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Enhanced stereoselectivity of α -mannosylation under thermodynamic control using trichloroacetimidates

Shu-jié Hou and Pavol Kováč^{*}

NIDDK, LBC, National Institutes of Health, Bethesda, MD 20892-0815 (U.S.A.)

Abstract

O-Specific polysaccharides of *Vibrio cholerae* O1, serotype Inaba and Ogawa, consist of α -(1 \rightarrow 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.

Keywords

O-Specific polysaccharide; cholera; *Vibrio cholerae* O1; oligosaccharide synthesis; glycosylation; β -mannosylation

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 \rightarrow 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 *Vibrio 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 shown¹⁰ that immunization of mice with a conjugate made from the synthetic hexasaccharide that mimics

^{*}Corresponding author. Tel.: 301-496-3569; fax: 301-480-5703. kpn@helix.nih.gov (P. Kováč).

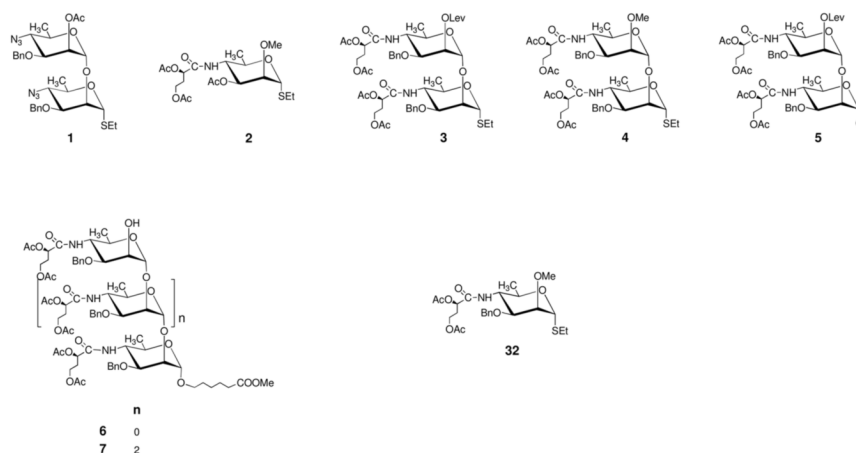
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the upstream terminus of the Ogawa O-PS conferred protection. Therefore, we have recently focused our efforts on improving the synthesis of that segment in the *V. cholerae* O1 series.

Our recent blockwise synthesis¹¹ 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 \rightarrow 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 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 (block-wise) strategy than by a linear (step-wise) approach. Also, in the assembly of large *N*-acyl-hexamine-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 *Vibrio 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. That approach,¹⁴ however, could not be extended to making higher α -(1 \rightarrow 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 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 be 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 \rightarrow 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 *Vibrio 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 precedence²¹ for highly stereoselective formation of α -mannosyl linkage from the fully assembled donor **2**, we used¹¹ donors **3–5** for glycosylation in the assembly of the Ogawa hexasaccharide **27**. It turned out¹¹ 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 later 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 temp) to 5:1 (refluxing toluene).¹¹ A high yield of the α product is the prerequisite for efficient syntheses of *Vibrio 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 corresponding trichloroacetimidate, we synthesized imidate **14** (Scheme 1) and treated it with alcohols **15** and **18** (Scheme 2). When the reaction of **14** and **15** was carried out in DCM at -35°C , a considerable amount of the β -linked trisaccharide **17** was formed (α : β ~2.5:1, NMR, unpublished results). The proclivity of this transformation to thermodynamic control became evident when the same synthons were allowed to react either at room temperature or at 100°C , in toluene. Thus, while at room temperature the trisaccharides were formed in the α : β ratio of 10:1, the reaction in hot toluene led to compounds **16** and **17** in the ratio of 20:1. It should be noted that the glycosylation yields in these three reactions were high (86–95%). Reaction of **14** and alcohol **18** under thermodynamic control gave pentasaccharide **19** α,β with the same high stereoselectivity (α : β ratio, ~20:1, NMR). Separation of the two pentasaccharides was difficult and the mixture of anomers was resolved after removal of the levulinoyl group, to give **20** and **21**.

Synthesis of the hexasaccharide fragments of the O-PS of *Vibrio cholerae* O1, serotype Inaba and Ogawa required extension of pentasaccharide **20** at O-2 with glycosyl donor **10** and its 2-O-methyl analog **25**, respectively.

To obtain imidate **25**, the known⁸ glycoside **22** was subjected sequentially to acetolysis (\rightarrow **23**), anomeric deacetylation, and conversion of the free sugar **24** thus formed to trichloroacetimidate **25**. We have previously observed²¹ high stereoselectivity of formation of the α -perosaminyl linkage under conventional, not thermodynamically controlled conditions (room temperature, CH_2Cl_2 as solvent) using a different fully assembled monosaccharide glycosyl donor, namely thioglycoside **32**. Therefore, alcohol **20** was treated with each of imidates **10** and **25** (Scheme 4) under the same conditions. Both reactions were very slow, and

some unchanged glycosyl donor was still present in both reaction mixtures after 16 h of reaction time. The β -linked hexasaccharides could not be found among the reaction products (NMR spectra of isolated, minor by-products), indicating excellent stereoselectivity of formation of **26** and **27**, which were isolated in 73 and 69% yield, respectively. Hydrogenolysis of the foregoing fully protected intermediates gave alcohols **28** and **30**, respectively. Treatment of the former with NaOMe in MeOH effected simultaneous removal of protecting acetyl and levulinoyl groups, to give the fully deprotected hexasaccharide **29**. Treatment of the Ogawa compound **30** with ethylenediamine effected simultaneous deacetylation of the side chains and amidation of the spacer, to give the final hexasaccharide **31**, which is amenable for conjugation by squaric acid chemistry.²²

3. Conclusions

Results of this study confirm our previous¹¹ observation that the presence of the 3-deoxy-L-glycero-tetronamido side chain in glycosyl donors derived from perosamine significantly affect the stereoselectivity of glycosylation. When such glycosyl donors bear a *nonparticipating* group (*O*-alkyl or *O*-glycosyl) at the position vicinal to the active glycosidic center, the stereochemical outcome of perosaminylations – conducted by us^{7,11,15,21,23} and elsewhere^{16,17,24} – can be summarized as follows. Firstly, the formation of α -glycosidic linkage is highly favored when *mono*- and *oligosaccharide* glycosyl donors having azido group at O-4 are employed. Secondly, the α glycosidic linkage can also be stereoselectively formed from *monosaccharide* glycosyl donors where the 4-*N*-side chain is already in place (e.g. **2** or **32**), regardless of the *O*-2 substituent. Thirdly, and in contrast to their monosaccharide counterparts, the stereoselectivity of glycosylation conducted at conventional reaction conditions is poor when the glycosyl donor, a thioglycoside, glycosyl chloride or trichloroacetimidate, is an *oligosaccharide* made from α -(1 \rightarrow 2)-linked perosamine having the 4-(3-deoxy-L-glycero-tetronamido) side chain already in place. In such situations, the α stereoselectivity can be markedly improved by conducting the reaction under thermodynamic control. The benefit of the thermodynamic control is more pronounced when glycosylation is effected by the use of trichloroacetimidate, which are preferred for this purpose, to the corresponding thioglycosides or glycosyl chlorides.¹¹

4. Experimental

4.1 General Methods

Unless stated otherwise, optical rotations were measured at ambient temperature with a Perkin–Elmer automatic polarimeter, Model 341. All reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 coated glass slides. Column chromatography was performed by elution from columns of silica gel with the CombiFlash Companion Chromatograph (Isco, Inc.) or Isolera Flash Chromatograph (Biotage). Solvent mixtures less polar than those used for TLC were used at the onset of separation. Nuclear Magnetic Resonance (NMR) spectra were measured at 300 MHz (¹H) and 75 MHz (¹³C) with a Varian Gemini or Varian Mercury spectrometer, or at 600 MHz (¹H) and 150 MHz (¹³C) with a Bruker Avance 600 spectrometer. Assignments of NMR signals were made by homonuclear and heteronuclear 2-dimensional correlation spectroscopy, run with the software supplied with the spectrometers. Assignment of ¹³C NMR spectra of some higher oligosaccharides was aided by comparison with spectra of related substances reported previously from this laboratory. When the latter approach was used, to aid in the ¹³C NMR signal-nuclei assignments, advantage was taken of variations of line intensity expected for oligosaccharides belonging to the same homologous series.^{25,26} Thus, spectra showed close similarity of chemical shifts of equivalent carbon atoms of the internal residues, and an increase in the relative intensity of these signals with the increasing number of D-perosamine residues in the molecule. Nevertheless, often only incomplete assignment was achieved because of the overlap of resonances. When reporting

assignment of NMR signals, nuclei associated with the 4-amido side chain are denoted with a prime and those with the spacer (linker) are denoted with a double prime. When reporting assignments of NMR signals, sugar residues in oligosaccharides are serially numbered, beginning with the one bearing the aglycon, and are identified by a Roman numeral superscript in listings of signal assignments. Signals for sugar ring nuclei lacking Roman numeral assignment may belong to any of the rings. Only resonances that could be confidently assigned are listed in signal assignments. When reporting preparation of known substances, their identity was confirmed by comparison of NMR characteristics with those published previously. Liquid Chromatography–Electron Spray–Ionization Mass Spectrometry (ESI-MS) was performed with a Hewlett–Packard 1100 MSD spectrometer. Attempts have been made to obtain correct combustion analysis data for all new compounds. However, some compounds tenaciously retained traces of solvents, despite exhaustive drying, and analytical figures for carbon could not be obtained within 0.4%. Structures of these compounds follow unequivocally from the mode of synthesis, NMR spectroscopic data and *m/z* values found in their mass spectra, and their purity was verified by TLC and NMR spectroscopy. 5% Palladium-on-charcoal catalyst (Escat| 103) was purchased from Engelhard Industries. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDAC) was purchased from ACROS Organics. Rubber septa used to close reaction flasks containing organic solvents were protected with a thin Teflon| sheet, to avoid leaching. Solutions in organic solvents were dried with anhydrous Na₂SO₄, and concentrated at 40 °C/2 kPa.

4.2 1-*O*-Acetyl-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy- α -*D*-mannopyranose (11)

A solution of hydrazine acetate (4.10 g, 44.6 mmol) in MeOH (50 ml) was added to a stirred mixture of **8**¹¹ (18.4 g, 31.9 mmol) in DCM (500 ml). The stirring was continued until TLC (3:1 toluene–acetone) showed that the starting material was completely consumed (~6 h). After concentration, chromatography (3:1 hexane–EtOAc) afforded **11** (13.2 g, 86%), [α]_D +16 (*c* 0.5, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ : 7.40–7.26 (m, 5 H, Ph), 6.14 (d, *J*_{1,2} = 1.2 Hz, H-1), 6.03 (d, *J* = 8.3 Hz, 1 H, NH), 5.14 (dd, *J* = 8.0 Hz and 4.9 Hz, 1 H, H-2'), 4.67–4.51 (AB_q, *J* = 12.0 Hz, 2 H, PhCH₂), 4.18–3.99 (m, 6 H, 2-OH, H-3, H-4, H-5, H-4'), 3.85 (m, 1 H, H-2), 2.12–2.02 (m, 11 H, 3 COCH₃, H-4'), 1.24 (d, *J*_{5,6} = 6.4 Hz, 3 H, H-6); ¹³C NMR (150 MHz, CDCl₃) δ : 95.4 (C-1), 137.2 (C_q), 92.8 (C-1), 74.9 (C-3), 71.4 (PhCH₂), 71.1 (C-2'), 68.9 (C-5), 66.3 (C-4), 59.9 (C-4'), 52.7 (C-2), 30.9 (C-3'), 21.0, 20.9, 20.8 (3 CH₃CO), 17.9 (C-6); TOF-MS *m/z*: 504 [M+Na]⁺; Anal. Calcd. for C₂₃H₃₁NO₁₀: C, 57.37; H, 6.49. Found C, 57.11; H, 6.55.

4.3 1-*O*-Acetyl-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy-2-*O*-levulinoyl- α -*D*-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy- α -*D*-mannopyranose (12)

Piperidine (54 ml, 549 mmol) was added with stirring to a solution of **8**²⁷ (15.7 g, 27.1 mmol) in THF (250 ml) and the stirring was continued at room temperature until TLC (2:1 hexane–acetone) showed that the reaction was complete. The mixture was diluted with DCM (1000 ml) and washed successively with ice-cooled 0.5 M HCl (2 × 250 mL), satd NaHCO₃ (200 ml) and brine (200 ml), the organic phase was dried and concentrated. Chromatography (2:1, hexane–acetone) gave 3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy-2-*O*-levulinoyl- α , β -*D*-mannopyranose (**9**, 13.8 g, 95%) as a mixture of anomers (α : β ~6:1). ¹H NMR (α product, 300 MHz, CDCl₃) δ : 7.35–7.26 (m, 5 H, Ph), 5.82 (d, *J* = 7.9 Hz, 1 H, NH), 5.40 (t, *J* = 2.06 Hz, 1 H, H-2), 5.20–5.16 (m, 2 H, H-1 and H-2'), 4.66–4.33 (AB_q, *J* = 12.0 Hz, 2 H, PhCH₂), 4.16–3.90 (m, 5 H, H-3, H-4, H-5, H-4'), 2.76–2.64 (m, 4 H, CH₂CH₂), 2.20–2.00 (m, 11 H, 3 COCH₃ and H-3'), 1.21 (d, *J*_{5,6} = 6.0 Hz, H-6); ¹³C NMR (75 MHz, CDCl₃) δ : 172.2 (CO), 169.8 (CO), 169.6 (CO), 137.9 (C_q), 92.5 (C-1), 73.2 (C-3), 71.3 (C-2'), 70.8 (PhCH₂), 67.8 (C-2 and C-5), 60.2 (C-4'), 53.0 (C-4), 38.2 (CH₂CO), 31.1

(C-3'), 30.0 (OCOCH₂), 28.3 (COCH₃), 21.0 (2 CH₃CO), 18.3 (C-6); TOF-HRMS *m/z*: [M+H]⁺ Calcd for C₂₆H₃₆NO₁₁: 538.2288. Found 538.2278.

A solution of compound **9** (600 mg, 1.19 mmol) in DCM (10 ml) was treated at 0 °C with Cl₃CCN (1.2 ml, 12 mmol) in the presence of DBU (0.09 ml, 0.6 mmol) for 2 h, and concentrated. Chromatography (3:2 hexane–EtOAc) gave 3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy-2-*O*-levulinoyl- α -D-mannopyranose 1-*O*-trichloroacetimidate (**10**, 540 mg, 71%). ¹H NMR (300 MHz, CDCl₃) δ : 8.71 (s, 1 H, NH), 7.35–7.28 (m, 5 H, Ph), 6.23 (d, *J*_{1,2} = 1.92 Hz, H-1), 5.80 (d, *J* = 7.4 Hz, 1 H, NH), 5.51 (t, *J* = 2.2 Hz, 1 H, H-2), 5.17 (dd, *J* = 7.9 Hz and 4.8 Hz, 1 H, H-2'), 4.66–4.37 (AB_q, *J* = 12.0 Hz, 2 H, PhCH₂), 4.15–3.93 (m, 5 H, H-3, H-4, H-5, H-4'), 2.79–2.71 (m, 4 H, CH₂CH₂), 2.18–2.05 (m, 11 H, 3 COCH₃ and H-3'), 1.25 (d, *J*_{5,6} = 5.6 Hz, H-6); ¹³C NMR (75 MHz, CDCl₃) δ : 95.4 (C-1), 72.5 (C-3), 71.3 (C-2'), 71.09, 70.6 (PhCH₂), 66.25 (C-2 and C-5), 60.1 (C-4'), 52.7 (C-4), 38.1 (CH₂CO), 31.1 (C-3'), 30.0 (OCOCH₂), 28.2 (COCH₃), 21.0 (2 CH₃CO), 18.3 (C-6); TOF-HRMS *m/z*: [M+H]⁺ Calcd for C₂₈H₃₆N₂O₁₁Cl₃: 681.1385. Found 681.1388.

A mixture of **10** (9.8 g, 14.4 mmol), **11** (6.29 g, 13.0 mmol) and 4 Å MS (2.1 g) in toluene-DCM (3:1, v/v 150 ml) was stirred under N₂ at room temperature for 30 min. TMSOTf (16 μ l, 0.65 mmol) was added, and the stirring was continued for 4 h when TLC (3:2 hexane–acetone) showed that the donor was completely consumed. After addition of Et₃N (1.0 ml), the mixture was filtered through Celite pad, the filtrate was concentrated, and chromatography (3:1→3:2 hexane–acetone) gave **12** (10.0 g, 76%). [α]_D⁻¹²⁰ (c 0.92, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ : 7.30–7.17 (m, 10 H, 2 Ph), 6.02 (d, *J* = 8.7 Hz, 1 H, NH^I), 5.99 (d, *J*_{1,2} = 2.2 Hz, 1 H, H-1^I), 5.68 (d, *J* = 9.0 Hz, 1 H, NH^{II}), 5.41 (t, *J* = 2.5 Hz, H-2^{II}), 5.09 (m, 2 H, H-2^{I,II}), 4.85 (d, *J*_{1,2} = 1.8 Hz, H-1^{II}), 4.60–4.30 (m, 4 H, 2 PhCH₂), 4.04–3.95 (m, 7 H, H-3^I, H-5^I, H-4^{II}, H-4^{I,II}), 3.85 (t, *J* = 2.5 Hz, 1 H, H-2^I), 3.71–3.67 (m, 3 H, H-4^I, 3^{II}, H-5^{II}), 2.62 (m, 4 H, CH₂CH₂), 2.10–1.92 (m, 22 H, 6 COCH₃, H-3^{I,II}), 1.15 (m, 6 H, H-6^{I,II}); ¹³C NMR (150 MHz, CDCl₃) δ : 137.6 (C_q), 137.5 (C_q), 99.4 (C-1^{II}), 92.5 (C-1^I), 74.2 (C-3^I), 73.3 (C-3^{II}), 72.6 (C-2^I), 71.9 (PhCH₂), 71.3 and 71.2 (C-2^{I,II}), 70.7 (PhCH₂), 69.6 (C-5^{II}), 69.0 (C-5^I), 67.2 (C-2^{II}), 60.1 and 60.0 (C-4^{I,II}), 53.4 (C-4^I), 51.9 (C-4^{II}), 38.0 (CH₂CO), 31.0 and 30.9 (C-3^{I,II}), 29.8 (COCH₃), 28.2 (OCOCH₂), 21.0, 20.9, 20.8 (5 C, 5 COCH₃), 18.1 and 17.9 (C-6^{I,II}); TOF-HRMS *m/z*: [M+H]⁺ Calcd for C₄₉H₆₅N₂O₂₀: 1001.4131. Found 1001.4139. Anal. Calcd. for C₄₉H₆₄N₂O₂₀: C, 58.79; H, 6.44. Found C, 58.60; H, 6.48.

4.4 5-(Methoxycarbonyl)pentyl 3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy-2-*O*-levulinoyl- α -D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy- α -D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy- α -D-mannopyranoside (**16**)

Piperidine (3.6 ml, 36 mmol) was added with stirring at 0 °C to a solution of **12** (1.32 g, 1.3 mmol) in THF (20 ml) and the mixture was allowed to warm up to room temperature. After 24 h, the mixture was diluted with DCM (200 ml) and washed with 0.5 M HCl (50 ml) and saturated NaHCO₃ aq (50 ml), dried, concentrated, and chromatography (3:1→95:5 EtOAc–Hexane) gave 3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy-2-*O*-levulinoyl- α -D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy- α -D-mannopyranose (**13**, 940 mg, 75%). ¹H NMR (600 MHz, CDCl₃) δ : 7.36–7.27 (m, 10 H, 2 Ph), 5.91 (d, *J* = 8.3 Hz, 1 H, NH^I), 5.82 (d, *J* = 9.2 Hz, 1 H, NH^{II}), 5.49 (t, *J* = 2.5 Hz, H-2^{II}), 5.20–5.15 (m, 3 H, H-1^I, H-2^{I,II}), 4.87 (d, *J*_{1,2} = 1.9 Hz, H-1^{II}), 4.65–4.38 (m, 4 H, 2 PhCH₂), 4.20–3.90 (m, 9 H, H-2^I, H-3^I, H-4^I, H-5^I, H-4^{II}, H-4^{I,II}), 3.81–3.74 (m, 2 H, 3^{II}, H-5^{II}), 3.10 (brs, 1 H, 1-OH), 2.76–2.60 (m, 4 H,

CH₂CH₂), 2.10–1.92 (m, 19 H, 5 COCH₃, H-3^{I,II}), 1.18 (d, $J_{5,6} = 5.8$ Hz, 3 H, H-6^I), 1.15 (d, $J_{5,6} = 6.3$ Hz, 3 H, H-6^{II}); ¹³C NMR (150 MHz, CDCl₃) δ: 137.8 (C_q), 137.6 (C_q), 99.5 (C-1^{II}), 93.4 (C-1^I), 74.5 (C-2^I, C-3^I), 72.9 (C-3^{II}), 71.3 (PhCH₂), 71.2 and 71.0 (C-2^{I,II}), 70.4 (PhCH₂), 68.5 (C-5^{II}), 67.9 (C-5^I), 67.1 (C-2^{II}), 60.0 (C-4^{I,II}), 52.6 (C-4^I), 51.9 (C-4^{II}), 38.0 (CH₂CO), 31.0 and 30.9 (C-3^{I,II}), 29.8 (COCH₃), 28.2 (OCOCH₂), 20.9 (2 COCH₃), 20.8 (2 COCH₃), 18.1 and 17.9 (C-6^{I,II}); TOF-HRMS m/z : [M+H]⁺ Calcd for C₄₇H₆₃N₂O₁₉: 959.4052. Found 959.4014.

DBU (0.18 ml, 0.9 mmol) was added with stirring at 0 °C to a mixture of **13** (9.0 g, 9.4 mmol), CNCCl₃ (4.7 ml, 47.0 mmol) and DCM (150 ml), the mixture was allowed to warm up to temperature and stirred overnight. After concentration and chromatography (3:1→3:2 hexane–acetone) gave 3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy-2-*O*-levulinoyl- α -D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy- α -D-mannopyranose-1-*O*-trichloroacetimidate (**14**, 8.7 g, 84%). ¹H NMR (600 MHz, CDCl₃) δ: 8.62 (s, 1 H, OCNHCCl₃), 7.37–7.27 (m, 10 H, 2 Ph), 6.15 (d, $J_{1,2} = 2.0$ Hz, H-1^I), 5.89 (d, $J = 7.8$ Hz, 1 H, NH^I), 5.74 (d, $J = 9.0$ Hz, 1 H, NH^{II}), 5.51 (bt, $J = \sim 2.4$ Hz, H-2^{II}), 5.23–5.17 (m, 2 H, H-2^{I,II}), 4.91 (d, $J_{1,2} = 1.7$ Hz, H-1^{II}), 4.68–4.40 (m, 4 H, 2 PhCH₂), 4.20–3.97 (m, 9 H, H-4^{I,II}, H-4^{II}, H-2^I, H-5^I, H-3^I, H-4^I), 3.78–3.76 (m, 2 H, 3^{II}, H-5^{II}), 2.75–2.65 (m, 4 H, CH₂CH₂), 2.10–1.92 (m, 19 H, 5 COCH₃, H-3^{I,II}), 1.24 (d, $J_{5,6} = 6.3$ Hz, 3 H, H-6^I), 1.19 (d, $J_{5,6} = 6.3$ Hz, 3 H, H-6^{II}); ¹³C NMR (150 MHz, CDCl₃) δ: 137.7 (C_q), 137.4 (C_q), 99.8 (C-1^{II}), 96.5 (C-1^I), 74.2 (C-3^I), 73.1 (C-3^{II}), 72.6 (C-2^I), 71.9 (PhCH₂), 71.3 and 71.2 (C-2^{I,II}), 70.8 (C-5^I), 70.7 (PhCH₂), 69.1 (C-5^{II}), 67.2 (C-2^{II}), 60.1 (C-4^{I,II}), 52.7 (C-4^{II}), 51.9 (C-4^I), 38.2 (CH₂CO), 31.2 and 31.0 (C-3^{I,II}), 30.0 (COCH₃), 28.3 (OCOCH₂), 21.1 (2 COCH₃), 21.0 (2 COCH₃), 18.3 and 18.0 (C-6^{I,II}); TOF-MS m/z : 1127 [M+Na]⁺.

A solution of TMSOTf in toluene (0.03 M, 10 ml) was added at 100 °C under N₂ to a stirred mixture of **14** (7.5 g, 6.79 mmol), 5-(methoxycarbonyl)pentyl 3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy- α -D-mannopyranoside (**15**,¹¹ 3.5 g, 6.17 mmol), 4 Å molecular sieves (10 g) and toluene (300 ml). After 5 h, when TLC (3:2 hexane–acetone) showed that donor **14** was consumed, the mixture was cooled to room temperature and Et₃N (0.8 ml) was added. The mixture was filtered through a Celite pad, the filtrate was concentrated, and chromatography (3:1→3:2 hexane–acetone) gave the unchanged glycosyl acceptor **15** (0.83 g) and a mixture of known⁸ α -linked compound **16** and 5-(methoxycarbonyl)pentyl 3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy-2-*O*-levulinoyl- α -D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy- β -D-mannopyranosyl-(1→2)-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy- α -D-mannopyranoside (**17**) (combined yield, 7.8 g, glycosylation yield 84%, **16**:**17** ~20:1). The mixture was rechromatographed (12:1 toluene–2-propanol), to give **16** (6.22 g) and **17** (0.21 g). A small amount of unresolved mixture of **16** and **17** was also obtained.

17: ¹H NMR (600 MHz, CDCl₃) δ: 7.92 (d, $J = 9.0$ Hz, 1 H, NH^{III}), 7.36–7.08 (m, 15 H, 3 Ph), 6.43 (d, $J = 10.0$ Hz, 1 H, NH^I), 6.25 (d, $J = 7.4$ Hz, 1 H, NH^{II}), 5.47 (d, $J_{1,2} = 1.3$ Hz, 1 H, H-1^{III}), 5.44 (dd, $J_{1,2} = 1.3$ Hz, $J_{2,3} = 3.1$ Hz, 1 H, H-2^{III}), 5.26 (dd, $J = 5.4$ Hz, $J = 7.6$ Hz, 1 H, H-2^I), 5.02 (m, 2 H, H-2^I), 4.80 (d, $J = 10.3$ Hz, 1 H, PhCH₂), 4.75 (d, $J_{1,2} = 1.6$ Hz, 1 H, H-1^I), 4.67 (d, $J = 11.3$ Hz, 1 H, PhCH₂), 4.56 (s, 1 H, H-1^{II}), 4.36 (d, $J_{2,3} = 2.2$ Hz, H-2^{II}), 4.28 (d, $J = 11.3$ Hz, 1 H, PhCH₂), 4.24–4.21 (m, 2 H, H-2^I, H-4^{III}), 4.10–3.98 (m, 13 H, PhCH₂, H-4^I, H-3^{II}, H-5^{II}, H-5^{III}, H-4^{I,II,III}), 3.77 (dd, $J_{2,3} = 3.1$ Hz, $J_{3,4} = 10.9$ Hz, H-3^{III}), 3.64–3.61 (m, 6 H, OCH₃, H-1^I, H-3^I and H-5^I), 3.37–3.34 (m, 2 H, H-4^{II}, H-1^I), 2.56–2.51 (m, 4 H, CH₂CH₂), 2.29 (t, $J = 7.5$ Hz, 2 H, H-5^I), 2.02–1.97 (m, 27 H, 5 COCH₃, H-3^{I,II,III}), 1.62–1.54 (m, 4 H, H-2^{I,II}, H-4^{I,II}), 1.28 (m, 2 H, H-3^{I,II}), 1.26 (d, $J_{5,6} = 6.3$ Hz, 3 H, H-6^I), 1.19 (d, $J_{5,6} = 6.3$ Hz, 3 H, H-6^{II}); ¹³C NMR (150 MHz, CDCl₃) δ: 97.4 (2 C, C-1^I, C-1^{II}), 96.9

(C-1^{III}), 77.3 (C-3^{III}), 74.2 (C-3^I), 71.7 (C-2'), 71.6 (PhCH₂), 71.5 (PhCH₂), 71.1 (C-2'), 70.8 (C-2'), 69.6 (C-2^I, C-5^{III}), 69.4 (PhCH₂), 69.3 (C-5^{II}), 68.8 (C-2^{III}), 67.2 (C-5^I), 76.1 (C-1''), 66.9 (C-2^{II}), 60.9 (C-4'), 60.1 (C-4'), 59.9 (C-4'), 55.7 (C-4^{II}), 51.8 (C-4^I, C-4^{II}), 38.4 (2 C, CH₂CH₂), 34.0 (C-5''), 31.3 (C-3'), 31.2 (C-3'), 30.6 (C-3'), 28.8 (C-2''), 25.6 (C-3''), 24.4 (C-4''), 21.0 (2 COCH₃), 20.9 (2 COCH₃), 20.8 (2 COCH₃), 18.4, 18.1 and 18.0 (C-6^{I,II,III}); TOF-HRMS *m/z*: [M+Na]⁺ Calcd for C₇₅H₁₀₁N₃O₂₉Na: 1530.6418. Found 1530.6324.

4.5 5-(Methoxycarbonyl)pentyl 3-O-benzyl-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-4,6-dideoxy- α -D-mannopyranosyl-(1 \rightarrow 2)-3-O-benzyl-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-4,6-dideoxy- α -D-mannopyranosyl-(1 \rightarrow 2)-3-O-benzyl-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-4,6-dideoxy- α -D-mannopyranoside (18)

Compound **16** (6.2 g, 4.1 mmol) was treated with hydrazine acetate, as described for preparation of **11**, to give trisaccharide **18** (5.0 g, 87%), which was identical (TLC, NMR) with the known, independently synthesized¹¹ substance.

4.6 5-(Methoxycarbonyl)pentyl 3-O-benzyl-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-4,6-dideoxy- α -D-mannopyranosyl-(1 \rightarrow 2)-3-O-benzyl-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-4,6-dideoxy- α -D-mannopyranosyl-(1 \rightarrow 2)-3-O-benzyl-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-4,6-dideoxy- α -D-mannopyranosyl-(1 \rightarrow 2)-3-O-benzyl-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-4,6-dideoxy- α -D-mannopyranoside (20)

A solution of TMSOTf in toluene (0.03 M, 6 mL) was added with stirring at 100 °C under N₂ to a mixture of **18**¹¹ (5.0 g, 3.5 mmol), **14** (4.69 g, 4.25 mmol), and 4 Å molecular sieves (5.8 g) in toluene (110 mL). After 3 h, when TLC (1:1 hexane–acetone) showed that the reaction was virtually complete, the mixture was cooled to room temperature and Et₃N (0.8 ml) was added. The mixture was filtered through Celite pad, the filtrate was concentrated, and chromatography (6:1 \rightarrow 3:1 hexane–acetone) gave an anomeric mixture of fully protected pentasaccharides **19 α , β** (6.0 g, total yield 75%). TOF-MS *m/z*: 2374 [M+Na]⁺. The foregoing anomeric mixture (6.0 g, 2.5 mmol) was treated with hydrazine acetate (202 mg, 3.0 mmol) in MeOH (400 ml) overnight. Work-up, as described above for a similar reaction, and chromatography (5:1 DCM–acetone), gave first the β -linked product, 5-(methoxycarbonyl)pentyl 3-O-benzyl-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-4,6-dideoxy- α -D-mannopyranosyl-(1 \rightarrow 2)-3-O-benzyl-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-4,6-dideoxy- β -D-mannopyranosyl-(1 \rightarrow 2)-3-O-benzyl-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-4,6-dideoxy- α -D-mannopyranosyl-(1 \rightarrow 2)-3-O-benzyl-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-4,6-dideoxy- α -D-mannopyranoside (**21**, 220 mg, 4%). ¹H NMR (600 MHz, CDCl₃) δ : 7.74 (brs, 1 H, NH), 7.37–7.21 (m, 25 H, 5 Ph), 6.40–6.12 (m, 4 H, 4 NH), 5.45 (brs, 1 H, H-1), 5.28 (dd, *J* = 4.8, 8.2 Hz, 1 H, H-2'), 5.18–5.12 (m, 4 H, 4 \times H-2'), 5.04 (brs, 1 H, H-1), 4.90 (brs, 1 H, H-1), 4.78 (d, overlapped with CHPh, H-1), ~4.30 (m, H-1^{IV}, overlapped), 2.30 (t, *J* = 7.2 Hz, H-5''_{a,b}), 2.17–1.92 (m, 40 H, H-3^{I–V}, 10 COCH₃), 1.67–1.63 (m, 4 H, H-2''_{a,b}, H-4''_{a,b}), 1.38 (m, 2 H, H-3''_{a,b}), 1.26–1.08 (m, 15 H, H-6^{I–V}); ¹³C NMR (150 MHz, CDCl₃) δ : 100.9 (C-1, *J*_{C-1,H-1} 172 Hz), 99.6 (br, C-1, *J*_{C-1,H-1} not determined), 98.8 (C-1, *J*_{C-1,H-1} 169.6 Hz), 98.6 (C-1, *J*_{C-1,H-1} 172 Hz), 98.1 (C-1, *J*_{C-1,H-1} 154 Hz), 20.9 and 20.8 (5 C, C-6^{I–V}); TOF-MS *m/z*: 2275 [M+Na]⁺.

Eluted next was the all- α -linked pentasaccharide **20** (5.0 g, 87%), [α]_D –9.2 (c 1.08, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ : 7.40–7.24 (m, 25 H, 5 Ph), 6.53–6.13 (m, 4 H, 4 NH), 5.18 (m, 5 H, H-2^{I–V}), 5.08 (d, *J*_{1,2} = 2.0 Hz, H-1), 4.09 (brs, 2 H, 2 H-1), 4.87 (brs, 1 H, H-1), 4.71 (brs, 1 H, H-1), 4.64–4.58 (m, 10 H, 5 PhCH₂), 4.20–4.08 (m, 20 H, H-2^{I–V}, H-4^{I–V},

H-4^{I-V}), 3.86–3.32 (m, 15 H, H-3^{I-V}, H-5^{I-V}, OCH₃, H-1^{a,b}), 2.46 (brs, 1 H, OH-2V), 2.32 (t, $J = 7.2$ Hz, H-5^{a,b}), 2.21–1.95 (m, 40 H, H-3^{I-V}, 10 COCH₃), 1.66–1.53 (m, 4 H, H-2^{a,b}, H-4^{a,b}), 1.44–1.32 (m, 2 H, H-3^{a,b}), 1.14–1.08 (m, 15 H, H-6^{I-V}). ¹³C NMR (150 MHz, CDCl₃) δ : 100.7 (C-1), 99.9 (3 C, 3 C-1), 98.8 (C-1), 18.4 (C-6), 18.3 (C-6), 18.2 (2 C, 2 C-6), 17.9 (C-6). TOF-HRMS m/z : [M+H]⁺ Calcd for C₁₁₂H₁₅₀N₅O₄₃: 2252.9705. Found 2252.9724; Anal. Calcd. for C₁₁₂H₁₄₉N₅O₄₃: C, 59.70; H, 6.66; N, 3.11. Found C, 59.41; H, 6.70; N, 3.08.

4.7 1-O-Acetyl-3-O-benzyl-4-(2,4-di-O-acetyl-3-deoxy-L-glycero-tetronamido)-4,6-dideoxy-2-O-methyl- α -D-mannopyranose (23)

Methyl 3-*O*-Benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy-2-*O*-methyl- α -D-mannopyranose (**22**,⁸ 7.0 g, 15 mmol) was treated at room temperature with Ac₂O-HOAc-H₂SO₄ (10–4–0.1, 140 ml) for 1.5 h, and NaOAc.3H₂O (2.0 g) was added to terminate the reaction. After concentration and chromatography (1:1 hexane–EtOAc) gave **23** (6.7 g, 91%), mp 104–105 °C (EtOH); [α]_D –2 (c 1.1, CHCl₃); ¹H NMR (600 MHz, CDCl₃) δ : 7.36–7.27 (m, 5 H, Ph), 6.13 (d, $J_{1,2} = 1.9$ Hz, 1 H, H-1), 6.08 (d, $J = 8.1$ Hz, 1 H, NH), 5.16 (dd, $J = 8.0$ Hz and 4.7 Hz, 1 H, H-2'), 4.65–4.51 (AB_q, $J = 11.7$ Hz, 2 H, PhCH₂), 4.18–4.07 (m, 4 H, H-3, H-5 and H-4'), 3.80 (m, 1 H, H-4), 3.50 (brs, 4H, H-2 and OCH₃), 2.19 (m, 1 H, H-3_a'), 2.11–2.04 (m, 10 H, 3 CH₃CO and H-3_b'), 1.22 (d, $J_{5,6} = 6.3$ Hz, 3 H, H-6). ¹³C NMR (150 MHz, CDCl₃) δ : 91.5 (C-1), 75.7 (C-2), 74.4 (C-3), 71.6 (PhCH₂), 71.3 (C-2'), 69.5 (C-5), 60.1 (C-4'), 59.3 (OCH₃), 53.8 (C-4), 31.1 (C-3'), 21.2, 21.0, 20.9 (3 COCH₃), 18.6 (C-6); TOF-HRMS m/z : [M+H]⁺ Calcd for C₂₄H₃₄NO₁₀: 496.2183. Found 496.2178; Anal. Calcd. for C₂₄H₃₃NO₁₀: C, 58.17; H, 6.71; N, 2.83. Found C, 58.36; H, 6.76; N 2.78.

4.8 (2-Aminoethylamido)carbonylpentyl 4-(3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy- α -D-mannopyranosyl-(1→2)-tetrakis[4-(3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy- α -D-mannopyranosyl]-4-(3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy-2-*O*-methyl- α -D-mannopyranoside (31)

Piperidine (4.0 ml, 40 ml) was added dropwise to a solution of **23** (1.0 g, 2.0 mmol) in THF (10 ml). After 3 h, when TLC (3:1 DCM–acetone) showed that the reaction was complete, the mixture was concentrated and chromatography (10:1 DCM–acetone) gave the intermediate **24**, 3-*O*-Benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy-2-*O*-methyl- α,β -D-mannopyranose (800mg, 75%). TOF-MS: m/z : 476.1 [M+Na]⁺.

DBU (0.08 ml, 0.5 mmol) was added with stirring at 0° C to a solution of **24** (2.2 g, 4.9 mmol) and CCl₃CN (2.5 ml, 24 mmol) in DCM (30 ml), and the mixture was allowed to warm up to room temperature. After 3 h, the mixture was concentrated and chromatography (3:1, hexane–acetone with 1% Et₃N) afforded 3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy-2-*O*-methyl- α -D-mannopyranose-1-*O*-trichloroacetimidate (**25**, 2.5 g, 86%). ¹H NMR (600 MHz, CDCl₃) δ : 8.58 (s, 1 H, NHCCl₃), 7.36–7.27 (m, 5 H, Ph), 6.27 (d, $J_{1,2} = 2.0$ Hz, 1 H, H-1), 5.95 (d, $J = 8.3$ Hz, 1 H, NH), 5.16 (dd, $J = 8.0$ Hz and 4.7 Hz, 1 H, H-2'), 4.64–4.54 (AB_q, $J = 12.0$ Hz, 2 H, PhCH₂), 4.16–4.06 (m, 4 H, H-3, H-5 and H-4'), 3.93 (m, 1 H, H-4), 3.63 (t, $J = 2.5$ Hz, 1 H, H-2), 3.53 (s, 3 H, OCH₃), 2.18–2.03 (m, 8 H, 2 COCH₃ and H-3'), 1.23 (d, $J_{5,6} = 6.4$ Hz, 3 H, H-6); ¹³C NMR (150 MHz, CDCl₃) δ : 95.3 (C-1), 74.9 (C-2), 74.1 (C-3), 71.5 (PhCH₂), 71.0 (C-2'), 70.2 (C-5), 59.2 (C-4'), 59.2 (OCH₃), 52.9 (C-4), 30.8 (C-3'), 20.8 20.7 (2 COCH₃), 18.0 (C-6); TOF-HRMS, m/z : Calcd for C₂₄H₃₁N₂O₉NaCl₃ [M+Na]⁺: 619.0993. Found 619.0969.

A solution of TMSOTf in toluene (0.03 M, 11 ml) was added with exclusion of moisture to a stirred mixture of **25** (1.6 g, 2.68 mmol), **20** (4.0 g, 1.77 mmol), 4 Å molecular sieves (6.5 g) in DCM (100 ml), and the stirring was continued overnight, when TLC (3:1 DCM–acetone)

showed that only small amount of the imidate **25** was present. The mixture was neutralized with Et₃N (1.0 ml), filtered, the filtrate was concentrated, and chromatography (5:1 DCM–acetone) gave the known¹¹ hexasaccharide, 5-(methoxycarbonyl)pentyl 3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy- α -D-mannopyranosyl-(1 \rightarrow 2)-tetrakis [-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy- α -D-mannopyranosyl]-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy-2-*O*-methyl- α -D-mannopyranoside **27** (3.3 g, yield 69%).

A stirred solution of **27** (3.3 g) in DCM-MeOH (1: 10, 70 ml) was treated overnight with hydrogen in the presence 5% Pd/C (2.0 g). After filtration and concentration, the residue was chromatographed (12:1 EtOAc–EtOH) to afford 5-(methoxycarbonyl)pentyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy- α -D-mannopyranosyl-(1 \rightarrow 2)-tetrakis [-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy- α -D-mannopyranosyl]-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy-2-*O*-methyl- α -D-mannopyranoside **30** (2.25 g, 87%). TOF-MS *m/z*: 1074 [M]²⁺, 2148 [M]⁺.

The foregoing hexasaccharide **30** (2.23 g, 1.03 mmol) was treated with ethylenediamine (17 ml) at 50 °C overnight. After concentration and chromatography (1:1:0.1 MeOH–DCM–25% NH₄OH) afforded the known²⁰ **31** (1.3 g, 75%). ¹H NMR (400 MHz, D₂O) δ : 5.12–4.79 (m, 6 H, H-1^{I-VI}), 4.21–4.18 (m, 6 H, H-2^{I-VI}), 4.08–3.95 (m, 10 H, H-2^{II-V}, H-3^{I-VI}), 3.86–4.74 (m, 13 H, H-2^I, H-4^{I-VI}, H-5^{I-VI}), 3.59–3.54 (m, 1 H, H-2^{VI}, H-4^{I-VI}, H-1^a), 3.46 (m, 1 H, H-1^b), 3.40 (s, 3 H, OCH₃), 3.34 (t, *J* = 6.0 Hz, H-6^{II}), 2.95 (t, *J* = 6.0 Hz, H-7^{II}), 2.20 (t, *J* = 7.6 Hz, H-5^{II}), 1.98–1.92 (m, 6 H, H-3^a^{I-VI}), 1.80–1.72 (m, 6 H, H-3^b^{I-VI}), 1.52 (m, 4 H, H-2^{II} and H-4^{II}), 1.27 (m, 2 H, H-3^{II}), 1.12–1.08 (m, 18 H, H-6^{I-VI}). ¹³C NMR (100 MHz, D₂O) δ : 100.8–98.3 (6 C, C-1^{I-VI}), 78.8, 77.7, 77.4, 77.1 (2 C), 68.9 (6 C, C-2^{I-VI}), 57.8 (6 C, C-4^{I-VI}), 16.9–16.7 (6 C, C-6^{I-VI}); TOF-MS *m/z*: 1670.8 [M]⁺.

4.9 5-(Methoxycarbonyl)pentyl 3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy-2-*O*-levulinoyl- α -D-mannopyranosyl-(1 \rightarrow 2)-tetrakis[-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy- α -D-mannopyranosyl]-3-*O*-benzyl-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy- α -D-mannopyranoside (**26**)

A solution of TMSOTf in toluene (0.03 M, 2 ml) was added with stirring and exclusion of moisture to a mixture of **20** (500 mg, 0.22 mmol), **10** (300 mg, 0.44 mmol), 4 Å molecular sieves (300 mg) in dichloromethane (10 ml). The stirring was continued overnight, when TLC (2:1 toluene–acetone) showed that only small amount of the imidate remained unchanged. After neutralization with Et₃N (0.2 ml), filtration and concentration of the filtrate, the residue was chromatographed (3:1 toluene–acetone) to give **26** (450 mg, 73%). ¹H NMR (600 MHz, CDCl₃) δ : 6.24–5.93 (m, 5 H, 5 NH), 5.40 (brs, 1 H, H-2^{VI}), 5.20–5.15 (m, 6 H, H-2^{I-VI}), 5.05–4.97 (3 s, 4 H, H-1^{II-V}), 4.69 (s, 2 H, H-1^{I,VI}), 4.63–4.43 (m, 12 H, 6 PhCH₂), 4.30–4.01 (m, 22 H, H-2^{II-V}, H-4^{I-VI}, H-4^{I-VI}), 3.86 (brs, 1 H, H-2^I), 3.80–3.60 (m, 16 H, H-3^{I-VI}, H-5^{I-VI}, OCH₃, H-1^a), 3.35 (m, 1 H, H-1^b), 2.72–2.61 (m, 4 H, CH₂CH₂), 2.33 (t, *J* = 7.2 Hz, H-5^{a,b}), 2.26–1.99 (m, 51 H, 13 COCH₃, H-3^{I-VI}), 1.67–1.55 (m, 4 H, H-2^{a,b}, H-4^{a,b}), 1.43–1.31 (m, 2 H, H-3^{a,b}), 1.17–1.08 (m, 18 H, H-6^{I-VI}); ¹³C NMR (150 MHz, CDCl₃) δ : 137.9 (C_q), 137.8 (C_q), 137.7 (C_q), 137.6 (C_q), 137.5 (C_q), 137.4 (C_q), 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.6, 127.4, 100.5–99.6 (4 C, C-1^{II-V}), 98.6 (2 C, C-1^{I,VI}), 18.1–17.8 (6 C, C-6^{I-VI}); TOF-MS *m/z*: 1387 [M]²⁺, 2772 [M+H]⁺, 2795 [M+Na]⁺.

4.10 5-(Methoxycarbonyl)pentyl 4-(3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy- α -D-mannopyranosyl-(1 \rightarrow 2)-tetrakis[-4-(3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy- α -D-

mannopyranosyl]-4-(3-deoxy-L-glycero-tetronamido)-4,6-dideoxy- α -D-manno-pyranoside (29)

A solution of compound **26** (400 mg, 0.14 mmol) in DCM-MeOH (1:10, 10 ml) was stirred under H₂ with 5% Pd/C (400 mg) overnight, then filtered and concentrated. Without further purification, the residue was treated with 1.0 M NaOMe (1.0 ml) in MeOH (10 ml), to complete deacylation. After neutralization with Amberlite IR-120, H⁺-resin, the mixture was filtered, the filtrate was concentrated, and the residue was chromatographed (11 DCM-MeOH) to afford the known⁷ compound **29** (210 mg, 89%).

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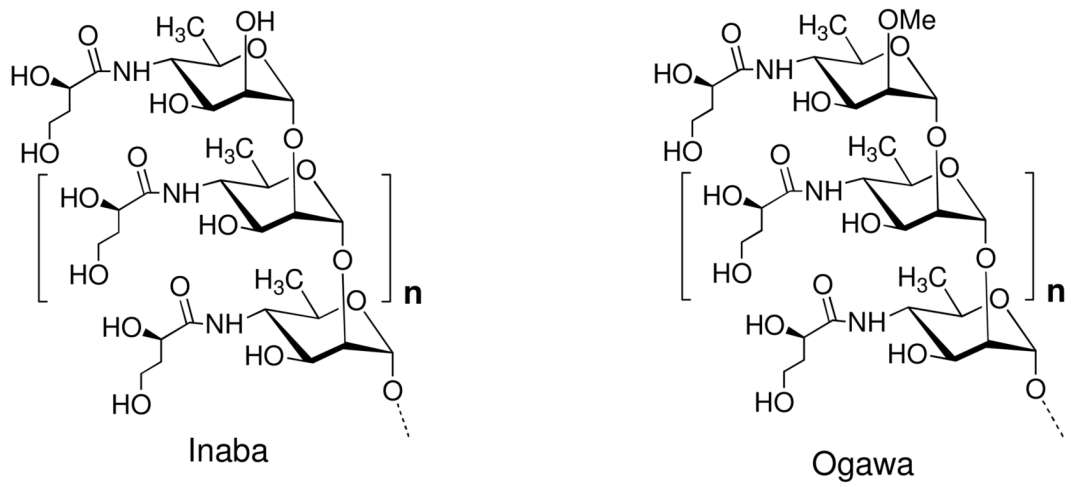
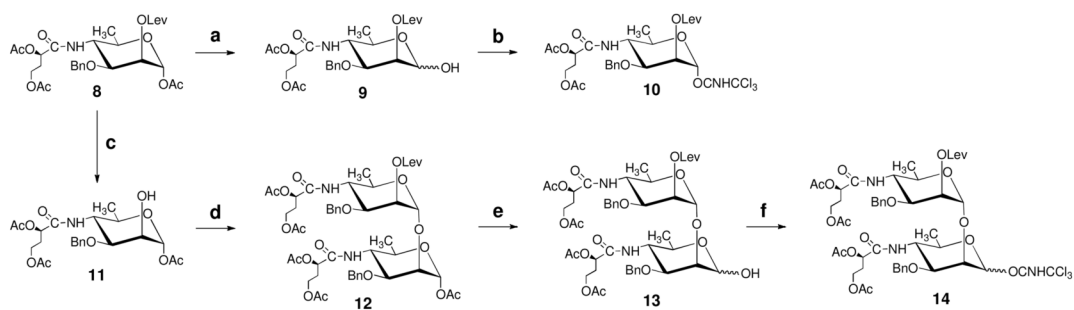
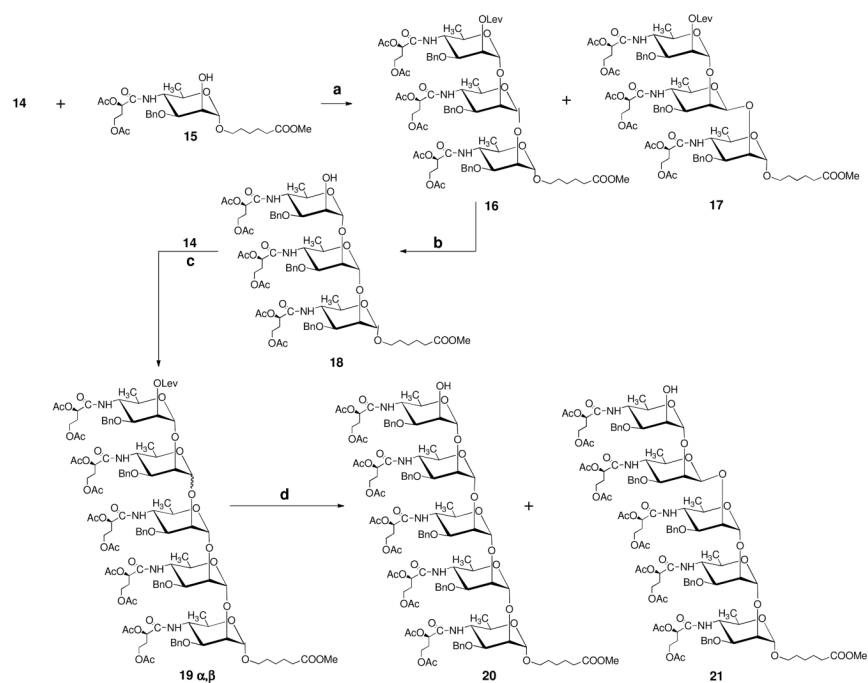


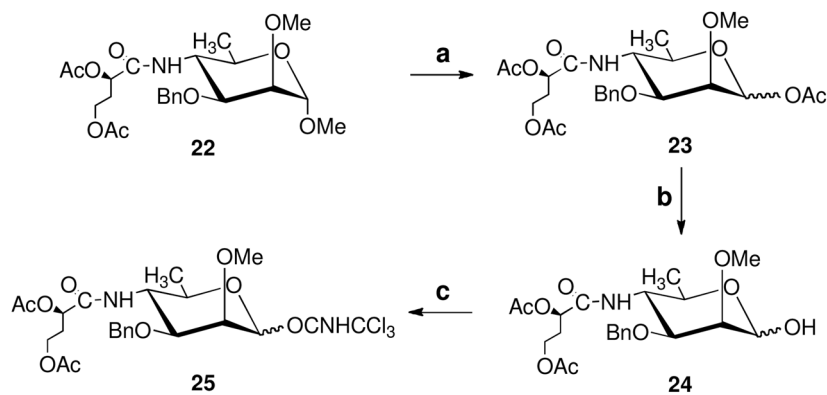
Fig 1.
Structure of the O-PSs of the two strains of *Vibrio cholerae* O1

**Scheme 1.**

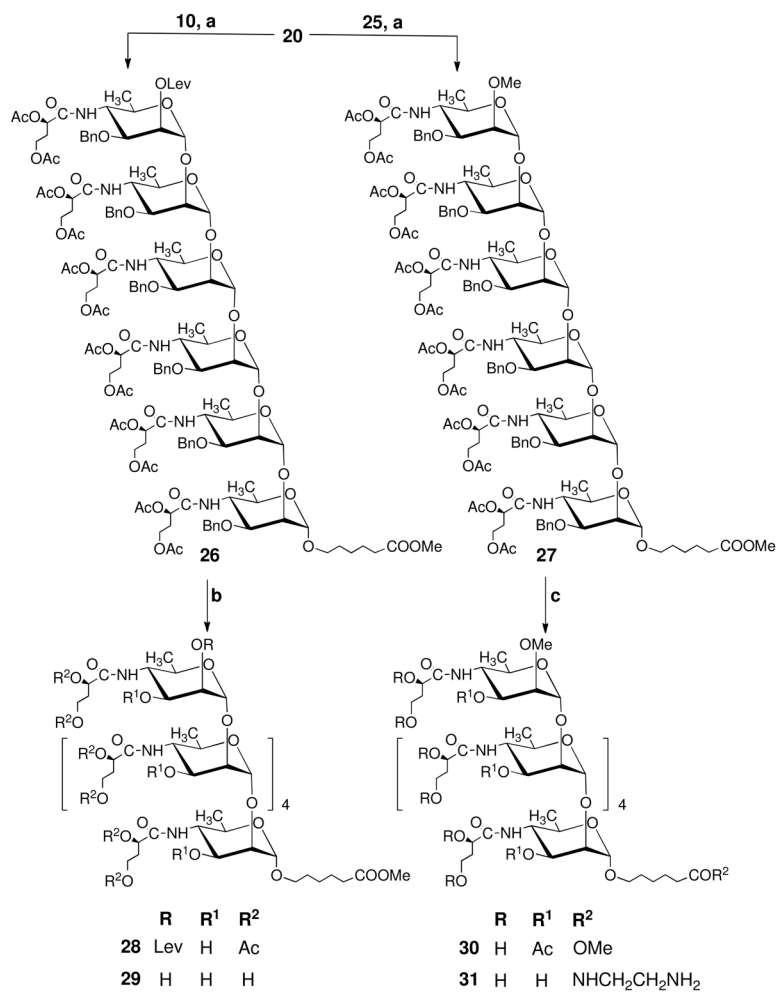
(a) Piperidine, THF, rt, 95%; (b) DBU, Cl_3CCN , DCM, 0 °C, 71%; (c) hydrazine acetate, DCM–MeOH (10:1, v/v), rt, 86%; (d) **10**, TMSOTf, 4 Å MS, toluene–DCM (3:1, v/v), rt, 76%; (e) piperidine, THF, 0 °C to rt, 75%; (f) DBU, Cl_3CCN , DCM, 0 °C to rt, 84%.

**Scheme 2.**

(a) TMSOTf, 4 Å MS, toluene, 100 °C, 86%; (b) hydrazine acetate, DCM–MeOH (10:1, v/v), rt, 87%; (c) **14**, TMSOTf, 4 Å MS, toluene, 100 °C, 75%; (d) hydrazine acetate, DCM–MeOH (10:1, v/v), rt, 91%.

**Scheme 3.**

(a) Ac_2O - HOAc - H_2SO_4 (10:4:0.1, v/v), rt, 91%; (b) piperidine, THF, rt, 75%; (d) DBU, Cl_3CCN , DCM, 0 °C to rt, 86%.

**Scheme 4.**

(a) TMSOTf, 4 Å MS, DCM, rt, 73% for **27** and 69% for **28**; (b) (i) 5% Pd/C, H₂, DCM–MeOH (1:10, v/v), rt; (ii) NaOMe, MeOH, rt. 89% over two steps; (c)(i) 5% Pd/C, H₂, DCM–MeOH (1:10, v/v), rt, 87%; (ii) H₂NCH₂CH₂NH₂, 50 °C, 75%.