Synthetic β-(1→3)-D-glucooligosaccharides: model compounds for the mechanistic study of β-(1→3)-D-glucan bioactivities and design of antifungal vaccines*

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Synthetic methods used for the preparation of linear β-(1→3)-D-glucooligosaccharides with three and more monosaccharide units and their conjugates with carrier proteins, as well as the application of such derivatives in the mechanistic study of bioactivites of natural β-(1→3)-D-glucans and in the design of conjugated antifungal vaccines are considered.

Key words: β-(1→3)-D-glucan, β-(1→3)-D-glucooligosaccharides, synthesis, glycoconjugates, antifungal vaccine, dectin-1.

β-(1→3)-D-Glucans are a group of natural polysaccharides consisting of D-glucose residues interlinked through β-(1→3)-glycosidic bonds. Frequently, the β-(1→3)-linked main chain of these polysaccharides is modified with glucose β-(1→6)- or β-(1→4)-branches. β-(1→3)-D-Glucans are fundamental structural members of cell wall or reserve polysaccharides of bacteria and fungi, including yeasts, algae, and higher plants. At the same time, β-(1→3)-D-glucans are absent in vertebrates, including humans.[1]

β-(1→3)-D-Glucans attracted great attention of researchers in the middle of XXth century when they were found to be capable of stimulating the immune system. Since that time, more than 6000 studies dedicated to various aspects of bioactivities of polysaccharides under discussion have been published. They were shown to possess anti-inflammatory, anticancer, antimicrobial, antidiabetic, and some other bioactivities.[2—5]

In the last decade, another trend in the study of β-(1→3)-D-glucans is in progress. These polysaccharides are known to be components of the cell walls of fungi, including such dangerous pathogens as *Candida albicans* and *Aspergillus fumigatus*, and they are not produced by mammals. This makes β-(1→3)-D-glucans and structurally related oligosaccharide ligands promising components of conjugated carbohydrate-protein vaccines for prevention and treatment of mycotic infections,[6] which are one of the most common hospital acquired diseases with a high mortality rate.[7]

β-(1→3)-D-Glucans, especially those isolated from different sources, may vary considerably from each other in molecular weight and number and position of branches along the main chain, which has a significant effect on their secondary and tertiary structures, water solubility, and other properties. These differences, as well as differences in the degrees of purity and homogeneity complicate the studies of molecular mechanisms responsible for β-(1→3)-D-glucan bioactivities, which gives rise to a great number of contradicting literature data.[8] These obstacles raised a demand for synthetic oligosaccharides corresponding to the β-(1→3)-D-glucan fragments and possessing a rigorously defined structure and a high degree of purity from researchers dealing with the study of glucan bioactivities and design of antimycotic vaccine preparations. At the same time, the oligosaccharide types under discussion provoke interest of synthetic chemists in these objects. The present review considers the works on the synthesis of linear β-(1→3)-D-glucooligosaccharides and their use in the mechanistic study of β-(1→3)-D-glucan bioactivities and in the design of conjugated antifungal vaccines.

1. Chemical synthesis of β-(1→3)-D-glucooligosaccharides

Virtually all presently known chemical syntheses of β-(1→3)-D-glucooligosaccharides with three and more monosaccharide units can be represented by the generalized sequence of chemical transformations shown in Scheme 1. Molecule **1** is a glycosyl donor, which is activated and the group X is replaced during the glycosylation reaction, *i.e.* the glycosyl moiety is transferred to the free

* On the occasion of the 100th anniversary of the birth of Academician N. K. Kochetkov (1915—2005).

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hydroxyl group in the 3 position of molecule **2** referred to as glycosyl acceptor to form the glycosylation product **3**. The glycosyl donor **1** contains the so called "permanent" protective groups R^2, R^4, and R^6 at the O(2), O(4), and O(6) atoms, respectively, *i.e.* the groups which remain in the molecule throughout elongation of the oligosaccharide and are removed only at the final preparation step of free oligosaccharide. It should be emphasized that the permanent protective group R^2 is acyl providing β-stereospecificity of glycosylation due to the anchimeric assistance. The hydroxyl group at the C(3) atom in the glycosyl donor **1** is protected by the "temporary" protective group R^3 which can be removed selectively without affecting the permanent protective groups. The number of monosaccharide residues in the glycosyl donor (*n*) and glycosyl acceptor (*m*) can vary from 1 to 4. The removal of the temporary protective group R^3 in the glycosylation product **3** affords the "next-generation" glycosyl acceptor **4** with a longer oligosaccharide chain which also can enter the glycosylation reaction with the glycosyl donor **1**. The *k*-fold repetition of glycosylation and temporary protective group removal results in the formation of oligosaccharide **5** containing $m + n(k + 1)$ glucose residues.

The preparation of a representative series of β-(1→3)-D-glucooligosaccharides up to octasaccharide was described for the first time in the first half of 1990s by Japanese authors (Scheme 2).[9] In this work, the permanent protective groups were 2-*O*-benzoyl and 4,6-*O*-benzylidene groups. The chloroacetyl group was used as the temporary protection at the O(3) atom. The disaccharide glycosyl chloride **9** served as the glycosyl donor for chain extension. All oligosaccharides were assembled starting from the single monosaccharide precursor, *viz.*, thioglycoside **6**.

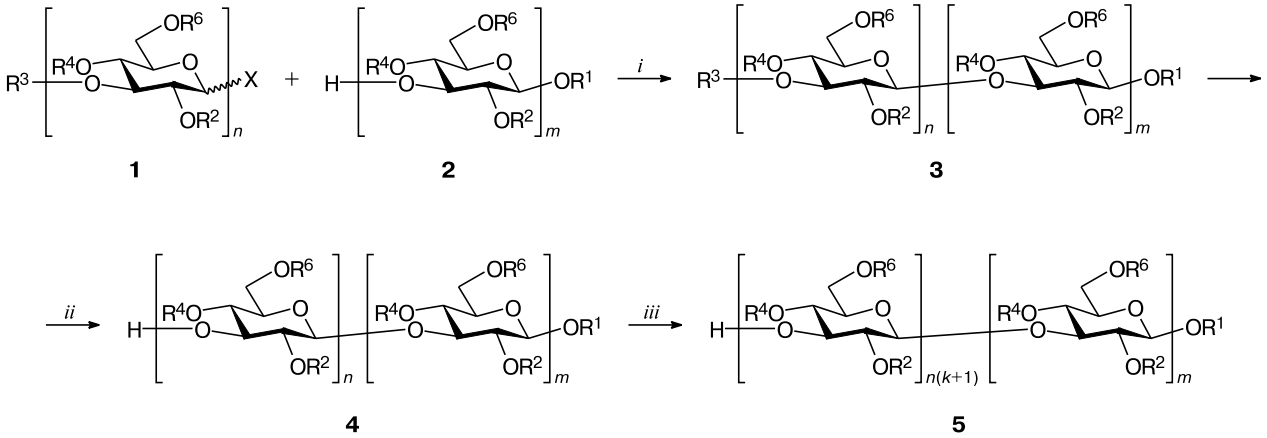
Chloroacetylation of compound **6** followed by chlorine treatment afforded glucosyl chloride **7** whose condensation with thioglycoside **6** in the presence of AgOTf and DTBMP* leads to disaccharide **8**. In compound **8**, the ethylthio group was substituted for the chlorine atom to form glycosyl chloride **9**, which is the main glycosyl donor used for extension of the oligoglucoside chain. The reaction of chloride **7** with methanol followed by removal of the temporary chloroacetyl group yielded methyl glucoside **10**, which is the glycosyl acceptor for the preparation of a series of oligoglucosides with odd number of monosaccharide residues. For example, glycosylation of **10** with chloride **9** afforded trisaccharide **13** where removal of the chloroacetyl group resulted in the "second-generation" glycosyl acceptor **14**.

Subsequent iterative glycosylation and temporary chloroacetyl group removal procedures led to oligomers containing five and seven glucose residues. Oligosaccharides with even number of monosaccharide units were prepared starting from the disaccharide glycosyl acceptor **12**, which, in turn, was synthesized by glycosylation of methyl glucoside **10**(→ **11**) with chloride **7** followed by removal of the chloroacetyl group. The extension of chain in acceptor **12** using glycosyl donor **9** resulted in a series of oligoglucosides containing four, six, and eight glucose residues (in all cases, glycosylation proceeded in 80—85% yields). The target unprotected methyl glycosides **15** were obtained after deprotection.

Attempts were made to obtain linear β-(1→3)-D-glucooligosaccharides using glycosyl donors and glycosyl acceptors where all permanent protective groups R^2, R^4, and R^6 were acyls. This approach is attractive, since it allows

* DTBMP is 2,6-di-*tert*-butyl-4-methylpyridine.

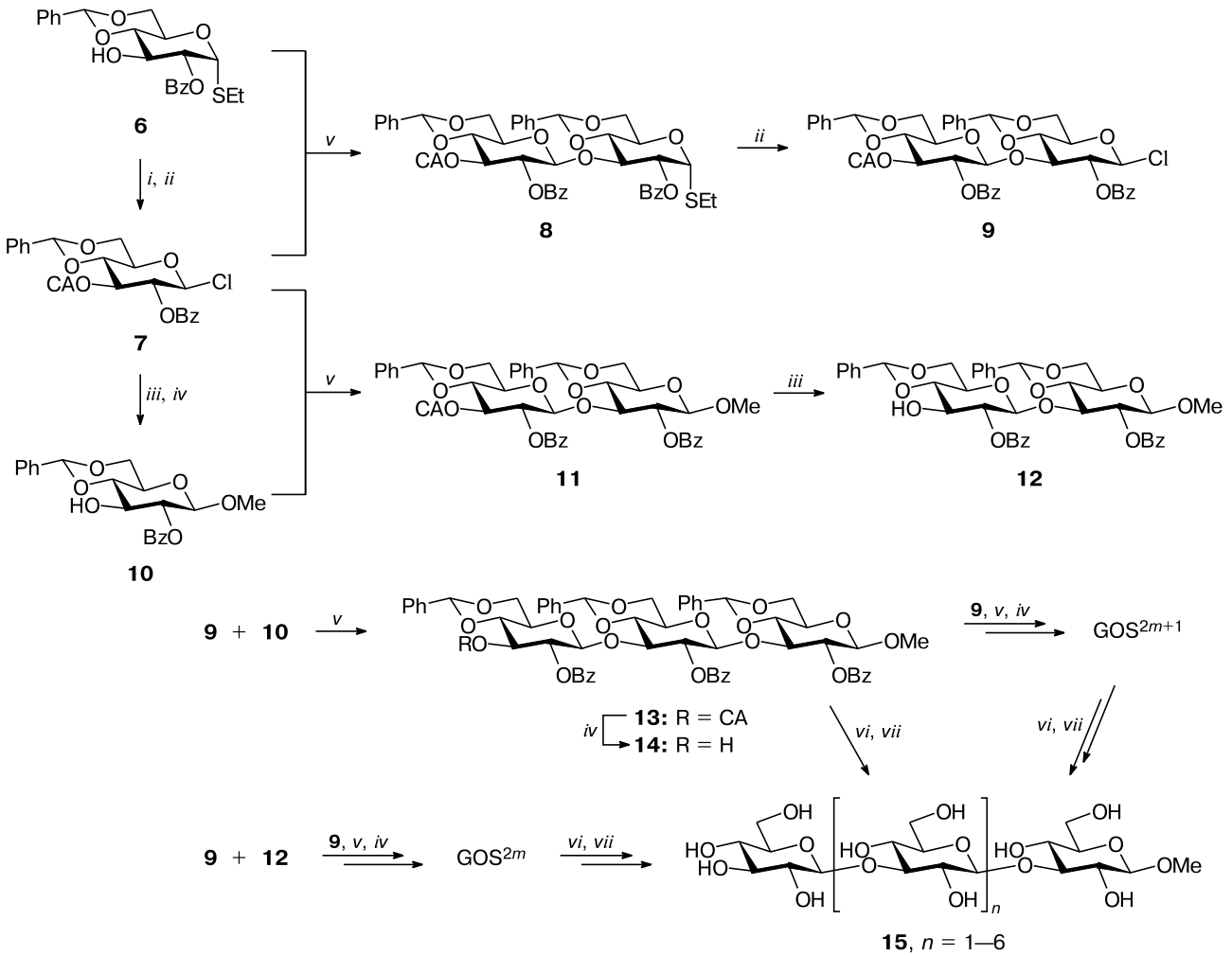
Scheme 1



R^2, R^4, and R^6 are permanent protective groups, R^3 is a temporary protective group, and X is a leaving group.

i. Glycosylation. *ii*. Removal of the temporary protective group R^3. *iii*. *k*-Fold repetition of glycosylation and protective group removal.

Scheme 2

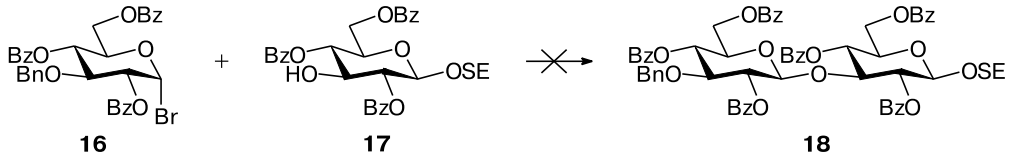


Reagents and conditions: *i*. $ClCH_2COCl$, CH_2Cl_2—Py; *ii*. Cl_2, $CHCl_3$; *iii*. MeOH, Ag_2CO_3, $AgClO_4$, MS 4 E, CH_2Cl_2; *iv*. $(H_2N)_2CS$, 2,6-lutidine, CH_2Cl_2—MeOH; *v*. AgOTf—PhMe, DTBMP, MS 4 Å, CH_2Cl_2; *vi*. ethylene glycol, $TsOH \cdot H_2O$, MeCN; *vii*. MeONa, MeOH.

Note. GOS^{2m+1} are glucooligosaccharides with odd number of monosaccharide residues (**5**, **7**) (m = 2, 3); GOS^{2m} are glucooligosaccharides with even number of monosaccharide residues (4, 6, 8) (m = 2, 3, 4); and CA is chloroacetyl.

efficient one-step removal of all protecting groups in the final products by a common alkaline treatment. However, the hydroxyl group at the C(3) atom was found to have a low reactivity in the case when it is in close vicinity to the acyl protective groups at the O(2) and O(4) atoms. For example, in the above-considered Ref. 9, the model glycosylation of glycosyl bromide **16** with the 2,4,6-tri-*O*-benzoylated glycosyl acceptor **17** in the presence of AgOTf and DTBMP afforded no anticipated disaccharide **18** (Scheme 3).

Scheme 3



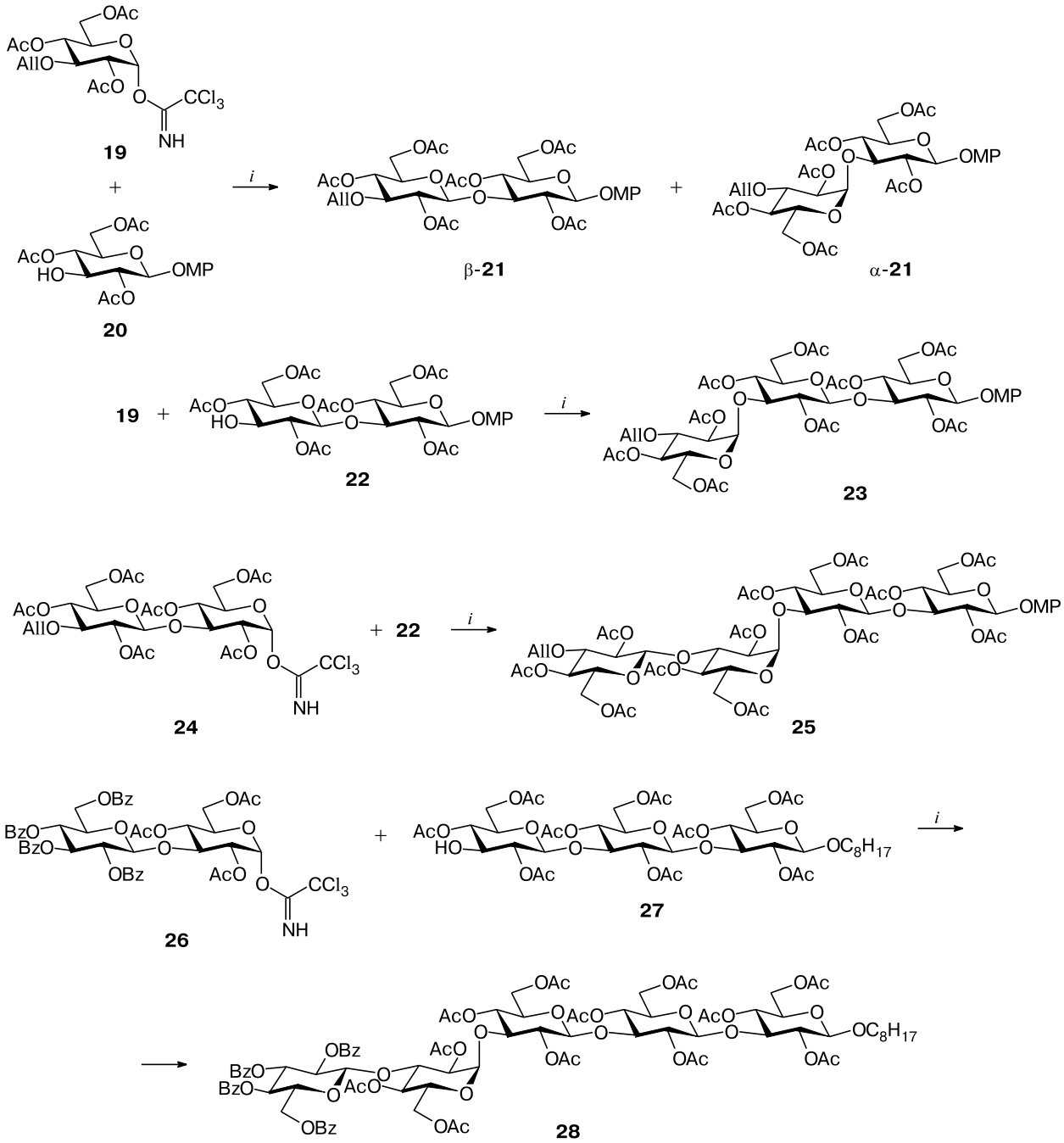
Reagents and conditions: AgOTf/PhMe, DTBMP, MS 4 Å, CH_2Cl_2.

Note. SE is 2-(trimethylsilyl)ethyl.

To compensate for the low reactivity of a glycosyl acceptor, more efficient glycosyl donors, for example, glycosyl trichloroacetimidates, should be used. However, glycosyl acceptors of this type were found to have a significant disadvantage. Glycosylation of poorly reactive acceptors often proceeds with a loss of β-stereospecificity and results in the formation of a mixture of α- and β-glycosides or even in the formation of α-glycoside alone despite the use of glycosyl donors with the participating acyl group at the O(2) atom. Some examples of such unusual stereochemical results are given in Scheme 4.

For example, the TMSOTf-catalyzed glycosylation of triacetyl glycosyl acceptor **20** with glycosyl imidate **19** affords a mixture of disaccharides **α-21** and **β-21** in the

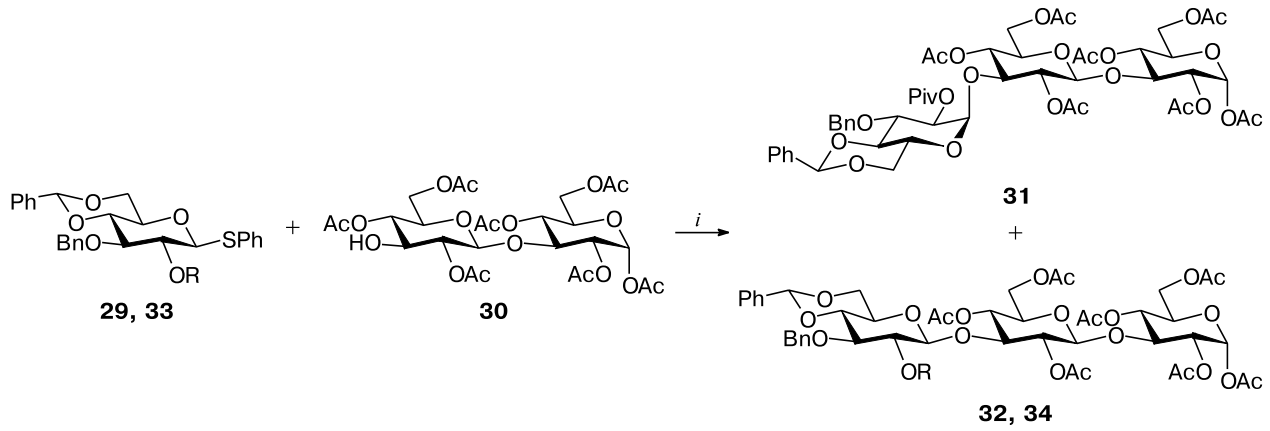
Scheme 4



Reagents and conditions: *i*. TMSOTf, CH_2Cl_2.

Note. MP is *p*-methoxyphenyl; and Piv is pivaloyl.

Scheme 5



R = Piv (**29**, **32**), $AcOCH_2CH_2C(CH_3)_2CO$ (**33**, **34**)

Reagents and conditions: *i*. NIS, AgOTf.

ratio of 7 : 3, whereas the analogous reaction of imidate **19** with the disaccharide glycosyl acceptor **22** yielded exclusively the α-linked trisaccharide **23** (see Refs 10 and 11). Similarly, the reaction of acceptor **22** with the disaccharide glycosyl donor **24** affords only α-tetrasaccharide **25**.[11] Another example is glycosylation of the trisaccharide glycosyl acceptor **27** with the disaccharide imidate **26**, which also leads to the α-linked product **28**.[12]

The formation of undesirable α-anomer during glycosylation of glycosyl acceptors with acyl protective groups at the O(2), O(4), and O(6) atoms was avoided by the use of glycosyl donors with the 4-acetoxy-2,2-dimethylbutyryl group at the O(2) atom (Scheme 5).

For example, the reaction between thioglycoside **29** containing the 2-*O*-pivaloyl protective group and glycosyl acceptor **30** in the presence of *N*-iodosuccinimide (NIS) and AgOTf afforded a mixture of α- and β-trisaccharides **31** and **32** in the ratio of 1 : 3, whereas glycosylation using glycosyl donor **33** with the 4-acetoxy-2,2-dimethylbutyryl group afforded β-trisaccharide **34** (83%) only.[13] On the authors´ opinion, 4-acetoxy-2,2-dimethylbutyrl group stabilizes the bicyclic intermediate **35** and facilitates the attack of glycosyl acceptor R´OH only from the β-side. Despite the fact that the use of glycosyl donors with the 4-acetoxy-2,2-dimethylbutyryl group allows avoiding the formation of undesirable α-products upon glycosylation of 2,4,6-tri-*O*-acylated glycosyl acceptors, the latter found no application for the preparation of β-(1→3)-D-glucooligosaccharides.

In all successful syntheses of oligosaccharides under consideration (with one exception), the benzylidene group was used for protection of hydroxyl groups at the C(4) and C(6) atoms. An example of such approach is the described[14] synthesis of pentasaccharide **42** (Scheme 6) by sequential extension of oligosaccharide chain by one monosaccharide residue using glycosyl donor **37**, which was obtained in seven steps from the available 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose **36**.

At the first step of oligosaccharide chain assembly, donor **37** was transformed by the reaction with benzyl alcohol in the presence of NIS and triethylsilyl trifluoromethane sulfonate (TESOTf) into benzyl glycoside **38** followed by removal of the naphthylmethyl group under the action of DDQ to afford **39**, on which the oligosaccharide chain was then assembled. Glycosylation of the glycosyl acceptor **39** with the glycosyl donor **37** led to disaccharide **40**, which then underwent three iterations of the reaction sequence consisting of removal of the temporary naphthylmethyl group and glycosylation to yield the protected pentasaccharide **41**. At the final step, all protective groups in pentasaccharide **41** were removed to give the deblocked β-(1→3)-D-glucopentaose **42**.

In some works, the assembly of relatively short oligosaccharide chains (5 or 6 monosaccharide residues) was performed using a complex approach where di- or trisaccharide glycosyl donors and glycosyl acceptors were as-

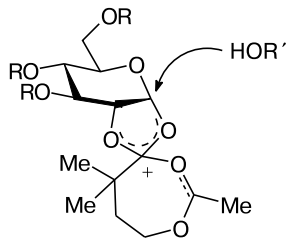


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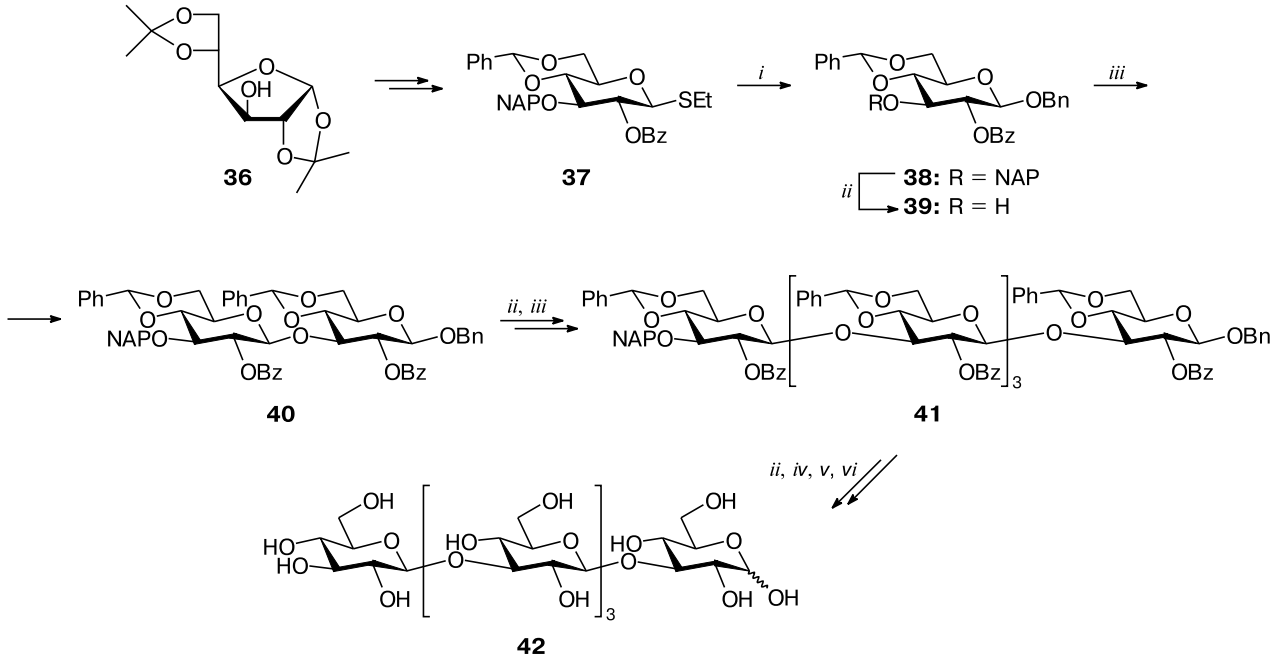
Fig. 1. Probable mechanism of the effect of 4-acetoxy-2,2-dimethylbutyryl group on the stereochemical result of glycosylation.

Scheme 6



Reagents and conditions: *i*. BnOH, NIS, TESOTf, MS 4 E, CH_2Cl_2; *ii*. DDQ, CH_2Cl_2—MeOH; *iii*. **37**, NIS, TESOTf, MS 4 Å, CH_2Cl_2; *iv*. camphorsulfonic acid, acetone—MeOH—water; and *v*. MeONa, MeOH; *vi*. H_2, $Pd(OAc)_2$, 50% aq. MeOH.

Note. NAP is 2-naphthylmethyl; and TES is triethylsilyl.

sembled first of monosaccharide blocks and then interlinked at the final glycosylation step. In one of these works,[15] the temporary protective group at the C(3) atom was acetyl (Scheme 7).

Oligosaccharide blocks were synthesized from β- and α-allyl glucosides **43** and **45** using imidate **44** as the single glycosyl donor. Condensation of compounds **43** and **44** in the presence of TMSOTf afforded disaccharide **46** (53%) which was converted into the the disaccharide acceptor **47** by selective deacetylation with HBF_4. Repetition of this reaction sequence afforded the trisaccharide acceptor **48**. The reaction between imidate **44** and glycosyl acceptor **45** yielded disaccharide **49** (53%), where replacement of the anomeric allyl group with trichloroacetimidoyl led to imidate **50**. At the final step, pentasaccharide **51** was obtained by condensation of **48** + **50**. Subsequent removal of protective groups and epoxidation of the allyl group afforded the target product **52**. However, the described selective removal of the acetyl group under acidic conditions (HBF_4, step *ii*) in the presence of acid-labile benzylidene groups in compound **46** (→**47**, 80%) and in the precursor of trisaccharide **48** is arguable.

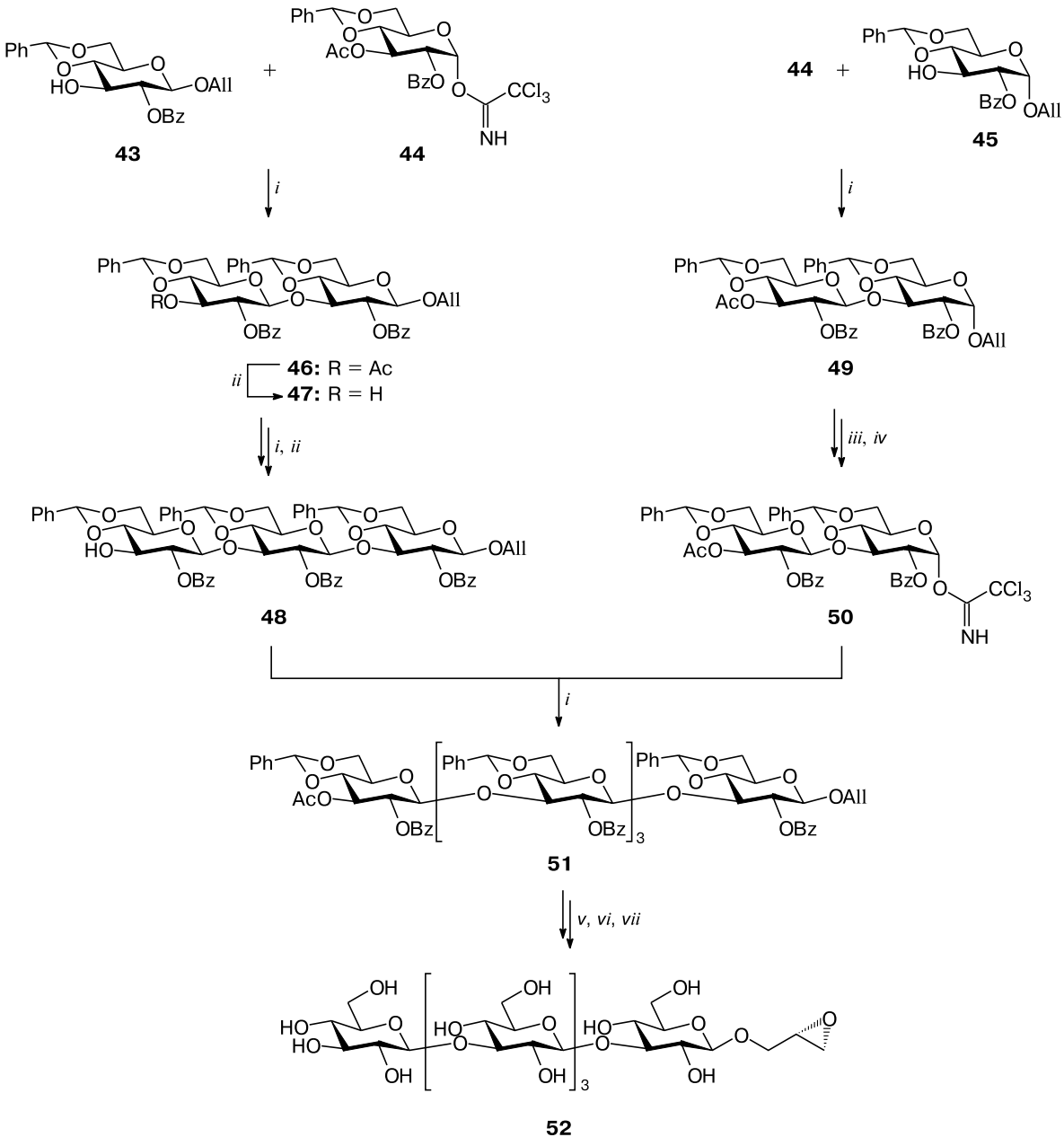
The analogous approach (Scheme 8) was used in Ref. 16 for the preparation of hexasaccharide **60**. As in Ref. 15, the formation of the oligoglucoside chain was carried out starting from the monosaccharide glycosyl acceptor **43** using the glycosyl donor **53** with a levulinoyl group (Lev)* as the temporary protective group at O(3). The first glycosylation afforded disaccharide **54** (75%), which was converted into the disaccharide glycosyl acceptor **55** by removal of the temporaty protective group. Further glycosylation of (**53** + **55**) yielded trisaccharide **56** (53%). Similarly, trisaccharide **56** was converted into the trisaccharide glycosyl acceptor **57** by removal of the levulinoyl group and also into the trisaccharide glycosyl donor **58** by replacement of the anomeric allyl group with trichloroacetimidoyl. Their condensation gave the protected hexasaccharide **59** (54%), which was deprotected and after radical addition of cysteamine to the allyl group afforded the free glucohexaoside **60** with an amino group in aglycon.

In some works, the β-(1→3)-D-oligoglucoside chain was elongated by sequential glycosylation with disaccharide glycosyl donors. For example, the thioglycoside donor was used in Ref. 17 (Scheme 9). The temporary protective group at the O(3′) atom in **63** was TBS and the O(2) atom in the glycosylating monosaccharide residue was protected by the above-considered 4-acetoxy-2,2-dimethylbutyryl group.

At the first step, the disaccharide glycosyl donor **63** (92%) was synthesized by condensation of glycosyl donor

* Lev is levulinoyl.

Scheme 7

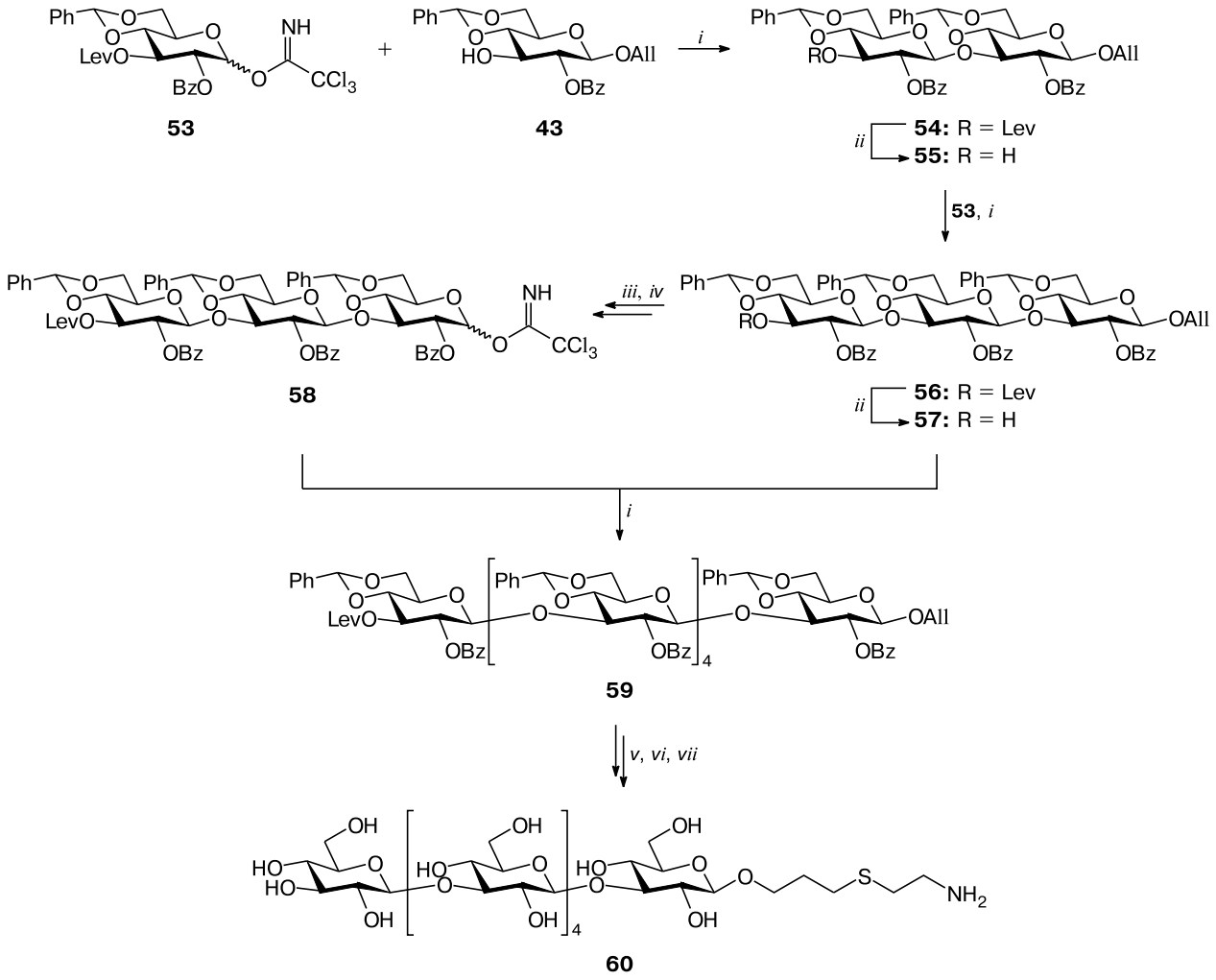


Reagents and conditions: *i*. TMSOTf, CH_2Cl_2; *ii*. HBF_4, THF; *iii*. $PdCl_2$, MeOH; *iv*. CCl_3CN, K_2CO_3, CH_2Cl_2; *v*. MCPBA, CH_2Cl_2; *vi*. 90% aq. AcOH; *vii*. MeONa, MeOH.

61 with the thioglycoside glycosyl acceptor **62** in the presence of NIS and AgOTf. In addition, the disaccharide glycosyl acceptor **66** was obtained by the reaction between thioglycoside **64** and glycosyl acceptor **65** followed by removal of the temporary silyl protection. Two successive cycles of oligosaccharide chain elongation via glycosylation of glycosyl acceptor **66** with donor **63** gave the protected hexasaccharide **67** (78%). Notably, the patent application (Ref. 18) describes the similar approach to the preparation of longer oligomers, in particular, the protected decasaccharide **68** was synthesized by four cycles of oligosaccharide chain elongation. The removal of all protective groups in derivatives **67** and **68** afforded β-(1→3)-glucohexaose **69** and β-(1→3)-glucodecaose **70**.

The patent application (Ref. 19) describes a similar approach (Scheme 10) without experimental details (in

Scheme 8



Reagents and conditions: *i*. TMSOTf, CH_2Cl_2; *ii*. $H_2NCH_2CH_2NH_2 \cdot AcOH$, CH_2Cl_2; *iii*. (cycloocta-1,5-diene)bis(methyldiphenylphosphine)iridium hexafluorophosphate, aq. THF; I_2; *iv*. CCl_3CN, DBU, CH_2Cl_2; *v*. NaOMe, MeOH; *vi*. 90% aq. AcOH; *vii*. $HSCH_2CH_2NH_2$, *hν*, aq. MeOH.

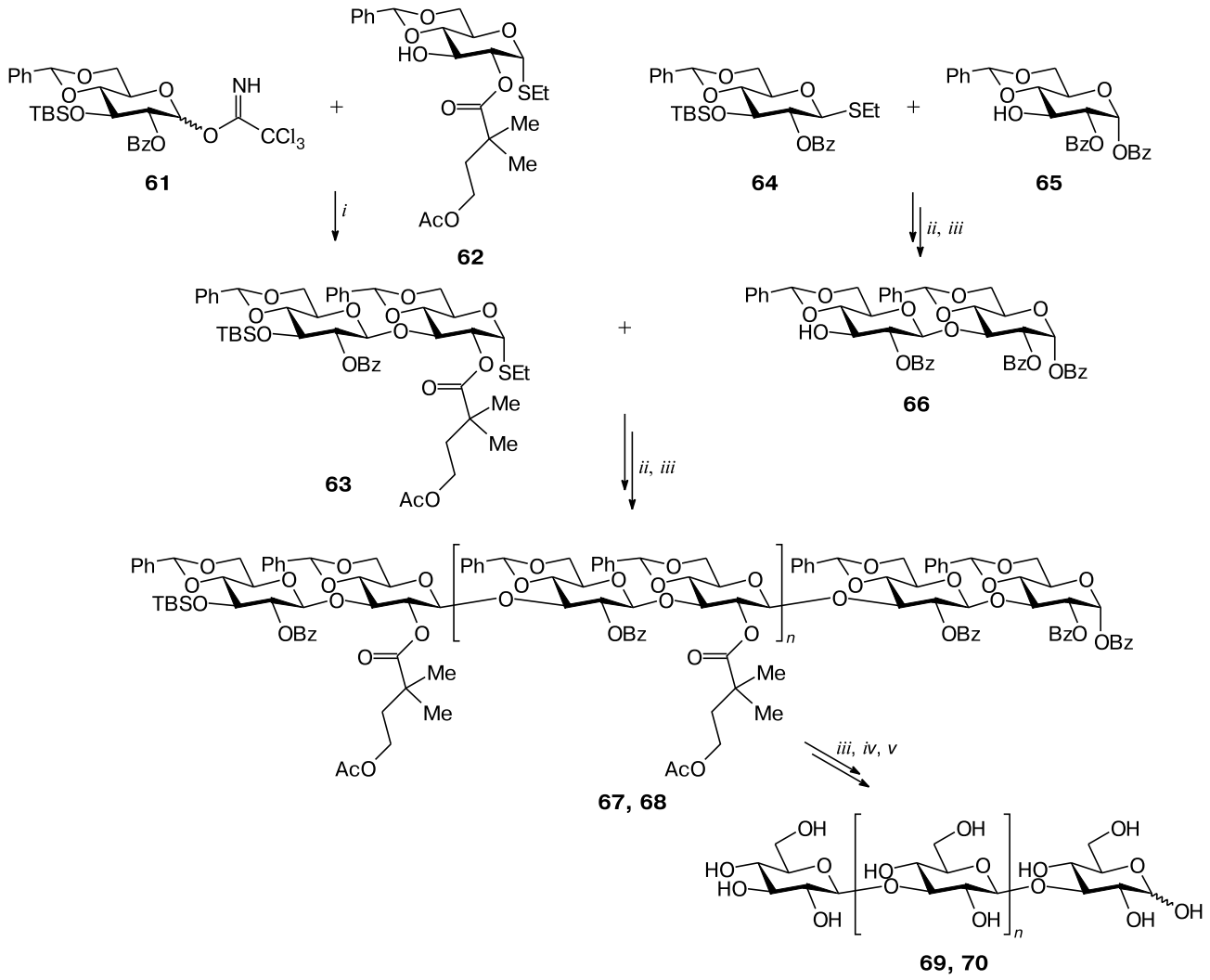
particular, reaction conditions and yields) for the preparation of linear β-(1→3)-D-glucopentadecaoside **81**. In this case, the disaccharide glycosyl trichloroacetimidate donor **75** with a pivaloyl protective group at O(2) and a chloroacetyl group as a temporary protection of the hydroxyl group at C(3′) was used. Imidate **75** was obtained by condensation of the monosaccharide glycosyl acceptor **71** and glycosyl donor **72** followed by replacement of the anomeric allyl group with trichloroacetimidoyl in the condensation product **74**.

The monosaccharide glycosyl acceptor **77**, on which the oligosaccharide chain was grown, was synthesized by condensation of imidate **72** with alcohol **73** followed by removal of the temporary chloroacetyl group in product **76**. The first cycle of oligosaccharide chain elongation involved preparation of trisaccharide **78** by glycosylation of **77** with glycosyl donor **75** followed by removal of the temporary chloroacetyl group to give the trisaccharide glycosyl acceptor **79**. Six iterations of this reaction cycle afforded the protected pentadecasaccharide **80** which was converted to β-(1→3)-glucopentadecaoside **81** by removal of protective groups and reduction of the azide group in the aglycon.

The largest linear synthetic oligo-β-(1→3)-D-glucoside, hexadecasaccharide, was synthesized (Scheme 11) using the tetrasaccharide glycosyl donor **89** and glycosyl acceptor **90** (see Refs 20 and 21). The use of large synthetic blocks of this type allowed assembly of the target oligosaccharides in a relatively small number of steps.

In addition, the authors made another important improvement, which is based on the β-(1→3)-selectivity[22–25] of glycosylation of 4,6-*O*-benzylidene glucopyranose deriva-

Scheme 9



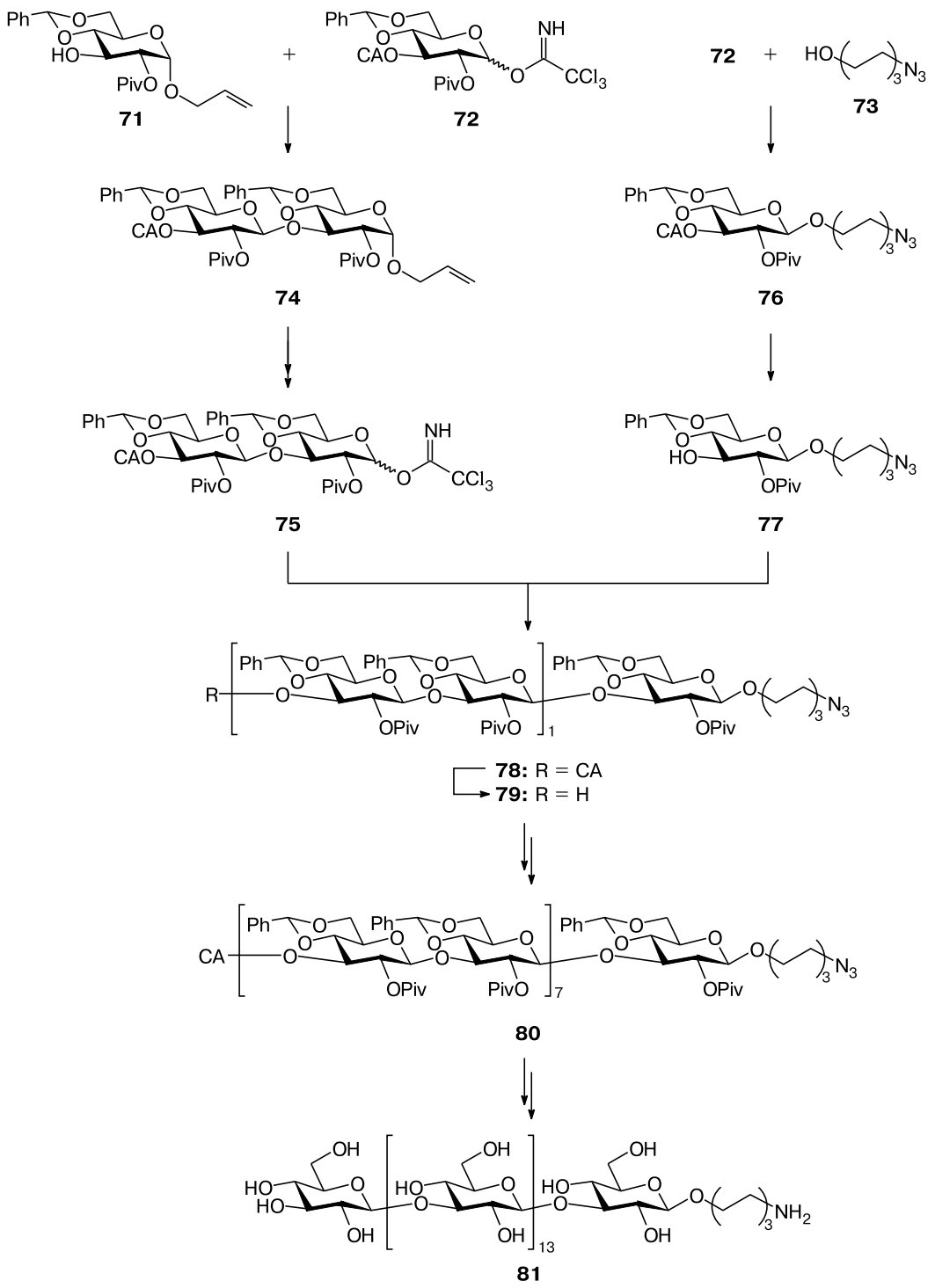
n = 1 (**67**), 3 (**68**), 4 (**69**), 8 (**70**)

Reagents and conditions: *i*. TMSOTf, MS 4 Å, CH_2Cl_2; *ii*. NIS, AgOTf—PhMe, MS 4 Å, CH_2Cl_2; *iii*. HF·Py, Py—THF; *iv*. H_2, Pd/C, MeOH—THF—AcOH; and *v*. MeONa, MeOH.

tives with unprotected hydroxyl groups at C(2) and C(3). As a result, there was no need for differentiation of protective groups at the O(2) and O(3) atoms, *i.e.* selective introduction of temporary protective group at the O(3) atom and permanent protective group at the O(2) atom. This simplified significantly the synthesis of both glycosyl donors and glycosyl acceptors and increased noticeably the total efficiency of synthetic scheme. In the work under consideration, the temporary protective group at the O(2) and O(3) atoms was levulinoyl which can be removed selectively in the presence of other acyl groups. At the first step of synthesis, the tetrasaccharide glycosyl donor **89** and glycosyl acceptor **90** were assembled starting from two readily available monosaccharide precursors, *viz.*, thioglycoside **82** and trichloroacetimidate **83**. Their condensation in the presence of TMSOTf afforded the β-(1→3)-glycosylation product in yield of 69%. In addition, the isomeric (1→2)-linked product was isolated in yield of 6%. This result shows that, even in the case of acceptor **82** with the anomeric thioethyl group, which does not cause sterical hindrance for 2-*O*-glycosylaiton, the reaction proceeds selectively to form the β-(1→3)-bond.

In the resulted disaccharide **84**, the free hydroxyl group at the C(2) atom was benzoylated to form product **85**, which was then transformed to the disaccharide glycosyl donor **86** and glycosyl acceptor **87** by replacement of the anomeric thioethyl group for trichloroacetimidate and removal of the levulinoyl groups, respectively. The reaction

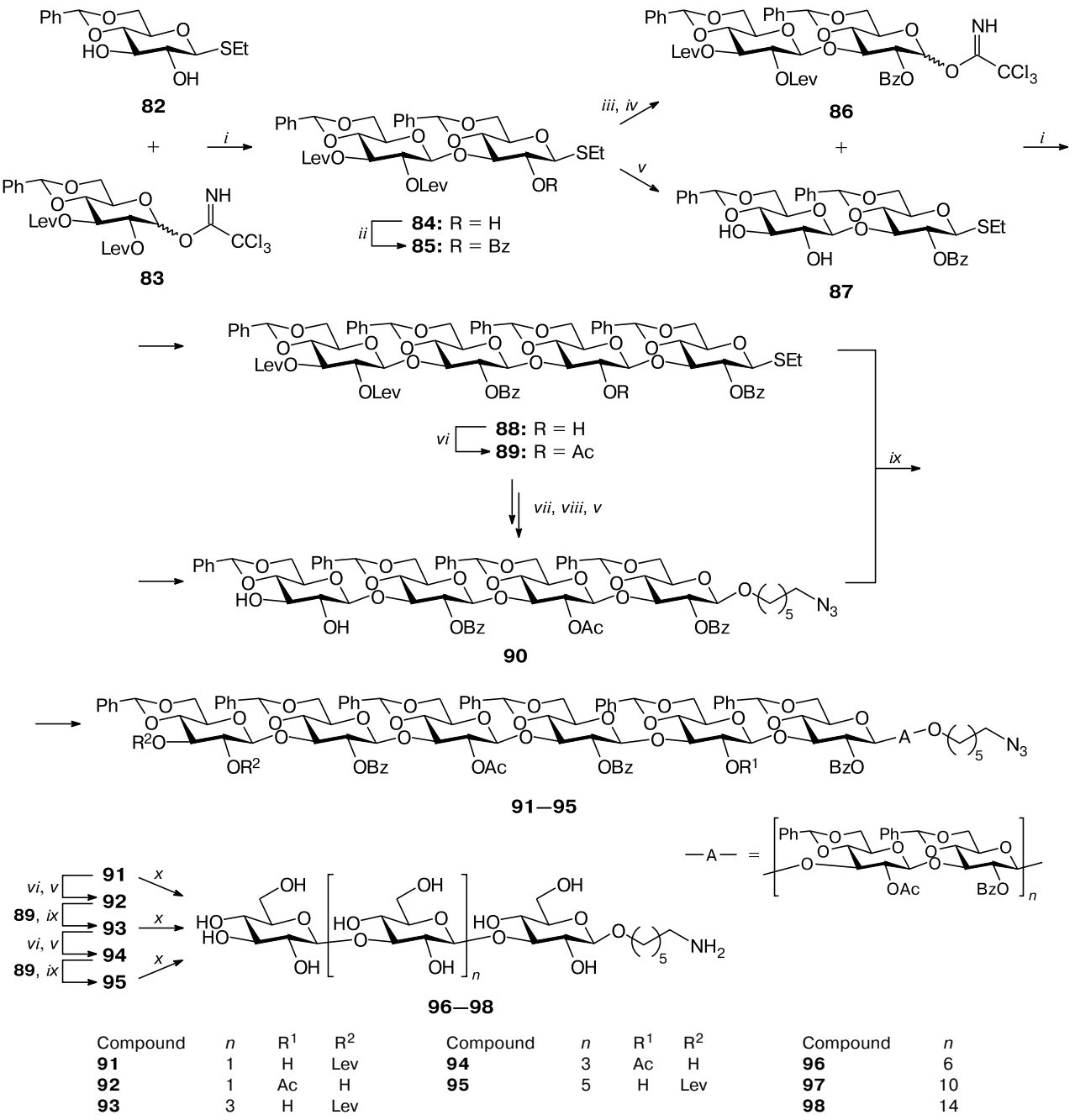
Scheme 10



between imidate **86** and diol **87** in the presence of TMSOTf afforded tetrasaccharide **88** in 90% yield. Thus, the presence of a bulky carbohydrate substituent in the anomeric position of glucose residue to be glycosylated inhibits its completely 2-*O*-glycosylation.

Acetylation of the free hydroxyl group in tetrasaccharide **88** yielded the tetrasaccharide glycosyl donor **89** which was then used for the oligosaccharide chain extension. Also, glycosylation of the hexanol derivative with donor **89** followed by removal of the levulinoyl groups afforded

Scheme 11

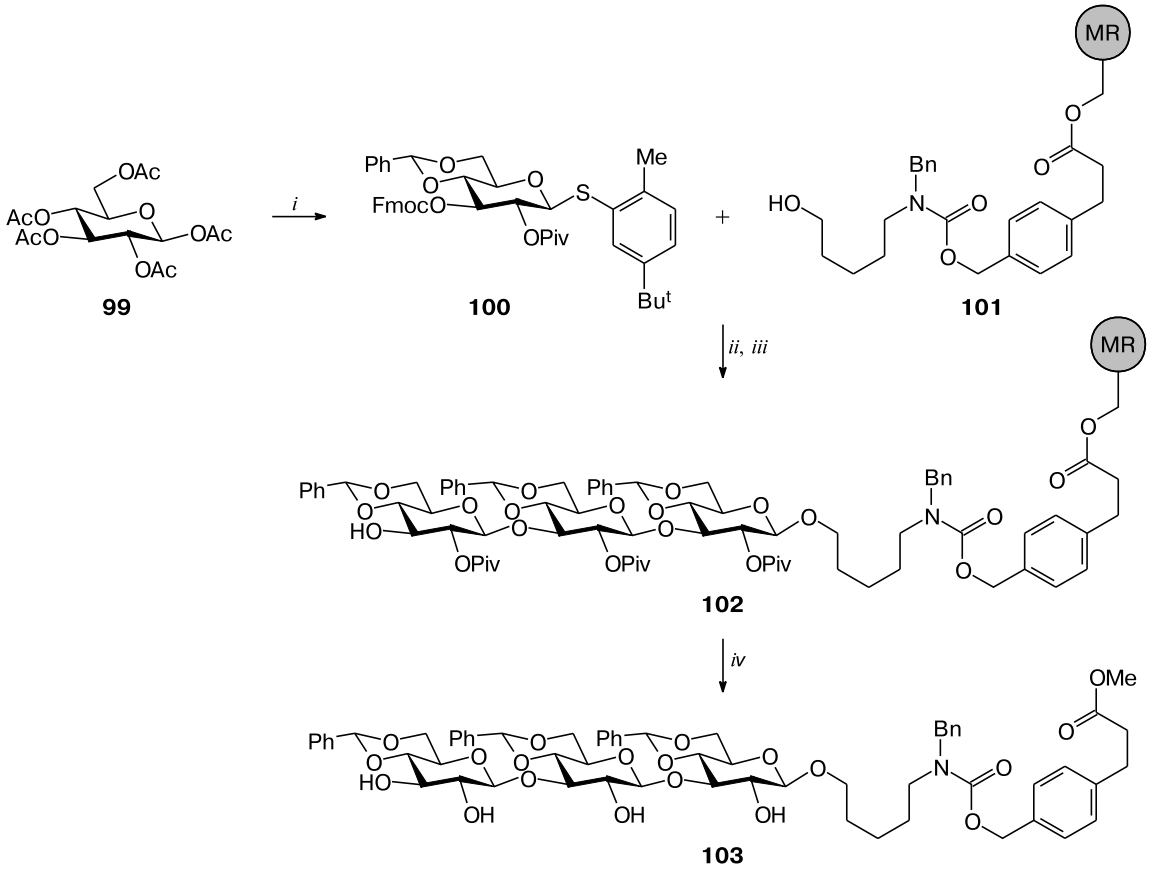


Compound	*n*	R^1	R^2	Compound	*n*	R^1	R^2	Compound	*n*
91	1	H	Lev	**94**	3	Ac	H	**96**	6
92	1	Ac	H	**95**	5	H	Lev	**97**	10
93	3	H	Lev					**98**	14

Reagents and conditions: *i*. TMSOTf, MS 4 Å, CH_2Cl_2; *ii*. BzCl, DMAP, Py; *iii*. *N*-(phenylsulfinyl)morpholine, Tf_2O, CH_2Cl_2, then Et_3N; *iv*. CCl_3CN, Cs_2CO_3, CH_2Cl_2; *v*. $NH_2NH_2 \cdot H_2O$, AcOH, THF; *vi*. Ac_2O, DMAP, Py; *vii*. $HO(CH_2)_6OTs$, NIS, TfOH, MS 4 Å, CH_2Cl_2; *viii*. NaN_3, DMF; *ix*. NIS, TfOH, MS 4 Å, CH_2Cl_2; and *x*. Li, liq. NH_3— THF—EtOH; and then MeOH.

glycosyl acceptor **90**. The first glycosylation of this compound with donor **89** in the presence of NIS and TfOH resulted in the formation of octasaccharide **91** which after acetylation of the remaining hydroxyl group and removal of the temporary levulinoyl protective groups was transformed into glycosyl acceptor **92**. Further glycosylation of **92** with donor **89** resulted in dodecasaccharide **93**, from which the corresponding glycosyl acceptor **94** was obtained. The last glycosylation (**94** + **89**) afforded hexadecasaccharide **95**. At the final step, all protective groups were removed and the azide group in aglycon was reduced to yield the free β-(1→3)-oligoglucosides **96**—**98**. High

Scheme 12



Reagents and conditions: *i*. Seven steps. *ii*. NIS, TfOH, CH_2Cl_2—dioxane; *iii*. piperidine, DMF; *iv*. NaOMe, MeOH—CH_2Cl_2.

Note. MR is the Merrifield resin; and Fmoc is 9-fluorenylmethoxycarbonyl.

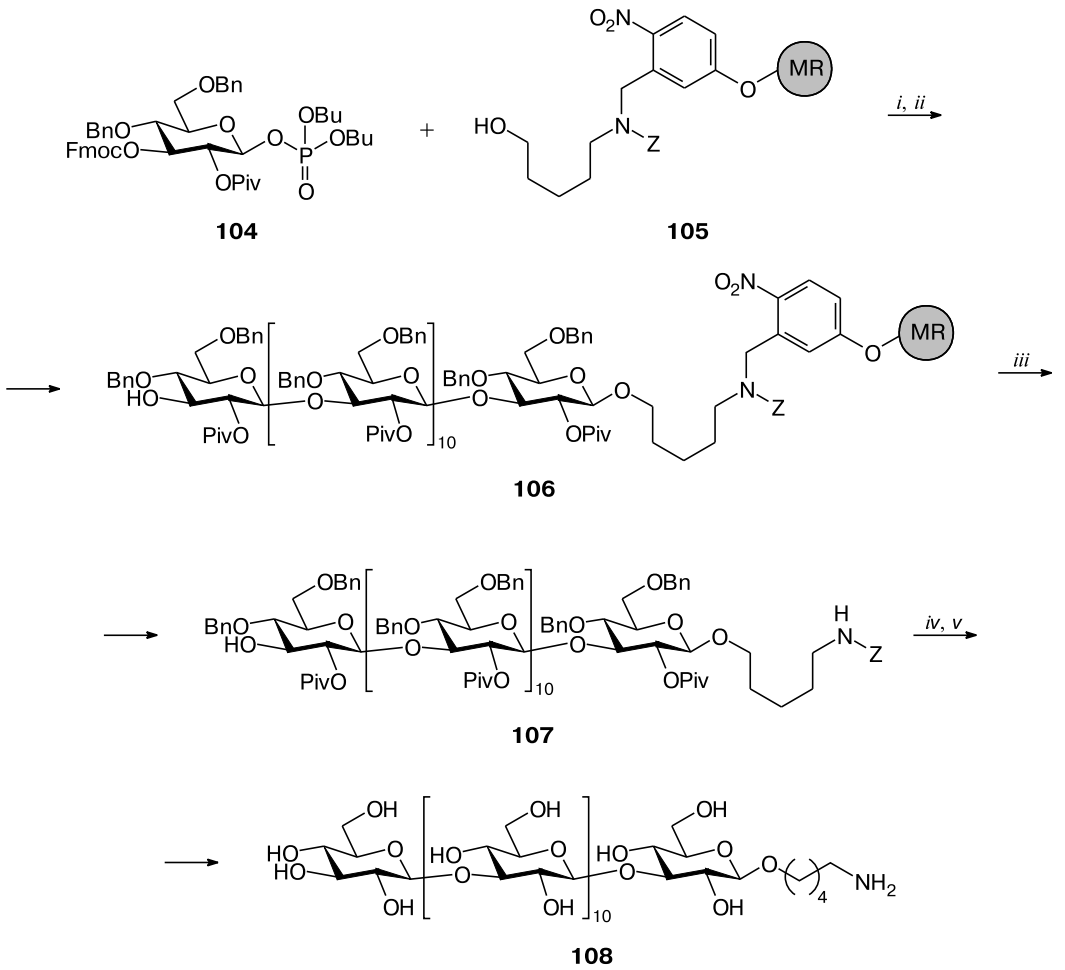
yields of products in the glycosylation reactions shoud be noted: oligosaccharides **91**, **93**, and **95** were obtained in yields of 97, 88, and 82%, respectively.

Recently, the automated solid-phase synthesis of β-(1→3)-D-glucododecaoside was described[26] (Scheme 12). The oligosaccharide chain was extended by one monosaccharide residue using the monosaccharide glycosyl donor. The permanent protective group at the O(2) atom was pivaloyl and the temporary protective group at the O(3) atom was 9-fluorenylmethoxycarbonyl (Fmoc). In the first version of synthesis, the benzylidene group was used for protection of the O(4) and O(6) atoms and the leaving group in glycosyl donor **100** was 5-*tert*-butyl-2-methylthiophenyl. Glycosyl donor **100** was synthesized in seven steps from the available glucose pentaacetate **99**.

The growth of oligosaccharide chain was performed on the Merrifield funcitonalized resin **101**; the linker binding the resin to the oligosaccharide that formed contained the ester group which was cleaved simulaneously with removal of the pivaloyl protecing group in the oligosaccharide upon treatment with sodium methoxide. After each glycosylation reaction, the Fmoc group at the O(3) atom was removed almost quantitatively under the action of piperidine in DMF to release the hydroxyl group for subsequent glycosylation. Three cycles of glycosylation with donor **100** resulted in the formation of polymer-bound trisaccharide **102** whose treatment with sodium methoxide afforded trisaccharide **103**. However, all attempts to extend further the chain in product **102** were unsuccessful. In authors´ opinion, the benzylidene group causes conformational distortion of the pyranose ring, which affects the reactivity of hydroxyl group at the C(3) atom in trisaccharide **102**.

Therefore, the second version of synthesis was based on application of glycosyl donor **104** where the 4,6-*O*-benzylidene group was replaced with the benzyl groups and the leaving group was dibutylphosphate[26] (Scheme 13). Since the conditions for activation of the dibutylphosphate group are incompatible with linker **101**, the second version of synthesis used the photolabile linker **105**. Twelve cycles of glycosylation with **104** resulted in the formation of polymer-linked dodecasaccharide **106** whose UV irradiation resulted in the linker cleavage and formation of the

Scheme 13



Reagents and conditions: *i*. TMSOTf, CH_2Cl_2; *ii*. piperidine, DMF, 12 glycosylation cycles; *iii*. *hν*, CH_2Cl_2; *iv*. MeONa, MeOH—CH_2Cl_2; and *v*. H_2, $Pd(OH)_2$, aq. THF—AcOH.

Note. Z is benzyloxycarbonyl.

protected dodecasaccharide **107**. The removal of all protective groups in compound **107** yielded the target free β-(1→3)-glucododecaoside **108**. The average yield at each of twelve glycosylation steps was 88%.

The main drawback of the above-considered approach for the preparation of β-(1→3)-oligoglucosides is the need for use of glycosyl donors in large excesses to achieve a high yield of the glycosylation product. For example, each of twelve glycosylation steps involved three successive treatments of the corresponding polymer-linked glycosyl acceptor with three equivalents of the glycosyl donor **104** in the presence of TMSOTf activator (see Scheme 13). Thus, the nine-fold excess of glycosyl donor was used at each glycosylation step. This work contains no infromation on the regeneration of the excess of glycosyl donor.

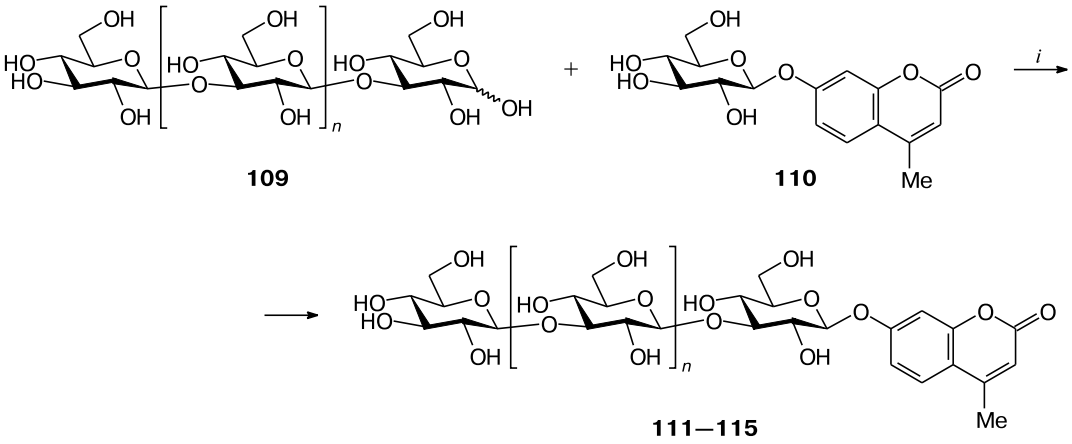
The reviewed works allow us to resume that the most efficient approach for the preparation of large oligo-β-(1→3)-D-glucosides is the block synthesis, which involves oligosaccharide glycosyl donor for the oligosaccharide chain extension. 4,6-*O*-Benzylidene derivatives with free hydroxyl groups at the C(2) and C(3) atoms should be used as glycosyl acceptors.

2. Enzymic and chemical enzymic syntheses of oligo-β-(1→3)-D-glucosides

Transglycosylation catalyzed with β-(1→3)-D-glucanases was used for the preparation of a series of oligo-β-(1→3)-glucosides with different degrees of oligomerization (Scheme 14).[27]

Polysaccharides of the general formula **109**, *viz.*, laminarin from the *Laminaria digitata* brown alga, which is a linear β-(1→3)-D-glucan with single β-(1→6)-branches (not shown in Scheme 14), and curdlan from the *Alcali-*

Scheme 14



i. β-1,3-D-Glucanase.

n = 0 (**111**), 1 (**112**), 2 (**113**), 3 (**114**), 4 (**115**)

genes faecalis bacterium, which is a linear β-(1→3)-D-glucan containing no branches, were used as glucose donors. The glycosyl acceptor was 4-methylumbelliferryl-glucoside (**110**). Two *endo*-β-(1→3)-D-glucanases isolated from *Oerskovia* sp. bacteria and *Spisula sachalinensis* seashell were studied. The composition of oligomer mixture formed depended on the natures of both glucose donor and enzyme. When glucanase from *Oerskovia* was used, a mixture of oligomers **111**—**113** with predomination of di- and trisaccharides **111** and **112** forms irrespective of the nature of polysaccharide donor. The use of enzyme from *S. sachalinensis* in combination with curdlan results in the formation of di- and trisaccharides **111** and **112** only and the use of this enzyme in combination with laminaran results in a mixture of oligomers **111**—**115** whose yields were 30, 20, 15, 8, and 6%, respectively.

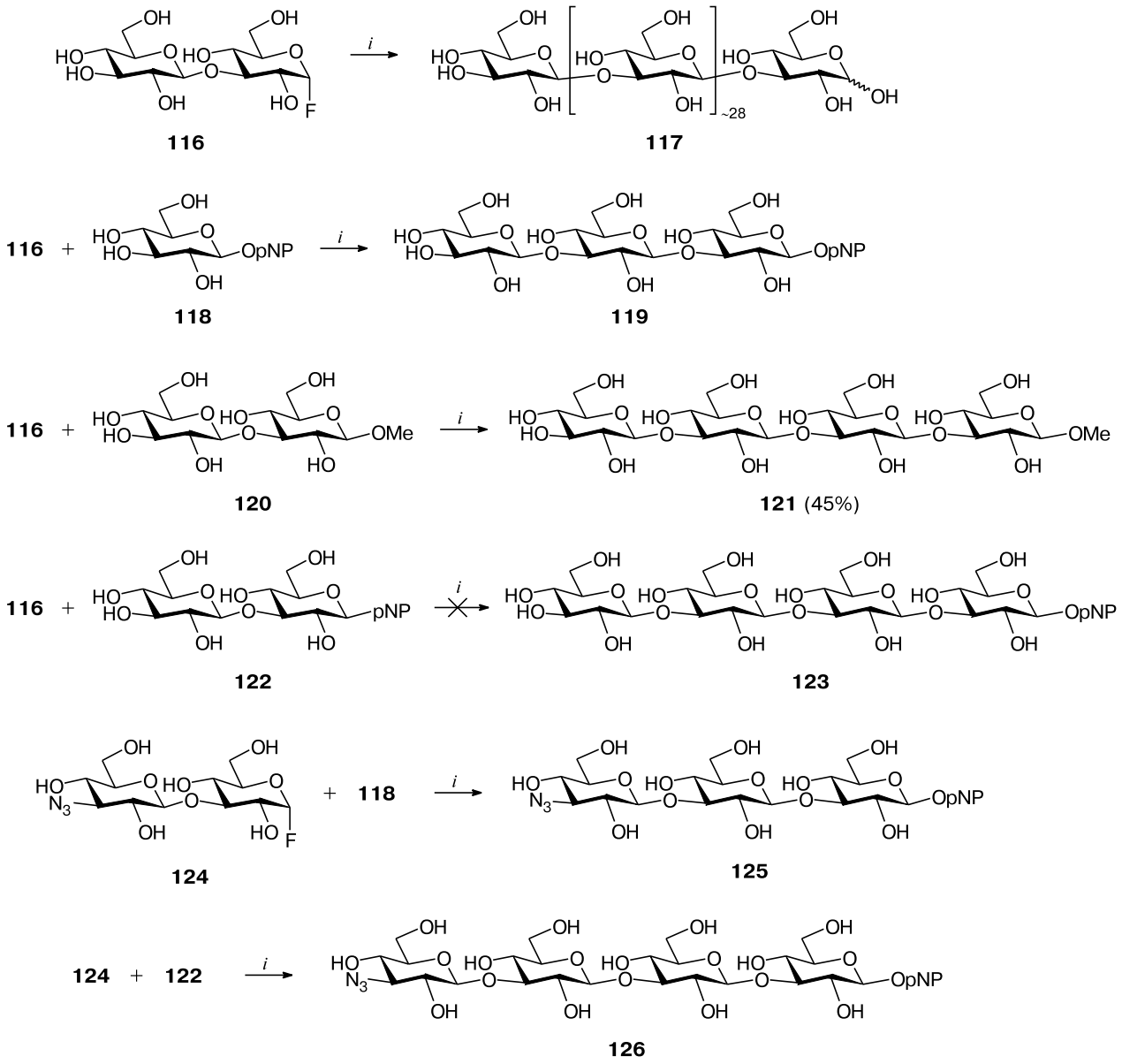
There are works where attempts were made to carry out the chemical enzymic synthesis of oligo-β-(1→3)-D-glucosides using a glycosynthase technique. This technique is based on the use of recombinant glycosyl hydrolases (glycosynthases) which act with the retention of the anomeric configuration of substrate in the reaction product; the replacement of amino acid functioning as a catalytic nucleophile in the active site results in inhibition of the hydrolase activity of these enzymes, but their ability to transfer glycosyl fluorides to the corresponding glycosyl acceptor is preserved. (1→3)-β-D-Glucan-endohydrolase from barley where glutamic acid 231 was replaced with glycine ((1→3)-β-D-glycosynthase, Glu231Gly) was shown to be capable of efficient condensation of α-laminaribiosyl fluoride **116** to form β-(1→3)-D-glucan **117** containing about 30 glucose residues.[28] This enzyme can also transfer glycosyl fluoride **116** to mono- and disaccharide glycosyl acceptors (Scheme 15).[29]

The reaction of fluoride **116** with *p*-nitrophenylglucoside **118** upon their mixing at equimolar concentrations affords mainly glucan **117** due to self-condensation of **116**. At higher concentration of glycosyl acceptor **118** and the ratio of **116** : **118** = 1 : 5, self-condensation of donor **116** is suppressed to result in the predominant formation of trisaccharide **119** in 90% yield. In the reaction of fluoride **116** with the disaccharide methylglycoside **120**, the corresponding tetrasaccharide **121** was obtained; however, its yield was not high (45%). Donor **116** underwent no transfer to *p*-nitrophenylglucoside **122** and no formation of tetrasaccharide **123** was observed even at the donor-acceptor ratio of 5 : 1, the reaction product being glucan **117** (see Scheme 15). According to authors, the absence of the reaction between **116** and **122** can result from their competition for the position of glycosyl donor in the active site of the enzyme. Inhibition of the formation of β-(1→3)-D-glucan by self-condensation of glycosyl donor can be achieved by protection of hydroxyl at the C(3′) atom or its replacement with the group which cannot adopt the glycosyl residue. For example, the 3′-azido derivative **124** undergoes efficient transfer to the monosaccharide acceptor **118** to form trisaccharide **125** at the equimolar reagent ratio; however, there was no transfer to the disaccharide acceptor **122** at such reagent ratio. Only, when the five-fold excess of donor **124** was used, tetrasaccharide **126** was obtained in high yield.

Attempts were made to use the glycosynthase technique also for the preparation of oligo-β-(1→3)-D-glucoside with specified number of monosaccharide units.[30] The general view of approach used is represented in Scheme 16.

Laminaribiosyl fluoride **130** where hydroxyl at the C(3′) atom is protected with the allyl group was used as the glycosyl donor. The glycosynthase-catalyzed transfer

Scheme 15

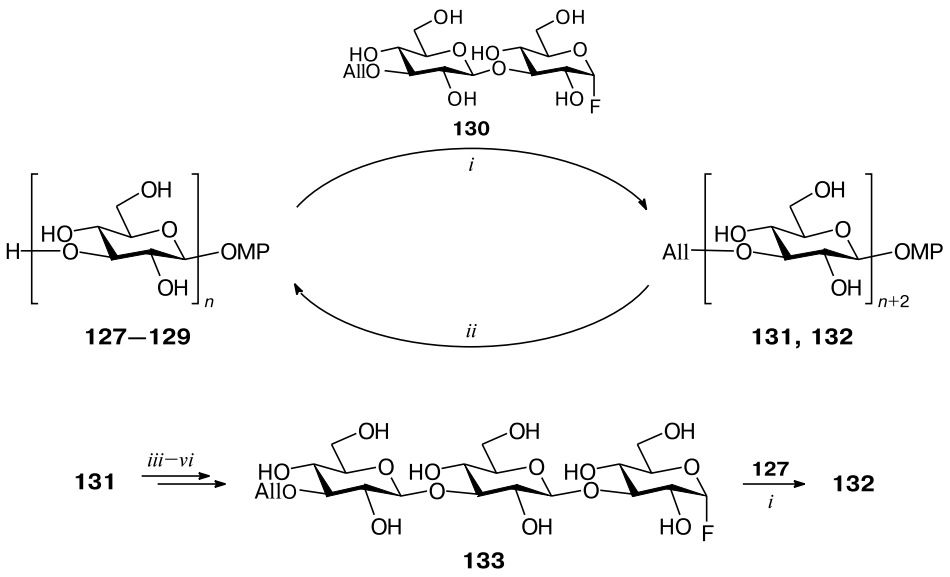


i. Glycosynthase.

Note. pNP is *p*-nitrophenyl.

of donor **130** to the monosaccharide glycosyl acceptor **127** was expected to result in trisaccharide **131** from which the trisaccharide acceptor **129** was obtained by chemical removal of the protective allyl group. Repetition of enzymic glycosylation and chemical removal of the protective group resulted in a series of oligo-β-(1→3)-D-glucosides with odd number of monosaccharide residues. In the case when the disaccharide glycosyl acceptor **128** was used, the first act of enzymic glycosylation resulted in tetrasaccharide **132** and, then, in a series of oligomers with even number of glucose residues. Indeed, only the transfer of donor **130** to the monosaccharide acceptor **127** proceeded smoothly to afford trisaccharide **131** in 70% yield, whereas the attempts to carry out the enzymic glycosylation of di- and trisaccharide acceptors **128** and **129** were unsuccessful. To obtain oligomers with more than three monosaccharide units, the authors of Ref. 30 also studied an alternative approach consisting in extension of oligosaccharide chain on the monosaccharide glycosyl acceptor **127** using larger glycosyl donors. For this purpose, prod-

Scheme 16



n = 1 (**127**, **131**), 2 (**128**, **132**), 3 (**129**)

Reagents and conditions: *i*. Glycosynthase; *ii*. $PdCl_2$, MeOH, allyl group removal; *iii*. Ac_2O, Py; *iv*. $(NH_4)_2Ce(NO_3)_6$, PhMe—MeCN—H_2O; *v*. Et_2NSF_3, CH_2Cl_2; and *vi*. MeONa, MeOH.

uct **131** was transformed in four chemical steps into the trisaccharide glycosyl donor **133**. The enzymic glycosylation of **127** with donor **133** afforded tetrasaccharide **132** in 70% yield. The authors do not report on the efficiency of this approach for the synthesis of larger oligo-β-(1→3)-D-glucosides.

Considering Refs 27—30, it may be concluded that the current enzymic methods cannot compete with chemical synthesis in a solution to the preparation problem of sufficiently large oligo-β-(1→3)-D-glucosides with specified number of monosaccharide units.

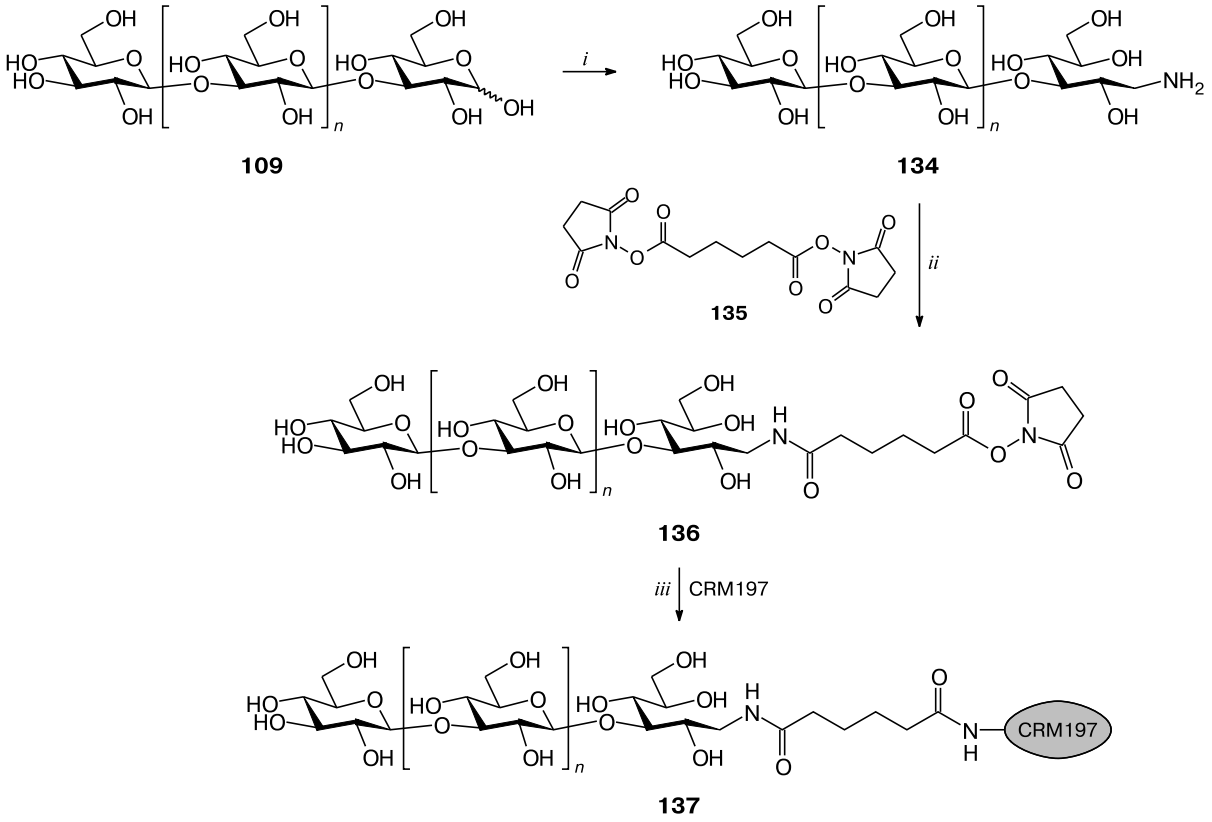
3. Methods of conjugation of synthetic oligo-β-(1→3)-D-glucosides and natural β-(1→3)-D-glucans with carrier proteins

To date, the preparation of several conjugates of natural β-(1→3)-D-glucans and synthetic oligo-β-(1→3)-D-glucosides with carrier proteins, which are used as potential antifungal vaccines, has been described (see Section 5). Scheme 17 shows the preparation of conjugates of natural β-(1→3)-glucans of the general formula **109** with the CRM197 (recombinant diphteria toxoid) protein.[31,32] The above-mentioned laminaran containing about 30 glucose residues and partially hydrolyzed curdlan containing the close number of monosaccharide residues were used as the starting glucans. For transformation of polysaccharide to the active form necessary for conjugation with carrier proteins, they were subjected to reductive amination with ammonium acetate and cyanoborohydride to form aminated derivatives **134**. Then, amines **134** were treated with the excess of bifunctional reagents, *viz.*, bis(*N*-succinimidyl) adipate **135**. The resulted monoamides **136** underwent reaction with the CRM197 protein; the activated ester in derivatives **136** reacted with the amino groups of lysine residues in the protein to form conjugates of the general formula **137**. According to the analysis data, the carbohydrate—protein weight ratio in conjugates **137** varied from 0.2 to 0.75.

The analogous approach was used also for the preparation of conjugates of synthetic hexa- and pentadecasaccharides **60** and **81** containing the amino group in aglycon required for conjugation[16,32] (Scheme 18). For example, the sequential reactions of compounds **60** and **81** with bifunctional reagent **135** and CRM197 protein afforded conjugates **138** and **139**. In the case of relatively short hexasaccharide, on the average 17 oligosaccharide residues were included into conjugate **138**; in the case of longer pentasaccharide, the conjugate contained on the average 7.5 oligosaccharide residues per one protein molecule.

In the above-described examples, the activated oligo- and polysaccharide forms were added to the lysine amino groups of the protein molecule. An alternative approach based on conjugation to tyrosine residues is known. Since the number of tyrosine residues available for modification in the protein is signficantly lower than that of lysine residues, the resulting conjugate should possess a more definite structure with regard to the addition sites of carbohydrate ligands, which facilitates the reproducibility of preparation of conjugates and their analytical characterization.[33]

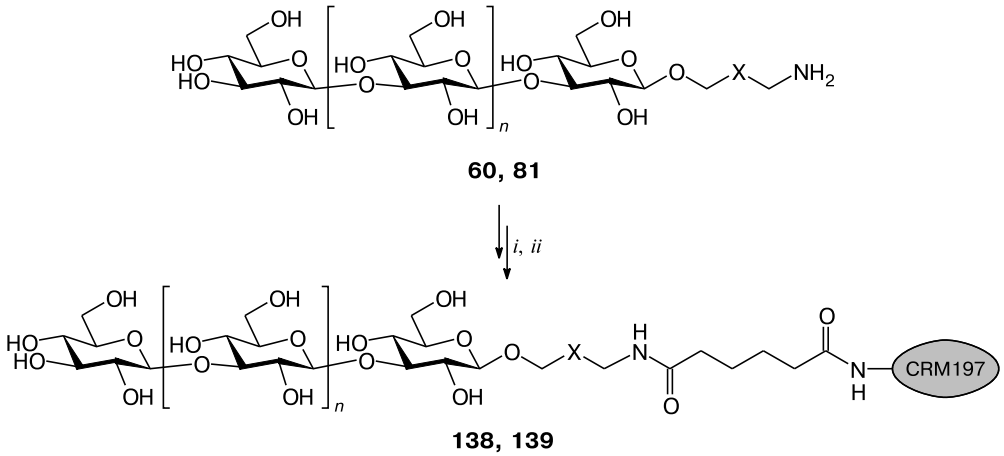
Scheme 17



Reagents and conditions: *i*. $AcONH_4$, $NaBH_3CN$, water, pH 7.5; *ii*. Et_3N, aq. DMSO; and *iii*. phosphate buffer, pH 7.2.

The proposed method is based on the nucleophilic addition of the tyrosine phenol group to the 1,2,4-triazoline-3,5-dione derivatives (Scheme 19). At the first step, the tyrosine residues of the CRM197 protein were modified by the reaction with the 4-phenyl-1,2,4-triazoline-3,5-dione derivative **140** containing the acetylenic fragment for fur-

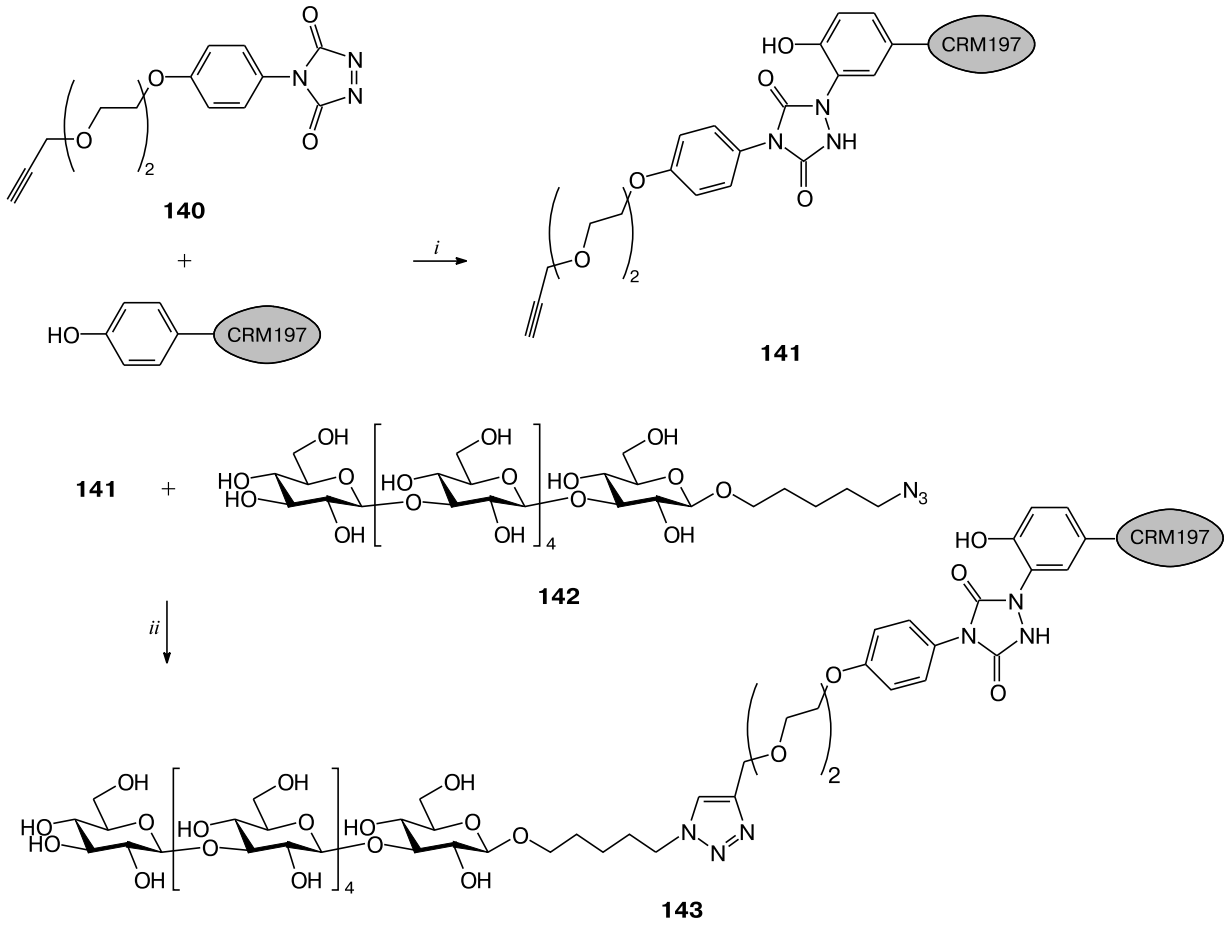
Scheme 18



n = 4, X = $CH_2CH_2SCH_2$ (**60**, **138**); n = 13, X = $CH_2CH_2CH_2CH_2$ (**81**, **139**)

Reagents and conditions: *i*. bis(*N*-succinimidyl) adipate (**135**), Et_3N, aq. DMSO; *ii*. CRM197, phosphate buffer, pH 7.0.

Scheme 19



Reagents and conditions: *i*. 0.5 *M* Tris buffer · HCl, pH 7.4; *ii*. $CuSO_4 \cdot 5H_2O$, sodium ascorbate, tris(3-hydroxypropyltriazolylmethyl)-amine, 0.1 *M* phosphate buffer, pH 7.0.

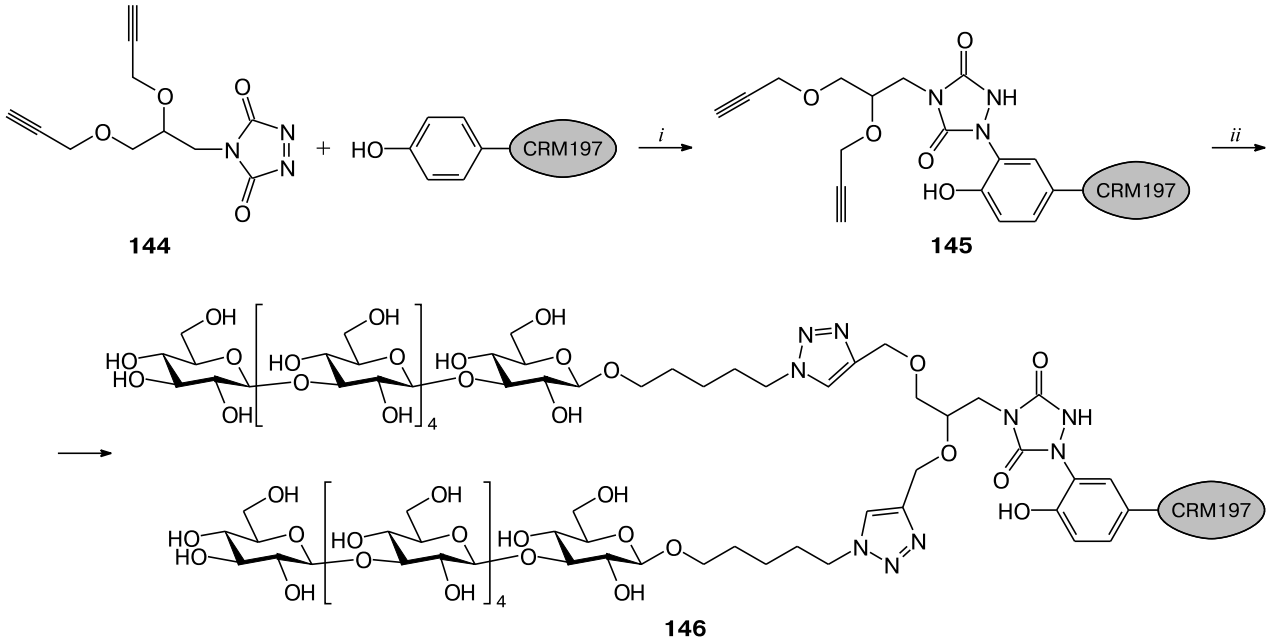
Note. Tris is 2-amino-2-hydroxymethylpropane-1,3-diol.

ther addition of the oligosaccharide ligand. The resulted modified protein **141** underwent reaction with hexasaccharide **142** containing the azide group in aglycon. 1,3-Dipolar cycloaddition of the azide group to acetylene ("click reaction") afforded conjugate **143** which contained about 3.5 hexasaccharide ligands per one protein molecule linked mainly through the tyrosine residues Y27, Y46, Y358, and Y380.

Similarly, the CRM197 protein was modified with 1,2,4-triazoline-3,5-dione derivative **144** containing two propargyl groups (Scheme 20).[34] The analysis of modified protein **145** showed the inclusion of three linker groups linked also with the tyrosine residues Y27, Y46, Y358, and Y380. The subsequent click reaction with hexasaccharide **142** afforded conjugate **146** containing six hexasaccharide residues, each of modified tyrosine residues binding to two oligosaccharide chains.

The same team of authors studied the relative availabilities of lysine amino group in the CRM197 protein for modification.[35] CRM197 was acylated with activated pentynoic ester **147** (Scheme 21) and the modified protein **148** was then subjected to partial hydrolysis using proteolytic enzymes. The resulted mixture of peptides, including those modified with the pentynoic acid residue, was then studied by the mass-spectrometry methods. It was shown that at the molar ratio of **147** : CRM197 = 5 : 1, three residues of pentynoic acid ($n = 3$) are attached to the CRM197 protein, mainly to the lysine residues K103, K221, and K242. With an increase in the **147** : CRM197 ratio to 10 : 1, conjugate **148** containing six acid residues ($n = 6$) formed; besides the above-mentioned three lysine residues, mainly the lysine residues K236, K498, and K526 entering into the reaction. The modified protein **148** ($n = 6$) underwent click reaction with hexasaccharide **142** which afforded conjugate **149** containing six hexasaccharide ligands linked predominantly with the above-mentioned lysine residues. The results of this work suggest that, at certain modifying reagent—protein ratios, con-

Scheme 20

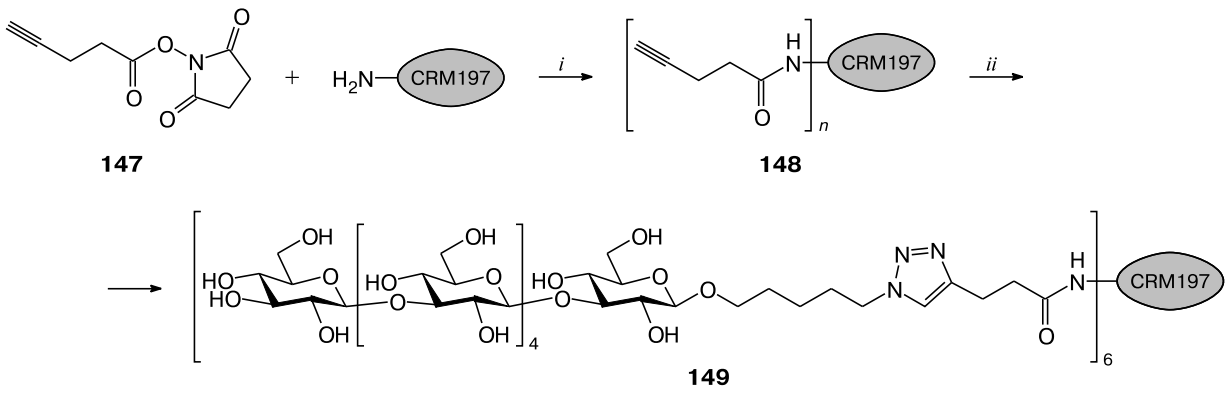


Reagents and conditions: *i*. 0.2 *M* Tris buffer · HCl, pH 7.4; *ii*. **142**, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, tris(3-hydroxypropyltriazolylmethyl)amine, 0.1 *M* phosphate buffer, pH 7.0.

jugation to lysine residues can also produce conjugates with quite definite positions of addition of carbohydrate ligands.

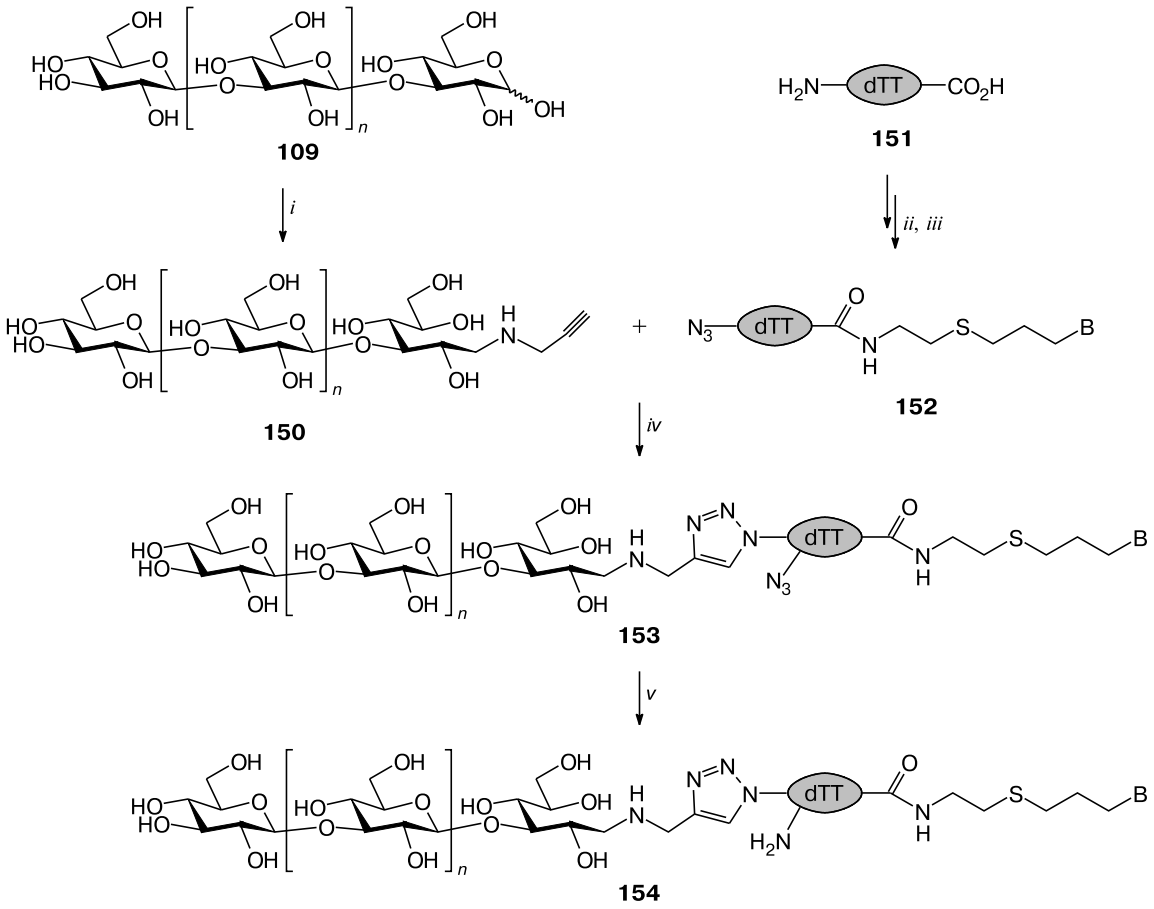
The click reaction was used also for the preparation (Scheme 22) of three-component conjugate based on detoxified tetanus toxoid (dTT) **151**.[36] One of the carbohydrate components was β-(1→2)-D-mannotrioside used for induction of protective antibodies against *C. albicans* whose cell wall contains oligo-β-(1→2)-D-mannoside chains. The second carbohydrate component was β-(1→3)-D-glucan, *viz.*, laminaran (**109**, the β-(1→6)-linked side chains being present in its structure are not shown in Scheme 22). This glucan is the ligand of dectin-1 receptor which is expressed at the surfaces of monocytes, phagocytes, and dendritic cells. In authors´ opinion, the presence of glucan chains should provide a more efficient binding of conjugate to dendritic cells due to interaction with dectin-1 and, as a consequence, a more efficient presentation of the β-(1→2)-D-mannotrioside ligand and subsequent production of homologous antibodies.

Scheme 21



Reagents and conditions: *i*. 0.4 *M* phosphate buffer — DMSO; and *ii*. **142**, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, tris(3-hydroxypropyltriazolylmethyl)amine, 0.1 *M* phosphate buffer, pH 7.0.

Scheme 22



B — β-(1→2)-mannotrioside.

Reagents and conditions: *i*. propargyl amine, $NaBH_3CN$, 0.2 *M* phosphate buffer, pH 6.0; *ii*. imidazol-1-sulfonyl azide hydrochloride, $CuSO_4 \cdot 5H_2O$, 0.5 *M* carbonate-bicarbonate buffer, pH 9.8; *iii*. β-(1→2)-D-mannotrioside-$O(CH_2)_3SCH_2CH_2NH_2$, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride, 100 *mM* 4-methylmorpholine, pH > 8.0; *iv*. bathophenanthroline—Cu^+, 0.2 *M* Tris buffer · HCl, pH 8.0; and *v*. Me_3P, 0.5 *M* sodium carbonate—THF.

Laminarin **109** underwent reductive amination with propargyl amine to form the modified polysaccharide **150** containing the acetylene fragment required for the subsequent click reaction. The amino groups of lysine in the dTT protein was replaced with the azide ones by the diazo-transfer reaction with imidazolyl-1-sulfonyl azide. Then, the β-(1→2)-D-mannotrioside derivative containing the amino group in aglycon was conjugated (step *iii* in Scheme 22) with the carboxyl groups of glutamic acid and aspartic acid residues of the protein. Conjugate **152** obtained in such way underwent click reaction with the modified laminaran **150** to result in the formation of **153**. At the final step, unreacted azide groups were reduced to yield the target three-component conjugate **154** containing ~20 trimannoside chains and two-three glucan chains per one protein molecule. The data on the bioactivity of this product are considered in Section 5 of the present review.

4. Study of bioactivities of synthetic β-(1→3)-D-glucooligosaccharides

β-(1→3)-D-Glucans belong to the group of evolutionary conserved macromolecules called pathogen-associated molecular patterns (PAMPs), which are components of the cell surface of pathogenic microorganisms not occurring in mammals. For this reason, PAMPs are the most important structures which are recognized by the innate immune system.[37] During evolution, the immune system have produced the so called pattern recognition receptors (PRRs),whose interaction with PAMPs is the first step in the response of innate immune system to microbial invasion.

Dectin-1 was shown to be the most important PRR recognizing β-(1→3)-D-glucans.[38] This receptor is expressed at the surfaces of monocytes, neutrophiles, macrophages, dendritic cells, and some T-lymphocytes and me-

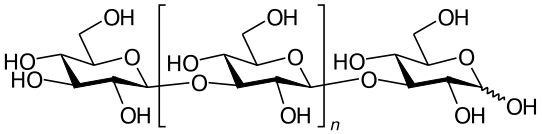
diate phagocytosis and secretion of inflammation mediators being involved in the development of immune response to mycotic pathogens. For this reason, synthetic β-(1→3)-D-glucooligosaccharides were used to determine the structure of epitope in the composition of β-(1→3)-D-glucans, which is responsible for specific binding to dectin-1.

For example, the capability of synthetic linear and branched β-(1→3)-D-glucooligosaccharides[18] **109** and **155** to inhibit the interaction between the murine recombinant dectin-1 and the biosensor surface-immobilized sclero-glucan, *viz.*, branched β-(1→3)-D-glucan from the *Sclerotium rolfsii* fungus with a molecular weight of ~1000 kDa, was studied in Ref. 39 by surface plasmon resonance.

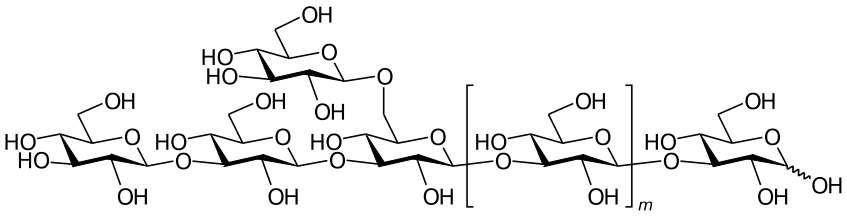
The strength of oligomer binding to dectin-1 depends on the length of oligosaccharide chain and the presence of β-(1→6)-branches. The linear oligomers **109** containing from seven to nine monosaccharide residues (n = 5—7) interacted weakly with dectin-1 (the half maximal inhibitory concentration IC_{50} was in the range of 1.3—2.6 mmol L^{-1}). The linear decasaccharide **109** (n = 8) exhibited a slightly higher affinity (IC_{50} 0.7 *mM*). Introduction of β-(1→6)-branch into the oligosaccharide molecule resulted in a significant increase in the affinity for dectin-1. For example, the branched decasaccharide **155** (m = 5) was found to be 25-fold more active inhibitor (IC_{50} 29 μmol L^{-1}) than the linear decasaccharide **109** (n = 8) and almost by two orders of magnitude more active than nonasaccharide **109** (n = 7). For comparison, laminaran (low-molecular-weight polysaccharide consisting of ~30 glucose residues and containing only one branch per ~10 glucose residues in the main chain) has IC_{50} = 22 nmol L^{-1}.

Inhibition of the interaction between the soluble dectin-1 form and schizophyllan, β-(1→3)-D-glucan from *Schizophyllum commune* with a molecular weight of ~450 kDa bringing β-(1→6)-branches on each third glucose residue of the main chain, was studied by enzyme-linked immunoassay.[21] The linear 16-mer **98** (see Scheme 11) and branched 17-mer **156** (m = 12) were found to inhibit the dectin—schizophyllan interaction to the same extent, their inhibiting ability being only about 10-fold lower than that of schizophyllan taken alone. The shorter linear oligomers **96** and **97** as the branched ones **156** (m = 4, 8) were significantly weaker inhibitors.

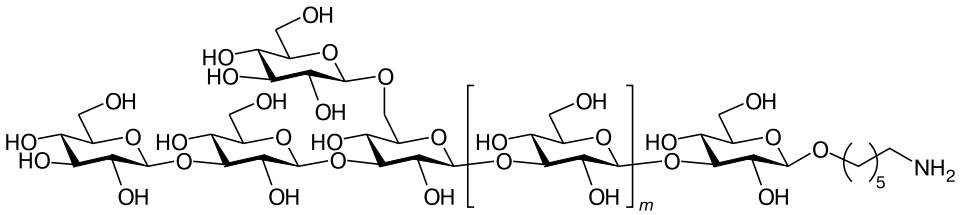
In the same Ref. 21 and later study,[40] the interaction between the linear 16-mer **98** and the lectin-like C-type domain of dectin-1 was studied by saturation transfer difference (STD) NMR spectroscopy. The oligosaccharide was shown to link with the protein through its hydrophobic α-side, so that mainly the H(1) and H(3) protons of the glucose residues within the oligosaccharide chain get involved in interaction. The conclusion of that the glucan chain—dectin interaction has a hydrophobic nature was confirmed using STD also by the example of laminaran in Ref. 41. The same works also showed that the linear hexasaccharide **69** (see Scheme 9) does not interact with the receptor. Thus, the use of synthetic oligosaccharides allowed one to ascertain that, for efficient recognition with dectin-1, β-(1→3)-D-glucooligosaccharide must contain at least 12—13 monosaccharide residues.



109 (n = 5—8)



155



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However, the mechanism of β-(1→3)-D-glucan bioactivity mediated by the interaction with dectin-1 is obviously not the only possible one,[41] which follows from the presence of immunostimullatory activity in short β-(1→3)-D-oligoglucosides incapable of being recognized with dectin-1 and binding thereto. The immunostimulatory properties of pentasaccharide **42** (see Scheme 6) compared to phycarine (commercial name of laminaran) were studied in Ref. 14. Upon intraperitoneal injection, the reducing pentasaccharide **42** and phycarine stimulate the increase in the blood levels of monocytes and granulocytes and the abdominal level of macrophages to the comparable extent. In addition, compound **42** demonstrated the ability to stimulate the phagocytic activity of granulocytes and macrophages and secretion of IL-1β cytokines comparable with that of phycarine. The abilities of **42** and phycarine to inhibit the breast tumor growth in mice were also close. The mechanisms of bioactivities of short β-(1→3)-D-glucooligosaccharides have not been clarified yet.

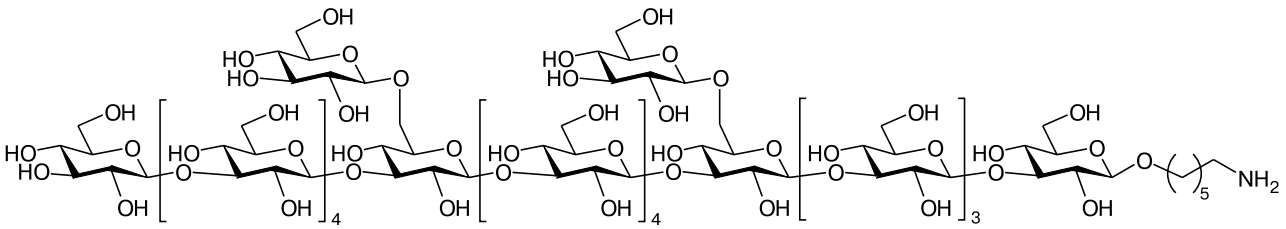
5. Antifungal conjugate vaccines based on natural β-(1→3)-D-glucans and synthetic β-(1→3)-D-glucooligosaccharides

Conjugates of natural β-(1→3)-D-glucans and synthetic β-(1→3)-D-glucooligosaccharides whose preparation has been described in Section 3 are being studied extensively as key components of potential antifungal vaccines. The main prerequisite defining the applicability of carbon-protein conjugate as vaccine is its ability to stimulate the body production of IgG antibodies specific for carbohydrate ligands as a part of conjugate. The above-described conjugates of natural and synthetic glucans **137**, **138**, **139**, **143**, **146**, and **149** (see Schemes 17—21) can induce the production of IgG antibodies specifically recognizing the β-(1→3)-D-glucan chains. The immunological potencies of conjugates depending on the length of carbohydrate ligands, their number in the composition of conjugate (carbohydrate loading), and pattern of binding to carrier protein (random or directed) were studied. For example, conjugate **138** containing a relatively short hexasaccharide ligand, but having a high carbohydrate loading (17 oligosaccharide chains per one molecule of the CRM197 carrier protein) induced a higher titer of antiglucan IgG antibodies than that induced by the laminaran-based conjugate **137** (~30 glucose residues) containing on the average only 7.5 glucan chains.[16] At the same time, the conjugate of partially hydrolyzed curdlan (~25 glucose residues) **137** (the number of glucan chains in the conjugate is not specified) was found to be more immunogenic than conjugate **139** containing 7.5 pentadecasaccharide ligands.[32] These data show that the formation of antiglucan IgG antibodies in comparable amounts can be induced by conjugates with different content of carbohydrate component, which allows no unambiguous conclusion of that the immunogenicity of preparations depend on the length of conjugated oligosaccharide fragments or the number of these fragments in the conjugate composition.

In Ref. 34, the immunogenicity of conjugates **137** (based on laminaran) and **138** (based on synthetic hexasaccharide) obtained by random conjugation to the lysine residues of carrier protein were compared with those of conjugates **143**, **146**, and **149** where the hexasaccharide ligands bind to certain tyrosine or lysine residues. The abilities of conjugates to induce the formation of antiglucan IgG antibodies varied in the order as follows: **138** > **143** ≈ **146** > **149** ≈ **137**, and the authors concluded that conjugation to tyrosine residues results in more immunogenic derivatives compared to conjugation to lysine residues. However, introduction of two oligosaccharide chains through one tyrosine residue (conjugate **146**) did not increase the immonological potency. Besides the difference in the immunological potency, conjugates **137**, **138**, **143**, **146**, and **149** differed in the ability to invoke antibodies which inhibit the adhesion of *C. albicans* cells to human epithelial cells. The highest inhibiting activity was exhibited by the serum obtained upon immunization with conjugate **149**.

The animal models[31,32] showed that conjugates **137** (based on laminaran and partially hydrolyzed curdlan) and **139** have protective activities, *i.e.* the ability to decrease the mortality rate upon infection with a lethal dose of infectious agent. If the infection with the lethal dose of *C. albicans* was preceded by immunization with the laminaran-based conjugate **137**, about 80% of laboratory animals survived, whereas the mortality rate in the control group reached 90%. The laminaran-based conjugate **137** also possessed protective activity against other fungal pathogen, *viz.*, *A. fumigatus*. The protective activity seems to be mediated by antiglucan antibodies, since only the whole immune serum and isolated fraction of IgG antibodies, as well as monoclonal IgG2b anbibodies specific for β-(1→3)-D-glucan possessed protective properties upon passive immunization with conjugate **137**.

The immune serum obtained upon immunization of with conjugate **137** can also inhibit the growth of *C. albicans* and *A. fumigatus*.[31] The protective activities of conjugate **137** based on curdlan and conjugate **139** based on synthetic pentadecasaccharide were close to that of laminaran-based conjugate **137**.[32] It should be noted that the conjugate of branched synthetic heptadecasaccharide **157**[19] containing β-(1→6)-branches in the fifth and tenth glucose residues possessed no protective properties.[32] At first sight, this result is in contradiction with a high protective activity of laminaran-based conjugate **137** which also contains β-(1→6)-branches. However, it was assumed[42] based on the study of anti-glucan monoclonal antibodies that the protective epitope is a linear β-(1→3)-glucose sequence containing no β-(1→6)-branches and consisting of eight glucose residues. It is obvious that oligosaccharide **157**



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contains no such sequence, while such sequence is present in laminaran where there are on the average ~10 glucose residues of the main chain per one branch.

The three-component conjugate **154** (see Scheme 22) in contrast to the conjugate containing only β-(1→2)-D-mannotrioside ligands can stimulate immature dendritic cells and increase secretion of some cytokines, including TGF-β and IL-6, which are activators of Th17 cells. Immunization of mice with conjugate **154** induced the formation of β-(1→2)-mannotrioside ligand-specific antibodies with titers being 5-10-fold higher than those upon immunization with the conjugate containing no glucan chains.[36] Thus, all of this confirms the author assumption of that introduction of the glucan ligands, which can interact with the dectin-1-bringing immune cells and activate them, into the composition of vaccinal conjugates causes a stronger immune response.

The results under consideration suggest the validity of assumption of that, since β-(1→3)-D-glucan is a component of cell walls of all clinically imporatnt mycotic pathogens, conjugates based on synthetic β-(1→3)-D-glucooligosaccharides are of great practical promise for application as pluripotential antifungal vaccines. This additionally shows why the preparation of oligosaccharide derivatives being related to linear and branched β-(1→3)-D-glucan chains are of great interest among synthetic chemists.

The data considered in this review show that the current methods and approaches allow efficient synthesis of quite large β-(1→3)-D-glucooligosaccharide derivatives comparable in length with short naturally occuring β-(1→3)-D-glucans, for example, laminaran. This creates a reliable base for the preparation of various model oligosaccharides required for the mechanistic study of immunostimulatory activities of natural β-(1→3)-D-glucans and ligands for the design of conjugated antifungal vaccines. Yet another promising field of application of β-(1→3)-D-glucooligosaccharide ligands and their protein conjugates is their use as components of clinical and ecological diagnostic preparations for detection of β-(1→3)-D-glucans and homologous antibodies.[43–48]

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