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Synthesis of the dodecasaccharide fragment representing the O-polysaccharide of *Vibrio cholerae* O:1, serotype Ogawa, bearing an aglycon offering flexibility for chemical linking to proteins*

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Two azidohexasaccharide building blocks, of which the glycosyl acceptor was the 5-(methoxycarbonyl)pentyl glycoside, were coupled using the trichloroacetimidate technology. The 12 azido functions present in the dodecasaccharide thus formed were then converted to amino groups using hydrogen sulfide as a reducing reagent. Subsequent *N*-acylation with 4-*O*-benzyl-L-*glycero*-tetronic acid, followed by catalytic debenzylation yielded the desired spacer-equipped, title dodecasaccharide.

***Keywords*: *Vibrio cholerae* O-antigen, dodecasaccharide, neoglycoconjugate, 4-*O*-benzyl-3-deoxy-L-*glycero*-tetronic acid**

Introduction

The internal part of the O-polysaccharide (O-PS) of *Vibrio cholerae* O:1, serotype Ogawa, consists of a relatively short chain of about 15 (1 → 2)-linked moieties of 4-amino-4,6-dideoxy-α-D-mannopyranose (D-perosamine), the amino groups of which are acylated with 3-deoxy-L-*glycero*-tetronic acid [2–4]. The upstream, terminal (non-reducing end) perosaminyl residue has its H*O*-2 methylated. The O-PS of *Vibrio cholerae* O:1, serotype Inaba, has an identical structure, but the terminal perosamine residue remains unmethylated [5]. We are interested in preparing neoglycoconjugates that could be used as imunogens for *Vibrio cholerae* O:1 antibodies. In the initial stages of our effort to develop a semisynthetic vaccine against cholera we synthesize ligands related to the O-antigen of *Vibrio cholerae* O:1 suitable for conjugation to carrier proteins. We have already reported on synthesis of glycosides of di- and hexasaccharide fragments of the O-PS of both *Vibrio cholerae* O:1 serotypes, whose aglycons make these substances amenable for linking to suitable carriers [1, 6]. By varying the size of the O-antigenic fragment, and other details in the architecture of the carbohydrate antigen-protein combinations, and studying the immunochemical properties of such molecules, we expect to unravel factors that affect the immune response to the *V. cholerae* O:1 pathogen. Conjugation of these molecules, and evaluation of the immunogenicity of the neoglycoproteins thus obtained is currently in progress. Here we describe the synthesis of the 5-(methoxycarbonyl)pentyl [7] glycoside of a dodecasaccharide belonging to the Ogawa series. Such an oligosaccharide has a molecular length that approaches that of the entire O-PS. The methoxycarbonyl group present in its aglycon offers considerable flexibility of choice of the chemistry for conjugation to proteins [8–10]. The immunochemical properties of neoglycoproteins derived from the described ligand will be described in a separate communication.

Materials and methods

General methods

Instruments and laboratory techniques used were described in a previous part in this series [21]. Unless stated otherwise, optical rotations were measured at ambient temperature for solutions in chloroform ($c \sim 1$). All reactions were monitored by thin-layer chromatography (TLC) on Silica gel 60 coated glass slides (Whatman or Analtech). The following solvent mixtures were used: *A*, 4:1 hexane-EtOAc; *B*, 50:1 CH_2Cl_2–MeOH; *C*, 5:1 CH_2Cl_2-MeOH; *D*, 20:1

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CH_2Cl_2–MeOH; *G*, 1:5:0.1 CH_2Cl_2-MeOH-NH_4OH. For column chromatography, solvent mixtures slightly less polar than those used for TLC were engaged at the onset of development. Assignments of NMR signals (measured at 300 MHz for ^1H and 75 MHz for ^{13}C) were made by first-order analysis of the spectra, and by comparison with spectra of related substances. When the latter approach was used to aid in the ^{13}C NMR signal-nuclei assignments, advantage was taken of variations of line intensity expected for oligosaccharides belonging to the same homologous series [22, 23]. 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. When feasible, the asignments were supported by homonuclear decoupling experiments or homonuclear and heteronuclear two-dimensional correlation spectroscopy, run with the software supplied with the spectrometers. When reporting assignments of NMR signals, sugar residues in oligosaccharides are serially numbered, beginning with the one bearing the aglycone, and are identified by a Roman numeral superscript in listings of signal assignments. Nuclei-assignments without a superscript notation indicates that those signals have not been individually assigned. Thus, for example, in a spectrum of a disaccharide, a resonance denoted H-3 can be that of H-3 of either sugar residue. Palladium (5%)-on charcoal catalyst (ESCAT 103) was a product of Engelhard Industries.

2-*O*-Acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1 → 2)-[4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1 → 2)]$_4$-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranose (**2**)

A mixture of 2-(trimethylsilyl)ethyl 2-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1 → 2)-[4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1 → 2)]$_4$-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside [1] (**1**, 3 g) and trifluoroacetic acid (TFAA, 60 ml) was stirred at room temperature for 2 h (TLC, solvent *A*). The solution was concentrated, and chromatography gave **2** (2.26 g, 80%) as a mixture of anomers where the α-anomer largely predominated. ^1H NMR ($CDCl_3$): δ 5.41 (dd, 1 H, $J_{1,2}$ 1.8, $J_{2,3}$ 3.4 Hz, H-2^{VI}), δ 5.05 (d, 1 H, $J_{1,2}$ 2 Hz, H-1^{I}), δ 4.95 (d, 1 H, $J_{1,2}$ 2 Hz, H-1), 4.88–4.85 (m, 4 H, 4 H-1, incl H-1^{VI}), 4.75–4.52 (m, 12 H, 6 CH_2Ph), 3.88–3.15 (m, 23 H, incl m for H-2^{I} at ~3.83, H-2$^{II–V}$, 3$^{I–VI}$, 4$^{I–VI}$, 5$^{I–VI}$), 2.10 (s, 3 H, $COCH_3$), 1.26–1.11 (m, 18 H, H-6$^{I–VI}$); ^{13}C NMR ($CDCl_3$): δ 100.27, 100.12, 100.09, 100.04 (C-1$^{II–V}$), 99.10 (C-1^{VI}), 93.39 ($J_{C,H}$ 171.4 Hz, C-1^{I}), 76.91, 76.80, 76.65, 76.57, 76.55 (C-3$^{I–V}$), 75.40 (C-3^{VI}), 73.93 (C-2^{I}), 73.52 (2 C), 73.46, 73.39 (C-3$^{II–V}$), 72.17 (4 C), 72.04, 71.54 (6 *C*H_2Ph), 67.81 (3 C), 67.76, 67.65 (5 C-5), 67.15 (2 C, C-5, 2^{VI}), 64.41, 64.20 (3 C), 64.05, 63.83 (C-4$^{I–VI}$), 20.95 ($COCH_3$), 18.64, 18.58, 18.48 (3 C), 18.37 (C-6$^{I–VI}$); FABMS, *m/z* 1759.7 [(M + Cs)]$^+$.

Analytical data. Calculated for $C_{80}H_{94}N_{18}O_{20}$: C, 59.03; H, 5.82; N, 15.49. Found: C, 59.00; H, 5.88; N, 15.38.

2-*O*-Acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1 → 2)-[4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1 → 2)]$_4$-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl trichloroacetimidate (**3**)

DBU (0.1 g, 0.65 mmol) was added to a solution of **2** (2.2 g, 1.35 mmol) and CCl_3CN (9.7 g, 65 mmol) in CH_2Cl_2 (40 ml). The mixture was stirred at room temperature for 30 min (TLC, solvent *B*), and directly chromatographed to give amorphous **3** (2.06 g, 87%), $[\alpha]_D$ + 72°, ^1H NMR ($CDCl_3$): δ 6.03 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1^{I}), 5.41 (dd, 1 H, $J_{1,2}$ 1.9, $J_{2,3}$ 3.4 Hz, H-2^{VI}), 4.96 (d, 1 H, $J_{1,2}$ 1.8, H-1), 4.90 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.89, 4.88 (2 d, partially overlapped, $J_{1,2}$ ~1.8 Hz, 2 H-1), 4.85 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1^{VI}), 4.76–4.52 (m, 12 H, 6 CH_2Ph), 3.89–3.17 (m, 23 H, 2 H-2, 3$^{I–VI}$, 4$^{I–VI}$, 5$^{I–VI}$, incl 2 dd at 3.88 and 3.73 for 2 H-2, and dd at 3.83, overlapped, for H-2^{I}), 2.11 (s, 3 H, $COCH_3$), 1.30, 1.27 (2 d, 3 H each, $J_{5,6}$ 6.2 Hz, 2 H-6), 1.22–1.17 (m, 12 H, 4 H-6); ^{13}C NMR ($CDCl_3$): δ 100.38, 100.16, 100.12, 100.07 (C-1$^{II–V}$), 99.11 (C-1^{VI}), 96.18 ($J_{C,H}$ 178.8 Hz, C-1^{I}), 76.81, 76.57 (3 C), 76.40 (C-3$^{I–V}$), 75.42 (C-3^{VI}), 73.49 (2 C), 73.40 (2 C, C-2$^{II–V}$), 72.47, 72.35, 72.20, 72.18, 72.05, 71.55 (6 *C*H_2Ph), 72.01 (C-2^{I}), 70.09 (C-5^{I}), 68.05, 67.87 (2 C), 67.84, 67.66 (5 C-5), 67.16 (C-2^{VI}), 64.20, 64.10, 64.07 (2 C), 63.85, 63.69 (C-4$^{I–VI}$), 20.96 ($CO*C*H_3$), 18.63, 18.50 (3 C), 18.46, 18.38 (C-6$^{I–VI}$); FABMS: *m/z* 1902.5 ([M + Cs]$^+$).

5-(Methoxycarbonyl)pentyl 2-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1 → 2)-[4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1 → 2)]$_4$-4-azido-3-*O*-benzyl-4,6-dideoxy-α- (**4**) and β-D-mannopyranoside (**14**)

A catalytic amount of TESOTf was added to a solution of **3** (2.0 g, 1.13 mmol), methyl 6-hydroxyhexanoate [15] (800 mg, 5.65 mmol) and 4 Å molecular sieves in CH_2Cl_2 (100 ml). The mixture was stirred at room temperature for 20 min (TCL, solvent *B*), and directly chromatographed to give first the α-isomer **4** (1.05 g, 53%), $[\alpha]_D$ + 90°. ^1H NMR ($CDCl_3$): δ 5.41 (dd, 1 H, $J_{1,2}$ 2.0, $J_{2,3}$ 3.3 Hz, H-2^{VI}), 4.96 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1), 4.88 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.86–4.83 (m, 3 H, 3 H-1, incl H-1^{VI}), 4.75–4.50 (m, 13 H, H-1^{I}, 6 CH_2Ph), 3.88 (bdd, 1 H, H-2), 3.84 (bdd, 1 H, H-2), 3.82–3.13 (m, 27 H, 4 H-2, H-3$^{I–VI}$, 4$^{I–VI}$, 5$^{I–VI}$ OCH_2, incl s 3.67 $COOCH_3$, dd ~3.78, H-3^{VI}), 2.31 (t, 2 H, $COCH_2$), 2.10 (s, 3 H, $COCH_3$), ~1.68–1.57 (m, partially overlapped, C$H_2$$CH_2$CO), ~1.60–1.49 (m, partially overlapped, OCH_2CH_2), ~1.38–1.24 (m, partially overlapped, OCH_2CH_2CH_2), 1.26–1.12 (6 d, partially overlapped, H-6$^{I–VI}$); ^{13}C NMR ($CDCl_3$): δ 100.36, 100.12 (2 C), 100.05, (C-1$^{II–V}$), 99.10 (C-1^{VI}), 98.60 (C-1^{I}), 77.43, 76.82, 76.67, 76.57 (2 C, C-3$^{I–V}$), 75.42 (C-3^{VI}), 74.06 (C-2^{I}), 73.61, 73.47 (2 C), 73.39 (C-2$^{II–V}$), 72.17 (3 C), 72.13, 72.05, 71.55 (6CH_2Ph), 67.83 (3 C),

67.74, 67.66, 67.07 (C-$5^{I–VI}$), 67.50 (OCH_2), 67.16 (C-2^{VI}), 64.38, 64.28, 64.22 (2 C), 64.07, 63.84 (C-$4^{I–VI}$), 51.48 (CH_2COCH_3), 33.90 (CH_2CO), 29.01 (OCH_2CH_2), 25.66 ($OCH_2CH_2CH_2CH_2$), 24.64 ($CH_2CH_2CH_2CO$), 20.96 ($COCH_3$), 18.58 (2 C), 18.49 (3 C), 18.38 (C-$6^{I–VI}$); FABMS: *m/z* 1887.6 ($[M + Cs]^+$).

Analytical data. Calculated for $C_{87}H_{106}N_{18}O_{22}$: C, 59.51; H, 6.08; N, 14.36. Found: C, 59.35; H, 6.13; N, 14.23.

Eluted next was the amorphous *β*-isomer **14**, (520 mg, 26%), $[\alpha]_D$ + 38°. Structurally significant signals in the ^{1}H NMR ($CDCl_3$) spectrum were at *δ* 6.60, 6.20 (2 bs, 3 H each, 6 NH), 5.41 (dd, 1 H, $J_{1,2}$ 1.9, $J_{2,3}$ 3.3 Hz, H-2^{VI}), 5.00, 4.93, 4.88, 4.86 (4 d, 1 H, each, $J_{1,2}$ 1.9 Hz, H-$1^{II–V}$), 4.84 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1^{VI}), 4.75–4.51 (m, 6 CH_2Ph), 4.20 (bs, partially overlapped, H-1^{I}), 4.01 (bd, partially overlapped, H-2^{I}), 3.88–3.61 (m, incl dd at 3.74, $J_{2,3}$ 3.3, $J_{3,4}$ 11.7 Hz, H-3^{VI}, m at ~3.68 for OCH_a and s at 3.67 for $COOCH_3$), 3.55–3.00 (m, incl dd at ~3.20 for H-3^{I} and m at ~3.05 for H-5^{I}), 2.32 (t, 2 H, *J* 7.4 Hz, CH_2CO), 2.10 (s, 3 H, $COCH_3$), 1.70–1.54 (m, 4 H, $OCH_2CH_2CH_2CH_2$), 1.45–1.28 (m, $OCH_2CH_2CH_2$ incl d, at 1.34, $J_{5,6}$ 5.9 Hz, H-6^{VI}), 1.25–1.08 (5 d, partially overlapped, H-$6^{I–V}$); ^{13}C NMR ($CDCl_3$): *δ* 100.05 ($J_{C,H}$ 171.9 Hz), 99.95 ($J_{C,H}$ 171.4 Hz), 99.88 ($J_{C,H}$ 171.4 Hz), 98.93 ($J_{C,H}$ 171.7 Hz, C-$1^{II–V}$), 99.60 ($J_{C,H}$ 154.1 Hz, C-1^{I}), 99.01 ($J_{C,H}$ 172.6 Hz, C-1^{VI}), 80.18 (C-3^{I}), 77.13, 76.75, 76.58 (2 C, C-$3^{II–V}$), 75.37 (C-3^{VI}), 73.57, 73.45, 73.40, 73.33 (C-$2^{II–V}$), 72.39, 72.08, 72.05, 71.96, 71.49, 69.49 (6 CH_2Ph), 71.12 (C-2^{I}), 70.98 (C-5^{I}), 67.75 (3 C), 67.58, 66.99 (C-$5^{II–VI}$), 67.11 (C-2^{VI}), 64.42, 64.18, (3 C), 63.97, 63.73 (C-$4^{I–VI}$), 62.46 (OCH_2), 51.47 ($COOCH_3$), 33.86 (CH_2CO), 28.23 (OCH_2CH_2), 25.55 ($OCH_2CH_2CH_2$), 24.60 ($OCH_2CH_2CH_2CH_2$), 20.91 ($COCH_3$), 18.42 (2 C), 18.34 (4 C, C-$6^{I–VI}$); FABMS: *m/z* 1887.6 ($[M + Cs]^+$).

5-(Methoxycarbonyl)pentyl 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1 → 2)-[4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1 → 2)]$_4$-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (**5**)

Compound **4** (1 g) was deacetylated (Zemplén), to give **5** (732 mg, 75%), $[\alpha]_D$ + 107°. ^{1}H NMR ($CDCl_3$): *δ* 4.98 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1^{VI}), 4.96, 4.88 (2 d, 1 H each, $J_{1,2}$ ~1.9 Hz, 2 H-1), 4.84 (2 d, overlapped, 2 H, $J_{1,2}$ ~1.9 Hz, 2 H-1), 4.72–4.58 (m, 13 H, 6 CH_2Ph, incl bd at ~4.59 for H-1^{I}), 4.00 (bt, 1 H, H-2^{VI}), 3.94, 3.84, 3.80, 3.79 (4 bt, partially overlapped, 4 H, H-$2^{II–V}$), 3.76 (bt, 1 H, H-2^{I}), 3.70–3.60 (m, partially overlapped, H-$3^{I–VI}$, incl s at 3.67 for $COOCH_3$), 3.58–3.13 (m, partially overlapped, 14 H, H-$4^{I–VI}$, $5^{I–VI}$, OCH_2), 2.32 (m, 2 H, CH_2CO), 1.68–1.58 (m, partially overlapped, ~2 H, CH_2CH_2CO), 1.58–1.49 (m, partially overlapped, ~2 H, OCH_2CH_2), 1.37–1.26 (m, partially overlapped, ~2 H, $OCH_2CH_2CH_2$), 1.27–1.12 (6 d, partially overlapped, C-$6^{I–VI}$); ^{13}C NMR ($CDCl_3$): *δ* 100.47, 100.37, 100.21, 100.12 (2 C, C-$1^{II–VI}$), 98.59 (C-1^{I}), 77.67, 77.42, 76.97, 76.60, 76.53 (2 C, C-3-$^{I–VI}$), 74.06, 73.61, 73.45 (2 C), 73.28 (C-$2^{I–V}$), 67.82 (3 C), 67.73, 67.34, 67.06 (C-$5^{I–VI}$), 67.50 (OCH_2), 67.15 (C-2^{VI}), 64.37, 64.21 (4 C), 63.84 (C-$4^{I–VI}$), 51.49 ($COOCH_3$), 33.90 (CH_2CO), 29.01 (OCH_2CH_2), 25.66 ($OCH_2CH_2CH_2$), 24.64 (CH_2CH_2CO), 18.59 (2 C), 18.50 (3 C), 18.31 (C-$6^{I–VI}$); FABMS: *m/z* 1845.7 ($[M + Cs]^+$).

Analytical data. Calculated for $C_{85}H_{104}N_{18}O_{21}$: C, 59.57; H, 6.12; N, 14.71. Found: C, 59.31; H, 6.09; N, 14.62.

4-Azido-3-*O*-benzyl-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-(1 → 2)-[4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1 → 2)]$_4$-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranose (**7**)

Compound **7** (1.87 g, 81%) was prepared from 2-(trimethylsilyl)ethyl 4-azido-3-O-benzyl-4,6-dideoxy-2-O-methyl-α-D-mannopyranosyl-(1 → 2)-[4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1 → 2)]$_4$-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (**6** [1], 2.5 g), as described for the preparation of **2**. It was obtained as a mixture of anomers where the α-anomer largely predominated. ^{1}H NMR ($CDCl_3$): *δ* 5.04 (bdd, H-1^{I}), 4.95–4.90 (m, 3 H, 3 H-1), 4.89–4.84 (m, 2 H, 2 H-1), 4.83 (bd, 1 H, H-1), 4.78–4.52 (m, 12 H, 6 CH_2Ph), 3.20 (s, overlapped, OCH_3), 2.68 (bd, $J_{1,OH}$ ~4 Hz, OH), 1.75–1.20 (m, 18 H, H-$6^{I–VI}$); ^{13}C NMR ($CDCl_3$): *δ* 100.29, 100.23, 100.14, 100.09, 98.78 (C-$1^{II–VI}$α), 93.39 (C-1^{I}α), 100.62, 100.37, 100.26, 100.18, 98.80 (C-$1^{II–VI}$*β*), 93.15 ($J_{C,H}$ 161.3 Hz, C-1^{I}*β*); FABMS, *m/z* 1731.7 ($[M + Cs]^+$).

Analytical data. Calculated for $C_{79}H_{94}N_{18}O_{19}$: C, 59.31; H, 5.92; N, 15.76. Found: C, 59.22; H, 5.95; N, 15.66.

4-Azido-3-*O*-benzyl-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-(1 → 2)-[4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1 → 2)]$_4$-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl trichloroacetimidate (**8**)

The trichloroacetimidate **8** (1.71 g, 87%) was obtained from **7** (1.8 g) as described for preparation of **3**, $[\alpha]_D$ + 91°. ^{1}H NMR ($CDCl_3$): *δ* 6.03 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1^{I}), 4.91 (bd, partially overlapped, ~3 H, 3 H-1), 4.90, 4.88 (2 d, partially overlapped, ~2 H, 2 H-1), 4.76–4.59 (m, 12 H, 6 CH_2Ph), 3.97 (bt, 1 H, H-2), 3.86–3.82 (m, 4 H, H-2^{I}, 3 H-2), 3.74–3.15 (m, 22 H, H-$3^{I–VI}$, $4^{I–VI}$, $5^{I–VI}$, incl bt at 3.39 for H-2^{VI}, and s at 3.20 for OCH_3), 1.30, 1.27, 1.18 (2 d, 3 H, each, d 12 H, H-$6^{I–VI}$); ^{13}C NMR ($CDCl_3$): *δ* 100.39, 100.27, 100.19, 100.13 (C-$1^{II–V}$), 98.82 (C-1^{VI}), 96.19 (C-1^{I}), 77.46, 77.19, 77.64, 76.58 (2 C), 76.40 (2 C, C-$3^{I–VI}$,2^{VI}), 73.48, 73.39, 73.29, 73.20 (C-$2^{II–V}$), 72.48, 72.40, 72.35, 72.19 (2 C), 72.07 (6 CH_2Ph), 72.04 (C-2^{I}), 70.09 (C-5^{I}), 68.10, 67.89 (2 C), 67.87, 67.79 (C-$5^{II–VI}$), 64.35, 64.22 (2 C), 64.12 (2 C), 63.70 (C-$4^{I–VI}$), 58.93 (OCH_3), 18.64, 18.52 (3 C), 18.46, 18.41 (C-$6^{I–VI}$); FABMS: *m/z* 1874.6 ($[M + Cs]^+$).

5-(Methoxycarbonyl)pentyl 4-azido-3-*O*-benzyl-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-(1 → 2)-[4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1 → 2)]$_{10}$-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (**9**)

Coupling of **5** (700 mg, 0.41 mmol) and **8** (1.06 g, 0.61 mmol), as described for the preparation of **4**, gave the dodecasaccharide **9** (876 mg, 65%), $[\alpha]_D + 85°$. Structurally significant signals in the ^{1}H NMR ($CDCl_3$) were at δ 4.95–5.55 (m, 36 H, H-1$^{I–XII}$, 12 CH_2Ph), 3.67 (s, overlapped, COOCH_3), 3.27 (s, overlapped, OCH_3), 2.32 (t, 2 H, *J* 7.6 Hz, CH_2CO), 1.70–1.49 (m, 4 H, OCH$_2$CH_2CH$_2$CH_2, 1.40–1.20 (m, partially overlapped, OCH$_2$CH$_2$CH_2), 1.30–1.05 (m, overlapped, H-6$^{I–XII}$); ^{13}C NMR ($CDCl_3$) δ: 100.34 (2 C), 100.24, 100.15, 100.08 (6 C, H-1$^{II–XI}$), 98.79 (C-1^{XII}), 98.58 (C-1^{I}), 77.42, 77.17, 76.57, 76.38 (13 C total, C-2^{XII}, C-3$^{I–XII}$), 74.05, 73.61, 73.47, 73.27, 73.17 (11 C total, C-2$^{I–XI}$), 72.38, 72.14 (10 C), 72.05 (12 *C*H$_2$Ph), 67.82 (11 C), 67.06 (C-5$^{I–XII}$), 67.49 (OCH$_2$), 64.35, 64.22 (10 C), 64.12 (C-4$^{I–XII}$), 58.90 (OCH$_3$), 51.47 (COO*C*H$_3$), 33.88 (*C*H$_2$CO), 28.99 (OCH$_2$*C*H$_2$), 25.65 (OCH$_2$CH$_2$*C*H$_2$), 24.62 (*C*H$_2$CH$_2$CO), 18.57, 18.49 (10 C), 18.40 (C-6$^{I–XII}$); FABMS: *m/z* 3428.5 ([M + Cs]$^+$).

Analytical data. Calculated for $C_{164}H_{196}N_{36}O_{39}$: C, 59.77; H, 5.99; N, 15.30. Found: C, 59.67; H, 6.06; N, 15.20.

5-(Methoxycarbonyl)pentyl 3-*O*-acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-(1 → 2)-[3-*O*-acetyl-4(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1 → 2)]$_{10}$-3-*O*-acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (**12**)

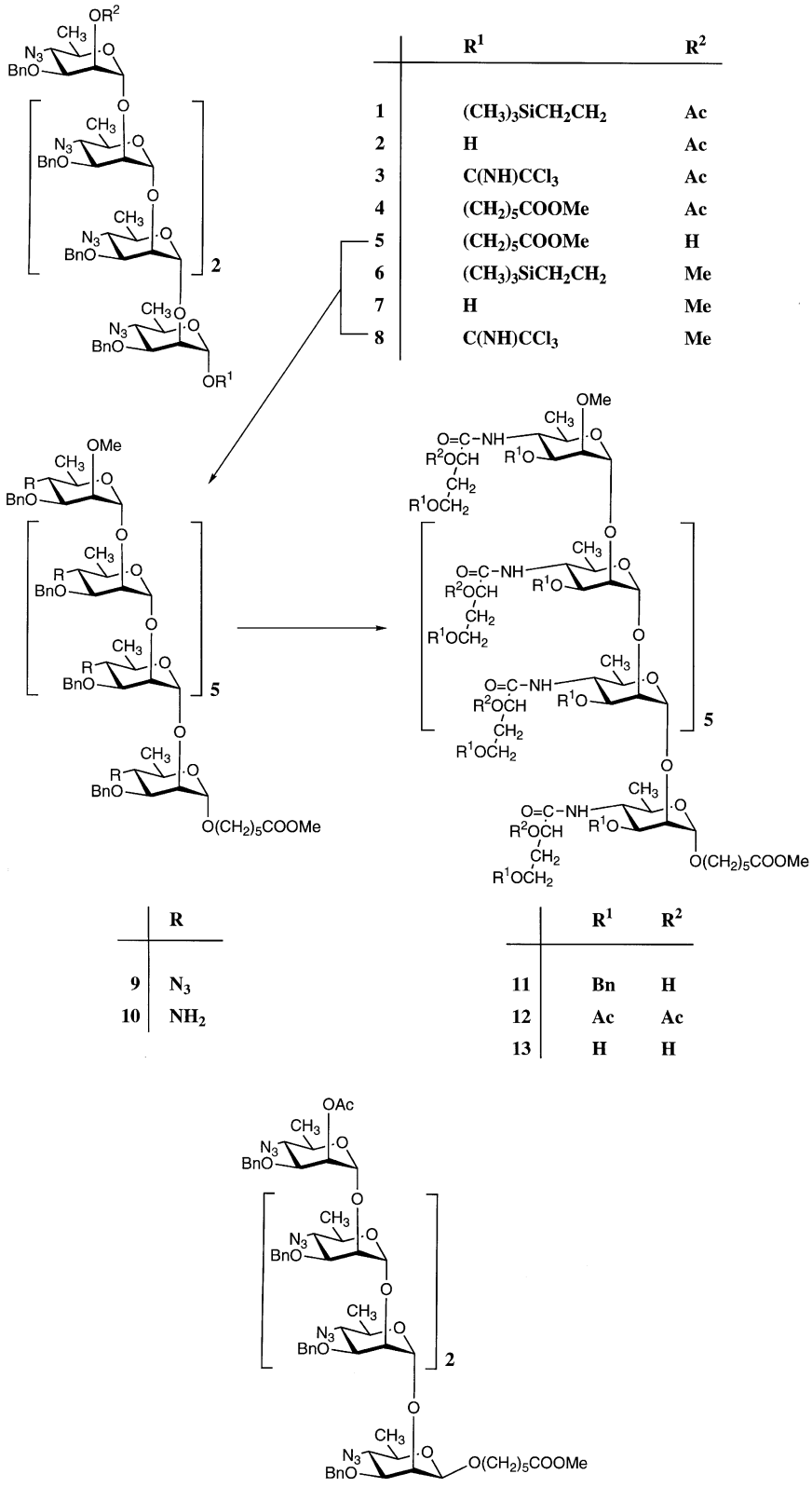
Hydrogen sulfide was passed for 1 h through a solution of **9** (800 mg) in 7:3 pyridine-triethylamine (20 ml), and the mixture was kept overnight at room temperature (TLC, solvent *C*). After concentration, the residue was chromatographed to give amine **10** (350 mg, 48%), FABMS: *m/z* 2982.5 ([M + 1]$^+$).

A solution of the foregoing compound **10** (350 mg, 0.11 mmol), 4-*O*-benzyl-3-deoxy-L-*glycero*-tetronic acid [11] (443 mg, 2.1 mmol), WSC (400 mg, 2.1 mmol), and hydroxybenzotriazole (280 mg, 2.1 mmol) in DMF (10 ml) was stirred overnight at room temperature (TLC, solvent *D*). After addition of chloroform the solution was washed successively with 2 N HCl, aqueous hydrogen carbonate, and NaCl solutions, dried, and concentrated. Chromatography of the residue gave crude **11** (320 mg).

A solution of the foregoing compound **11** (320 mg) in 90% acetic acid was hydrogenolysed overnight at room temperature in the presence of 5% palladium-on-charcoal catalyst (300 mg). After conventional processing, the crude material was treated with excess of pyridine-Ac_2O, and after processing, the crude product was chromatographed (solvent *D*). Analysis by ^{1}H NMR spectroscopy revealed the presence of resonances at δ 7.45–7.20, indicative of incomplete hydrogenolysis of benzyl groups present in **11**. The treatment with hydrogen followed by acetylation was repeated, as described above, whereupon the desired compound **12** (17 mg, 6%, based on **10**) was obtained. Structurally significant, confidently assignable signals in the ^{1}H NMR ($CDCl_3$) were at δ 6.85–6.32 (m, 12 H, 12 NH), 5.34–5.26 (m, 12 H, H-3$^{I–XII}$), 4.92 (bd, 1 H, H-1), 4.74 (bd, 1 H, H-1^{I}), 3.93 (bs, 1 H, H-2^{I}), 3.71 (s, overlapped, COOCH_3), 3.56 (s, 3 H, OCH_3), 3.50–3.36 (m, 1 H, OCH), 2.40–2.30 (m, partially overlapped, CH_2CO), 1.84–1.56 (m, partially overlapped, OCH$_2$CH_2CH$_2$CH_2), 1.50–1.35 (m, partially overlapped, OCH$_2$CH$_2$CH_2) 1.30–1.05 (m, 36 H, H-6$^{I–XII}$); structurally significant, confidently assignable signals in the ^{13}C NMR ($CDCl_3$) were at δ 100.40, 100.06, 99.75, 99.72 (total of 11 C, C$^{II–XI}$), 98.65 (C-1^{I}), 77.84 (C-2^{XII}), 75.55–74.25 (total of 11 C, C-2$^{I–XI}$), 70.95 (12 C, C-2′$^{I–XII}$), 70.88 (10 C), 69.74 (7 C), 68.99 (7 C, 3$^{I–XII}$, 5$^{I–XII}$), 67.05 (OCH$_2$), 59.99 (7 C), 59.93 (5 C, C-4′$^{I–XII}$), 59.73 (OCH$_3$), 51.80 (7 C), 51.69 (4 C), 51.45 (2 C, C-4$^{I–XII}$, COO*C*H$_3$), 33.88 (CH_2CO), 30.62 (12 C, C-3′$^{I–XII}$), 28.66, 24.35 (OCH$_2$*C*H$_2$CH$_2$*C*H$_2$), 25.42 (OCH$_2$CH$_2$*C*H$_2$), 17.97 (9 C), 17.86 (3 C, C-6$^{I–XII}$); FABMS: *m/z* 4641.7 ([M + 1]$^+$), 4663.9 ([M + Na]$^+$).

5-(Methoxycarbonyl)pentyl 4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranosyl-(1 → 2)-[4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranosyl-(1 → 2)]$_{10}$-4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (**13**)

Deacetylation (Zemplén) of **12** (27 mg) gave (TLC, solvent *E*) after freeze drying, the target dodecasaccharide derivative **13** as a white solid, (17 mg, 94%), $[\alpha]_D - 3°$ (*c* 0.8, 3:1 MeOH–H_2O). Structurally significant, confidently assignable signals in the ^{1}H NMR (~ 5:1 CD_3OD-D_2O): δ 3.67 (s, overlapped, COOCH_3), 3.48 (s, overlapped, OCH_3), 2.36 (t, 2 H, CH_2CO), 2.10–1.94, 1.92–1.72 (2 m, 24 H, H-3′$^{I–XII}$), 1.68–1.56 (m, 4 H, OCH$_2$CH_2CH$_2$CH_2), 1.47–1.37 (m, 2 H, OCH$_2$CH$_2$CH_2), 1.30–1.04 (m, 36 H, H-6$^{I–XII}$); ^{13}C NMR (~ 5:1 CD_3OD–D_2O): δ 102.30 (2 C), 102.24 (8 C, C-1$^{II–XI}$), 100.34 (C-1^{XII}), 100.05 (C-1^{I}), 80.43 (C-2^{XII}), 79.45 (C-2^{I}), 78.81 (10 C, C-2$^{II–XI}$), 70.45 (12 C, C-2′$^{I–XII}$), 69.70, 69.45 (12 C), 69.24 (2 C), 69.12 (8 C), 68.99 (C-3$^{I–XII}$, 5$^{I–XII}$), 68.78 (OCH$_2$), 59.23 (12 C, C-4′$^{I–XII}$), 58.66 (OCH$_3$), 54.39 (12 C, C-4$^{I–XII}$), 52.76 (COO*C*H$_3$), 37.84 (12 C, C-3′$^{I–XII}$), 34.65 (*C*H$_2$CO), 29.76, 25.46 (OCH$_2$*C*H$_2$CH$_2$*C*H$_2$), 26.49 (OCH$_2$CH$_2$*C*H$_2$), 18.14 (12 C, C-6$^{I–XII}$); FABMS: *m/z* 3127.9 ([M + 1]$^+$).



	R^1	R^2
1	$(CH_3)_3SiCH_2CH_2$	Ac
2	H	Ac
3	$C(NH)CCl_3$	Ac
4	$(CH_2)_5COOMe$	Ac
5	$(CH_2)_5COOMe$	H
6	$(CH_3)_3SiCH_2CH_2$	Me
7	H	Me
8	$C(NH)CCl_3$	Me

	R
9	N_3
10	NH_2

	R^1	R^2
11	Bn	H
12	Ac	Ac
13	H	H

14

Scheme. 1

Results and discussion

In the course of syntheses of fragments of O-PS of *Vibrio cholerae* O:1, this laboratory has built up an extensive library of useful building blocks. Of those, most useful for construction of the complex dodecasaccharide **13** appear to be the hexasaccharide trimethylsilylethyl (SE) glycosides **1** and **6**, and the *N*-acylating reagent derived from 3-deoxy-L-*glycero*-tetronic acid, 4-*O*-benzyl-3-deoxy-L-*glycero*-tetronic acid. These substances, prepared in connection with our previous syntheses (see [1, 6, 11, 12] and papers cited therein) are now incorporated in the reaction pathway (Scheme 1) leading to the title dodecasaccharide. Accordingly, compound **1** was treated with neat trifluoroacetic acid at room temperature, to affect deprotection of the anomeric position (**1** → **2**). We have found these conditions more suitable for clean removal of the anomeric 2-(trimethylsilylethyl) group in higher oligosaccharides than the more commonly used procedure that utilizes dichloromethane as a solvent [13]. The hemiacetal **2** was then converted [14] to the trichloroacetimidate **3** which was treated with methyl 6-hydroxyhexanoate [7, 15] to give, after chromatography, the desired 5-(methoxycarbonyl)pentyl α-glycoside **4**. Some material, identified by NMR spectroscopy as the corresponding β-anomer (**14**), was also obtained. It had a more negative specific rotation than that of **4**, and diagnostic for the assignment of the anomeric configuration to **14** were the $J_{C\text{-}1,H\text{-}1}$ values found in the ^{13}C NMR spectrum of **14**. Also, the resonance for H-1^{I} observed in the ^{1}H NMR spectrum of **14** was shifted upfield, when compared with the analogous signal observed in the spectrum of **4**. To obtain a glycosyl acceptor for the subsequent chain-alongation, compound **4** was deacetylated (**4** → **5**).

The previously prepared [1] SE-glycoside **6** was converted to the glycosyl trichloroacetimidate **8**, and this was coupled with **5** under conditions known to produce mainly 1,2-*trans*-glycosides. The resulting dodeca-azido derivative **9** was obtained in 65% yield in the analytically pure state. Such pure material was used for further conversions. This comprised the reduction with H_2S of the azido groups to amino functions [16] (**9** → **10**), subsequent *N*-acylation with 4-*O*-benzyl-3-deoxy-L-*glycero*-tetronic acid (**10** → **11**), and debenzylation by catalytic hydrogenolysis. At this stage of the synthesis, purification of the desired substance **13** and verification of the completeness of debenzylation was difficult. Therefore, the crude material was acetylated, purified by chromatography, and the absence of benzyl group in **12** was ascertained by ^{1}H NMR spectroscopy and mass spectrometry. Deacetylation of **12** (Zemplén) then gave the desired, title glycoside **13**.

In the past, construction of *N*-acyl-perosamine-containing oligosaccharides up to and including hexasaccharides (*cf* [1, 17–19]) has been very successful using azidosaccharide building blocks, followed by further conversion of the resulting higher azido-oligosaccharides. This strategy was originally developed by Bundle *et al.* [19]. During this work, the construction of the dodecasaccharide **12** was realized in a similar way, by coupling of two azidohexasaccharide building blocks, and further conversion of the resulting azidododecasaccharide. While we have previously not experienced difficulties during similar transformations involving hexasaccharides [1], some of the conversions described here, leading from **9** to **12**, gave only moderate yields of desired products (for yields of products obtained in individual steps, see Experimental). This, perhaps, indicates that when high-molecular mass fragments ($\sim$ DP $>$ 10) of the O-PS of *Vibrio cholerae* O:1 are to be assembled, the strategy involving coupling of glycosyl donors and glycosyl acceptors having the 3-deoxy-L-*glycero*-tetronic acid side-chain already in place [12, 20, 21] might be more advantageous.

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