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Synthesis of the methyl α-glycoside of a trisaccharide mimicking the terminus of the O antigen of *Vibrio cholerae* O:1, serotype Inaba[☆]

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Abstract

Coupling of methyl 4-amino-4,6-dideoxy-2-*O*-4-methoxybenzyl-α-D-mannopyranoside, obtained from the corresponding 4-azido derivative by treatment with H_2S, with 3-deoxy-L-*glycero*-tetronolactone gave the crystalline methyl 4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-4-methoxybenzyl-α-D-mannopyranoside (**7**). Subsequent acetylation of **7**, followed by *O*-demethoxybenzylation of the **8** formed gave the crystalline methyl 3-*O*-acetyl-4,6-dideoxy-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranoside (**9**), which was used as the key intermediate in the construction of the title trisaccharide. To make a glycosyl donor allowing the extension of the oligosaccharide chain at O-2, compound **9** was converted, via conventional transformations, into 3-*O*-acetyl-2-*O*-bromoacetyl-4,6-dideoxy-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranosyl chloride (**12**). Condensation of **12** with **9** afforded the disaccharide **20** having a selectively removable protecting group at $O\text{-}2^2$. The latter was *O*-debromoacetylated, and the disaccharide nucleophile thus obtained was treated with 2,3-di-*O*-acetyl-4,6-dideoxy-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranosyl chloride to give, after *O*-deacetylation, the target, title trisaccharide. The constituent monosaccharide of the O-specific polysaccharide antigen of *Vibrio cholerae* serotype Inaba, 4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-D-mannopyranose (**18**), was obtained from the peracetate of its methyl α-glycoside by acetolysis, followed by *O*-deacetylation. The amorphous compound **18** was characterized by ^1H and ^{13}C NMR spectroscopy and through its crystalline α-per-*O*-acetyl derivative.

Keywords: Oligosaccharide; Trisaccharide; O-Antigen; *Vibrio cholerae* O:1

[☆] Synthesis of ligands related to the *Vibrio cholerae* O-specific antigen. Part 7. For Part 6, see ref. [1].

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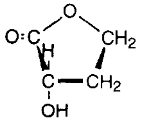
1. Introduction

The intracatenary part of the O-specific polysaccharide (O-SP) of the two main strains of *Vibrio cholerae* O:1, Ogawa and Inaba, are identical. It consists of a relatively short chain [2] 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 (shown as the lactone **1**). Only the Ogawa strain has its non-reducing D-perosaminyl group in the O-SP methylated at O-2 [3,4]. Perosamine as a constituent of a bacterial O-PS is not unique to *V. cholerae.* It is present also in other bacterial polysaccharides, such as of the Brucella [2,5], Yersinia [5] and Escherichia [6] families but in these the amino group is formylated or acetylated.



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In connection with our work on the binding of carbohydrate antigens and antibodies involving the O-PSs of *V. cholerae* O:1, we have synthesized a number of monosaccharide ligands [1,7–9] and a disaccharide [10]. Here, we describe a stepwise synthesis of the title trisaccharide using a strategy that allows the preparation of higher oligosaccharides in this series.

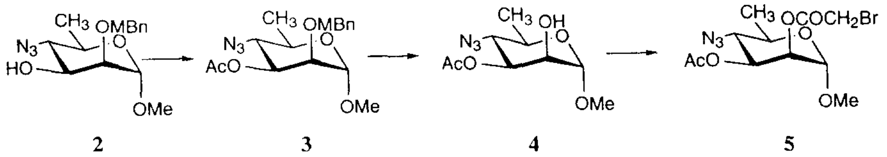
2. Results and discussion

Oligosaccharides composed of D-perosamine but bearing other *N*-acyl groups have been synthesized as a part of studies on different antigen–antibody systems. Those synthetic strategies involve [11–15] condensations of building blocks containing azido groups. The azido oligosaccharides of the required size were subsequently converted, via the corresponding 4-amino derivatives, into oligosaccharides containing the requisite 4-acylamido groups. This is the approach of choice when the acylating reagent to be used is an inexpensive, readily available commodity, and its use in large excess is not cost prohibitive. The present synthesis is based on the use of glycosyl donors and glycosyl acceptors having the *N*-3-deoxy-L-*glycero*-tetronyl group already in place. We have used such an approach in our preparation [10] of the intracatenary disaccharide repeating unit of the O-PS of *V. cholerae* O:1. The glycosyl donor used there did not, however, allow the extension of the oligosaccharide chain.

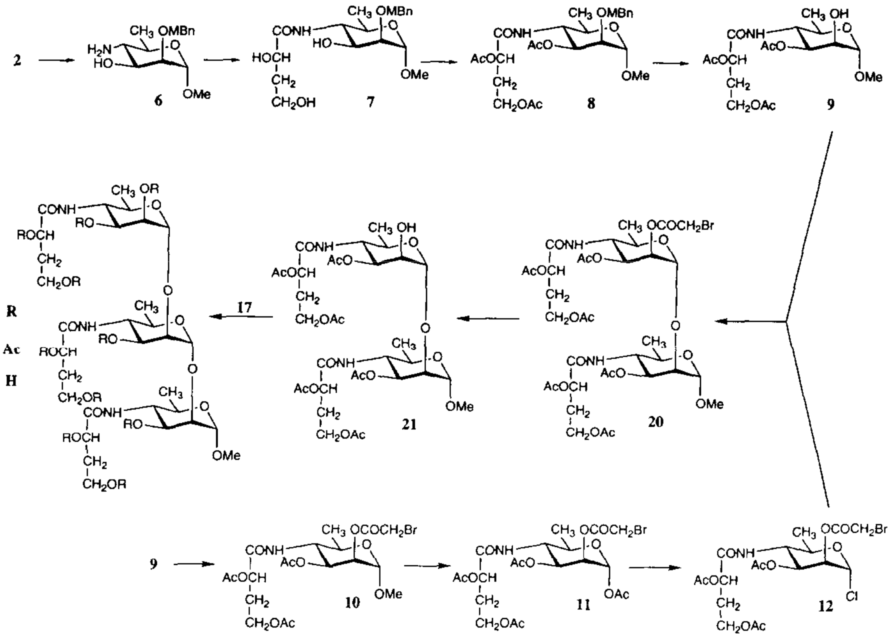
The synthesis of the title trisaccharide is based on the use of the *O*-acetyl group as a permanent protecting group and the *O*-4-methoxybenzyl and *O*-bromoacetyl groups as temporary protecting groups. We chose the *O*-bromoacetyl group, rather than the generally more commonly used *O*-chloroacetyl [16] group, because the former can be removed under milder conditions [17,18] and the migration of other *O*-acyl protecting groups during *O*-dehaloacetylation is, thus, less likely to occur.

Previously [13], selective (56%) protection of HO-2 in methyl 4-azido-4,6-dideoxy-*α*-D-mannopyranoside was achieved by its benzylation. Here we use the 4-methoxybenzyl group, because it could be introduced at O-2 more selectively (70%) [7]. Also, a hydroxyl group protected by the 4-methoxybenzyl group can be regenerated rather quickly under non-hydrogenolytic conditions making, again, the migration of *O*-acyl protecting groups during deprotection at O-2 less likely. In the synthesis of the intracatenary disaccharide repeating unit of either O-polysaccharide of *V. cholerae* O:1 [10], we used a fully benzoylated glycosyl chloride as a glycosyl donor, and a 3-*O*-benzylated, otherwise fully benzoylated, except HO-2, glycosyl acceptor. In our decision to use here the *O*-acetylated, rather then the *O*-benzoylated, glycosyl halides we have taken into consideration the experimentally more convenient introduction and removal of the *O*-acetyl groups. In addition, the often decreased solubility of materials containing a large number of *O*-benzoyl groups in the molecule is known. Such would be the case with higher oligosaccharides in this series, and it could complicate their handling and NMR spectroscopy. Also, the condensation of glycosyl acceptors that are protected with acetyl groups only, with fully acetylated and bromoacetylated glycosyl donors gives rise to acylated products devoid of benzoyl or benzyl groups. This simplifies the deprotection protocol, compared to the one previously applied [10].

Extensive migration of the acetyl group at O-2 or O-3 in D-mannose has been observed [19] under a variety of conditions. Therefore, we felt it important to ascertain that the anticipated chemical manipulations could be performed in a practicable way. Thus, at the onset of the synthesis, we investigated in model experiments (Scheme 1) the stability of the acetyl group at HO-3 during those chemical transformations that had to be carried out at O-2 according to the anticipated synthetic strategy (*O*-4-demethoxybenzylation, following *O*-bromoacetylation and *O*-debromoacetylation, Scheme 2). Accordingly, methyl 4-azido-4,6-dideoxy-2-*O*-4-methoxybenzyl-*α*-D-mannopyranoside (**2**) [7] was acetylated (→ **3**). Subsequent *O*-4-demethoxybenzylation effected with ammonium cerium(IV) nitrate (CAN) [20,21] gave a product that was homogeneous according to TLC. However, its NMR spectrum revealed the presence of two mono-*O*-acetyl derivatives, showing that the conversion was accompanied by *O*-acetyl group migration. On the other hand, treatment of **3** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) gave a single product, the desired 3-*O*-acetyl derivative **4**, as shown by NMR spectroscopy. Subsequent bromoacetylation with bromoacetyl bromide in dichloromethane in the presence of 2,4,6-trimethylpyridine gave **5**, and *O*-debromo-



Scheme 1.

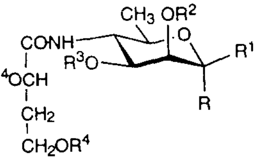


Scheme 2.

acetylation of the latter with thiourea regenerated **4**, all three reactions occurring without detectable (TLC, NMR) acetyl group migration.

Having verified the feasibility of the planned strategy, the 2-*O*-(4-methoxybenzyl) derivative **2** was converted (Scheme 2), by treatment with H_2S [22], to the corresponding amine **6**, and the latter was treated with 3-deoxy-L-*glycero*-tetronolactone [7,10] (**1**). In the past, such couplings have been most often carried out at 100–110°C using pyridine as solvent. Here we have carried out the reaction at 130°C (bath) in the presence of virtually only a catalytic amount of pyridine. In this way, the usual reaction times [9,10,23] of 24–72 h could be decreased to 2–3 h. A portion of the desired addition product **7** crystallized from the crude reaction mixture, and an additional amount of the same material was obtained by chromatography (total yield, ca. 85%). Acetylation of **7** (→ **8**), followed by *O*-demethoxybenzylation gave the key glycosyl acceptor **9**.

The stepwise construction of the title trisaccharide required a glycosyl donor allowing the extension of the chain at O-2. Such a synthon was obtained from compound **9** (Scheme 2), which was first bromoacetylated to give the fully protected glycoside **10**. Methyl glycosides of variously protected saccharides can be converted directly to the corresponding glycosyl halides using dihalogenomethyl methyl ether–$ZnCl_2$ reagents [24]. However, the anomeric position in compound **10** is highly deactivated towards such transformation due to the presence of the 2-*O*-bromoacetyl group [25] at the neighboring position. Consequently, when we attempted the direct conversion of **10** → **12** (not described in the Experimental), rather harsh reaction conditions had to be applied, and the formation of byproducts was extensive. Therefore, glycoside **10** was subjected to acetolysis, to give the 1-*O*-acetyl derivative **11** in admixture with a small proportion of the β-anomer **13**. When these were treated [24] with dichloromethyl methyl ether (DCMME) in the presence of a catalytic amount of freshly fused $ZnCl_2$, the complex glycosyl donor **12** was obtained in high yield. Coupling of the latter with the nucleophile **9** (Scheme 2) afforded the expected disaccharide **20**. *O*-Debromoacetylation of **20** with thiourea, conducted as for the conversion of **5** → **4** (which was found to occur without 3-*O*-Ac → 2-*O*-Ac group migration), yielded two products. In addition to the desired **21**, a small amount of a side product, presumably a product of acetyl group migration, was also formed, and it was removed by chromatography. The simple glycosyl chloride **17** required for the final condensation with **21** was obtained from the fully acetylated glycoside **14** [9] via the per-*O*-acetyl derivatives **15** and **16** by treatment with the DCMME–$ZnCl_2$ reagent. Silver trifluoromethanesulfonate-mediated coupling of pure **21** with **17** in the presence of 2,4,6-trimethylpyridine gave a mixture that was difficult to resolve by chromatography. Therefore, the fully protected trisaccharide **22** was not isolated pure at this stage of the synthesis. Instead, a mixture of products enriched in **22**, isolated by chromatography, was deacylated (Zemplén), and chromatography then yielded the target trisaccharide **23** which mimicks the terminal, upstream [10,26] portion of the O-PS of *V. cholerae*, serotype Inaba. For characterization, pure **22** was obtained from **23** by conventional acetylation with pyridine–acetic anhydride.



	R	R¹	R²	R³	R⁴
13	H	OAc	$COCH_2Br$	Ac	Ac
14	OMe	H	Ac	Ac	Ac
15	OAc	H	Ac	Ac	Ac
16	H	OAc	Ac	Ac	Ac
17	Cl	H	Ac	Ac	Ac
18	HOH		H	H	H
19	HOH		Ac	Ac	Ac

In the course of their structural studies of the *V. cholerae* O antigen, Kenne et al. [27] treated the polysaccharide successively with liquid hydrogen fluoride followed by mild acid hydrolysis. They isolated the monomeric constituent of the polysaccharide in ca. 50% yield, and reported some of its ^1H NMR characteristics. When we *O*-deacetylated (Zemplén) the per-*O*-acetyl derivative **15**, the ^1H and ^{13}C NMR characteristics of the product obtained were consistent with the structure **18**. In agreement with the data reported [27], the α-anomer slightly predominated in the anomeric mixture we obtained, as shown by the $J_{C,H}$ 170.5 Hz for the predominating anomer in contrast to $J_{C,H}$ 159.7 Hz found for the anomer present in a smaller amount. The ^{1}H NMR characteristics of the substance were essentially the same as those reported [27]. This establishes the identity of the material isolated from the natural source with that obtained by the chemical synthesis described herein.

3. Experimental

General methods.—Optical rotations were measured at ambient temperature for solutions in chloroform with a Perkin–Elmer automatic polarimeter, Model 241MC, unless indicated otherwise. Thin-layer chromatography (TLC) was performed with A, 2:1 hexane–EtOAc; B, 4:1 hexane–EtOAc; C, EtOAc; D, 1:2 hexane–EtOAc; E, 6:1 $CHCl_3$–acetone; F, 4:1 toluene–acetone; G, 4:1 $CHCl_3$–acetone; H, 2:1 $CHCl_3$–acetone; I, 20:1 $CHCl_3$–MeOH; J, 6:1 toluene–MeOH; and K, 2:1:0.2 $CHCl_3$–MeOH–25% NH_4OH. Detection was effected by charring with 5% H_2SO_4 in EtOH and with UV light or, when required, with iodine vapor. Preparative chromatography was performed by gradient elution from columns of Silica Gel 60 (particle size 0.04–0.063 mm) using, at the onset of development, a solvent mixture slightly less polar than that used for TLC. For chromatography of glycosyl halides the silica gel was dried at 160°C for 12 h, and elution solvents contained 0.05% of 2,4,6-trimethylpyridine or Et_3N. When elution solvent for preparative chromatography contained aqueous ammonia and/or a large proportion of methanol, prior to use the silica gel was washed successively with hot 1:1:0.1 (v/v) water–methanol–25% ammonia and water, and dried at 140°C for 16 h. NMR spectra were obtained at 300 MHz for ^{1}H and 75 MHz for ^{13}C. The measurements were done at ambient temperature, using a Varian XL 300 or a Varian Gemini spectrometer. The solvents used are given as required. Chemical shifts are reported in ppm downfield of the signal of Me_4Si; ^{1}H shifts determined in D_2O were measured relative to the signal of HOD (δ 4.78). The ^{13}C shifts were measured relative to the signal of $CDCl_3$ (δ 77.0), benzene (δ 128.0), or MeOH (δ 49.0). Assignments of NMR signals were made by first-order analysis of the spectra, and by comparison with spectra of related substances. When feasible, the assignments were supported by homonuclear decoupling experiments or homonuclear and heteronuclear two-dimensional correlation spectroscopy, using commercial software supplied with the spectrometers. Sugar residues in oligosaccharides are serially numbered, beginning with the one bearing the aglycone, and are identified by a superscript in listings of signal assignments. Nuclei assignments without a superscript notation have not been individually assigned. Thus, for example, in a spectrum of an oligosaccharide, a resonance denoted H-3 can be that of H-3 of any sugar residue. The original procedure for the preparation [10] of lactone (**1**) from its 2-*O*-acetyl derivative was modified in that the reaction was carried out at 70°C. After concentration, the residue was rid of any residual trifluoroacetic acid by concentation, several times, of its aqueous solution [7]. The product was stored as a solution in pyridine (1 mmol/mL) at ca. 5°C. Chemical-ionization mass spectra (CIMS) were measured using ammonia as the reactive gas. L-Homoserine was purchased from Bachem California and used as supplied. DCMME was purchased from Fluka Chemical

Company and used as supplied. Chloroform used in conjunction with DCMME was rid of alcohol by passing through activated neutral alumina (100 g/500 mL). Before final drying, the solutions of analytical samples were filtered through Anotop 10 Plus 0.1 μm syringe filters (Whatman, Inc.). We have found these devices superior to other, same-porosity filtration devices for removal of coloidal material sometimes present. Solutions in organic solvents were dried with anhydrous sodium sulfate and concentrated at or below 40°C/2 kPa.

Methyl 3-O-acetyl-4-azido-4,6-dideoxy-2-O-4-methoxybenzyl-α-D-mannopyranoside (3).—The alcohol 2 [7] was acetylated with acetic anhydride–pyridine and, after conventional processing, the title product was obtained in virtually theoretical yield: mp 60–60.5°C (from MeOH, twice); $[\alpha]_D$ +45° (*c* 1); ^{1}H NMR ($CDCl_3$): δ 5.06 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 10.0 Hz, H-3), 4.62 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.58, 4.50 (2 d, 1 H each, ^2J 12.0 Hz, CH_2Ph), 3.80 (m, 4 H, H-2, PhOCH_3), 3.60 (t, partially overlapped, H-4), 3.58 (m, partially overlapped, H-5), 3.32 (s, 3 H, OCH_3), and 1.35 (d, 3 H, $J_{5,6}$ 5.9 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 170.00 (CO), 98.87 (C-1), 74.48 (C-2), 72.85 (CH_2Ph), 72.61 (C-3), 66.84 (C-5), 62.73 (C-4), 55.28 (PhOCH_3), 54.91 (OCH_3), 20.86 (COCH_3), and 18.39 (C-6); CIMS: *m/z* 383 $[M + 18]^+$. Anal. Calcd for $C_{17}H_{23}N_3O_6$: C, 55.88; H, 6.35; N, 11.50. Found: C, 55.67; H, 6.28; N, 11.63.

Methyl 3-O-acetyl-4-azido-4,6-dideoxy-α-D-mannopyranoside (4).—(a) Ammonium cerium(IV) nitrate (CAN, 223 mg, 0.41 mmol) was added at 0°C to a solution of compound 3 (60 mg, 0.16 mmol) in 8:1 acetonitrile–water (1 mL). The mixture was stirred at 0°C for 2 h, when TLC (solvent A) showed that the reaction was complete. After concentration, the residue was chromatographed, to give a product that was homogeneous by TLC. ^{1}H NMR showed, however, that two compounds were present, one of which resulted from acetyl group migration.

(b) DDQ (752 mg, 3.3 mmol) followed by water (1.2 mL) was added to a solution of 3 (1.1 g, 3.01 mmol) in dichloromethane (22 mL). The mixture was stirred at room temperature for 4 h. TLC (solvent A) then showed that the reaction was complete. The mixture was partitioned between dichloromethane and water, the organic phase was dried, concentrated, and the residue was chromatographed to give pure 4 (0.7 g, 95%): $[\alpha]_D$ +164° (*c* 1.15); ^{1}H NMR ($CDCl_3$): δ 5.11 (dd, 1 H, $J_{2,3}$ 2.8, $J_{3,4}$ 9.8 Hz, H-3), 4.66 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.04 (m, 1 H, H-2), 3.62 (m, 1 H, H-5), 3.53 (t, 1 H, *J* 10.0 Hz, H-4), 3.37 (s, 3 H, OCH_3), 2.52 (d, 1 H, $J_{2,OH}$ 4.7 Hz, OH), 2.17 ($COCH_3$), and 1.36 (d, 3 H, $J_{5,6}$ 6.1 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 169.76 (CO), 100.49 (C-1), 72.81 (C-3), 68.41 (C-2), 66.66 (C-5), 62.41 (C-4), 54.99 (OCH_3), 20.86 (COCH_3), and 18.26 (C-6); CIMS: *m/z* 263 $[M + 18]^+$. Anal. Calcd for $C_9H_{15}N_3O_5$: C, 44.08; H, 6.17; N, 17.13. Found: C, 44.21; H, 6.15; N, 17.85.

(c) A solution of thiourea (19 mg, 0.25 mmol) in methanol 0.5 mL) was added to a solution of the bromoacetyl derivative 5 (30 mg, 0.08 mmol) in dichloromethane (1 mL). The mixture was stirred at room temperature for 1.5 h. TLC (solvent B) then showed that the reaction was complete, and that a single product was formed. After concentration, the residue was partitioned between dichloromethane and a mixture of aqueous sodium hydrogencarbonate and sodium chloride, the organic phase was dried and concentrated, and the residue was chromatographed. A ^{1}H NMR spectrum of the product (ca. 20 mg, ca. 100%) showed that only 4 was present.

Methyl 3-O-acetyl-4-azido-2-O-bromoacetyl-4,6-dideoxy-α-D-mannopyranoside (**5**).—Bromoacetyl bromide (0.32 mL, 3.67 mmol) was added at 0°C to a solution of **4** (0.6 g, 2.45 mmol) and 2,4,6-trimethylpyridine (0.65 mL, 4.9 mmol) in dichloromethene (20 mL), and the mixture was stirred at room temperature overnight. TLC (solvent B) showed that the reaction was complete. A few drops of methanol was added to destroy excess of the reagent, and, after concentration, the residue was partitioned between dichloromethane and aqueous sodium chloride. The organic phase was dried and concentrated. The NMR spectrum of the crude product obtained showed that the conversion was not accompanied by acetyl group migration. Chromatography gave **5** (818 mg, 90%): mp 70.5–71°C (from EtOH), $[\alpha]_D$ +53° (*c* 1.1); ^{1}H NMR ($CDCl_3$): δ 5.26 (dd, partially overlapped, $J_{1,2}$ 1.7, $J_{2,3}$ 3.3 Hz, H-2), 5.22 (dd, partially overlapped, $J_{3,4}$ 10 Hz, H-3), 4.64 (d, 1 H, H-1), 3.94, 3.88 (2 d, 1 H each, ^2J 12.0 Hz, CH_2Br), 3.66 (m, 1 H, H-5), 3.53 (t, 1 H, *J* 10 Hz, H-4), 3.38 (s, 3 H, OCH_3), 2.08 (s, 3 H, $COCH_3$), and 1.38 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 169.61, 166.42 (2 CO), 98.00 (C-1), 70.52 (C-2), 70.29 (C-3), 66.76 (C-5), 62.42 (C-4), 55.22 (OCH_3), 25.17 (CH_2Br), 20.67 ($COCH_3$), and 18.30 (C-6); CIMS: *m/z* 384 $[M + 18]^+$. Anal. Calcd for $C_{11}H_{16}BrN_3O_6$: C, 36.08; H, 4.40; Br, 21.82; N, 11.48. Found: C, 36.22; H, 4.37; Br, 21.72; N, 11.46.

Methyl 4-amino-4,6-dideoxy-2-O-4-methoxybenzyl-α-D-mannopyranoside (**6**).—Hydrogen sulfide was passed for 30 min through a solution of **2** (13.5 g) in a mixture of pyridine–triethylamine (7:3, 500 mL). The solution was kept in a septum-closed flask for 16 h, at which time TLC (solvent B) showed that the reaction was complete. After concentration, the residue was chromatographed to give **6** (10 g, 81%). The compound crystallized on standing, but recrystallization from common organic solvents failed. The crystals were triturated with ca. 1:5 diethyl ether–isopropyl ether, filtered, and washed successively with the same solvent, isopropyl ether, and hexane. The colorless material obtained after drying at 30°C/2 kPa showed mp 42–43°C and $[\alpha]_D$ −9.7° (*c* 1). ^{1}H NMR ($CDCl_3$): δ 4.72 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 4.68, 4.48 (2 d, 1 H each, ^2J 11.5 Hz, CH_2Ph), 3.80 (s, 3 H, $PhOCH_3$), 3.64 (dd, 1 H, $J_{2,3}$ 3.7 Hz, H-2), 3.53 (dd, partially overlapped, $J_{3,4}$ 10 Hz, H-3), 3.54–3.42 (m, partialy overlapped, H-5), 3.33 (s, 3 H, OCH_3), 2.67 (t, 1 H, *J* 9.8 Hz, H-4), and 1.22 (d, 3 H, $J_{5,6}$ 7.0 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 98.29 (C-1), 76.88 (C-2), 72.59 (CH_2Ph), 71.24 (C-3), 69.00 (C-5), 56.01 (C-4), 55.21 ($PhOCH_3$), 54.61 (OCH_3), and 17.87 (C-6); CIMS: *m/z* 298 $[M + 1]^+$. Anal. Calcd for $C_{15}H_{23}NO_5$: C, 60.59; H, 7.80; N, 4.71. Found: C, 60.40; H, 7.85; N, 4.67.

*Methyl 4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-O-4-methoxybenzyl-α-D-mannopyranoside* (**7**).—A solution of lactone **1** (6.9 g, 67.7 mmol) in pyridine was added to the 2-*O*-(4-methoxybenzyl) derivative **6** (13.4 g, 45 mmol), and the solution was concentrated to remove almost all of the solvent. The residue was heated at 125–130°C (bath) for 24 h, and TLC (solvent C) showed that all starting amine was consumed, and that virtually one product was formed. Crystallization and chromatography of the material that remained in the mother liquor gave **7** (15.5 g, 84.6%): mp 103.5–104°C (from acetone), $[\alpha]_D$ −2.2° (*c* 0.7, MeOH). ^{1}H NMR (CD_3OD): δ 4.65, 4.58 (2 d, 1 H each, ^2J 11.5 Hz, CH_2Ph), 4.65 (bd, partially overlapped, $J_{1,2}$ ~ 1.7 Hz, H-1), 4.16 (dd, 1 H, $J_{2',3'a}$ 3.9, $J_{2',3'b}$ 7.9 Hz, H-2'), 3.92 (t, 1 H, *J* 10.4 Hz, H-4), 3.84

(dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 10.4 Hz, H-3), 3.78 (s, 3 H, PhOCH_3), 3.75–3.64 (m, 3 H, H-5,4′a,b), 3.61 (dd, 1 H, H-2), 3.34 (s, 3 H, OCH$_3$), 1.99, 1.82 (2 m, 1 H each, H-3′a,b), and 1.15 (d, 3 H, $J_{5,6}$ 5.7 Hz, H-6); ^{13}C NMR (CD$_3$OD): δ 100.13 (C-1), 78.38 (C-2), 73.85 (CH$_2$Ph), 70.67 (C-2′), 69.93 (C-3), 68.45 (C-5), 59.42 (C-4′), 55.66 (PhOCH$_3$), 55.15 (OCH$_3$), 54.79 (C-4), 38.19 (C-3′), and 18.30 (C-6); CIMS: *m/z* 400 [M + 1]$^+$ and 417 [M + 18]$^+$. Anal. Calcd for $C_{19}H_{29}NO_8 \cdot H_2O$: C, 54.67; H, 7.49; N, 3.35. Found: C, 54.66; H, 7.53; N, 3.33.

*Methyl 3-O-acetyl-4,6-dideoxy-4-(2,4-di-O-acetyl-3-deoxy-*L-glycero-*tetronamido)-2-O-4-methoxybenzyl-α-*D-*mannopyranoside* (**8**).—The foregoing compound **7** (13.7 g) was treated overnight with a mixture of 2:5 acetic anhydride–pyridine (70 mL). After concentration, chromatography gave pure **8** (17.1 g, ca. 95%): $[\alpha]_D$ +31° (*c* 0.8); ^{1}H NMR (CDCl$_3$): δ 6.05 (d, 1 H, $J_{4,NH}$ 9.3 Hz, NH), 5.15 (dd, partially overlapped, $J_{2,3}$ 3.2, $J_{3,4}$ 11.1 Hz, H-3), 5.12 (dd, partially overlapped, $J_{2',3'a}$ 4.9, $J_{2',3'b}$ 7.5 Hz, H-2′), 4.64 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.62 (s, 2 H, CH_2Ph), 4.30 (m, 1 H, H-4), 4.21–4.02 (m, 2 H, H-4′a,b), 3.80 (s 3 H, PhOCH_3), 3.67 (dd, 1 H, H-2), 3.61 (m, 1 H, H-5), 3.32 (s, 3 H, OCH$_3$), 2.16, 2.07, 2.05 (3 s, overlapped, 3 COCH$_3$), 2.4–1.8 (m, overlapped, H-3′a,b), and 1.23 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR (CDCl$_3$): δ 171.75, 170.79, 169.73, 169.35 (4 CO), 99.02 (C-1), 74.42 (C-2), 72.70 (CH$_2$Ph), 70.91 (C-2′), 70.82 (C-3), 68.34 (C-5), 59.82 (C-4′), 55.21 (PhOCH$_3$), 54.80 (OCH$_3$), 51.65 (C-4), 30.52 (C-3′), 20.91, 20.77, 20.71 (3 COCH$_3$), and 17.85 (C-6); CIMS: *m/z* 526 [M + 1]$^+$ and 543 [M + 18]$^+$. Anal. Calcd for $C_{25}H_{35}NO_{11}$: C, 57.13; H, 6.71; N, 2.67. Found: C, 56.85; H, 6.81; N, 2.62.

*Methyl 3-O-acetyl-4,6-dideoxy-4-(2,4-di-O-acetyl-3-deoxy-*L-glycero-*tetronamido)-α-*D-*mannopyranoside* (**9**).—DDQ (143 mg, 6.3 mmol), followed by water (0.3 mL), was added with stirring at room temperature to a solution of **8** (0.3 g, 0.57 mmol) in dichloromethane (5 mL), and the mixture was stirred for 4 h. TLC (solvent D) showed at the end of this time that the reaction was complete. Dichloromethane (10 mL) was added, and the mixture was washed with saturated NaCl solution. The organic phase was dried and concentrated, and the residue was chromatographed to give **9** (190 mg, 82%): mp 104.5–105°C (from EtOH–Et$_2$O), $[\alpha]_D$ +52° (*c* 1); ^{1}H NMR (CDCl$_3$): δ 6.05 (d, 1 H, $J_{4,NH}$ 9.2 Hz, NH), 5.17 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 11.0 Hz, H-3), 5.08 (dd, $J_{2',3'a}$ 4.6, $J_{2',3'b}$ 8.1 Hz, H-2′), 4.72 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.28–4.18 (m, partially overlapped, H-4), 4.20–4.06 (m, partially overlapped, H-4′a,b), 3.95 (ddd, 1 H, $J_{2,OH}$ 4.8 Hz, H-2), 3.70–3.61 (m, 1 H, H-5), 3.39 (s, 3 H, OCH$_3$), 2.34 (d, 1 H, OH), 2.27–2.05 (m, partially overlapped, H-3′a,b), 2.17, 2.13, 2.06 (3 s, overlapped, 3 COCH$_3$), and 1.23 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR (CDCl$_3$): δ 171.50, 169.85, 169.60 (C, C, 2 C, 4 CO), 100.39 (C-1), 71.00 (C-3), 70.94 (C-2′), 68.91 (C-2), 67.98 (C-5), 59.88 (C-4′), 55.02 (OCH$_3$), 51.18 (C-4), 30.57 (C-3′), 20.92, 20.81, 20.71 (3 COCH$_3$), and 17.77 (C-6); CIMS: *m/z* 406 [M + 1]$^+$ and 423 [M + 18]$^+$. Anal. Calcd for $C_{17}H_{27}NO_{10}$: C, 50.37; H, 6.71; N, 3.56. Found: C, 50.58; H, 6.75; N, 3.52.

*Methyl 3-O-acetyl-2-O-bromoacetyl-4,6-dideoxy-4-(2,4-di-O-acetyl-3-deoxy-*L-glycero-*tetronamido)-α-*D-*mannopyranoside* (**10**).—To a cold (0°C) solution of **9** (4.9 g, 12.1 mmol) and 2,4,6-trimethylpyridine (4.3 mL, 32.7 mmol) in dichloromethane (100 mL) was added bromoacetyl bromide (2.1 mL, 24.2 mmol), and the mixture was stirred at room temperature until all starting material was consumed (ca. 4 h, TLC, solvent D).

A few drops of MeOH were added, and the mixture was stirred for 2 h to decompose the excess reagent. The mixture was washed with NaCl solution, the organic phase was dried and concentrated, and the residue was chromatographed to give **10** (5.2 g, 82%): mp 104–105°C (from Et_2O), $[\alpha]_D$ +24° (*c* 0.9); ^1H NMR ($CDCl_3$): δ 6.10 (d, 1 H, $J_{4,NH}$ 9.2 Hz, NH), 5.29 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 11 Hz, H-3), 5.19 (dd, 1 H, $J_{1,2}$ 1.7 Hz, H-2), 5.09 (dd, 1 H, $J_{2',3'a}$ 4.8, $J_{2',3'b}$ 7.9 Hz, H-2′), 4.69 (d, 1 H, H-1), 4.21 (t, partially overlapped, $J_{4,5}$ 9.7 Hz, H-4), 4.20–4.06 (m, partially overlapped, H-4′a,b), 3.96 (s, 2 H, CH_2Br), 3.75–3.64 (m, 1 H, H-5), 3.39 (s, 3 H, OCH_3), 2.20–1.99 (m, overlapped, H-3′a,b), 2.17, 2.05 (2 s, overlapped, 3 $COCH_3$), and 1.25 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 171.27, 170.81, 169.79, 169.53, 166.61 (5 CO), 97.87 (C-1), 70.91 (C-2′), 70.87 (C-2), 68.20 (C-3), 68.01 (C-5), 59.78 (C-4′), 55.14 (OCH_3), 51.34 (C-4), 30.52 (C-3′), 25.47 (CH_2Br), 20.78, 20.68, 20.66 (3 $COCH_3$), and 17.69 (C-6); CIMS: *m/z* 543 $[M + 18]^+$, where the cluster of ion-peaks agreed with the anticipated isotopic composition. Anal. Calcd for $C_{19}H_{28}BrNO_{11}$: C, 43.36; H, 5.36; Br, 15.18; N, 2.66. Found: C, 43.38; H, 5.44; Br, 15.27; N, 2.71.

*1,3-Di-O-acetyl-2-O-bromoacetyl-4,6-dideoxy-4-(2,4-di-O-acetyl-3-deoxy-*L-glycero-*tetronamido)-α-* (**11**) *and -β-*D*-mannopyranose* (**13**).—A solution of **10** (4.5 g) in a mixture of 25:10:0.3 Ac_2O–HOAc–H_2SO_4 (75 mL) was kept at room temperature for 2 h, at the end of which time TLC (solvent E) showed that all of **10** was consumed. The mixture was poured into a mixture of ice and aq $NaHCO_3$ and stirred for 2 h to hydrolyze excess Ac_2O. The product was extracted with dichloromethane, dried and concentrated, and the residue was chromatographed to give 4.1 g (87%) of material consisting of **11** and **13**, where the α-anomer largely predominated. A portion (1 g) was rechromatographed to give first **11**, $[\alpha]_D$ +30° (*c* 2.3); ^1H NMR ($CDCl_3$): δ 6.07 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1), 6.04 (d, 1 H, $J_{4,NH}$ 9.0 Hz, NH), 5.33 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 11.1 Hz, H-3), 5.22 (dd, 1 H, H-2), 5.08 (dd, 1 H, $J_{2',3'a}$ 4.9, $J_{2',3'b}$ 7.7 Hz, H-2′), 4.29–4.18 (m, partially overlapped, H-4), 4.18–4.07 (m, partially overlapped, H-4′a,b), 3.97 (dd, 2 H, ^2J 12.5 Hz, CH_2Br), 3.88–3.79 (m, 1 H, H-5), 2.18, 2.17, 2.08, 2.06 (4 s, overlapped, 4 $COCH_3$), 2.18–2.06 (m, overlapped, H-3′a,b), and 1.25 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 90.30 (C-1, $J_{C,H}$ 177.0 Hz), 70.96 (C-2′), 70.45 (C-5), 69.76 (C-2), 67.86 (C-3), 59.74 (C-4′), 51.14 (C-4), 30.52 (C-3′), 25.13 (CH_2Br), 20.86, 20.72 (2 C each, 4 $COCH_3$), and 17.76 (C-6); CIMS: *m/z* 572 $[M + 18]^+$, where the intensity of the peaks in the cluster agreed with the expected isotopic composition. Anal. Calcd for $C_{20}H_{28}BrNO_{12}$: C, 43.33; H, 5.09; Br, 14.41; N, 2.83. Found: C, 43.28; H, 5.14; Br, 14.48; N, 2.55.

Eluted later was material containing almost entirely the β-1-*O*-acetyl derivative **13**. ^1H NMR ($CDCl_3$): δ 6.14 (d, 1 H, $J_{4,NH}$ 9.4 Hz, NH), 5.85 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 5.46 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 5.17 (dd, 1 H, $J_{3,4}$ 11.0 Hz, H-3), 5.05 (dd, 1 H, $J_{2',3'a}$ 5.0, $J_{2',3'b}$ 7.6 Hz, H-2′), 4.28–4.06 (m, 3 H, H-4,4′a,b), 4.00 (s, 2 H, CH_2Br), 3.68–3.58 (m, 1 H, H-5), 2.15, 2.10, 2.06, 2.05 (4 s, overlapped, 3 $COCH_3$), 2.15–2.05 (m overlapped, H-3′a,b), and 1.31 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 90.38 (C-1, $J_{C,H}$ 162.5 Hz), 72.85 (C-5), 70.99 (C-2′), 69.83 (C-2), 69.62 (C-3), 59.74 (C-4′), 50.97 (C-4), 30.50 (C-3′), 25.31 (CH_2Br), 20.77, 20.63, 20.51 (C, 2 C, C, 4 $COCH_3$), and 17.65 (C-6); CIMS: *m/z* 572 $[M + 18]^+$, where the intensity of the peaks in the cluster agreed with the expected isotopic composition.

An intermediate, mixed fraction of **11** and **13** was also obtained.

*Methyl [3-O-acetyl-2-O-bromoacetyl-4,6-dideoxy-4-(2,4-di-O-acetyl-3-deoxy-*L-glycero-*tetronamido)-α-*D-*mannopyranosyl]-(1 → 2)-3-O-acetyl-4,6-dideoxy-4-(2,4-di-O-acetyl-3-deoxy-*L-glycero-*tetronamido)-α-*D-*mannopyranoside* (**20**).—A mixture of **11** (1.1 g, 2 mmol), DCMME (0.35 mL, 4 mmol), alcohol-free $CHCl_3$, and freshly fused $ZnCl_2$ (ca. 100 mg) was heated at 50°C until TLC (solvent F) showed that only a trace of the starting material remained (ca. 30 min). After filtration through glass wool and concentration, chromatography gave pure (TLC, NMR) 3-*O*-acetyl-2-*O*-bromoacetyl-4,6-dideoxy-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranosyl chloride (**12**, 0.9 g, 86%). ^{1}H NMR ($CDCl_3$): δ 6.19 (d, 1 H, $J_{4,NH}$ 9.1 Hz, NH), 6.01 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 5.58 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 11.2 Hz, H-3), 5.35 (dd, 1 H, H-2), 5.08 (dd, 1 H, $J_{2',3'a}$ 4.9, $J_{2',3'b}$ 7.7 Hz, H-2′), 4.29 (m, 1 H, H-4), 4.22–4.08 (m, 2 H, H-4′a,b), 4.07–3.97 (m, 1 H, H-5), 3.95 (s, 2 H, CH_2Br), 2.18, 2.08, 2.06 (3 s, overlapped, 3 $COCH_3$), 2.18–2.06 (m, overlapped, H-3′a,b), and 1.28 (d, 3 H, $J_{5,6}$, 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 88.84 (C-1), 72.80 (C-2), 71.29 (C-5), 70.96 (C-2′), 66.84 (C-3), 59.77 (C-4′), 50.97 (C-4), 30.55 (C-3′), 25.02 (CH_2Br), 20.81, 20.71, 20.61 (3 $COCH_3$), and 17.38 (C-6); CIMS: *m/z* 547 $[M + 18]^+$, based on the monoisotopic mass, while the intensity of lines in the cluster of peaks agreed with the compound containing one atom each of chlorine and bromine.

A solution of **12** (0.9 g, 1.7 mmol) in CH_2Cl_2 (5 mL) was added dropwise at room temperature to a mixture of AgOTf (0.48 g, 1.9 mmol), **9** (0.68 g, 1.7 mmol), and 2,4,6-trimethylpyridine (0.25 mL, 1.9 mmol) in CH_2Cl_2 (5 mL), and the mixture was stirred for 30 min, at the end of which time TLC (solvent G) showed that all of the glycosyl donor was consumed. After filtration, the filtrate was washed with a mixture of aq $NaHCO_3$ and $Na_2S_2O_3$, dried and concentrated, and the residue was chromatographed to give 1.14 g (75%) of pure **20**: $[\alpha]_D$ +12° (*c* 0.8); ^{1}H NMR ($CDCl_3$): δ 6.35 (d, 1 H, $J_{4,NH}$ 9.2 Hz, NH^2), 6.17 (d, 1 H, $J_{4,NH}$ 9.1 Hz, NH^1), 5.32 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 11.0 Hz, H-3^2), 5.26 (dd, 1 H, $J_{1,2}$ 1.8, $J_{2,3}$ 3.3 Hz, H-2^2), 5.21 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 11.1 Hz, H-3^1), 5.10 (m, 2 H, H-2′^{1,2}), 4.95 (d, 1 H, H-1^2), 4.67 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1^1), 4.30–4.03 (m, 6 H, H-4^{1,2},4′^{1,2}a,b), 3.94 (s, 2 H, CH_2Br), 3.92 (dd, 1 H, H-2^1), 3.87 (m, 1 H, H-5^2), 3.70–3.60 (m, 1 H, H-5^1), 3.38 (s, 3 H, OCH_3), 2.22, 2.18, 2.12, 2.09, 2.06, 2.05 (6 s, overlapped, 6 $COCH_3$), 2.22–2.05 (m, overlapped, H-3′^{1,2}a,b), 1.24, and 1.21 (2 d, 6 H, $J_{5,6}$ 6.3 Hz, H-6^{1,2}); ^{13}C NMR ($CDCl_3$): δ 99.44 (C-1^1), 98.72 (C-1^2), 76.02 (C-2^1), 71.10 (C-2^2), 70.83 (2 C, C-2′^{1,2}), 69.70 (C-3^1), 69.04 (C-5^2), 68.11 (C-5^1), 67.75 (C-3^2), 59.75 (2 C, C-4′^{1,2}), 54.91 (OCH_3), 51.61 (C-4^1), 51.47 (C-4^2), 30.55, 30.42 (C-3′^{1,2}), 25.34 (CH_2Br), 17.75 (C-6^1), and 17.54 (C-6^2); CIMS: *m/z* 916 $[M + 18]^+$, where the cluster of peaks agreed with the isotopic composition. Anal. Calcd for $C_{35}H_{51}BrN_2O_{20}$: C, 46.73; H, 5.71; N, 3.11. Found: C, 47.02; H, 5.80; N, 2.99.

*Methyl [3-O-acetyl-4,6-dideoxy-4-(2,4-di-O-acetyl-3-deoxy-*L-glycero-*tetronamido)-α-*D-*mannopyranosyl]-(1 → 2)-3-O-acetyl-4,6-dideoxy-4-(2,4-di-O-acetyl-3-deoxy-*L-glycero-*tetronamido)-α-*D-*mannopyranoside* (**21**).—A solution of thiourea (295 mg, 2.6 mmol) and 2,4,6-trimethylpyridine (0.17 mL, 1.3 mol) in methanol (1 mL) was added at 0°C to a solution of the fully protected disaccharide **20** (0.77 g, 0.86 mmol) in CH_2Cl_2 (10 mL), and the mixture was stirred at 0°C until TLC (solvent H) showed that all starting material was consumed. The mixture was filtered, the filtrate was concentrated, and the residue was partitioned between water and CH_2Cl_2. The organic phase was dried and concentrated, and the residue was chromatographed to give pure **21** (0.55 g, 83%): $[\alpha]_D$ +40° (*c* 0.7); ^{1}H NMR ($CDCl_3$): δ 6.24 (d, 1 H, $J_{4,NH}$ 9.2 Hz, NH^1), 6.09 (d, 1 H, $J_{4,NH}$ 9.3 Hz, NH^2), 5.22 (dd, partially overlapped, $J_{2,3}$ 3.0, $J_{3,4}$ 11.1 Hz, H-3^1), 5.18 (dd, partially overlapped, $J_{2,3}$ 2.9, $J_{3,4}$ 11.0 Hz, H-3^2), 5.10–5.05 (m, 2 H, H-2$'^{1,2}$), 5.01 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1^2), 4.67 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1^1), 4.33–4.23 (m, 1 H, H-4^2), 4.20–4.06 (m, 6 H, H-2^2,4^1,4$'^{1,2}$a,b), 3.95 (dd, 1 H, H-2^1), 3.79–3.70 (m, 1 H, H-5^2), 3.68–3.59 (m, 1 H, H-5^1), 3.38 (s, 3 H, OCH_3), 2.62 (bd, 1 H, OH), 2.21, 2.17, 2.15, 2.10, 2.06, 2.05 (6 s, overlapped, 6 $COCH_3$), 2.21–2.05 (m, overlapped, H-3$'^{1,2}$a,b), 1.24, and 1.20 (2 d, 6 H, $J_{5,6}$ 6.3 Hz, H-6^{1,2}); ^{13}C NMR ($CDCl_3$): δ 101.14 (C-1^2), 99.67 (C-1^1), 75.02 (C-2^1), 70.87 (2 C, C-2$'^{1,2}$), 70.72, (C-3^2), 70.30 (C-3^1), 69.06 (C-5^2), 68.84 (C-2^2), 68.24 (C-5^1), 59.83 (2 C, C-4$'^{1,2}$), 54.96 (OCH_3), 51.57 (C-4^1), 51.19 (C-4^2), 30.60, 30.49 (C-3$'^{1,2}$), 17.78 (C-6^1), and 17.65 (C-6^2); CIMS: *m/z* 796 $[M + 18]^+$. Anal. Calcd for $C_{33}H_{50}N_2O_{19}$: C, 50.89; H, 6.47; N, 3.60. Found: C, 50.90; H, 6.49; N, 3.50.

*1,2,3-Tri-O-acetyl-4,6-dideoxy-4-(2,4-di-O-acetyl-3-deoxy-*L-glycero-*tetronamido)-α-* **(15)** *and -β-*D*-mannopyranose* **(16)**.—A solution of **14** (ref. [9], 0.9 g) in a 20:10:0.3 mixture of Ac_2O–HOAc–H_2SO_4 (5 mL) was kept at room temperature for 30 min, when TLC (solvent G) showed that the starting material was no longer present. The product showed slightly greater chromatographic mobility than **14**, and it appeared as a single spot. The mixture was poured into a mixture of ice and aq $NaHCO_3$ to neutralize the acids present and stirred for 1 h to hydrolyze the excess Ac_2O. The product was extracted with dichloromethane, and, after workup as usual, crystallization from ethanol gave **15**: mp 125–126°C, $[\alpha]_D$ +46° (*c* 1); ^{1}H NMR ($CDCl_3$): δ 6.07 (d, 1 H, $J_{4,NH}$ 9.3 Hz, NH), 6.04 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1), 5.29 (dd, 1 H, $J_{2,3}$ 3.4, $J_{4,5}$ 11.2 Hz, H-3), 5.17 (dd, 1 H, H-2), 5.08 (dd, 1 H, $J_{2',3'a}$ 5.0, $J_{2',3'b}$ 7.6 Hz, H-2′), 4.32–4.21 (m, partially overlapped, H-4), 4.20–4.06 (m, 2 H, partially overlapped, H-4′a,b), 3.85–3.75 (m, 1 H, H-5), 2.18, 2.17, 2.15, 2.07, 2.06 (5 s, overlapped, 5 $COCH_3$), 2.18–2.06 (m, overlapped, H-3′a,b), and 1.25 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 90.71 ($J_{C,H}$ 177.9 Hz, C-1), 70.91 (C-2′), 70.51 (C-5), 68.08 (C-2), 67.97 (C-3), 59.73 (C-4′), 51.15 (C-4), 30.49 (C-3′), and 17.75 (C-6); CIMS: *m/z* 476 $[M + 1]^+$ and 493 $[M + 18]^+$. Anal. Calcd for $C_{20}H_{29}NO_{12}$: C, 50.52; H, 6.15; N, 2.95. Found: C, 50.49; H, 6.13; N, 2.95.

The mother liquor was chromatographed to give an additional crop of **15** (total yield, 0.75 g, 78%).

Continued elution gave a mixture of **15** and **16** (50 mg), followed by the amorphous **16** (ca. 10 mg, total yield of the conversion **14** → **15** + **16**, 84%). ^{1}H NMR data for **16** ($CDCl_3$): δ 6.10 (d, 1 H, $J_{4,NH}$ 9.3 Hz, NH), 5.80 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 5.44 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 5.11 (dd, partially overlapped, $J_{3,4}$ 11.1 Hz, H-3), 5.06 (dd, partially overlapped, $J_{2',3'a}$ 5.0, $J_{2',3'a}$ 7.5 Hz, H-2′), 4.26–4.07 (m, 3 H, H-4,4′a,b), 3.63–3.54 (m, 1 H, H-5), 2.21, 2.15, 2.10, 2.06, and 2.05 (5 s, overlapped, 5 $COCH_3$), 2.15–2.05 (m, overlapped, H-3′a,b); ^{13}C NMR ($CDCl_3$): δ 90.63 ($J_{C,H}$ 161.5 Hz, C-1), 73.04 (C-5), 70.98 (C-2′), 69.90 (C-3), 67.97 (C-2), 59.76 (C-4′), 51.03 (C-4), 30.53 (C-3′), and 17.71 (C-6); CIMS: *m/z* 493 $[M + 18]^+$.

*Methyl [3-O-acetyl-4,6-dideoxy-4-(2,4-di-O-acetyl-3-deoxy-*L-glycero-*tetronamido)-2-O-acetyl-α-*D-*mannopyranosyl]-(1 → 2)-[3-O-acetyl-4,6-dideoxy-4-(2,4-di-O-acetyl-3-deoxy-*L-glycero-*tetronamido)-α-*D-*mannopyranosyl]-(1 → 2)-3-O-acetyl-4,6-dideoxy-4-(2,4-di-O-acetyl-3-deoxy-*L-glycero-*tetronamido)-α-*D-*mannopyranoside* (**22**) *and methyl [4,6-dideoxy-4-(3-deoxy-*L-glycero-*tetronamido)-α-*D-*mannopyranosyl]-(1 → 2)-[4,6-dideoxy-4-(3-deoxy-*L-glycero-*tetronamido)-α-*D-*mannopyranosyl]-(1 → 2)-4,6-dideoxy-4-(3-deoxy-*L-glycero-*tetronamido)-α-*D-*mannopyranoside* (**23**).—Compound **15** (0.35 g, 0.75 mmol) was treated with DCMME (0.13 mL, 1.5 mmol), as described for the preparation of **12**. Chromatography (solvent F) gave 2,3-di-*O*-acetyl-4,6-dideoxy-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranosyl chloride (**17**, 0.3 g, 90%). ^{1}H NMR ($CDCl_3$): δ 6.13 (d, 1 H, $J_{4,NH}$ 9.8, NH), 5.99 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 5.55 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 11.2 Hz, H-3), 5.30 (dd, 1 H, H-2), 5.10 (dd, 1 H, $J_{2',3'a}$ 4.9, $J_{2',3'b}$ 7.5 Hz, H-2′), 4.35–4.25 (m, 1 H, H-4), 4.22–4.08 (m, 2 H, H-4′a,b), 4.05–3.95 (m, 1 H, H-5), 2.19, 2.18, 2.07, 2.06 (4 s, overlapped, 4 $COCH_3$), 2.19–2.05 (m, overlapped, H-3′a,b), and 1.28 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 89.49 (C-1), 71.37 (2 C, C-2,5), 70.94 (C-2′), 66.97 (C-3), 59.77 (C-4′), 51.15 (C-4), 30.58 (C-3′), and 17.43 (C-6); CIMS: *m/z* 469 $[M + 18]^+$.

The glycosyl acceptor **21** (0.3 g, 0.39 mmol) was treated with the glycosyl donor **17** (0.24 g, 0.53 mmol) as described for the preparation of **20**, and the crude product was chromatographed (solvent I). Eluted first was a mixture of products enriched in the desired trisaccharide **22**, as shown by NMR spectroscopy.

Eluted next was the product of hydrolysis, **19**, of the glycosyl donor **17**, as shown by NMR spectroscopy and CIMS. ^{1}H NMR ($CDCl_3$) for the α-anomer, largely predominating: δ 6.32 (d, 1 H, $J_{4,NH}$ 9.3 Hz, NH), 5.35 (dd, 1 H, $J_{2,3}$ 2.7, $J_{3,4}$ 11.0 Hz, H-3), 5.20 (bs, 2 H, H-1,2), 5.08 (dd, 1 H, $J_{2',3'a}$ 4.9, $J_{2',3'b}$ 7.7 Hz, H-2′), 4.27–4.05 (m, 3 H, H-4,4′a,b), 4.02–3.92 (m, 1 H, H-5), 2.16, 2.05, 2.06 (3 s, overlapped, 4 $COCH_3$), 2.16–2.06 (m, overlapped, H-3′a,b), and 1.22 (d, 3 H, $J_{5,6}$ 6.1 Hz, H-6); ^{13}C NMR ($CDCl_3$) for the α-anomer, largely predominating: δ 91.92 (C-1), 71.03 (C-2′), 69.74 (C-2), 68.23 (C-3), 68.01 (C-5), 59.92 (C-4′), 30.59 (C-3′), and 17.81 (C-6); CIMS: *m/z* 434 $[M + 1]^+$, and 451 $[M + 18]^+$.

The material enriched in **22** was rechromatographed (solvent J), and the material further enriched in **22**, thus obtained, was *O*-deacetylated (Zemplén). Chromatography (solvent F) gave **23** (0.155 g, 64% over two steps): $[\alpha]_D$ +3.5° (*c* 0.7, H_2O); ^{1}H NMR (D_2O): δ 5.21 (bd, 1 H, $J_{1,2}$ ~ 1.1 Hz, H-1^2), 5.08 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1^3), 4.83 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1^1), 4.36–4.31 (m, 3 H, H-$2'^{1-3}$), 4.23–4.18 (m, 2 H, H-2,3), 4.15 (dd, 1 H, $J_{2,3}$ 3.3 H, H-2^3), 4.11–4.04 (m, 2 H, H-3,3), 4.01–3.84 (m, 7 H, H-2,4^{1-3},5^{1-3}), 3.91–3.74 (m, 6 H, H-$4'^{1-3}$a,b), 3.43 (s, 3 H, OCH_3), 2.13–1.82 (2 m, 6 H, H-$3'^{1-3}$a,b), 1.24 (2 d, overlapped, 6 H, H-6,6), and 1.19 (d, 3 H, $J_{5,6}$ 6.1 Hz, H-6); ^{13}C NMR (D_2O): δ 101.99 (C-1^3), 100.63 (C-1^2), 99.58 (C-1^1), 77.25 (2 C, C-$2^{1,2}$), 69.13 (C-2^3), 69.05 (3 C, C-$2'^{1-3}$), 68.22 (C-5), 68.01 (C-5), 67.73 (2 C, C-3,3), 67.42 (2 C, C-3,5), 57.92 (3 C, C-$4'^{1-3}$), 54.98 (OCH_3), 53.03, 52.97, 52.75 (C-4^{1-3}), 35.00 (3 C, C-$3'^{1-3}$), 16.97, and 16.89 (C, 2 C, C-6^{1-3}); FABMS: *m/z* 774 $[M + 1]^+$ and $[M + Na]^+$, 906 $[M + Cs]^+$.

For characterization, compound **23** (50 mg) was treated with 1:1 pyridine–acetic anhydride (2 mL), to give **22** in virtually theoretical yield: $[\alpha]_D$ +27° (*c* 0.8); ^{1}H NMR

($CDCl_3$): δ 6.47, 6.39, 6.24 (3 d, 1 H each, $J_{4,NH}$ 9.0 Hz, 3 NH); 5.31 (dd, 1 H, $J_{2,3}$ 3.4, $J_{3,4}$ 11 Hz, H-3^3), 5.25–5.18 (m, 3 H, H-2^3,3^1,3^2), 5.15–5.05 (m, 3 H, H-$2'^{1\ 3}$), 4.99 (d, 1 H, $J_{1,2}$ 2.1 Hz, H-1^2), 4.93 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1^3), 4.66 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1^1), 4.30–4.07 (m, 9 H, H-$4^{1\cdot 3}$,$4'^{1-3}$a,b) 4.04 (dd, 1 H, $J_{2,3}$ 3.4 Hz, H-2^2), 3.91 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-2^1), 3.87–3.74, 3.68–3.61 (2 m, 2 H, 1 H, H-$5^{1\ 3}$), 3.39 (s, 3 H, OCH_3), 2.23, 2.20, 2.17, 2.16, 2.15, 2.09, 2.08, 2.06 (~ double intensity), 2.05 (9 s overlapped, 10 $COCH_3$), 2.23–1.23 (m, overlapped, H-$3'^{1\ 3}$a,b), 1.21 (2 d, partially overlapped, $J_{5,6}$ ~ 6.6 Hz, 2 H-6), and 1.17 (d, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 100.54 (C-1^2), 99.62 (C-1^1), 99.48 (C-1^3), 76.75 (C-2^2), 75.81 (C-2^1), 70.92, 70.86 (2 C, C, C-$2'^{1-3}$), 70.10 (C-2^3), 69.41, 69.36 (C-$3^{1,2}$), 69.23, 68.23 (2 C, C, C-5^{1-3}), 68.04 (C-3^3), 59.83, 59.79 (2 C, C, C-$4'^{1\ 3}$), 55.07 (OCH_3), 51.90, 51.67, 51.51 (C-4^{1-3}), 30.65, 30.59, 30.55 (C-$3'^{1\ 3}$), 17.81, 17.76, and 17.70 (C-6^{1-3}); CIMS: m/z 1211 $[M + 18]^+$. Anal. Calcd for $C_{51}H_{75}N_3O_{29}$: C, 51.30; H, 6.73; N, 3.52. Found: C, 51.15; H, 6.36; N, 3.43.

*4,6-Dideoxy-4-(3-deoxy-*L-glycero-*tetronamido)-α,β-*D*-mannopyranose* (18).—Compound 15 was *O*-deacetylated (Zemplén), and, after conventional processing, elution from a short column of silica gel (solvent K) and subsequent freeze drying gave the product 18 (a mixture of anomers, $\alpha:\beta$ ca. 0.55:0.45) in virtually theoretical yield: $[\alpha]_{578}$ −13° (*c* 1.0, water); $[\alpha]_D$ −13° (*c* 1.0, water); lit. [27] $[\alpha]_{578}$ −6.5° (*c* 1.0, water); ^1H NMR (D_2O): δ 5.16 (d, $J_{1,2}$ 1.7 Hz, H-1α), 4.86 (d, $J_{1,2}$ 1.0 Hz, H-1β), 4.29 (dd, $J_{2',3'a}$ 3.9, $J_{2',3'b}$ 8.6 Hz, H-2'α,β), 4.40–3.83 (m, H-2α,2β,3α,4α,5α), 3.80–3.72 (m, H-3β,4β,4'α,β,a,b), 3.62–3.52 (m, H-5β), 2.08–1.94, 1.85–1.75 (2 m, H-3'α,β,a,b), 1.14 (d, $J_{5,6}$ 6.3 Hz, H-6β), and 1.11 (d, $J_{5,6}$ 6.3 Hz, H-6α); ^{13}C NMR (D_2O): δ 177.31 (COα,β), 94.06 ($J_{C,H}$ 170.5 Hz, C-1α), 93.62 ($J_{C,H}$ 159.7 Hz, C-1β), 70.94 (C-5β), 70.73 (C-2β), 70.66 (C-3β), 70.04 (C-2α), 69.10 (C-2'α,β), 67.76 (C-3α), 67.14 (C-5α), 57.94 (C-4'α,β), 53.06 (C-4α), 52.72 (C-4β), 36.03 (C-3'α,β), 17.07 (C-6α), and 17.01 (C-6β); CIMS: m/z 283 $[M + 18]^+$.

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