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Linear and cyclic oligo-β-(1 → 6)-D-glucosamines: Synthesis, conformations, and applications for design of a vaccine and oligodentate glycoconjugates*

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Abstract: Poly-β-(1 → 6)-*N*-acetyl-D-glucosamine is an exopolysaccharide secreted by numerous pathogenic bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Yersinia pestis*, *Bordetella pertussis*, *Acinetobacter baumannii*, *Burkholderia* spp., and others. A convergent approach was developed for the synthesis of oligosaccharide fragments consisting of 5, 7, 9, and 11 glucosamine or *N*-acetylglucosamine units and for the preparation of five nona-β-(1 → 6)-D-glucosamines with various *N*-acetylation patterns. Penta- and nona-β-(1 → 6)-D-glucosamines conjugated to protein carriers through a specially developed sulfhydryl linker proved to be highly immunogenic in mice and rabbits and elicited antibodies that mediated opsonic killing of multiple strains of *S. aureus* (including methicillin-resistant *S. aureus*, MRSA) and *E. coli*, and protected against *S. aureus* skin abscesses and lethal *E. coli* and *B. cenocepacia* peritonitis. These findings provide a basis for the construction of a unique semisynthetic vaccine against multiple bacterial targets.

Conformational studies by means of special NMR experiments and computer modeling revealed that the oligo-β-(1 → 6)-D-glucosamine chain exists mostly in a helix-like conformation, where the terminal monosaccharides are arranged close to each other. Owing to

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this feature, oligoglucosamines consisting of 2 to 7 residues easily form products of cycloglycosylation. Cyclooligo-β-(1 → 6)-D-glucosamines represent a new family of functionalized cyclic oligosaccharides. Owing to their molecular architectonics, these compounds are convenient scaffolds for the design of conjugates with defined valency, symmetry, flexibility, and function.

Keywords: carbohydrates; conformation; glycoconjugates; cyclooligosaccharides; vaccines.

INTRODUCTION

Poly-*N*-acetylglucosamine (PNAG), a β-(1 → 6)-linked polymer of *N*-acetyl-D-glucosamine (GlcNAc) [1] with some proportion of the amino groups lacking *N*-acetyl substituents, is a surface polysaccharide produced by a broad range of common pathogens and may be considered a promising target for vaccine development. Among important bacterial pathogens, PNAG is known to be produced by *Staphylococcus aureus* and *S. epidermidis* [2–4], *Escherichia coli* [5,6], *Bordetella pertussis* and *B. parapertussis* [7,8], *Aggregatibacter actinomycetemcomitans* [9], *Acinetobacter* spp. [10], *Burkholderia* spp. [11], and *Yersinia pestis* [12,13]. It was shown that antibodies to PNAG conjugated to a protein carrier can mediate in vitro opsonic killing and protect mice from *S. aureus* [2,4], *B. cenocepacia* [11], and *E. coli* [14] infections, but such immunity can only be engendered by first removing the majority of the *N*-acetyl groups from the PNAG polymer to produce *N*-deacetylated PNAG (dPNAG).

While dPNAG conjugate vaccines appear effective in animal studies for providing protective immunity, the lack of a defined chemical composition of this material and the need to produce it by chemical deacetylation of highly acetylated native PNAG resulting in variability in the final composition, limit the conclusions that can be drawn about optimal vaccine formulation. Native PNAG (consisting of 60–90 % GlcNAc units) has a certain amount of deacetylated glucosamine units (GlcN) but whether they are grouped together or interspersed throughout the molecule is not known. It is not established if preparations of native PNAG and dPNAG are either homogeneous or consist of a mixture of different molecules with various degrees of acetylation. To develop optimal vaccines that generate protective antibodies, the relative amounts of GlcN units and their spacing need to be determined, and these are not possible by chemical deacetylation due to random conversion of GlcNAc units to GlcN ones. Therefore, synthetic oligoglucosamines with glucosamine units bearing *N*-acetylated and free amino groups in definite positions are needed to determine the structure of active epitopes.

SYNTHESIS OF LINEAR OLIGO-β-(1 → 6)-D-GLUCOSAMINES

As a first step, we synthesized oligoglucosamines with either all *N*-unprotected or *N*-acetylated glucosamine residues. The first approach to the synthesis of these structures used the reaction of terminated oligomerization, which proved to be effective for assembly of additional oligo- and polysaccharide [15,16] fragments. However, the results of our experiments revealed a low efficiency of this method for the preparation of oligo-β-(1 → 6)-glucosamines [17]. In contrast, it was observed that bifunctional di- and tri-β-(1 → 6)-glucosamine blocks displayed a strong tendency to the formation of cyclic oligosaccharides. For example, an attempt at oligomerization of disaccharide **1** in the presence of terminating alcohol **2** led almost exclusively to cyclic product **3** instead of linear oligomers **4** (Fig. 1). Therefore, block-wise assembly of oligosaccharide chains proved to be a more reliable and scalable approach for the synthesis of linear oligo-β-(1 → 6)-glucosamines.

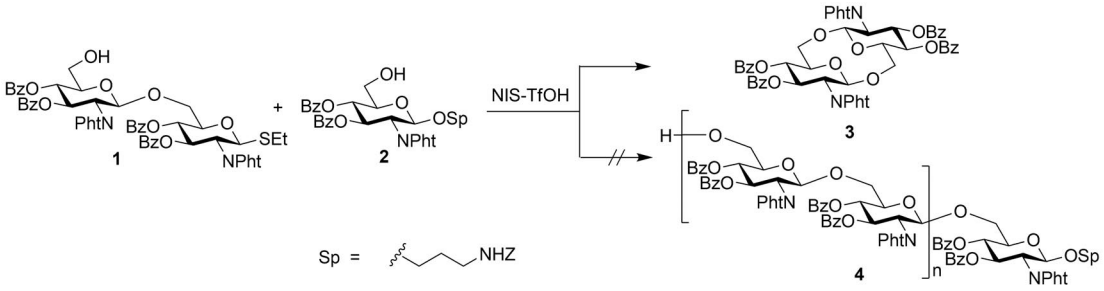

Fig. 1 Intramolecular cyclization vs. linear oligomerization.

To determine the length of oligosaccharide ligands sufficient for effective binding to antibodies to PNAG, a representative series of oligoglucosamines with a different number of glucosamine units was needed, therefore, oligoglucosamines with 5, 7, 9, and 11 monosaccharide residues both containing unsubstituted and *N*-acetylated amino groups were selected as the target structures. The protected precursors of the target oligosaccharides were synthesized as *Z*-amidopropyl glycosides suitable for further functionalization and glycoconjugate design. For their synthesis, we employed a convergent approach based on the use of a minimal number of relatively large building blocks. This approach is illustrated by the synthesis of pentamer **10** and nonamer **12** (Fig. 2) [18].

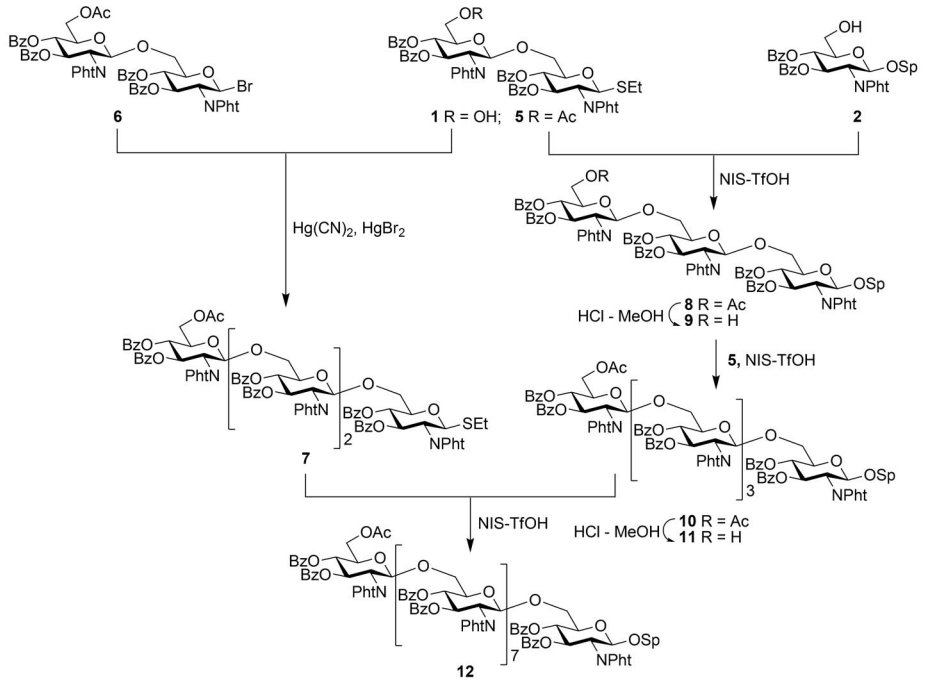

Fig. 2 Assembly of protected penta- and nonasaccharides.

Mono- and disaccharide blocks **1**, **2**, **5**, and **6** were used as starting compounds. Coupling of **2** with thioglycoside **5** afforded, after 6-*O*-deacetylation, trisaccharide acceptor **9**, and glycosylation of **1** with bromide **6** provided tetrasaccharide donor **7**. Further elongation of **9** with **5** gave the first of the target oligomers, pentasaccharide **10**, which yielded upon deacetylation pentasaccharide acceptor **11**.

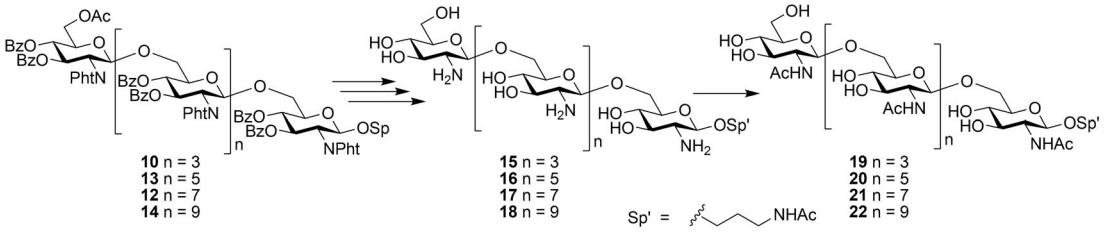

Fig. 3 Synthesis of free and completely *N*-acetylated nona-β-(1 → 6)-D-glucosamines.

Final coupling of **11** with **7** afforded desired nonamer **12**. Hepta- and undecasaccharides **13** and **14** were prepared similarly. Replacement of the *Z* group in the aglycon of protected precursors **10**, **12–14** by acetyl one (Fig. 3) followed by total *N*,*O*-deprotection in glucosamine units produced non-*N*-acetylated oligomers **15–18**. A series of fully *N*-acetylated compounds **19–22** was obtained after subsequent *N*-acetylation.

In order to ascertain if a combination of GlcN and GlcNAc units may form a protective epitope on dPNAG, a series of five nonasaccharides **23–28** containing two GlcNAc units separated by 1–5 GlcN residues was prepared (Fig. 4) [19]. The degree of *N*-acetylation in this case is about 20 %, and theoretically these synthetic nonasaccharides may represent specific fragments of dPNAG.

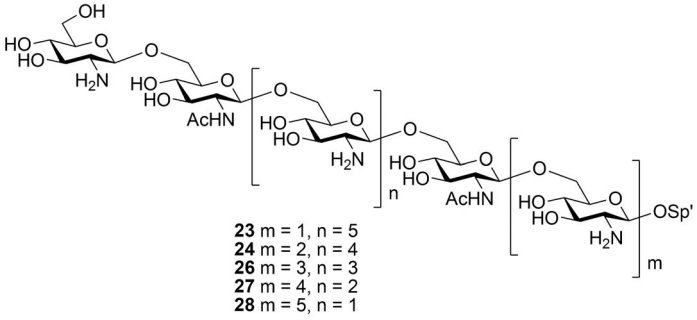

Fig. 4 Structures of partially *N*-acetylated nona-β-(1 → 6)-D-glucosamines.

A prerequisite for the successful synthesis of nonasaccharides **23–28** was the proper choice of a precursor for *N*-acetylglucosamine units ("pre-NAc-block"). The pre-NAc-block has to be readily available, effective as a β-stereoselective glycosyl donor, and easily convertible to the corresponding *N*-acetyl derivative. To meet these requirements, the derivative of *N*-acetylglucosamine itself was chosen as the pre-NAc block. The main advantage of this approach was the possibility of avoiding the steps of selective deprotection and further *N*-acetylation. Based on the successful synthesis of the homo-oligosaccharide ligands, we planned to use again *N*-phthaloyl groups for pre-NH_2 blocks, and an *N*-benzyloxycarbonyl group for the spacer derived from 3-aminopropanol. Synthesis of building blocks containing *N*-acetylated glucosamine residues is presented in Fig. 5.

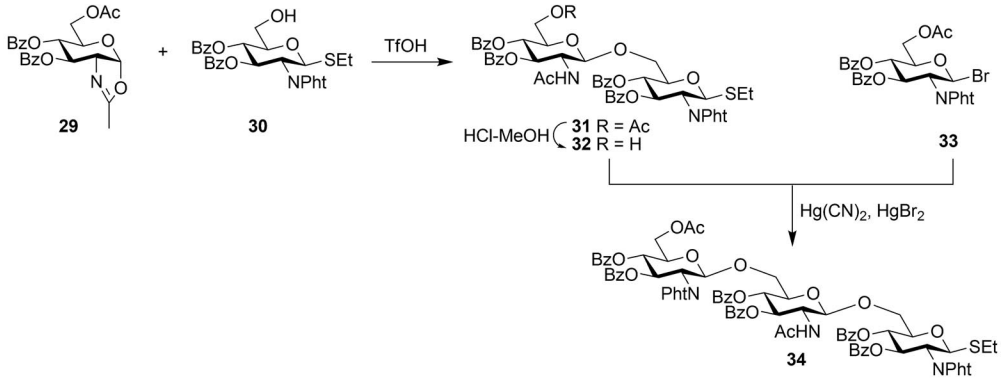

Fig. 5 Synthesis of di- and trisaccharide building blocks containing *N*-acetylglucosamine residues.

Oxazoline **29** was employed as a glycosyl donor for introduction of *N*-acetylglucosamine into the oligosaccharide chain: triflic acid-catalyzed coupling of **29** with thioglycoside acceptor **30** provided disaccharide **31**, the first of two *N*-acetylated synthetic blocks used for the assembly of the target nonasaccharides. Selective *O*-deacetylation of **31** followed by glycosylation of obtained **32** with bromide **33** afforded the second synthetic block, trisaccharide **34**. The assembly of partially *N*-acetylated nonasaccharides exemplified by the synthesis of nonasaccharide **23** is depicted in Fig. 6.

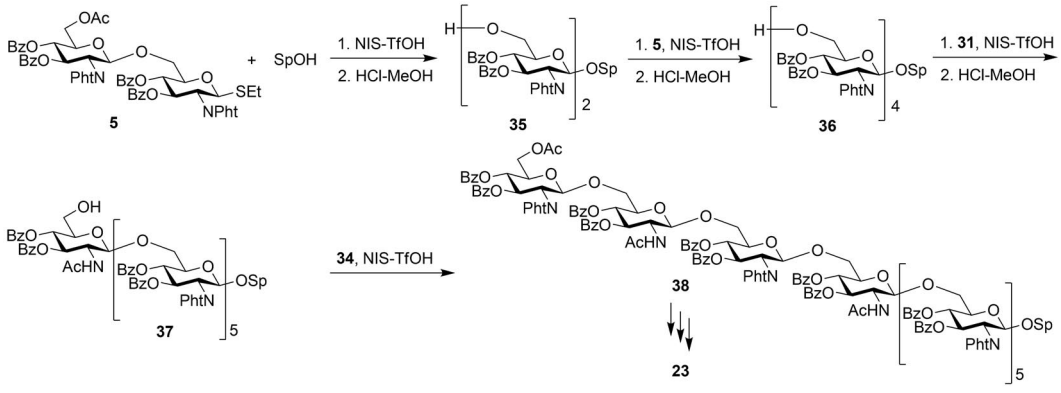

Fig. 6 An example of the assembly of partially *N*-acetylated nonasaccharide.

Z-Protected 3-aminopropanol SpOH was glycosylated first with disaccharide thioglycoside **5** to give, after 6-*O*-deacetylation, acceptor **35**. Its repeated glycosylation with **5** followed by *O*-deacetylation afforded tetrasaccharide acceptor **36**. Further elongation of the oligosaccharide chain was carried out using *N*-acetylated disaccharide **31**; coupling of **36** with **31** and subsequent removal of the acetyl group furnished hexasaccharide **37**. At the final step of the synthesis, glycosylation of **37** with thioglycoside **34** afforded target nonamer **38** containing two *N*-acetylglucosamine units. Modification of the aglycon and removal of *N*-Phth and *O*-protecting groups gave nonasaccharide **23**. The syntheses of compounds **24–28** were implemented similarly and involved at the final steps "3 + 6"-glycosylation with trisaccharide **34**.

PREPARATION AND EVALUATION OF VACCINE CONJUGATES

To define more precisely the immune response elicited to different epitopes on the PNAG molecule, oligoglucosamines containing either 5 or 9 fully acetylated (5GlcNAc and 9GlcNAc) or fully non-acetylated (5GlcNH$_2$ and 9GlcNH$_2$) monosaccharides were conjugated to a protein carrier (tetanus toxoid, TT) and used to immunize mice and rabbits. Only the synthesis of nonasaccharide conjugates is described below in detail, as the main immunological results were obtained with these vaccine preparations.

The *N*-protecting group in the spacer arm of nonasaccharide **12** was selectively removed, and a thiol linker was introduced as shown in Fig. 7 [20a]. A new linker molecule **39** based on 4-mercaptobutanoic acid was employed because the conventional linker derived from thioglycolic acid underwent some side reactions on further deprotection of the nonasaccharide ligand. After complete deprotection of **40**, ligand **41**, which existed mainly as the disulfide, was obtained. The latter arose upon spontaneous oxidation of the SH-derivative with atmospheric oxygen. Suitable reducing agents were used for reliable exposure of sulfhydryl group for further derivatization of **41**. Thus, with the use of dithiothreitol and acetic anhydride, *N*- and *S*-acetylated derivative **42** was produced.

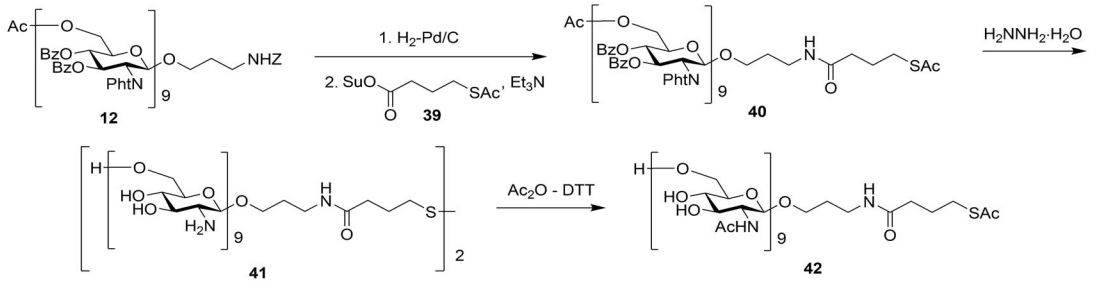

Fig. 7 Conversion of protected nonasaccharide **12** into ligands for conjugation with a protein carrier.

Conjugates of the resultant nonasaccharide ligands with TT were prepared as depicted in Fig. 8. Compounds **41** and **42** were converted into sulfhydryl derivatives **43** and **44**, respectively. Carrier protein was activated by reaction with *N*-hydroxysuccinimidyl 3-(bromoacetamido)propionate **45**, and

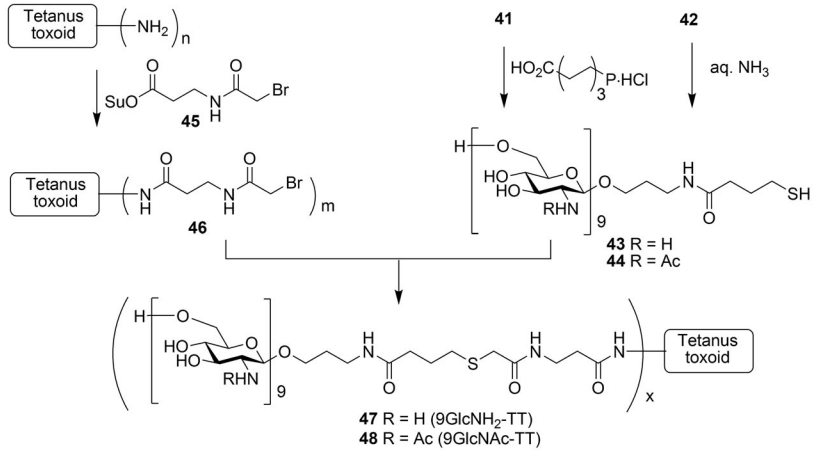

Fig. 8 Preparation of vaccine conjugates.

resulting **46** was incubated with carbohydrate ligands **43** and **44**. As a result, conjugates **47** and **48**, containing 74 and 71 nonasaccharides respectively, were obtained.

The conjugates were used to immunize mice. Mice immunized with a 10-μg dose, based on carbohydrate content, of 9GlcNH$_2$-TT **47** made a robust IgG response that bound to both native PNAG and dPNAG. Lower doses of the 9GlcNH$_2$-TT vaccine gave lesser immune responses. Mice immunized with the 9GlcNAc-TT conjugate **48** made an excellent response to the native PNAG molecule. However, no binding of antibody to dPNAG was detected in the sera of mice immunized with the 9GlcNAc-TT conjugate. No immune response to either PNAG or dPNAG was detected in sera of mice immunized with a mix of TT and non-conjugated nonasaccharides, either *N*-acetylated or NH$_2$-free. When mouse sera were tested for opsonic killing activity in the presence of human polymorphonuclear neutrophils (PMN) and rabbit complement (C), the animals immunized with the 9GlcNH$_2$-TT conjugate **47** clearly had opsonically active antibody that mediated killing of *S. aureus* CP8 strain MN8, Newman (CP5), and a USA 300. The antibody raised to the 9GlcNAc-TT conjugate **48** had no killing activity. Overall, the synthetic oligosaccharides showed that the 9GlcNH$_2$-TT conjugate **47** elicited the best opsonic killing activity with specificity for epitopes on the homologous immunizing oligosaccharide antigen.

As the non-acetylated glycoform induced the desired opsonic killing activity in mice, conjugate **47** was used to immunize rabbits and test binding, opsonic killing, and protective activities of antibodies. Antibody obtained also bound to native PNAG and dPNAG isolated from *S. aureus*, as well as to the immunizing oligosaccharide **17**, but failed to bind to the fully acetylated oligosaccharide **21**. Antibodies in post-immunization rabbit antisera mediated dose-dependent opsonic killing of a variety of *S. aureus* strains, with different capsular polysaccharide (CP) types as well as to two USA 300 MRSA strains of *S. aureus* lacking CP antigens (strains LAC and SF8300). Similarly, these antibodies mediated opsonic killing of two *E. coli* strains previously shown to produce PNAG, but not a third strain lacking the *pga* genes encoding the biosynthetic enzymes for PNAG in *E. coli* [14].

In a murine model of skin abscesses, injection of 200 μl of immune sera 24 h prior to infection resulted in highly significant reductions in the bacterial cfu/abscess produced by three different infectious doses of *S. aureus* MRSA strain LAC when compared with animals given the normal rabbit serum (NRS). Similarly, the antiserum also significantly reduced the cfu/abscess formed by CP5 strain Newman and CP8 strain MN8. When tested against strains Newman and MN8 unable to make PNAG due to deletion of the *ica* biosynthetic locus there was no significant ($P > 0.05$) protective efficacy [20].

The protective efficacy of antibody to **47** was also tested in a lethal peritonitis model of *E. coli* infection. This antibody protected all immunized mice against infection caused by two PNAG-positive *E. coli* isolates whereas all controls receiving NRS did not survive. There was no protection afforded by antibody to 9GlcNH$_2$-TT antiserum against PNAG-negative *E. coli* strain. These results suggest a potential for vaccination against PNAG for *E. coli* infections, although further studies are warranted [20a].

Our findings indicate that relatively short oligo-β-(1 → 6)-glucosamines conjugated to a carrier protein can induce high titers of opsonic antibody that is also protective against experimental *S. aureus* skin infection and lethal peritonitis due to *E. coli*. If this antibody is truly protective against the range of pathogens [20b] producing PNAG as a surface molecule, then this material may be used as a component of vaccines for humans as long as it is immunogenic and safe. Currently, further preclinical evaluation of this vaccine's protective efficacy against various PNAG-producing pathogens is ongoing to validate the utility of eventual human trials of such a preparation.

SYNTHESIS OF CYCLIC OLIGO-β-(1 → 6)-D-GLUCOSAMINES

As mentioned above, activation of bifunctional disaccharide precursor **1** (Fig. 1) resulted in the almost quantitative formation of cyclic product **3**, while no formation of linear oligomers was surprisingly detected. This cycle represented a new type of cyclo-oligosaccharides and gave rise to a new investigation.

Despite a considerable research activity in the field of synthetic cyclic oligosaccharides, there have been no examples to date of those with D-glucosamine units and only a few discrete examples of cyclic oligosaccharides with (1 → 6)-glycoside linkages [21–24]. We have studied cyclization of oligo-β-(1 → 6)-D-glucosamine derivatives **49–53** containing up to 7 monosaccharide units. Regardless of the length of the linear precursor, NIS-TfOH-promoted activation of **49–53** afforded corresponding cyclic products **54–58** in good yields (Fig. 9) [25]. Practically no products of linear oligomerization were detected even at concentrations up to 10 times higher than those described for the cyclization of other linear oligosaccharides [21–24]. The strong predominance of intramolecular glycosylation over linear chain elongation may be explained by the conformational preorganization of the precursors in a shape providing spatial proximity of the terminal glycosyl donor and acceptor moieties. It was noticeable that, unlike cyclization of di- and trisaccharide precursors **1** and **49**, intramolecular glycosylation of compounds **50–53** resulted in the formation of minor amounts of (1 → 6)-α-linked cycles **55α–58α** along with expected symmetric cycles **55β–58β** as major products.

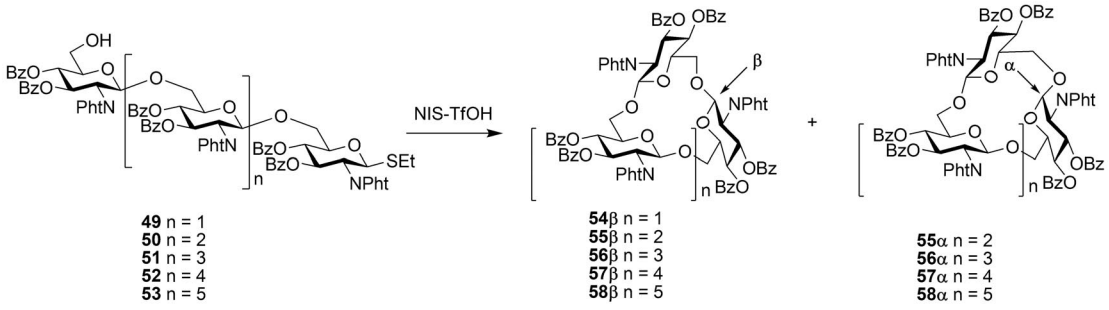

Fig. 9 Cyclization of linear bifunctional precursors **49–53**.

The formation of the α-products upon cyclization of bifunctional blocks **50–53** was unexpected because they contain a participating *N*-phthaloyl group which provides stereospecific β-glycosylation, especially if a primary OH group is involved. We hypothesized that the α-linked cycles are formed as a result of conformational features of the linear precursors, which were illustrated by the example of tetrasaccharide **50**. The data of computer modeling and conformational analysis in solution by means of special NMR techniques revealed the prevalence of a helical shape. Hence, compound **50** in its preferential conformation represents one turn of a right-handed spiral (Fig. 10), in which C-1 of the glycosylating unit A and 6-OH of the glycosyl acceptor residue D are spatially prearranged in a way favoring α-glycosylation. Presumably, a competition between the conformational α-stereocontrol and β-stereocontrol by the participating *N*-phthaloyl group determined the stereochemical outcome of cyclization.

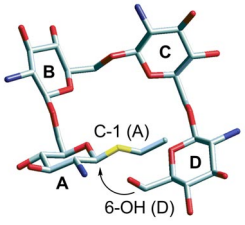

Fig. 10 Preferential conformation of linear precursor **50** in wire frame representation.

The formation of α-linked cycles **55α–58α** can be regarded as a specific case of "tethered" glycosylation. Currently, the influence of several factors such as reaction conditions, nature of the leaving group at anomeric center and substituent at C-2 of glycosylating unit on stereochemical outcome of cyclization of linear tetrasaccharide is under investigation. It was found that substitution of *N*-phthaloyl group in glycosylating unit by *O*-benzoyl group (substitution of D-glucosamine residue by D-glucose) leads to the formation of exclusively β-product of cycloglycosylation (no formation of α-cycle was detected) [26]. It is noteworthy that cyclic tetrasaccharide **55β** is characterized by unusually low mobility on silica gel (thin layer chromatography, TLC, and column chromatography) as compared to the linear tetrasaccharide precursor **50**. For instance, starting thioglycoside **50** has Rf of 0.5 (toluene/ethylacetate 3:1) while cycle **55β** has Rf of only 0.15. However, when protonic solvent is used for elution (e.g., toluene/methanol 3:1) the mobility of **50** and **55β** is identical (Rf 0.5). We assumed that hydroxyl groups on silica gel surface may penetrate through the inner space of tetrasaccharide cycle providing additional retention which is prevented by the use of methanol. Indeed, computer modeling confirmed that the hole of the cycle **55β** is large enough for a water molecule to pass through.

The one-step deprotection of cyclic oligosaccharides by hydrazinolysis provided a series of free amines **59–63**, which were isolated as acetate salts. Further *N*-acetylation resulted in the formation of corresponding *N*-acetylated derivatives **64–68** (Fig. 11).

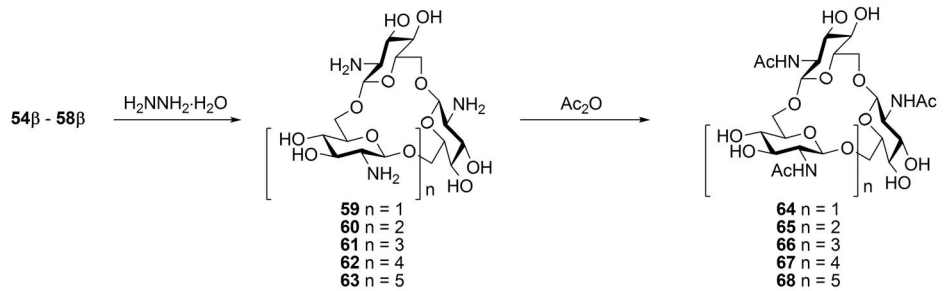

Fig. 11 Conversion of protected cycles into free (**59–63**) and *N*-acetylated (**64–68**) cyclic oligo-(1 → 6)-β-D-glucosamines.

CONFORMATIONAL STUDIES OF β-(1 → 6)-D-GLUCOSAMINES

The conformational investigation of the synthesized linear and cyclic oligosaccharides was performed by means of NMR and computer modeling techniques [27]. They included transglycosidic C–H spin–spin coupling constants ($^3J_{C,H}$) measurement and molecular dynamics (MD) calculations under different types of conditions varying in solvent consideration and temperature.

Analysis of the experimental $^3J_{C,H}$ values measured for linear oligoglucosamines revealed that they did not depend neither upon the oligosaccharide chain length nor the position of a linkage in the chain both in case of acetylated and non-acetylated compounds. On the contrary, the experimental coupling constants for small cycles changed significantly upon the enlargement of the molecules, and only for the cycles with five and more monosaccharide residues they behaved like $^3J_{C,H}$ in the linear structures and had similar absolute values. This fact along with the observation that the NMR spectra of the larger cycles had the same pattern as those for the internal units in the large linear oligoglucosamines led to the conclusion that the conformations of glycosidic linkages and monosaccharide units in the cyclic structures larger than pentasaccharides are close to those of the linear ones.

Theoretical simulation of cyclic structures using MD revealed that di-, tri-, and tetrasaccharides existed mostly in symmetrical ring-shaped conformations with very low degree of flexibility. Larger cycles tended to adopt more complicated shapes. After geometry optimizations of the structures from the MD trajectories, global minima were found for each cyclic molecule. The calculation of the

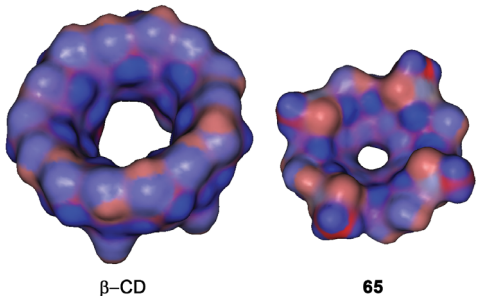

Fig. 12 Distribution of the hydrophilic (red) and hydrophobic (blue) areas in molecules of β-CD (outer diameter is 1.44 nm) and tetrasaccharide **65** (outer diameter is 1.16 nm).

hydrophobicity and hydrophilicity distribution exemplified by tetramer **65** (Fig. 12) showed that, unlike the well-known cyclodextrins (CDs), cyclo-oligo-(1 → 6)-β-D-glucosamines lack the distinct hydrophobic cavity.

All the linear structures exhibited rather high flexibility during MD simulations. Various clustering methods were employed for the conformational analysis of the three bonds that composed each linkage. Four conformers (**I–IV**) were found to be characteristic for these compounds, which are shown in Fig. 13.

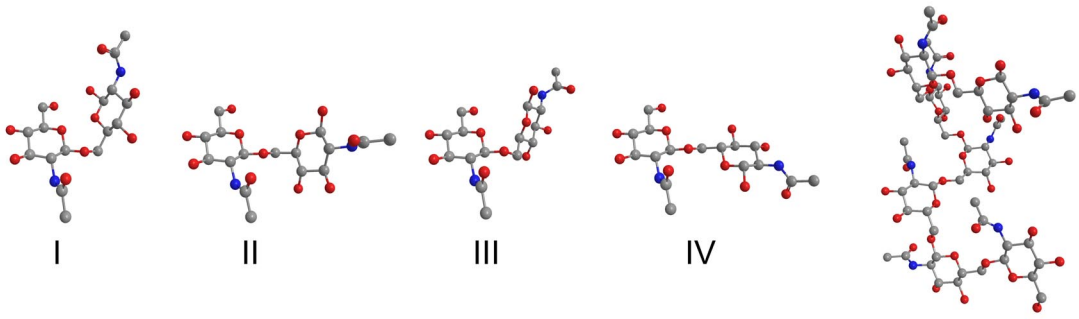

Fig. 13 Four main conformations of (1 → 6)-β-linkage in the studied saccharides and the energy minimum conformational shape of heptaglucosamine **16** (right structure).

The molecules constructed from the preferable conformations of glycosidic linkages are characterized by twisted shapes with some helical elements both for the acetylated and non-acetylated compounds (Fig. 13). The substituents at C-2 mostly protruded into the outer space and did not encounter any spatial hindrances hence their acetylation did not lead to a considerable conformational change. In addition, this fact could explain the influence of *N*-acetylation pattern on the biological activity of these compounds.

POSSIBLE APPLICATIONS OF CYCLIC OLIGO-β-(1 → 6)-D-GLUCOSAMINES

Lectins are carbohydrate-binding proteins with high specificity for their sugar ligand and play a role in biological recognition events involving cells and proteins. Although the interaction between a carbohydrate and a lectin is strongly specific, the affinity is usually weak with dissociation constants in the millimolar range for monovalent interactions. Therefore, Nature uses the so-called "glycoside cluster effect" to overcome this weak interaction by presenting several oligosaccharide ligands interacting with one or more of their receptor(s) at once. Many approaches have been designed to take advantage of

multivalency including glycoclusters, glycodendrimers, and glycopolymers [28]. These multivalent structures demonstrate a much stronger affinity for the lectin when compared to the monovalent one. Glycoclusters display in a very well predicted geometry a synthetically controlled and exact number of carbohydrate moieties at their surface. Even though it is now well established that valency increases the affinity of a multivalent ligand to a lectin [28], it is still obscure whether the geometry can induce selectivity and to what extent. It would be of interest to evaluate the influence of the three-dimensional geometry of glycoclusters on the binding selectivity to lectins. Due to the presence of amino groups, which can be easily subjected to a variety of highly chemoselective modifications, cyclic oligo-(1 → 6)-β-D-glucosamines allow construction of multivalent glycoclusters with defined spatial organization, and, therefore, these structures may help to answer the questions posed above. Modern chemical, spectral, and computer calculation methods permits detail modeling of carbohydrate shape and interaction between carbohydrate-ligand to protein-receptor [29,30] and even predict the structure of carbohydrate binding domain when its parameters are not known yet [31]. Due to the position of amino groups facing outwards almost in the plane of the cycle (Fig. 14, as exemplified by cyclotetraglucosamine **60**) the resultant conjugates have substituents spread nearly horizontally. In contrast, substituted primary alcohol groups of CDs (Fig. 14) provide dense bundle organization. Investigations toward the synthesis of multivalent glycoclusters of type **69** (Fig. 15) and study of their binding to some bacterial lectins are currently in progress [32].

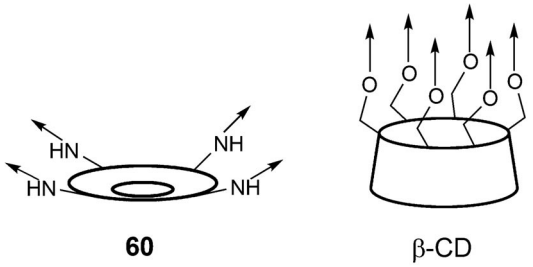

Fig. 14 Schematic representation of orientation of substituents at amino groups in cyclic tetrasaccharide **60** and primary hydroxyl groups of β-CD.

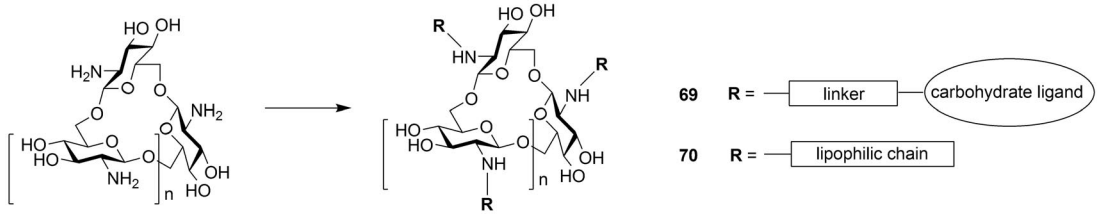

Fig. 15 Application of cyclic oligo-(1 → 6)-β-D-glucosamines for the synthesis of multivalent glycoclusters and artificial ion channels.

Another possible use of cyclic oligo-(1 → 6)-β-D-glucosamines may be construction of artificial ion channels. Ion channel proteins are pervasive in living systems and capable of performing the complex tasks of the passage of ions. This ion-conducting ability synchronized with the ion selectivity is crucial to a wide variety of biological processes, such as signal transduction and cellular regulation. Selectivity of ion channel proteins towards specific ion is very important for their functions, and mutation of important amino acid residues often cause many diseases, e.g., hyper- and hypokalemic periodic paralysis, epilepsy, Alzheimer's disease, Parkinson's disease, schizophrenia, blindness, etc. There has long been interest in elucidating the mechanisms by which channel proteins mediate selective ion trans-

port. Due to the complexity of channel proteins, several research groups have been trying to develop artificial analogues capable of mimicking the structures and functions of natural ones. Creating ion channel forming molecules with efficient non-gated/gated ion transporting ability and high selectivity remains an active area of research.

It is known that the β-CD-based monomolecular ion channel [33] resembles a Hofmeister anion selectivity sequence [34]. However, synthesis of cyclic oligosaccharides with either larger or smaller rings is often challenging, thereby modulation of ion selectivity by varying the oligosaccharide ring size has not been explored. Therefore, cyclic oligo-(1 → 6)-β-D-glucosamines of various ring sizes may also serve as a basis for ion channels. Smaller cycles **70** (Fig. 14; n = 0, 1, 2) with structural rigidity are expected to be ideal for ion channel formation in that, when incorporated into cell membranes, could sustain the membrane pressure. However, larger cycles ($n \geq 3$) being flexible [25,27] would collapse by membrane pressure and are expected to form poorly active pores. As described above, MD simulation has shown the presence of a hydrophilic cavity in cyclic oligo-(1 → 6)-β-D-glucosamines in contrast to the hydrophobic cavity created by β-CD. This characteristic may help to overcome the desolvation process during ion transport which is expected to be present in the case of β-CD ion channel due to hydrophobicity. Thus, smart creation of synthetic ion channels simply by altering the macrocycle diameter would provide defined ion selectivities and potential application as antibacterial agents. The preparation of cycles **70** of various sizes, having different lipophilic *N*-substituents as potential pore-forming molecules, is now being carried out.

In conclusion, a complex of synthetic methods was developed to prepare cyclic and linear oligoglucosamines having spiral-like conformations which are suitable for further design of spatially arranged neoglycoconjugates thereof for a variety of glycobiology applications.

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