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SYNTHESIS OF A FUNCTIONALIZED HEXASACCHARIDE PRECURSOR OF A GLYCOCONJUGATE VACCINE AGAINST CHOLERA[1]

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Abstract. A hexasaccharide fragment of the O-polysaccharide of *Vibrio cholerae* O:1, serotype Inaba was constructed from mono and disaccharides carrying azido groups. After the azido→amino group conversion, the requisite *N*-acylation was achieved using as the reagent 4-*O*-benzyl-L-*glycero*-tetronic acid, prepared from the corresponding derivative of L-homoserine by deamination.

Cholera is known to cause epidemics and periodic worldwide pandemics causing enormous disease-related problems. The organism recognized as the etiologic agent of cholera in humans is *Vibrio cholerae* O:1.[3] The structure of the O-specific polysaccharide (O-SP or the O-antigen) of *Vibrio cholerae* O:1, strain Inaba, consists[4] of a relatively short chain of less than twenty (1→2)-linked moieties of 4-amino-4,6-dideoxy-α-D-mannopyranose (perosamine), the amino groups of which are acylated with 3-deoxy-L-*glycero*-tetronic acid (Fig.1.)

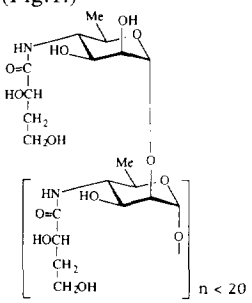
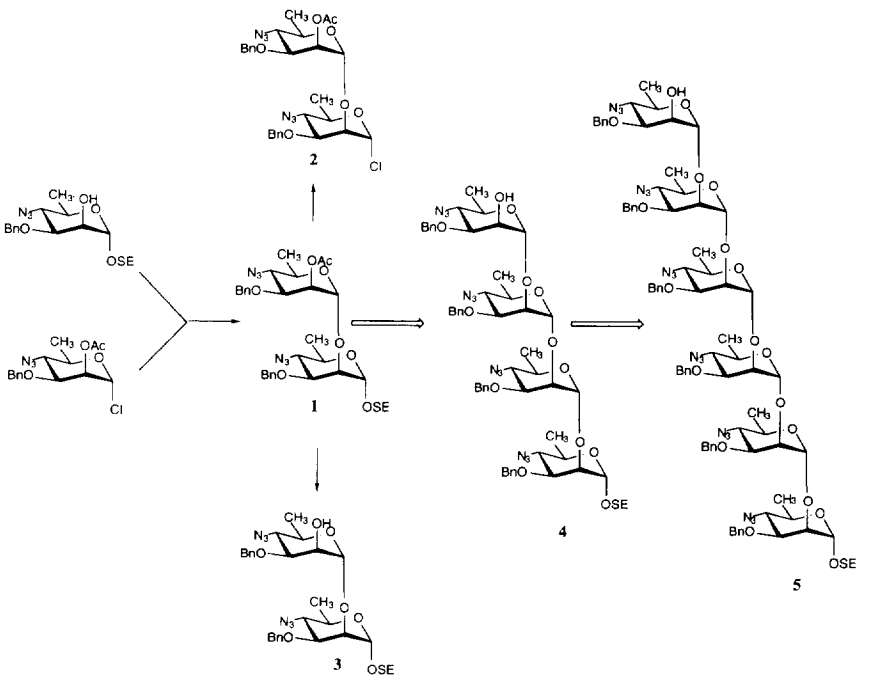


Fig. 1

Our laboratory has a long history of studying the interaction of carbohydrate antigens and antibodies. From binding studies on many antigen/antibody systems[5] we have obtained a great deal of information on binding at the molecular level. Following these theoretical studies, as a logical next step, we now apply the proven methods to systems where the antibodies are specific for the O-PSs of bacterial strains that are responsible for existing infectious diseases. Systematic prevention of cholera by immunization has not yet been achieved because of lack of a protective vaccine. Conjugates of the detoxified lipopolysaccharide of *Vibrio cholerae* O:1, serotype Inaba and cholera toxin have been described.[6] In our efforts to develop a vaccine against cholera based on a synthetic carbohydrate antigen we are exploring various synthetic routes to fragments of the O-PS of *Vibrio cholerae* O:1. Basically, two principally different strategies for the assembly of *N*-acylated, perosamine-containing oligosaccharides have been proposed. The strategy involving the use of glycosyl donors and glycosyl acceptors which have the required *N*-3-deoxy-L-*glycero*-tetronamido groups already in place was recently[7-9] developed in this laboratory. It is a variation of the approach, originally introduced by Bundle *et al.*,[10-14] which involves the construction of the corresponding azido oligosaccharide and its subsequent conversion, *via* the corresponding 4-amino derivative, into the oligosaccharide containing the requisite 4-acylamido group. We have used the latter chemistry in one of our

syntheses of the methyl α-glycoside of the disaccharide intracatenary repeating unit of the O-PS of *Vibrio cholerae* O:1,[7] and the same strategy is applied here.

An important consideration in the construction of a higher oligosaccharide that is to be subsequently attached covalently to a protein carrier is the selection of a protective group for the reducing end of the initial glycosyl acceptor. Such group should allow, after the product of the required size has been built, the conversion of the oligosaccharide into a glycosyl donor useful for coupling with a carrier, either by way of a spacer or without one. For this purpose, we have selected the trimethylsilylethyl (SE) group,[15,16] which offers the chemical flexibility required. Accordingly†, the known[13,17] methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside was converted, *via* its 2-*O*-benzoyl[18] derivative* ($\delta_{H\text{-}1}$ 4.77; $[\alpha]_D$ -31°) into 2-(trimethylsilyl)ethyl 4-azido-2-*O*-benzoyl-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside* [mp 73-75 °C, $[\alpha]_D$ -1° (*c* 1, $CHCl_3$)]. It was debenzoylated, and the 2-hydroxy derivative* thus formed was coupled¶ with 2-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl chloride[13] to give **1*** [$\delta_{C\text{-}1^1}$ 98.16, $\delta_{C\text{-}1^2}$ 99.36]§ (Scheme 1). Compound **1** was treated with dichloromethyl methyl ether[19] to give **2**, or deacetylated to give the

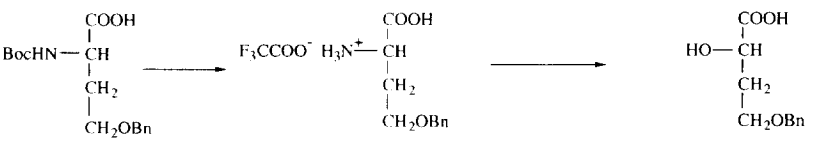


†All compounds produced NMR spectra consistent with the anticipated structure. ^{1}H- and ^{13}C-NMR spectra were obtained at 300 and 75 MHz, respectively, for solutions in $CDCl_3$, using TMS as the internal standard.
*Compounds marked with an asterisks produced correct elemental analysis.
¶ All glycosylation reactions were mediated with silver trifllate in the presence of 2,4,6-trimethylpyridine. The yields of desired products, based on the amounts of glycosyl acceptors used and performed with 1.2–1.5 molar excess of glycosyl donors, were 82-95%.
§ When reporting NMR data, sugar residues in oligosaccharides are serially numbered, beginning with the one bearing the aglycone, and are identified by a superscript in listings of signal assignments.

Scheme 1

disaccharide glycosyl acceptor **3**. The latter compound was coupled again with **2** to give, after deacetylation, **4*** ($\delta_{C\text{-}1^1}$ 98.22, $\delta_{C\text{-}1^{2,3}}$ 100.34, 100.19, $\delta_{C\text{-}1^4}$ 100.44). This sequence of reactions was repeated again to give **5*** [$\delta_{C\text{-}1^1}$ 98.20, $\delta_{C\text{-}1^{2-6}}$ 100.44, 100.30, 100.18, 100.09 (2 C)]. The azido groups in the building blocks **3** - **5** were then transformed, by treatment[20-22] with H_2S, to amino functions to give the corresponding amines. Succesful azido → amino conversions were verified by NMR and mass spectroscopy.

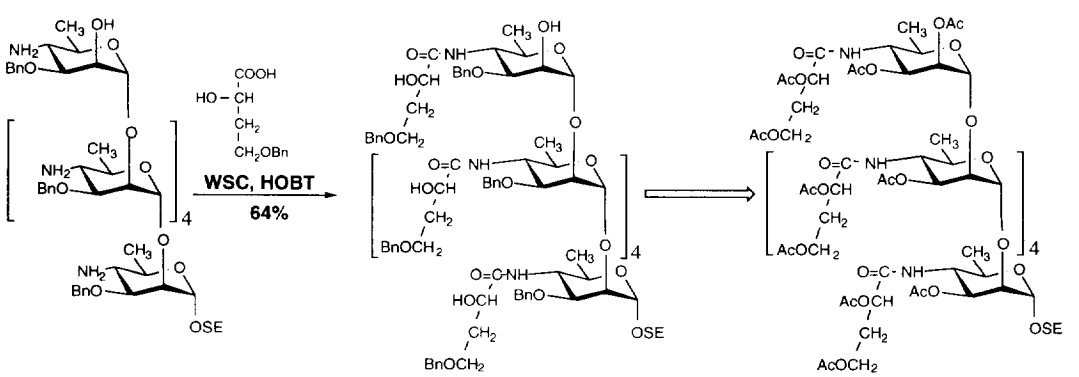
The next task was to introduce the *N*-3-deoxy-L-*glycero*-tetronoyl groups into the hexasaccharide. Initially, our reagent of choice was 3-deoxy-L-*glycero*-tetronolactone[7,23,24] which we have successfully used in previous, related syntheses.[7,8,23,25-27] However, treatment of the oligosaccharide amines with that reagent under conditions we commonly apply met with mixed success. As the size of the starting amine increased, the yield of the desired 4-(3-deoxy-tetronamido) derivative decreased dramatically and no desired product could be isolated from the reaction of the hexasaccharide. Consequently, a more efficient reagent for *N*-3-deoxy-L-*glycero*-tetronoylation of higher (1→2)-linked oligosaccharides composed of 4-amino-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranose had to be sought. Such a compound, 4-*O*-benzyl-3-deoxy-L-*glycero*-tetronic acid **7*** {mp 70-72 °C, $[\alpha]_D$ -12° (*c* 0.9, $CHCl_3$)}, was prepared from the commercially available 4-*O*-benzyl-*N*-Boc-L-homoserine **6** by deamination (Scheme 2). The presence of a stable protecting group at HO-4, such as in **7**, prevents the otherwise readily occurring, spontaneous 1,4-lactonization of 3-deoxy-L-*glycero*-tetronic acid



6 **7**

Scheme 2

during chemical manipulations. By the coupling of **7** with **8** in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC) and *N*-hydroxybenzotriazole (HOBT), the desired functionalized hexasaccharide **9*** {$[\alpha]_D$ -5° (*c* 1, $CHCl_3$), $\delta_{C\text{-}1^{1-6}}$ 100.55, 99.82 (2 C), 99.52 (2 C),98.35} was obtained in



8 **9** **10**

Scheme 3

the acceptable yield of 64%. Compound **9** was further converted (Scheme 3) to the functionalized, crystalline hexasaccharide **10*** {mp 112-113 °C, $[\alpha]_D$ +30° (*c* 1, $CHCl_3$); $\delta_{C\text{-}1^{1-6}}$ 100.21, 99.97, 99.77 (2 C), 99.18, 97.04}, which can be potentially converted to a neoglycoconjugate using one of the available technologies.[28]

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