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Synthesis of terminal disaccharide elements corresponding to the Ogawa and Inaba antigenic determinant from *Vibrio cholerae* O1 [1]

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Abstract

Vibrio cholerae O1 LPS terminal mono- and disaccharide elements were synthesized by reduction of the azido group in several 4-amino-4,6-dideoxy-D-mannose mono- and disaccharide derivatives, followed by coupling with 2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronic acid in the presence of 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline. This compound represents a useful model in order to elucidate the size of the epitopes which define Ogawa and Inaba serotypes from *Vibrio cholerae* O1.

Keywords: Vibrio cholerae, disaccharide; Ogawa serotype, synthesis; Inaba serotype

1. Introduction

Recently, the interest in cholera has been intensified by the occurrence of epidemics in Latin America [1] and Asia [2]. Taking into account the structure of the lipopolysaccharide (LPS), *Vibrio cholerae* causing cholera in man has been divided into three serotypes: O1 Ogawa, O1 Inaba, and O139 Bengal [2,3]. The LPSs belonging to the Ogawa and Inaba species are linear homopolymers composed of α-(1 $\rightarrow$ 2)-linked *N*-(3-deoxy-L-*glycero*-tetronyl)-perosamine (perosamine 4-amino-4,6-dideoxy-D-mannose) [4,5]. Furthermore, for the LPS of the Ogawa serotype 2-*O*-methyl-perosamine was demonstrated to occur at the nonreducing terminus [6,7], suggesting that its antigenic determinant is associated with the methyl group.

Several syntheses of the monosaccharide [8], the disaccharide with an α-(1 $\rightarrow$ 2) linkage [9] and even larger fragments [10] containing the 3-deoxy-L-*glycero*-tetronamide unit were reported. In all the previous examples, the amide linkage was formed by

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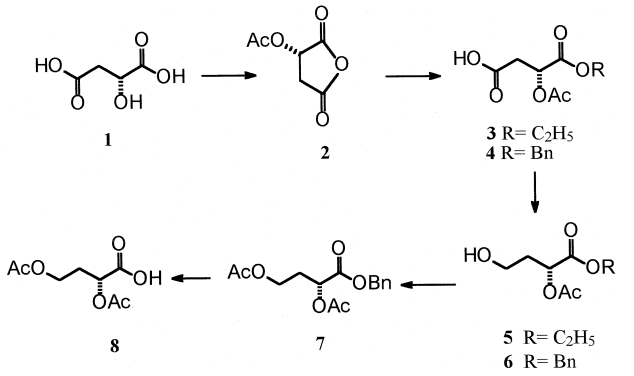


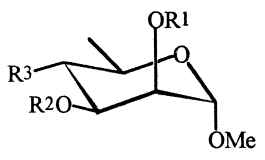
Scheme 1.

direct acylation with the corresponding lactone. Our strategy [11] is based on the synthesis of a peracetylated L-*glycero*-tetronyl donor and its activation with 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) for the coupling with perosamine oligosaccharidic derivatives.

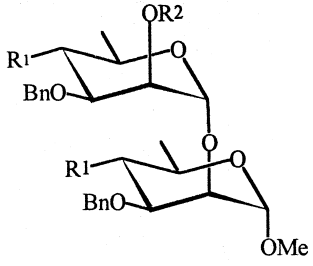
In the present paper we describe full experimental data for the synthesis of 2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronic acid, and its use in the synthesis of all of the possible terminal mono- and disaccharides from *Vibrio cholerae* O1 LPS.

2. Results and discussion

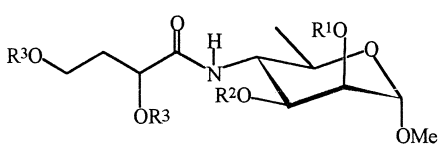
3-Deoxy-L-*glycero*-tetronic acid with a suitable protective group was prepared from L-malic acid **1** by the sequence of reactions as described in Scheme 1. Reaction of **1** with acetyl chloride gave the corresponding anhydride **2,** which was treated directly with excess ethanol to afford the C-1 ester **3** (79% over two steps). The C-4 carboxylic function of **3** was then reduced with diborane in tetrahydrofuran (→ **5**, 86%), but attempts to remove the ethyl ester by using either acidic or basic conditions proceeded with a significant decomposition and probably the formation of several lactonic byproducts. To overcome this problem, we turned then to the benzyl ester **4** that was obtained in a similar fashion from **2**. After



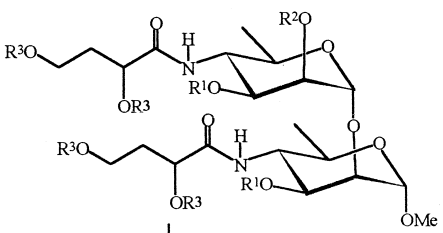
	R^1	R^2	R^3
9	CMe_2		N_3
10	H	Bn	N_3
11	Me	Bn	N_3
12	CMe_2		NH_2
13	Me	Bn	NH_2



	R^1	R^2
15	N_3	Ac
16	N_3	H
17	N_3	Me
18	NH_2	H
19	NH_2	Me



	R^1	R^2	R^3
20	CMe_2		Ac
21	Me	Bn	Ac
24	H	H	H
25	Me	H	H



	R^1	R^2	R^3
22	Bn	H	Ac
23	Bn	Me	Ac
26	H	H	H
27	H	Me	H

Scheme 2.

reduction (→ **6**, 53% over two steps), the resulting hydroxyl group was protected by acetylation (→ **7**, 83%) to avoid lactonization. Hydrogenolysis of **7** afforded **8** in quantitative yield.

Two different 4-amino-4,6-dideoxy-D-mannose derivatives **12** and **13** were selected for the synthesis of terminal monosaccharide derivatives as occurring in *V. cholerae* O1 LPS and two others **18** and **19** for the synthesis of corresponding disaccharide elements (Scheme 2). We reproduced with minor changes the previously described [12] route to the perosamine derivatives **9** and **10**. The methylation of **10** with methyl iodide–sodium hydride in *N,N*-dimethylformamide afforded **11** in 95% yield.

Disaccharide derivative **15** [12] was obtained by condensation of 2-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-*α*-D-mannopyranosyl chloride **14** [12] and acceptor **10** in a silver triflate promoted glycosylation reaction (60%). The presence of a small quantity of a transacetylated acceptor was also detected [13]. Disaccharide derivative **17** was obtained from **15** after deacetylation (→ **16**) and methylation with methyl iodide–sodium hydride in *N,N*-dimethylformamide (→ **17,** 80% overall yield).

The azido groups in derivatives **9**, **11**, **16** and **17** were hydrogenated over 5% Pd–C in ethanol for 24 h to afford the corresponding amines **12**, **13**, **18** and **19**. The transformation was ascertained by the shielding of the C-4 ^{13}C NMR signal (**9** → **12**, δ 64.1 → 54.3; **11** → **13**, δ 64.2 → 53.6; **16** → **18**, δ 63.8, 63.7 → 53.6, 53.2; **17** → **19**, δ 64.9, 64.8 → 53.7, 53.6). It is noteworthy that under these conditions [11] the adjacent benzyl group in compounds **11**, **16**, and **17** remained unchanged as recently was also found [14]. The hydrogenolysis proceeded more cleanly for compound **16** than for the corresponding O-2 acetyl derivative **15**.

Condensation of **12**, **13**, **18** or **19** with 2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronic acid **8** in the presence of EEDQ in dichloromethane afforded the expected amides (→ **20,** 81%; → **21,** 85%; → **22,** 57%; → **23,** 75%). Their structures were confirmed by NMR spectroscopy which showed in the ^{1}H NMR spectra one (**20**, **21**) or two (**22**, **23**) doublets at 5.80–6.50 ppm (J 5–6 Hz) corresponding to the NHCO proton and a doublet of doublet corresponding to the tetronamide H-2 at 5.19–5.22 ppm. Hydrogenolysis of the benzyl groups of **21, 22, 23** with 5% Pd–C or acid hydrolysis of the isopropylidene groups (**20**) followed by Zemplen deacetylation afforded **24**, **25**, **26**, and **27**, respectively.

The ability of synthetic model compounds to inhibit the reaction of mouse polyclonal antibodies was studied by an enzyme-linked immunosorbent assay (ELISA). The antibodies obtained against the Ogawa serotype were absorbed with Inaba LPS in order to eliminate the fraction of antibodies directed to a common antigen. The reaction in ELISA between the remaining fraction and Ogawa LPS was then separately inhibited with compounds **24**, **25**, **26** and **27.** As can be seen from Fig. 1, the reaction was inhibited by the monosaccharide **25** and very strongly by the disaccharide **27**, demonstrating that **27** represents the epitope recognized by most anti-Ogawa antibodies.

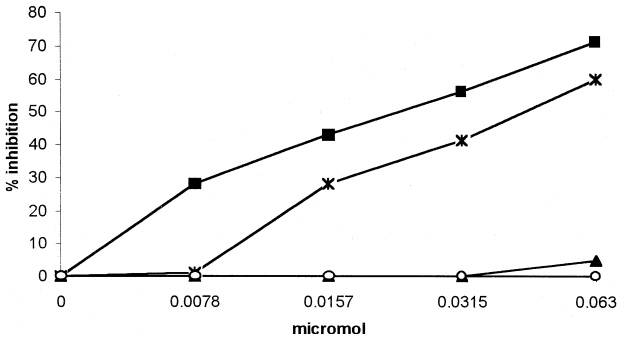


Fig. 1. Inhibition with synthetic fragments **27** (-■-), **25** (-*x*-), **26** (-▲-), **24** (-*o*-) of the reaction between anti-Ogawa polyclonal serum and the homologous lipopolysaccharide.

3. Experimental

General procedures.—Optical rotations were measured at 25 °C with a POLAMAT A automatic polarimeter, using a 5-cm 5-mL cell. NMR spectra were recorded at 25 °C with a Bruker AC-250F spectrometer. ^{1}H and ^{13}C assignments were made on the basis of homo- and heteronuclear correlation experiments. Chemical shifts (δ) are given in ppm relative to the signal for internal Me_4Si for ^{1}H, and indirectly to $CDCl_3$ (δ 77.03) for ^{13}C. The following notation was used to define the NMR signals: **p** for perosamine, **p′** or just ′ for the second perosamine unit, t and t′ for the L-*glycero*-tetronyl moieties.

All compounds characterized were purified by column chromatography on Kieselgel 60 (Fluka, < 230 mesh ASTM) and fractions were monitored by TLC on Kieselgel $60F_{254}$ (Merck). Detection was effected by charring with aq 50% H_2SO_4 after examination under UV light. Evaporations were conducted under reduced pressure at 50 °C (bath).

(S)-*3-Acetoxy-3-ethoxycarbonyl propionic acid*

(**3**).—A solution of L-malic acid (**1**; 1 g, 7.46 mmol) in AcCl (6 mL) was boiled under reflux for 24 h, then concentrated and co-concentrated with toluene (4 × 2 mL) to give 2-*O*-acetyl-L-malic anhydride **(2).** The residue was immediately dissolved in EtOH (6 mL) and stirred for 24 h at room temperature. The mixture was concentrated and distilled at 80–105 °C to afford **3** (1.20 g, 79%) as a low melting solid; $[\alpha]_D$ −20.6° (*c* 1, $CHCl_3$); NMR ($CDCl_3$): ^{1}H, δ 5.45 (t, 1 H, $J_{2,3}$ 6.0 Hz, H-3), 4.23 (q, 2 H, OCH_2), 2.94 (d, 2 H, H-2a,b), 2.15 (s, 3 H, Ac), 1.28 (t, 3 H, CH_3CH_2); ^{13}C, δ 174.7 (C-1), 170.0 and 168.7 (C=O), 68.0 (C-3), 61.9 (OCH_2), 35.8 (C-2), 20.4 (CH_3CO), 13.9 (CH_3). Anal. Calcd for $C_8H_{12}O_6$: C, 46.07; H, 5.93. Found: C, 46.32; H, 5.78.

(S)-*3-Acetoxy-3-benzyloxycarbonyl propionic acid* (**4**).—To a solution of freshly prepared 2-*O*-acetyl-L-malic anhydride **2** (3 g, 22.4 mmol) in dry CH_2Cl_2 (3 mL) was added benzyl alcohol (2.3 mL, 2.23 mmol). The mixture was stirred for 24 h and concentrated. Column chromatography (2:1 hexane–EtOAc) of the residue afforded **4**, isolated as a syrup (2.5 g, 83%); $[\alpha]_D$ −28.5° (*c* 1, $CHCl_3$); R_f 0.5 (3:1 $CHCl_3$–acetone); NMR ($CDCl_3$): ^{1}H, δ 7.35–7.30 (m, 5 H, C_6H_5), 5.51 (t, 1 H, H-3), 5.18 (s, 2 H, $PhCH_2$), 2.93 (d, 2 H, H-2a,b), 2.12 (s, 3 H, Ac); ^{13}C, δ 174.5 (C-1), 170.0 and 168.6 (C=O), 134.9, 128.6, 128.5, and 128.1 (C_6H_5), 67.9 (C-3), 67.5 ($PhCH_2$), 35.7 (C-2), 20.4 (CH_3CO). Anal. Calcd for $C_{13}H_{14}O_6$: C, 58.64; H, 5.30. Found: C, 58.50; H, 5.65.

Ethyl 2-O-*acetyl-3-deoxy*-L-glycero-*tetronate* (**5**).—Compound **3** (0.2 g, 0.98 mmol) was dried in vacuo for 2 h. A solution of borane tetrahydrofuran complex (1 M; 3.4 mL, 3.43 mmol) was added under Ar, and the mixture was stirred for 1 h. The reaction was quenched with EtOH (1 mL), and the mixture was concentrated and co-concentrated with EtOH (2 mL) and toluene (2 × 2 mL) to afford **5**, isolated as a syrup (0.16 g, 86%); $[\alpha]_D$ −25.5° (*c* 0.9, $CHCl_3$); NMR ($CDCl_3$): ^{1}H, δ 5.16 (dd, 1 H, H-2), 4.22 (q, 2 H, OCH_2), 3.85–3.65 (m, 2 H, CH_2OH), 2.15 (s, 3 H, Ac), 2.13–2.05 (m, 2 H, H-3a,b), 1.28 (t, 3 H, CH_3); ^{13}C, δ 170.6 (C=O), 69.7 (C-2), 61.6 (OCH_2), 58.2 (CH_2OH), 33.9 (C-3), 20.6 (CH_3CO), 14.1 (CH_3). Anal. Calcd for $C_8H_{14}O_5$: C, 50.51; H, 5.93. Found: C, 50.20; H, 5.86.

Benzyl 2-O-*acetyl-3-deoxy*-L-glycero-*tetronate* (**6**).—Compound **4** (0.3 g, 1.12 mmol) was reduced as described for the preparation of **5**. Column chromatography (1:1 hexane–EtOAc) of the residue afforded **6**, isolated as a syrup (0.183 g, 64%); $[\alpha]_D$ −51.8° (*c* 1.08, $CHCl_3$); R_f 0.42 (1:1 hexane–EtOAc); NMR ($CDCl_3$): ^{1}H, δ 7.37–7.25 (m, 5 H, C_6H_5), 5.20 (dd, 1 H, H-2), 3.76–3.60 (m, 2 H, CH_2OH), 2.10 (s, 3 H, Ac), 2.09–2.00 (m, 2 H, H-3a,b); ^{13}C, δ 170.4 and 170.2 (C=O), 135.0, 128.4, 128.2, and 127.9 (C_6H_5), 69.4 (C-2), 66.9 ($PhCH_2$), 57.7 (CH_2OH), 33.6 (C-3), 20.3 (CH_3CO).

Benzyl 2,4-di-O-*acetyl-3-deoxy*-L-glycero-*tetronate* (**7**).—To a solution of **6** (3.74 g, 14.8 mmol) in pyridine (50 mL) cooled to 0–5 °C was added Ac_2O (10 mL). The mixture was stirred for 24 h, concentrated and co-concentrated with toluene (3 × 10 mL). Column chromatography (1:1 hexane–EtOAc) of the residue afforded **7**, isolated as a syrup (3.60 g, 83%); $[\alpha]_D$ −29.7° (*c* 1, $CHCl_3$); R_f 0.5 (2:1 hexane–EtOAc); NMR ($CDCl_3$): ^{1}H, δ 7.40–7.30 (m, 5 H, C_6H_5), 5.18 (s, 2 H, $PhCH_2$), 5.16 (dd, 1 H, H-2), 4.22–4.09 (m, 2 H, H-4a,b), 2.13 and 2.00 (2 s, each 3 H, 2 Ac), 2.24–1.97 (m, 2 H, H-3a,b); ^{13}C, δ 170.6, 170.1, 169.5 (C=O), 135.0, 128.5, 128.3, and 128.0 (C_6H_5), 68.9 (C-2), 67.1 ($PhCH_2$), 59.6 (C-4), 30.0 (C-3), 20.6 and 20.5 (CH_3CO). Anal. Calcd for $C_{15}H_{18}O_6$: C, 61.21; H, 6.16. Found: C, 61.23; H, 6.46.

2,4-Di-O-*acetyl-3-deoxy*-L-glycero-*tetronic acid* (**8**).—A suspension of **7** (0.3 g, 1.09 mmol) and 5% Pd–C (45 mg) in EtOH (5 mL) was stirred under H_2 for 20 h, filtered, and concentrated to yield **8** (0.208 g, 94%); $[\alpha]_D$ −23.2° (*c* 1.12, $CHCl_3$); NMR ($CDCl_3$): ^{1}H, δ 7.12 (bs, 1 H, COOH), 5.12 (dd, 1 H, H-2), 4.24–4.18 (m, 2 H, H-4a,b), 2.27–2.05 (m, 2 H, H-3a,b), 2.15 and 2.05 (2 s, each 3 H, 2 Ac); ^{13}C, δ 173.6 (COOH), 171.3 and 170.6 (C=O), 68.8 (C-2), 59.8 (C-4), 30.0 (C-3), 20.7 and 20.4 (CH_3CO). Anal. Calcd for $C_8H_{12}O_6$: C, 47.06; H, 5.93. Found: C, 47.35; H, 5.90.

Methyl 4-azido-3-O-*benzyl-4,6-dideoxy-2*-O-*methyl-α*-D-*mannopyranoside* (**11**).—To a solution of **10** [12] (0.05 g, 0.17 mmol) in DMF (0.5 mL) was added NaH (8 mg, 0.34 mmol, oil dispersion) at 0 °C. After 30 min, CH_3I (13 μL, 0.204 mmol) was added at 0 °C, and the mixture was stirred for an additional period of 15 min at 25 °C. Then MeOH was added to destroy the excess of NaH. After concentration, a solution of the residue in $CHCl_3$ (5 mL) was washed with water (2 mL), dried (Na_2SO_4), and concentrated. Column chromatography (toluene) of the residue afforded **11**, isolated as a syrup (0.049 g, 95%); $[\alpha]_D$ +105.3° (*c* 1.1, CH_2Cl_2); R_f 0.56 (3:1 hexane–EtOAc); NMR ($CDCl_3$): ^{1}H, δ 4.68 (s, 1 H, H-1), 3.71 (dd, 1 H, H-3), 3.50–3.48 (m, 2 H, H-2,4), 3.47 (s, 3 H, OMe), 3.46–3.44 (m, 1 H, H-5), 3.32 (s,

3 H, OMe), 1.31 (d, 3 H, 3 H-6); ^{13}C, δ 137.7, 128.4, 127.9, and 127.8 (C_6H_5), 98.4 (C-1), 78.2 (C-3), 76.4 (C-2), 71.9 (Ph*C*H$_2$), 66.9 (C-5), 64.2 (C-4), 59.3 (C-2–O*C*H$_3$), 54.8 (C-1–O*C*H$_3$), 18.4 (C-6).

Methyl 4-amino-4,6-dideoxy-2,3-O-*isopropylidene-α*-D-*mannopyranoside* (**12**).—A solution of **9** [12] (660 mg, 3.04 mmol) in EtOH (5 mL) was stirred in the presence of 5% Pd–C (200 mg) under H_2. After 24 h, TLC (4:1 EtOAc–MeOH) revealed a complete conversion of the starting material into **12**, isolated as a syrup (492 mg, 83%); R_f 0.33 (1:3 hexane–EtOAc, ninhydrine positive); NMR ($CDCl_3$): ^{1}H, δ 4.88 (s, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 4.06 (d, 1 H, H-2), 3.85 (dd, 1 H, $J_{2,3}$ 5.5, $J_{3,4}$ 8.5 Hz, H-3), 3.50 (dq, 1 H, $J_{4,5}$ 10.4, $J_{5,6}$ 6.4 Hz, H-5), 3.35 (s, 3 H, OMe), 2.60 (dd, 1 H, H-4), 1.50 and 1.30 (2 s, each 3 H, C*Me*$_2$), 1.20 (d, 3 H, 3 H-6); ^{13}C, δ 109.0 (*C*Me$_2$), 98.0 (C-1), 56.5 (C-4), 54.6 (OCH$_3$), 28.0 and 26.1 [C(*C*H$_3$)$_2$], 17.3 (C-6). Anal. Calcd for $C_{10}H_{19}NO_4$: C, 55.28; H, 8.81. Found: C, 55.63; H, 9.12.

Methyl 4-amino-3-O-*benzyl-4,6-dideoxy-2*-O-*methyl-α*-D-*mannopyranoside* (**13**).—A solution of **11** (70 mg, 0.23 mmol) in EtOH was hydrogenated as for the preparation of **12**. Work-up then gave **13** (48 mg, 75%); R_f 0.35 (1:3 hexane–EtOAc, ninhydrine positive); NMR ($CDCl_3$): ^{1}H, δ 7.40–7.20 (m, 5 H, C_6H_5), 4.75 (d, 1 H, $J_{1,2}$ 1.1 Hz, H-1), 4.63 (dd, 2 H, PhC*H*$_2$), 3.47 (s, 3 H, OMe), 3.34 (s, 3 H, OMe), 2.94 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4), 1.25 (d, 3 H, 3 H-6); ^{13}C, δ 138.1, 128.5, 128.0, and 127.9 (C_6H_5), 98.6 (C-1), 79.6 (C-3), 76.5 (C-2), 71.4 (Ph*C*H$_2$), 59.2 (C-2–O*C*H$_3$), 54.7 (C-1–O*C*H$_3$), 53.6 (C-4), 18.1 (C-6).

Methyl (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-*mannopyranoside* (**17**).—To a solution of **15** [12] (90 mg, 1.60 mmol) in dry MeOH (1 mL) was added NaOMe to pH 9, and the mixture was stirred for 30 min. Then the solution was neutralized with Dowex-50 (H^+) resin, filtered, and concentrated to afford **16**. The residue was methylated as for the preparation of **11** using CH_3I (12 μL, 1.9 mmol) and DMF (8 mg, 3.20 mmol, 80% oil dispersion) in Me_2NCHO (1 mL). Column chromatography (20:1 toluene–acetone) of the residue afforded **17**, isolated as a syrup (74 mg, 80%); $[\alpha]_D$ +58.5° (*c* 1.06, $CHCl_3$); R_f 0.56 (20:1 toluene–acetone); NMR ($CDCl_3$): ^{1}H, δ 7.37–7.26 (m, 10 H, 2 C_6H_5), 4.89 (d, 1 H, $J_{1',2'}$ 1.8 Hz, H-1′), 4.70 (d, 2 H, PhC*H*$_2$), 4.63 (d, 2 H, PhC*H*$_2$), 4.49 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 3.92 (dd, 1 H, $J_{2,3}$ 2.4 Hz, H-2), 3.71 (dd, 1 H, $J_{3,4}$ 9.6 Hz, H-3), 3.49 (m, 1 H, H-5′), 3.47 (t, 1 H, $J_{4',5'}$ 8.5 Hz, H-4′), 3.45 (m, 1 H, H-5), 3.31 (s, 3 H, OMe), 3.22 (t, 1 H, $J_{4,5}$ 9.7 Hz, H-4), 3.18 (s, 3 H, OMe), 1.31 (d, 3 H, 3 H-6), 1.30 (d, 3 H, 3 H-6′); ^{13}C, δ 99.9 (C-1′), 99.1 (C-1), 78.2 (C-3′), 77.0 (C-3), 76.4 (C-2′), 73.4 (C-2), 67.7 (C-5′), 66.9 (C-5), 64.4 (C-4), 64.1 (C-4′), 58.9 (C-2–O*C*H$_3$), 54.9 (C-1–O*C*H$_3$), 18.6 and 18.5 (C-6,6′). Anal. Calcd for $C_{28}H_{36}O_7N_6$: C, 59.14; H, 6.38; N, 14.78. Found: C, 59.42; H, 6.18; N, 14.85.

Methyl (4-amino-3-O-*benzyl-4,6-dideoxy-α*-D-*mannopyranosyl)-(1 → 2)-4-amino-3*-O-*benzyl-4,6-dideoxy-α*-D-*mannopyranoside* (**18**).—A solution of **16** [12] (40 mg, 0.079 mmol) in EtOH was hydrogenated as for the preparation of **12**. Work-up then gave **18** (30 mg, 83%); R_f 0.60 (1:4 MeOH–EtOAc, ninhydrine positive); NMR ($CDCl_3$): ^{1}H, δ 7.38–7.20 (m, 10 H, 2 C_6H_5), 5.02 (d, 1 H, $J_{1'2'}$ 2.0 Hz, H-1′), 4.70 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1), 4.09 (dd, 1 H, H-2′), 3.90 (1 H, H-2), 3.69–3.67 (m, 2 H, H-3,3′), 3.52–3.51 (m, 2 H, H-5,5′), 3.32 (s, 3 H, OMe), 2.90 (dd, 1 H, H-4′), 2.85 (dd, 1 H, H-4), 1.35 and 1.30 (2 d, each 3 H, 3 H-6,6′); ^{13}C, δ 137.7, 137.5, 128.5, 128.0 and 127.9 (C_6H_5), 101.0 and 100.2 (C-1,1′), 54.7 (OCH$_3$), 53.6 and 53.2 (C-4,4′), 18.2 and 18.0 (C-6,6′).

Methyl (4-amino-3-O-*benzyl-4,6-dideoxy-2*-O-*methyl-α*-D-*mannopyranosyl)-(1 → 2)-4-amino-3*-O-*benzyl-4,6-dideoxy-α*-D-*mannopyranosyde* (**19**).—A solution of **17** (52 mg, 0.1 mmol) in EtOH was hydrogenated as for the preparation of **12**. Work-up then gave **19**, isolated as a syrup (29 mg, 61%); R_f 0.43 (1:4 MeOH–EtOAc, ninhydrine positive); NMR ($CDCl_3$): ^{1}H, δ 7.40–7.25 (m, 10 H, 2 C_6H_5), 5.02 (d, 1 H, $J_{1'2'}$ 1.9 Hz, H-1′), 4.73 (d, 1 H, PhC*H*), 4.70 (d, 1 H, PhC*H*), 4.65 (d, 1 H, $J_{1'2'}$ 2.0 Hz, H-1), 4.55 (d, 1 H, PhC*H*), 3.30 (s, 3 H, OMe), 3.29 (s, 3 H, OMe), 2.98–2.80 (m, 2 H, H-4,4′), 1.33 and 1.29 (2 d, each 3 H, 3 H-6,6′); ^{13}C, δ 138.0, 137.9, 128.5, 128.0, and 127.8 (C_6H_5), 100.4 and 99.1 (C-1,1′), 58.9 (C-2–O*C*H$_3$), 54.6 (C-1–O*C*H$_3$), 53.8 and 53.6 (C-4,4′), 18.2 and 18.1 (C-6,6′).

Methyl 4-(2,4-di-O-*acetyl-3-deoxy*-L-glycero-*tetronamido)-4,6-dideoxy-2,3*-O-*isopropylidene-α*-D-*mannopyranoside* (**20**).—To a solution of **12** (228 mg, 0.97 mmol) and **8** (398 mg, 1.94 mmol) in CH_2Cl_2 (4 mL) was added EEDQ (479 mg, 1.94 mmol), and the mixture was stirred for 15 min. Then, TLC (1:3 hexane–EtOAc) showed the absence of **12** (R_f 0.33) and a new spot (R_f 0.50). After concentration, column chromatography (1:1 hexane–EtOAc) of the residue afforded **20**, isolated as a syrup (335 mg,

81%); $[\alpha]_D$ −37.7° (*c* 1.06, CH_2Cl_2); R_f 0.50 (3:1 EtOAc–hexane); NMR ($CDCl_3$): ^{1}H, δ 6.37 (d, 1 H, NHCO), 5.23 (dd, 1 H, H-2t), 4.85 (s, 1 H, $J_{1p,2p}$ 1.4 Hz, H-1p), 4.23–4.07 (m, 3 H, H-3p,2p,4t), 3.86 (dd, 1 H, H-4p), 3.74 (dq, 1 H, H-5p), 3.40 (s, 3 H, OMe), 2.35–2.16 (m, 1 H, H-3t), 2.15 and 2.05 (2 s, each 3 H, 2 Ac), 1.53 and 1.33 (2 s, each 3 H, CMe_2) 1.24 (d, 3 H, 3 H-6p); ^{13}C, δ 170.8, 169.6, and 169.1 (C=O), 109.6 (CMe_2), 98.3 (C-1p), 74.7 (C-3p), 74.4 (C-2p), 71.1 (C-2t), 65.8 (C-5p), 59.9 (C-4t), 55.0 (OCH_3), 52.9 (C-4p), 30.7 (C-3t), 27.5 and 25.9 (2 CH_3), 20.7 (CH_3CO), 18.2 (C-6p). Anal. Calcd for $C_{18}H_{29}O_9N$: C, 53.59; H, 7.24; N, 3.47. Found: C, 53.32; H, 7.82; N, 3.22.

*Methyl 4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-3-*O*-benzyl-4,6-dideoxy-2-*O*-methyl-α-*D*-mannopyranoside* (**21**).—The reaction was performed as for the preparation of **20**, using **13** (54 mg, 0.19 mmol), **8** (0.75 mg, 0.37 mmol), and EEDQ (93 mg, 0.37 mmol) in CH_2Cl_2 (1 mL). Column chromatography (1:1 hexane–EtOAc) of the residue afforded **21**, isolated as a syrup (76 mg, 85%); mp 161.1–163.6 °C; $[\alpha]_D$ +3.17° (*c* 0.63, $CHCl_3$); R_f 0.55 (4:1 EtOAc–hexane); NMR ($CDCl_3$): ^{1}H, δ 7.50–7.22 (m, 10 H, 2 C_6H_5), 5.90 (d, 1 H, NHCO), 5.17 (dd, 1 H, H-2t), 4.73 (d, 1 H, $J_{1p,2p}$ 1.3 Hz, H-1p), 4.68–4.45 (2 d, each 1 H, PhCH_2), 4.10 (m, 2 H, H-4t), 3.95–3.80 (m, 3 H, H-3p,4p,5p), 3.59 (s, 1 H, H-2p), 3.50 and 3.30 (2 s, each 3 H, 2 OMe), 2.15 (m, 2 H, H-3t), 2.10 and 2.00 (2 s, each 3 H, 2 Ac), 1.22 (d, 3 H, 3 H-6p); ^{13}C, δ 170.8, 169.5, and 169.2 (C=O), 138.1, 128.4, 128.0, and 127.7 (C_6H_5), 98.7 (C-1p), 76.1 (C-2p), 75.6 (C-3p), 71.1 (C-2t and PhCH_2), 67.3 (C-5p), 60.0 (C-4t), 59.3 (C-4p), 59.2 (C2–OCH_3), 54.3 (C1–OCH_3), 30.9 (C-3t), 20.8 and 20.7 (2 CH_3CO), 18.0 (C-6p). Anal. Calcd for $C_{23}H_{33}O_9N$: C, 59.09; H, 7.11; N, 3.00. Found: C, 58.90; H, 7.35; N, 2.91.

*Methyl [4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-3-*O*-benzyl-4,6-dideoxy-α-*D*-mannopyranosyl]-(1 → 2)-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-3-*O*-benzyl-4,6-dideoxy-α-*D*-mannopyranoside* (**22**).—The reaction was performed as for the preparation of **20**, using **18** (70 mg, 0.14 mmol), **8** (113 mg, 0.55 mmol), and EEDQ (136 mg, 0.55 mmol) in CH_2Cl_2 (2 mL). Column chromatography (1:4 hexane–EtOAc) of the residue afforded **22**, isolated as a syrup (300 mg, 57%); $[\alpha]_D$ +49° (*c* 1, $CHCl_3$); R_f 0.33 (2:1 CH_2Cl_2–acetone); NMR ($CDCl_3$): ^{1}H, δ 7.40–7.20 (m, 10 H, 2 C_6H_5), 6.11 and 5.91 (2 d, each 1 H, 2 NHCO), 5.20 and 5.18 (2 dd, each 1 H, H-2t,2t′), 4.98 (d, 1 H, $J_{1',2'}$ 1.8 Hz, H-1p′), 4.70 (d, 1 H, $J_{1p,2p}$ 1.8 Hz, H-1p), 4.68 (d, 1 H, *J* 11.3 Hz, PhCH_2), 4.62 (d, 1 H, *J* 11.5 Hz, PhC*H*), 4.52 (d, *J* 11.5 Hz, PhC*H*), 4.46 (d, 1 H, *J* 11.3 Hz, PhCH_2), 4.22–3.98 (m, 9 H, H-2p,2p′,3p,4p,4p′,4t,4t′), 3.90–3.60 (m, 3 H, H-3p,5p,5p′), 3.30 (s, 3 H, OMe), 2.35–2.10 (m, 4 H, H-3t,3t′), 2.05–1.90 (m, 12 H, 4 Ac), 1.20 and 1.05 (2 d, each 3 H, 3 H-6p,6p′); ^{13}C, δ 171.0, 169.7, and 169.6 (C-1t,1t′ and 4CH_3CO), 137.6, 128.6, 128.0, and 127.8 (C_6H_5), 101.1 and 100.1 (C-1p,1p′), 68.1 and 67.8 (C-2t,2t′), 60.1 and 60.0 (C-4t,4t′), 55.1 (OCH_3), 52.3 and 52.2 (C-4p,4p′), 31.0 and 30.9 (C-3t,3t′), 20.9, 20.8, and 20.6 (CH_3CO), 18.0 and 17.8 (C-6p,6p′). Anal. Calcd for $C_{43}H_{58}O_{18}N_2$: C, 59.03; H, 6.68; N, 3.20. Found: C, 58.75; H, 6.80; N, 3.29.

*Methyl[4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-3-*O*-benzyl-4,6-dideoxy-2-*O*-methyl-α-*D*-mannopyranosyl]-(1 → 2)-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-3-*O*-benzyl-4,6-dideoxy-α-*D*-mannopyranoside* (**23**).—The reaction was performed as for the preparation of **20**, using **19** (70 mg, 0.14 mmol), **8** (110 mg, 0.54 mmol), EEDQ (134 mg, 0.54 mmol) in CH_2Cl_2 (2 mL). Column chromatography (1:3 hexane–EtOAc) of the residue afforded **23**, isolated as a syrup (90 mg, 75%); $[\alpha]_D$ −17.8° (*c* 1, CH_2Cl_2); R_f 0.73 (2:1 CH_2Cl_2–acetone); NMR ($CDCl_3$): ^{1}H, δ 7.45–7.20 (m, 10 H, 2 C_6H_5), 5.88 and 5.85 (2 d, each 1 H, NH, NH′), 5.20 (dd, 2 H, H-2t,2t′), 4.96 (d, 1 H, $J_{1p',2p'}$ 2.5 Hz, H-1p′), 4.72 (d, 1 H, *J* 11.25 Hz, PhC*H*), 4.66 (d, 1 H, $J_{1p,2p}$ 2.5 Hz, H-1p), 4.66 (d, 1 H, *J* 11.25 Hz, PhC*H*), 4.20–4.00 (m, 8 H, H-3p′,4p,4p′,2p′,4t,4t′), 3.83–3.60 (m, 4 H, H-2p,3p,5p,5p′), 3.33 and 3.30 (2 s, each 3 H, 2 OMe), 2.40–2.20 (m, 4 H, H-3t,3t′), 2.15, 2.08, 2.00, and 1.95 (4 s, each 3 H, 4 Ac), 1.29 and 1.19 (2 d, each 3 H, 3 H-6p,6p′); ^{13}C, δ 171.0, 169.9, and 169.5 (C=O), 138.0, 137.9, 128.5, 128.0, and 127.6 (C_6H_5), 100.2 and 99.6 (C-1p,1p′), 60.1 and 59.9 (C-4t,4t′), 59.2 (C2–OCH_3), 55.1 (C1–OCH_3), 52.4 and 52.1 (C-4p,4p′), 31.1 and 30.9 (C-3t,3t′), 20.9, 20.9, and 20.8 (CH_3CO), 18.0 (C-6p,6p′). Anal. Calcd for $C_{44}H_{60}O_{17}N_2$: C, 59.45; H, 6.80; N, 3.15. Found: C, 59.19; H, 7.19; N, 3.21.

*Methyl 4-(3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy-α-*D*-mannopyranoside* (**24**).—A solution of **20** (272 mg, 0.67 mmol) in aq 90% CF_3COOH was stirred at ambient temperature for 15 min. Then, TLC (3:1 EtOAc-hexane) showed the reaction to be complete. The mixture was concentrated and co-concentrated with toluene (3 × 5 mL). To a solution of the crude residue in dry MeOH (2 mL) was added

NaOMe to pH 9, and the mixture was stirred for 15 min. Then the solution was neutralized with Dowex-50 (H^+) resin, filtered, and concentrated. The residue was dissolved in water and freeze dried to give **24** (163 mg, 87%); $[\alpha]_D$ +20° (*c* 1, water), lit. +34° (water) [11,12]; R_f 0.33 (5:1 EtOAc–MeOH); NMR (D_2O): ^1H, δ 4.76 (s, 1 H, $J_{1p,2p}$ 1.5 Hz, H-1p), 4.35 (dd, 1 H, H-2t), 4.00 (d, 1 H, H-2p), 3.98–3.88 (m, 3 H, H-3p,4p,5p), 3.83 (t, 2 H, H-4t), 3.46 (s, 3 H, OMe), 2.20–2.02 (m, 1 H, H-3t_a), 2.02–1.84 (m, 1 H, H-3t_b), 1.26 (d, 3 H, 3 H-6p); ^{13}C, δ 102.1 (C-1p, $J_{C1,H1}$ 170.7 Hz), 70.4 (C-2p), 70.2 (C-2t), 69.2 (C-3p), 68.4 (C-5p), 59.1 (C-4t), 56.0 (O*C*H_3), 54.1 (C-4p), 37.2 (C-3t), 18.1 (C-6p).

*Methyl 4-(3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-*D*-mannopyranoside* (**25**).—A solution of **21** (154 mg, 0.33 mmol) in EtOH (2 mL) was stirred in the presence of 5% Pd–C (0.1 mg) under H_2. After 24 h, TLC (1:4 hexane–EtOAc) revealed a complete conversion of the starting material (R_f 0.9) into a new spot (R_f 0.21). The mixture was filtered, concentrated, and dissolved in dry MeOH (1 mL), NaOMe was added to pH 9, and the mixture was stirred for 15 min. Then the solution was neutralized with Dowex-50 (H^+) resin, filtered, and concentrated. The residue was dissolved in water and freeze dried to give **25** (0.090 g, 93%); $[\alpha]_D$ +16.6° (*c* 0.96, MeOH); R_f 0.36 (5:1 EtOAc–MeOH); NMR (D_2O): ^1H, δ 5.00 (d, 1 H, $J_{1p,2p}$ 1.7 Hz, H-1p), 4.38 (dd, 1 H, H-2t), 4.04 (dd, 1 H, H-3p), 3.93–3.81 (m, 4 H, H-5p,4p,4t), 3.67 (dd, 1 H, H-2p), 3.59 and 3.51 (2 s, each 3 H, 2 OMe), 2.20–2.05 (m, 1 H, H-3t_a), 2.02–1.85 (m, 1 H, H-3t_b), 1.35 (d, 3 H, 3 H-6p); ^{13}C, δ 178.4 (C=O), 99.0 (C-1p), 80.3 (C-2p), 70.2 (C-2t), 68.9 (C-3p), 68.3 (C-5p), 60.1 (*C*-2–OCH_3), 59.1 (C-4t), 56.1 (*C*-1–OCH_3), 54.5 (C-4p), 37.2 (C-3t), 18.1 (C-6p).

*Methyl [4-(3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy-α-*D*-mannopyranosyl]-(1 → 2)-4-(3-deoxy-*L*-glycero-tetronamido)- 4,6-dideoxy-α-*D*-mannopyranoside* (**26**).—The reaction was performed as for the preparation of **25**, using **22** (70 mg, 0.08 mmol) and 5% Pd–C (40 mg) in EtOH (2 mL) to afford **26** (35 mg, 83%); $[\alpha]_D$ +1.0° (*c* 1, water), lit. 0° [9]; NMR (D_2O): ^1H, δ 5.10 (s, 1 H, H-1p′), 4.85 (d, 1 H, $J_{1p,2p}$ = 2.0 Hz, H-1p), 4.35 (dd, 2 H, H-2t,2t′), 4.15 (dd, 1 H, H-2p′), 4.10–3.85 (m, 6 H, H-3p,3p′,2p,4p,5p,5p′), 3.80–3.70 (m, 2 H, H-4t,4t′), 3.95 (s, 3 H, OMe), 2.15–2.00 (m, 2 H, H-3t_a,3t'_a), 2.00–1.80 (m, 2 H, H-3t_b,3t'_b), 1.30 and 1.25 (2 d, each 3 H, 3 H-6p,6p′); ^{13}C, δ 178.5 (C-1t,1t′), 103.4 and 100.8 (C-1p,1p′), 79.0 (C-2p′), 70.4 (C-2p), 70.2 (C-2t,2t′), 69.3 (C-2p′), 68.7, 68.7, and 68.4 (C-3p,3p′,5p,5p′), 59.0 (C-4t,4t′), 56.1 (O*C*H_3), 54.2 (C-4p), 54.0 (C-4p′), 37.2 (C-3t′), 18.1 (C-6p,6p′).

*Methyl [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*-mannopyranoside* (**27**).—The reaction was performed as for the preparation of **25**, using **23** (30 mg, 34 μmol) and 5% Pd–C (15 mg) in EtOH (1 mL) to afford **27** (18 mg, 98%); $[\alpha]_D$ +1.1° (*c* 1, water), lit. +2.7° [10]; NMR (D_2O): ^1H, δ 5.22 (d, 1 H, $J_{1p',2p'}$ 1.7 Hz, H-1p′), 4.85 (d, 1 H, $J_{1p,2p}$ 2.0 Hz, H-1p), 4.35 (dd, 2 H, H-2t,2t′), 4.15 (dd, 1 H, H-3p′), 4.10–3.95 (m, 6 H, H-2p,5p′,3p′,4p,4p′,5p′), 3.85–3.71 (m, 3 H, H-2p′,4t,4t′), 3.55 and 3.45 (2 s, each 3 H, 2 OMe), 2.15–2.00 (m, 2 H, H-3t_a,3t'_a), 2.00–1.85 (m, 2 H, H-3t_b,3t'_b), 1.30 and 1.25 (2 d, each 3 H, 3 H-6p,6p′); ^{13}C, δ 178.6 and 178.4 (C-1t,1t′), 100.2 and 100.7 (C-1p,1p′), 80.1 (C-2p′), 79.1 (C-2p), 70.1 (C-2t,2t′), 69.1 (C-3p), 68.6 (C-3p′,5p,5p′), 59.9 (*C*-2–OCH_3), 59.0 (C-4t,4t′), 56.1 (*C*-1–OCH_3), 54.4 (C-4p′), 54.2 (C-4p), 37.1 (C-3t,3t′), 18.0 (C-6p,6p′).

Inhibition reaction.—A pool of sera obtained from male BALB/C mice, immunized with *V. cholerae* O1 serotype Ogawa (strain E 7946) [15] was absorbed with 0.25 mg/mL of LPS serotype Inaba (strain 569 B) by incubation at 37 °C for 2 h to eliminate cross reactions. The strains were from the Finlay Institute collection (Havana, Cuba).

Compounds **24**, **25**, **26** and **27** were dissolved in water, adjusted to the following concentration: 0.063, 0.0316, 0.0157, and 0.0078 μmol, and added to the pool of absorbed sera diluted 1/1000 in 1% skim milk in phosphate-buffered saline (PBS, pH 7.4)–Tween 20. The mixture was incubated for 2 h at 37 °C and then analyzed by ELISA. Briefly, the 96-well flat-bottom microdilution plates (Flow Laboratories) were coated with 0.1 mL of LPS (27 μg/mL, from both serotypes) in carbonate/bicarbonate buffer overnight at 37 °C. After the plates were washed four times with water–Tween, skim milk was added at a concentration of 2% in PBS and the plates were kept at 37 °C for 1 h. After the skim milk solution was discarded, the samples were added and incubated for 1 h at 37 °C. The plates were washed as described above, 100 μL of Peroxidase-conjugated rabbit anti-mouse immunoglobulin (Sigma) diluted 1/1000 in 1% skim milk/PBS was added and incubated for 1 h at 37 °C. Then, the plates were washed and 100 μL of substrate solution (H_2O_2–*o*-phenylenediamine in phosphate–citrate buffer, pH 5.0) was added to each well and incubated for 15 min at room temperature in

the dark. The reaction was stopped with 100 μL 2.5 M H_2SO_4. The optical density at 450 nm was measured in an ELISA reader (Multiskan, Titertek).

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