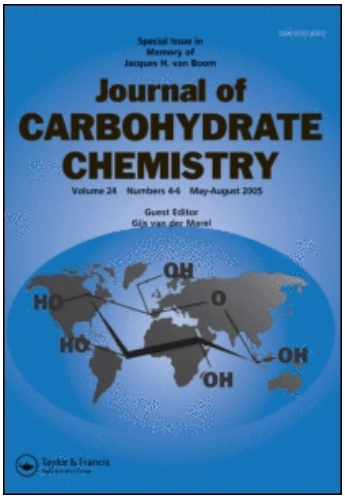

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Synthesis and Characterization of the Crystalline Methyl α-Glycoside of the Repeating Unit of the O-Polysaccharide of *Vibrio Cholerae* O:1

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COMMUNICATION

SYNTHESIS AND CHARACTERIZATION OF THE CRYSTALLINE METHYL α-GLYCOSIDE OF THE REPEATING UNIT OF THE O-POLYSACCHARIDE OF *VIBRIO CHOLERAE* O:1[1]

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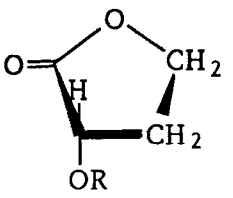
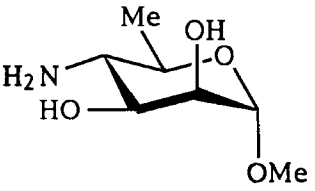
There are more than eighty serotypes of *Vibrio cholerae*, all causing disease with symptoms of Asian cholera. Systematic prevention of cholera by immunization has not yet been achieved because of a lack of a protective vaccine. *Vibrio cholerae* O:1 Gram-negative bacteria occur as two immunologically distinct strains: Ogawa and Inaba. The lipopolysaccharide (LPS) of both strains seem to contain the same O-polysaccharide antigen consisting[3,4] of (1→2)-α-linked 4-amino-4,6-dideoxy-α-D-mannopyranosyl residues the amino groups of which are acylated with 3-deoxy-L-*glycero*-tetronic acid. Although the chemical structure of the O-polysaccharides has been known[5] since 1979, the synthesis of its monomeric repeating unit was reported[6] only in 1988.

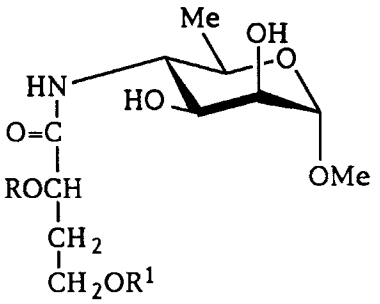
Studies of antigen-antibody interactions involving antibodies specific to the *Vibrio cholerae* O:1 polysaccharide would require, *inter alia*, a series of methyl α-glycosides of oligosaccharides related to the antigenic polymer. Such compounds have hitherto not been synthesized. Preparation of oligosaccharides in this series is hampered by the lack of an efficient synthesis of their monomeric constituent.

In addition to the original approach by Stevens *et al.*,[7] three syntheses of methyl 4-amino-4,6-dideoxy-α-D-mannopyranoside (methyl α-perosaminide, **3**) have been recently described,[6,8,9] and the compound has been fully characterized. The only

attempted synthesis of the corresponding 3-deoxy-L-*glycero*-tetronamide (5) is that by Kenne *et al.*,[6] in which acylation of **3** with ~3 molar equivalents of a 4:1 mixture of 3-deoxy-L-*glycero*-tetronolactone (**1**) and the corresponding carboxylic acid gave the amorphous methyl 4,6-dideoxy-4-(3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranoside (**5**) in 45 % yield. A method providing more efficient introduction of the 3-deoxy-L-*glycero*-tetronyl group into (**3**) would clearly be desirable.







	R
1	H
2	Ac

3

	R	R^1
4	Ac	H
5	H	H

We have now prepared the hitherto unknown, acetylated lactone **2** and tested its acylating ability to form **4**. Thus, commercially avilable L-homoserine was deaminated,[6] and the crude product was acetylated. TLC of the crude product (1:1 hexane-ethyl acetate, iodine-vapor detection) showed the presence of one product having chromatographic mobility. Distillation (95-100 °C/26 Pa, bath) gave virtually pure 2-*O*-acetyl-3-deoxy-L-*glycero*-tetronolactone (**2**, a pale yellow oil, 50-60%, based on homoserine), suitable for the reaction with **3**. The analytical sample of **2** (colorless oil), obtained from the crude product of acetylation by chromatography followed by distillation, showed[10] a peak at *m/z* 162 ($[M + 18]^+$) in its ammonia CI mass spectrum, $[\alpha]_D$ - 20.7° (*c* 0.9, chloroform); ^1H NMR ($CDCl_3$): δ 5.43 (dd, 1 H, $J_{2,3a}$ 8.7, $J_{2,3b}$ 9.2 Hz, H-2), 4.48 (m, 1 H, H-4a), 4.31 (m, 1 H, H-4b), 2.72 (m, 1 H, H-3a), 2.31 (m, 1 H, H-3b), 2.19 (s 3 H, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 172.53, 169.57 (2 CO), 67.60 (C-2), 65.01 (C-4), 28.90 (C-3), 20.62 ($COCH_3$).

The acylation of **3** with **2** (50% molar excess) was achieved in pyridine at elevated temperature. Reactions under identical conditions using 2,4,6-trimethylpyridine or 1,1,3,3-tetramethylurea gave the same results. In a typical conversion **3** → **4**, a solution of **3** (0.53 g, 3 mmol) and **2** (0.65g, 4.5 mmol) in pyridine (1.5 mL) was heated in a tightly closed screw-capped vial for 16 h at 110-115 °C. All of the amine **3** had been consumed (TLC, 10:1 CH_2Cl_2-MeOH) and one major and several minor products had

been formed. After concentration, chromatography (15:1 CH_2Cl_2-MeOH) gave methyl 4,6-dideoxy-4-[2-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido]-α-D-mannopyranoside (**4**), 0.7 g, 72.6 %), mp 129-130 °C (from ethyl acetate), $[\alpha]_D$ +48° (*c* 0.9, chloroform); The structure of **4** was confirmed by NMR spectroscopy: ^1H NMR (300 MHz, $CDCl_3$), δ 7.26 (δ, 1 H, $J_{4,NH}$ 8.8 Hz, NH), 4.70, (s, 1 H, H-1), 4.17 - 4.33 (m, 3 H, H-2',4'a, 4'b), 3.93 - 3.82 (m, 3 H, H-2,3,4), 3.74 - 3.82 (m, 1 H, H-5), 3.36 (s, 3 H, OCH_3), 2.17 - 2. 28 (m, 1 H, H-3'a), 2.07 (s 3 H, $COCH_3$), 1.85 - 1.39 (m, 1 H, H-3'b), 1.21 (d, 1 H, $J_{5,6}$ 5.9 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 175.22, 171.65 (2 CO), 100.89 (C-1), 69.97 (C-2), 69.22 (2 C, C-2',3), 66.74 (C-5), 61.16 (C-4'), 54.90 (OCH_3), 53.62 (C-4), 33.33 (C-3'), 21.05 ($COCH_3$), 17.90 (C-6); CIMS: *m/z* 322 ($[M + 1]^+$), 339 ($[M + 18]^+$).

Deacetylation of **4** (Zemplén) gave the target perosaminide **5** in virtually theoretical yield, mp 136-138 °C (from CH_3OH-acetone), $[\alpha]_D$ +34° (*c* 1.7, water), lit.[6] $[\alpha]_D$ +34° (*c* 2.1, H_2O); the NMR data agreed with those reported.[6]

The efficient preparation described herein of the title ligand **5** is simple and amenable to large scale work. This is expected to be helpful to advance further work on the *Vibrio cholerae* LPS.

REFERENCES AND NOTES

1. Synthesis of ligands related to the O-specific antigen of Vibrio cholerae, Part 1.

2. On leave from Pharmaceutical Research Center, Nihon Nohyaku Co. Ltd., 326-2 Oyamada-cho, Kawachinagano-shi, Osaka 586, Japan.

3. B. Lindberg, in *Carbohydrate Antigens*, ACS Symposium Series 519; P.J. Garegg, & A.A. Lindberg, Eds.; American Chemical Society, Washington, D.C., 1993, p 64.

4. L. Kenne, B. Lindberg, P. Unger, B. Gustafsson, and T. Holme, *Carbohydr. Res.*, **100**, 341 (1982).

5. L. Kenne, B. Lindberg, P. Unger, T. Holme, and J. Holmgren, *Carbohydr. Res.*, **68**, C14 (1979).

6. L. Kenne, P. Unger, and T. Wehler, *J. Chem. Soc. Perkin Trans. 1*, 1183 (1988).

7. C. L. Stevens, R. P. Glinski, K. G. Taylor, P. Blumberg, and S. K. Gupta, *J. Amer. Chem. Soc.*, **92**, 3160 (1970).

8. M. J. Eis and B. Ganem, *Carbohydr. Res.*, **176**, 316 (1988).

9. D. R. Bundle, M. Gerken, and T. Peters, *Carbohydr. Res.*, **174**, 239 (1988).

10. All new compounds gave the correct elemental analysis data.