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Note

Synthesis of the 2-deoxy analogue of the methyl α-glycoside of the monosaccharide repeating unit of the O-polysaccharide of *Vibrio cholerae* O:1 ☆

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The serological specificity of pathogenic Gram-negative organisms often resides in one of the structural constituents of their cell-surface lipopolysaccharides, namely the O-specific polysaccharide (O-SP), or the O-antigen. The intracatenary part of the O-SP of the two main strains of *Vibrio cholerae* O:1, Ogawa and Inaba, is identical and consists [2–4] of a relatively short [3] chain of (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. Only the Ogawa strain has its nonreducing residue in the O-SP methylated at O-2.

We are interested in understanding the interaction of polysaccharide antigens and antibodies on the molecular level. To obtain information on the involvement of hydrogen bonding in the binding process, we have synthesized specifically deoxygenated fragments of polysaccharides and studied their binding in systems involving, for example, (1 → 6)-β-D-galactan- and (1 → 6)-α-D-glucan-specific antibodies [5,6]. For similar investigations involving *Vibrio cholerae* O:1, we have previously synthesized [7] methyl α-glycosides of the intracatenary repeating unit of the O-PS deoxygenated at each of the theoretically possible positions, namely 3, 2′, and 4′. These substances are also useful as specifically deoxygenated models for the nonreducing end-group of both strains of the O-PS of *Vibrio cholerae* O:1. To be able to ascertain the occurrence of

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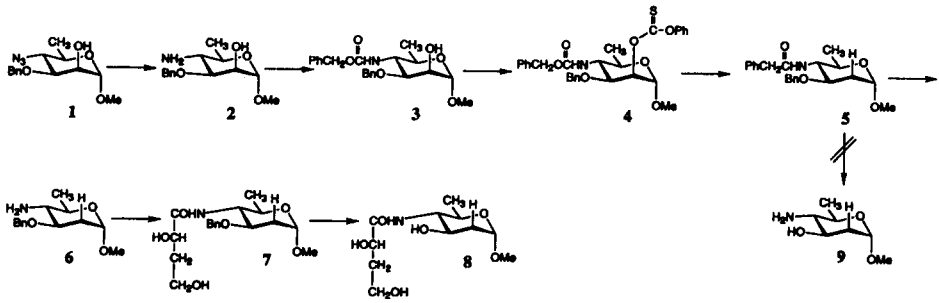

Scheme 1.

hydrogen bonding involving 2-O-H or 2-O-CH_3 present, respectively, in the nonreducing end-group in the O-SP of the Inaba and Ogawa strains, we have now synthesized the appropriately *N*-acylated analogue of methyl 4-amino-2,4,6-trideoxy-α-D-mannopyranoside.

Our reported synthesis of the 3-deoxy derivative of perosamine [7] was based on the conversion of methyl 4-azido-2-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside into the corresponding 4-acetamido derivative, followed by deoxygenation at C-3 via a 3-*O*-imidazol-1-ylthiocarbonyl derivative, and deprotection. Since the *N*-deacetylation is sometimes difficult, the synthetic strategy here used is slightly different. The use is made of the benzyloxycarbonyl group for temporary protection of the amino group. We expected this to make it possible to regenerate the 4-amino and the HO-3 groups simultaneously by catalytic hydrogenolysis (Scheme 1, **5** → **9**). Also, the phenoxythiocarbonyl group [8] is used here to activate the hydroxyl group to be deoxygenated more efficiently [8,9], as compared with the activation by way of the imidazol-1-ylthiocarbonyl group [7]. Accordingly (Scheme 1), the known [9–11] azide **1** was reduced with hydrogen sulfide [12], and the amorphous amine **2** formed, characterized via its crystalline acetamido derivative, was treated with benzyloxycarbonyl chloride, to give **3** in virtually theoretical yield. Compound **3** was readily converted to the 2-*O*-phenoxythiocarbonyl derivative **4** which, when treated with tributyltin hydride, gave a high yield of the 2-deoxy derivative of perosamine **5**. Subsequent treatment of **5** with hydrogen under conditions of common catalytic hydrogenolysis gave amine **6** in virtually theoretical yield, instead of the expected derivative **9**. That the *O*-benzyl protecting group was inert under our hydrogenolytic conditions may be due to reduced activity of the palladium-on-charcoal catalyst used.

The coupling of **6** with 3-deoxy-L-*glycero*-tetronolactone (→ **7**) under usual conditions (e.g., ref. [7]), as well as the final debenzylation (→ **8**) by hydrogenolysis, were uneventful and yielded the desired products in over 80% yields.

D-Perosamine containing different *N*-acyl groups occurs as a constituent of other O-SPs [13–15]. Thus, for evaluation of the role of hydrogen bonding in binding of such antigens with their homologous antibodies, there is a potential interest in other *N*-acyl derivatives of methyl α-D-perosaminide deoxygenated at specific positions. Amine **6**, the synthesis of which is described herein, can be used for the preparation of virtually any N-acyl derivative of methyl 4-amino-2,4,6-trideoxy-α-D-mannopyranose.

1. Experimental

General methods.—Unless stated otherwise, optical rotations were measured at 25°C for solutions in $CHCl_3$ (*c* 1) with a Perkin–Elmer automatic polarimeter, Model 241 MC. All reactions were monitored by thin-layer chromatography (TLC) on silica gel-coated glass slides (Whatman), performed with solvents of appropriately adjusted polarity consisting of dichloromethane–methanol. Detection was effected by charring with 5% sulfuric acid in ethanol, and, when applicable, by UV light. Preparative chromatography was performed by gradient elution from columns of Silica Gel 60 (Fluka, particle size 0.035–0.070 mm) using, at the onset of development, a solvent mixture slightly less polar than that used for TLC. Assignments of NMR signals, obtained at 300 MHz for ^1H and 75 MHz for ^{13}C at 25°C, were made by first-order analysis of spectra, and, when feasible, the assignments were supported by APT and/or DEPT experiments, homonuclear decoupling and/or homo- and hetero-nuclear 2-dimensional correlation spectroscopy. The commercial software supplied with the spectrometers (Varian Gemini or Varian XL 300) was used. Some assignments were aided by mutual comparison of the spectra, and by comparison with spectra of related [7,11,16] substances. Chemical ionization mass spectra (CIMS) were measured using ammonia as the reactive gas. The 10% palladium-on-charcoal catalyst was purchased from Fluka Chemical Co. Solutions in organic solvents were dried with anhydrous sodium sulfate, and concentrated at 40°C/2 kPa.

Methyl 3-O-benzyl-4-benzyloxycarbonylamino-4,6-dideoxy-α-D-mannopyranoside (**3**). —Hydrogen sulfide was passed for 1 h through a solution of the azido derivative (**1**, 2 g) [10,11] in 7:3 pyridine–triethylamine (10 mL), and the reaction mixture, in a loosely closed flask, was kept in the hood at room temperature overnight. TLC showed that the reaction was complete and that a slower moving product was formed. Concentration with coevaporation of toluene to remove pyridine gave the amine **2**, which was sufficiently pure for the next step. For characterization, a solution of **2** (~ 100 mg) in methanol (5 mL) was treated with acetic anhydride (1 mL). After 16 h at room temperature, TLC showed that the reaction was complete and that one product was formed. The volatiles were removed, with coevaporation of toluene to remove excess of acetic anhydride, and the solid residue was crystallized from dichloromethane–hexane to give methyl 4-acetamido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside in virtually theoretical yield: mp 172–174°C; $[\alpha]_D$ +35° (*c* 0.9); ^1H NMR ($CDCl_3$): δ 5.03 (d, 1 H, $J_{4,NH}$ 8.5 Hz, NH), 4.75 (bs, 1 H, H-1), 4.57 (dd, 2 H, ^2J 12.1 Hz, CH_2Ph), 4.01–3.89 (m, 2 H, H-2,4), 3.70–3.58 (m, 2 H, H-3,5), 3.35 (s, 3 H, OCH_3), 2.59 (bs, 1 H, OH), 1.90 (s, 3 H, $COCH_3$), 1.23 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 100.14 (C-1), 75.94 (C-3), 70.93 (*C*H_2Ph), 67.25 (C-5), 66.90 (C-2), 51.90 (C-4), 17.95 (C-6); CIMS: *m/z* 310 ($[M + 1]^+$), 327 ($[M + 18]^+$). Anal. Calcd for $C_{16}H_{23}NO_5$: C, 62.12; H, 7.49; N, 4.53. Found: C, 62.02; H, 7.50; N, 4.49.

A mixture of **2** (1.6 g, 5.46 mmol), benzyloxycarbonyl chloride (2.0 g, 11.76 mmol), dichloromethane (20 mL) and aq satd sodium carbonate (20 mL) was stirred vigorously for 1 h at room temperature. The layers were separated, and the organic phase was dried and concentrated. The material in the residue was chromatographed, to give **3** (2.2 g, 92%): mp 131–133°C (from ether–ethyl acetate); $[\alpha]_D$ +54° (*c* 1.1); ^1H NMR

($CDCl_3$): δ 5.16, 5.07 (2 d, 1 H each, ^2J 12.2 Hz, CH_2Ph), 4.73 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 4.63, 4.44 (2 d, 1 H each, ^2J 12.3 Hz, CH_2Ph), 4.35 (d, 1 H, $J_{4,NH}$ 7.3 Hz, NH), 4.00 (bt, 1 H, H-2), 3.70 (t, partially overlapped, J 9.5 Hz, H-4), 3.70–3.46 (m, 2 H, H-3,5), 3.33 (s, 3 H, OCH_3), 1.23 (bd, 3 H, H-6); ^{13}C NMR ($CDCl_3$): δ 100.15 (C-1), 76.21 (C-3), 71.21 (CH_2Ph), 67.16 (C-5), 67.09 (C-2), 66.77 [NC(O)OCH_2Ph], 54.91 (OCH_3), 53.91 (C-4), 17.85 (C-6); CIMS: m/z 419 ([M + 18]$^+$). Anal. Calcd for $C_{22}H_{27}NO_6$: C, 65.82; H, 6.78; N, 3.49. Found: C, 65.85; H, 6.72; N, 3.52.

*Methyl 3-O-benzyl-4-benzyloxycarbonylamino-2,4,6-trideoxy-α-*D*-mannopyranoside* (**5**).—To a solution of **3** (2.71 g, 6.75 mmol) and 4-dimethylaminopyridine (2.14 g, 17.5 mmol) in dichloromethane (80 mL) was slowly added phenoxythiochloroformate (1.51 g, 8.74 mmol). The mixture was stirred overnight at room temperature, washed successively with 2 M HCl, aq $NaHCO_3$, water, dried, and concentrated. Chromatography of the residue (solvent A) gave **4** (3.2 g, 89%): CIMS: m/z 555 ([M + 18]$^+$).

A mixture of **4** (3.2 g, 5.95 mmol), tributyltin hydride (4.4 g, 15.1 mmol), 2,2-azobis(2-methylpropionitrile) (200 mg, 1.2 mmol) and toluene (80 mL) was heated under reflux for 2 h. After concentration, chromatography gave **5** (1.83 g, 80%): mp 100–101°C (from ether–hexane); [α]$_D$ +32°; ^{1}H NMR ($CDCl_3$): δ 5.17, 5.08 (2 d, 1 H each, ^2J 12.3 Hz, CH_2Ph), 4.78 (bd, 1 H, H-1), 4.60, 4.40 (2 d, 1 H each, ^2J 12.0 Hz, CH_2Ph), 4.49 (bd, 1 H, $J_{4,NH}$ 8.2 Hz, NH), 3.69–3.58 (m, 2 H, H-3,5), 3.42 (t, 1 H, J 9.7 Hz, H-4), 3.29 (s, 3 H, OCH_3), 2.28 (ddd, 1 H, $J_{1,2a}$ 1.4, $J_{2a,3}$ 4.8, $J_{2a,2b}$ 12.9 Hz, H-2a), 1.72 (bdd, 1 H, H-2b), 1.25 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 98.34 (C-1), 73.89 (C-3), 70.61 (CH_2Ph), 67.35 (C-5), 66.76 [NC(O)OCH_2Ph], 58.55 (C-4), 54.64 (OCH_3), 35.56 (C-2), 18.13 (C-6); CIMS: m/z 403 ([M + 18]$^+$). Anal. Calcd for $C_{22}H_{27}NO_5$: C, 68.55; H, 7.06; N, 3.63. Found: C, 68.28; H, 7.04; N, 3.52.

*Methyl 4-amino-3-O-benzyl-2,4,6-trideoxy-α-*D*-mannopyranoside* (**6**).—A mixture of **5** (1.6 g) and 10% palladium-on-charcoal catalyst (~ 0.45 g) in ethanol (60 mL) was stirred in a hydrogen atmosphere, at room temperature and atmospheric pressure, for 6 h. TLC then showed that all starting material was consumed and that virtually one product, detected by charring with sulfuric acid and UV light, was formed. After conventional processing, chromatography gave **6** (0.88 g, 88%): [α]$_D$ +122°; ^{1}H NMR ($CDCl_3$): δ 4.80 (bd, 1 H, H-1), 4.54 (dd, 2 H, ^2J 11.5 Hz, CH_2Ph), 3.65–3.50 (m, 2 H, H-3,5), 3.31 (s, 3 H, OCH_3), 2.53 (t, 1 H, J 9.4 Hz, H-4), 2.29 (ddd, 1 H, $J_{1,2a}$ 1.2, $J_{2a,3}$ 4.8, $J_{2a,2b}$ 12.8 Hz, H-2a), 1.62 (ddd, 1 H, $J_{1,2b}$, 3.7, $J_{2b,3}$ 11.3 Hz, H-2b), 1.53 (bs, 2 H, NH_2), 1.26 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 98.56 (C-1), 77.01 (C-3), 70.82 (CH_2Ph), 68.68 (C-5), 58.89 (C-4), 54.42 (OCH_3), 34.79 (C-2), 18.22 (C-6); CIMS: m/z 252 ([M + 1]$^+$), 269 ([M + 18]$^+$). Anal. Calcd for $C_{14}H_{21}NO_3$: C, 66.91; H, 8.42; N, 5.57. Found: C, 67.01; H, 8.50; N, 5.47.

*Methyl 3-O-benzyl-2,4,6-trideoxy-4-(3-deoxy-*L*-glycero-tetronamido)-α-*D*-mannopyranoside* (**7**).—A mixture of **6** (0.86 g, 3.42 mmol), 3-deoxy-L-*glycero*-tetronolactone [1,11,16] (524 mg, 5.13 mmol) and pyridine (0.1 mL) was stirred overnight at 110°C. TLC showed that all **6** was consumed and that essentially one product was formed. The crude product was chromatographed to give amorphous **7** (1 g, 83%): [α]$_D$ +41° (*c* 0.8); ^{1}H NMR (D_2O): δ 4.90 (d, $J_{1,2}$ 3.0 Hz, H-1), 4.62, 4.49 (2 d, 1 H each, ^2J 11.3 Hz, CH_2Ph), 4.19 (dd, 1 H, $J_{2',3'a}$ 4.1, $J_{2',3'b}$ 9.2 Hz, H-2'), 3.97–3.78 (m, 2 H, H-3,5), 3.69–3.59 (m, 3 H, H-4,4'a,b), 3.32 (s, 3 H, OCH_3), 2.35 (bd, 1 H, $J_{2a,3}$ 4.8,

$J_{2a,2b}$ 13.0 Hz, H-2a), 2.0–1.88 (m, 1 H, H-3'a), 1.77–1.62 (m, 2 H, H-2b,3'b), 1.16 (d, 3 H, $J_{5,6}$ 6.4 Hz, H-6); ^{13}C NMR ($CDCl_3$: δ 177.06 (CO), 98.43 (C-1), 73.60 (C-3), 71.38 (CH_2Ph), 69.01 (C-2'), 67.10 (C-5), 58.05 (C-4'), 56.23 (C-4), 54.56 (OCH_3), 36.24 (C-3'), 34.91 (C-2), 17.05 (C-6); CIMS: m/z 354 ($[M + 1]^+$), 371 ($[M + 18]^+$). Anal. Calcd for $C_{18}H_{27}NO_6$: C, 61.12; H, 7.70; N, 3.96. Found: C, 60.90; H, 7.66; N, 3.79.

*Methyl 2,4,6-trideoxy-4-(3-deoxy-*L-glycero-*tetronamido)-α-*D-*mannopyranoside* (**8**). —A mixture of **7** (0.95 g) and 10% palladium-on-charcoal catalyst was stirred, in a hydrogen atmosphere at atmospheric pressure, overnight at 40°C. After conventional processing and chromatography, pure **8** (0.59g, 83%) was crystallized from ethyl acetate: mp 106–108°C, $[\alpha]_D$ +151° (*c* 0.9); ^{1}H NMR (D_2O): δ 4.89 (bd, 1 H, H-1), 4.28 (dd, 1 H, $J_{2',3'a}$ 3.9, $J_{2',3b}$ 8.6 Hz, H-2'), 3.93 (ddd, 1 H, $J_{2a,3}$ 5.1, $J_{2b,3}$ 15.0, $J_{3,4}$ 10.0 Hz, H-3), 3.87–3.78 (m, 1 H, H-5), 3.73 (m, 2 H, H-4'a,b), 3.52 (t, 1 H, $J \sim 10$ Hz, H-4), 3.34 (s, 3 H, OCH_3), 2.17 (ddd, 1 H, $J_{1,2a}$ 1.3, $J_{2a,b}$ 13.4 Hz, H-2a), 2.07–1.97 (m, 1 H, H-3'a), 1.89–1.78 (m, partially overlapped, H-3b), 1.76 (ddd, partially overlapped, $J_{1,2b}$ 3.7 Hz, H-3b), 1.17 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR (D_2O): δ 177.27 (CO), 98.37 (C-1), 69.06 (C-2'), 66.98 (C-5), 65.77 (C-3), 57.99 (C-4), 57.92 (C-4'), 54.54 (OCH_3), 37.33 (C-2), 36.06 (C-3'), 17.03 (C-6); CIMS: m/z 264 ($[M + 1]^+$), 281 ($[M + 18]^+$). Anal. Calcd for $C_{11}H_{21}NO_6$: C, 50.18; H, 8.04; N, 5.32. Found: C, 49.89; H, 7.87; N, 5.15.

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