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Enhanced stereoselectivity of α-mannosylation under thermodynamic control using trichloroacetimidates

Shu-jie Hou, Pavol Kováč *

NIDDK, LBC, National Institutes of Health, Bethesda, MD 20892-0815, USA

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ABSTRACT

O-Specific polysaccharides of *Vibrio cholerae* O1, serotypes Inaba and Ogawa, consist of α-(1→2)-linked *N*-(3-deoxy-L-*glycero*-tetronyl)perosamine (4-amino-4,6-dideoxy-D-mannose). The blockwise synthesis of larger fragments of such O-PSs involves oligosaccharide glycosyl donors that contain a nonparticipating 2-*O*-glycosyl group at the position vicinal to the anomeric center where the new glycosidic linkage is formed. Such glycosyl donors may bear at C-4 either a latent acylamino (e.g., azido) or the 3-deoxy-L-*glycero*-tetronamido group. While monosaccharide glycosyl donors, even those bearing a nonparticipating group at *O*-2 (e.g., methyl), *and* the 4-*N*-(3-deoxy-L-*glycero*-tetronyl) side chain form α-linked oligosaccharides with excellent stereoselectivity, α-mannosylation with analogous oligosaccharide donors in this series is adversely affected by the presence of the side chain. Consequently, the unwanted β-product is formed in a considerable amount. Conducting the reaction at elevated temperature under thermodynamic control substantially enhances formation of the α-linked oligosaccharide. This effect is much more pronounced when glycosyl trichloroacetimidates, rather than thioglycosides or glycosyl chlorides, are used as glycosyl donors.

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1. Introduction

Cholera in humans is caused by three strains of *Vibrio cholerae*—O1 Inaba, O1 Ogawa, and O139.[1] Progress in the development and practical relevance of a conjugate vaccine for cholera from a synthetic antigen depends, among other things, on the availability of oligosaccharides that mimic the O-specific polysaccharides (O-PSs) of the bacterial pathogens involved. The O-PSs of O1 Inaba and O1 Ogawa are very similar and consist of a (1→2)-α-linked perosamine (4-amino-4,6-dideoxy-D-mannose) whose amino group is acylated with 3-deoxy-L-*glycero*-tetronic acid. They differ[2,3] in that the Ogawa O-PS has a methyl group at O-2 of the upstream,[4] terminal perosamine residue (Fig. 1). We have been involved in the synthesis of oligosaccharides that mimic the O-PS of *V. cholerae* O1 and O139 for more than a decade.[5] The chemical syntheses are challenging and, despite several attempts to improve early approaches,[6–9] there is still a need to optimize the synthetic strategy. We have shown[10] that immunization of mice with a conjugate made from the synthetic hexasaccharide that mimics the upstream terminus of the Ogawa O-PS conferred protection. Therefore, we have recently focused our efforts on improving the synthesis of that segment in the *V. cholerae* O1 series.

Our recent blockwise synthesis[11] of the Ogawa hexasaccharide from disaccharide glycosyl donors bearing the 4-(3-deoxy-L-*glycero*-tetronamido) group in place (fully assembled glycosyl donors, as opposed to donors bearing 4-azido groups) has definite advantages over previous approaches, despite less than optimum stereoselectivity in the formation of the α-(1→2)-interglycosidic linkages. There,[11] we were able to improve the yield of the desired, α-linked product by reacting a disaccharide thioglycoside donor under thermodynamic control. Here we report on further improvement of the synthesis of the hexasaccharide sequence, which was accomplished by the use of relevant mono- and disaccharide

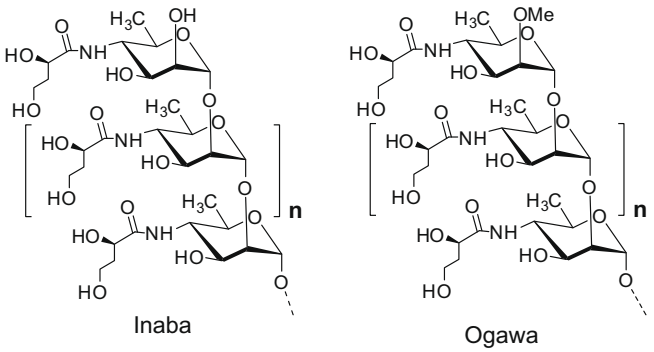

Figure 1. Structure of the O-PSs of the two strains of *Vibrio cholerae* O1.

* Corresponding author. Tel.: +1 301 496 3569; fax: +1 301 480 5703.
E-mail address: kpn@helix.nih.gov (P. Kováč).

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trichloroacetimidates as glycosyl donors. Under thermodynamic control, the stereoselectivity of formation of the α-mannosyl linkage markedly increased, compared to the use of thioglycosides as donors.[11]

2. Results and discussion

Generally, it is more efficient to synthesize higher oligosaccharides using a convergent (blockwise) strategy than by a linear (stepwise) approach. Also, in the assembly of large *N*-acyl-hexosamine-containing oligosaccharides, it is preferable to use intermediates where the *N*-acyl group is already installed (e.g., **3**–**5**) than those containing latent acylamino groups (e.g., **1**, where the azido group can be converted to an acylamino function at a later stage of the synthesis). Examples of such strategies can be found in the two different approaches to the tetrasaccharide side chain of the major glycoprotein of the *Bacillus anthracis* exosporium.[12,13] In our initial attempt to synthesize oligosaccharides in the *V. cholerae* O1 series,[14] we explored the feasibility of using fully assembled intermediates (cf., our synthesis of the Inaba disaccharide).[14] There, the formation of the α-mannopyranosyl linkage was highly stereoselective due to anchimeric assistance from a participating acyl group at *O*-2 in the monosaccharide glycosyl donor. This approach,[14] however, could not be extended to the synthesis of higher α-(1→2)-linked oligosaccharides because of the absence of a selectively removable protecting group in the product disaccharide. We have subsequently made a series of Inaba oligosaccharides by a stepwise approach from a fully assembled monosaccharide donor.[15] There, again, the stereoselectivity of glycosylation was not an issue because of the presence of the participating 2-*C*-acetyloxy group in the donor.

Seminal work by Peters and Bundle, within their synthetic work toward oligosaccharides that mimic the *Brucella* A polysaccharide, showed that large α-linked, perosamine-containing oligosaccharides can by synthesized in very good yields from donors that lack a participating moiety at C-2.[16,17] They used the C-4 azido group-containing (1→2)-linked disaccharide glycosyl donor **1** to synthesize α-mannopyranosyl linkages with high stereoselectivity (the pure α-products were obtained in >80% yields). Following their strategy, we have been able to prepare various oligosaccharides in the *V. cholerae* O1 series, including a dodecasaccharide.[7,18–20]

Encouraged by the high stereoselectivity of formation of the α-mannopyranosyl linkage in the absence of anchimeric assistance,[7,16,17] and by the precedence[21] for highly stereoselective formation of α-mannosyl linkage from the fully assembled donor **2**, we used[11] donors **3**–**5** for glycosylation in the assembly of the Ogawa hexasaccharide **27**. It turned out[11] that, unlike with glycosyl donors **1** and **2**, the presence of the side chain in the *oligosaccharide* donors **3**–**5** resulted in loss of the ability of these donors to form α-mannosyl linkage with high stereoselectivity. For example,[11] the reaction of thioglycoside **4** with methyl 6-hydroxyhexanoate in DCM at −20 °C gave mainly the unwanted β glycoside. Thus, paradoxically, although the synthesis of the β-mannosyl linkage is one of the most difficult glycosidic linkages to synthesize, we faced the uncommon task to minimize formation of that linkage. Conducting the glycosidation of methyl 6-hydroxyhexanoate at higher temperatures changed the situation considerably, as it resulted in increased relative amount of the desired α product formed, with the latter slightly predominating when the reaction was conducted at the temperature of refluxing toluene.[11] With oligosaccharide glycosyl acceptors **6** and **7** and donor **4**, the α:β ratio of products could be increased from 2:1 (DCM as solvent, room temperature) to 5:1 (refluxing toluene).[11]

A high yield of the α product is the prerequisite for efficient syntheses of *V. cholerae* O1 antigens. Guided by the increase of α-stereoselectivity of glycosylation under thermodynamic control using thioglycosides as glycosyl donors, we deemed it important to examine how trichloroacetimidate **14**, which is analogous to thioglycoside **3**,[11] would perform under similar conditions. To our knowledge, glycosylation under thermodynamic control with trichloroacetimidates has not been attempted, lest decomposition of the highly reactive donor might preclude glycosylation.

To compare the stereoselectivity of formation of an α-perosaminyl linkage from thioglycoside **3**[11] with that using the correspond-