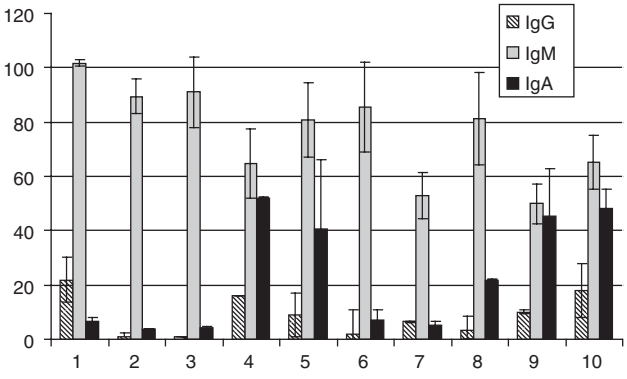

Fig. 16: An overview of sera anti-oligosaccharide specific Ig class antibody responses. Biotinylated derivatives of oligosaccharide ligands [***1*** – Galf(1→5)-[Galf(1→5)]$_3$-(1→6)-αMan; ***2*** – Galf(1→5)-[Galf(1→5)]$_3$-(1→3)-αMan; ***3*** – βGlc-(1→3)-βGlc-(1→3)-[βGlc-(1→6)]-βGlc-(1→3)-βGlc-(1→3)-βGlc; ***4*** – βGlc-(1→3)-[βGlc(1→3)]$_7$-βGlc; ***5*** – βMan-(1→2)-αMan-(1→3)-αMan-(1→2)-αMan-(1→3)-αMan-(1→2)-αMan-(1→2); ***6*** – αMan-(1→2)-αMan-(1→3)-[αMan-(1→6)]-αMan-(1→2)-αMan-(1→2)-αMan; ***7*** – αMan-(1→2)-[αMan-(1→3)]-αMan-(1→2)-αMan-(1→2)-αMan; ***8*** – αMan-(1→3)-αMan-(1→2)-αMan-(1→2)-αMan; ***9*** – αMan-(1→2)-αMan-(1→2)-αMan; ***10*** – βMan-(1→2)-αMan-(1→2)-αMan] were used as antigens. Study cohort comprised 32 patients with RVC (Dept. Clin. Immunol. Allergol., OISE, Bratislava, Slovakia). Sera levels were determined by modified ELISA anti-*Candida* (Biogema, Košice, Slovakia).

Prepared oligosaccharides **109** and **112** were conjugated to BSA using squarate procedure [30] and labeled with biotin. These glycoconjugates were used for generation of monoclonal antibodies and also as model ligands for determination of their ligand specificity.

The above synthetic approaches together with controlled O(5)→O(6) benzoyl migration were also applied [59] for the synthesis of tri- (**113**) and heptasaccharide **114** and to the synthesis of pentasaccharide **115** (Fig. 15) whose oligo-galactofuranosyl sequence contains not only β-(1→5)-bonds but also one β-(1→6)-bond which was detected in minor components of galactomannan [60].

Conclusion and prospects

Described syntheses considerably extend the repertoire of chemical approaches towards stereo- and regioselective assembling of homo- and heterosaccharide chains which are structurally related to polysaccharide components of fungal cell wall. The carbohydrate derivatives of this group are required for a variety of

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glycobiologic and pharmaceutical investigations including the screening of specificity of anti-carbohydrate Abs [46, 51, 61], design of immunogens and preparation MAbs for in vitro diagnostics for detecting sera Ig-isotypic antibodies (for example see Fig. 16 and [34]) or monitoring of the efficacy of anti-fungal treatment and disease progression. Synthetic spacered oligosaccharide ligands are also required in the development of therapeutic MAbs and vaccines (see also [62]), search for inhibitors of the polysaccharide biosynthesis and blockers of fungal adhesion to host cells. Synthetic oligosaccharides of defined structure representing different fragments of fungal polysaccharides are also indispensable models for different spectral investigations [63], including computer-assisted ones [64, 65] and conformational analysis to assess the details of 3D spatial organization of fungal carbohydrate antigens and their interaction with related receptors. β-(1→3)-Glucan related oligosaccharide ligands represent also an interest as potential vector components which may enhance vaccine efficiency by its targeting to dendritic cells carrying Dectin-1 [66, 67].

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