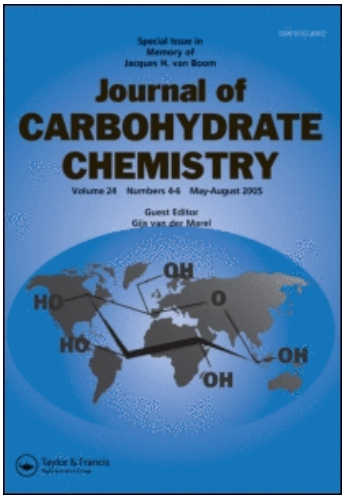

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SYNTHESIS OF THE METHYL α-GLYCOSIDE OF THE INTRACATENARY DISACCHARIDE REPEATING UNIT OF THE O-POLYSACCHARIDE OF *VIBRIO CHOLERAE* O:1. A COMPARISON OF TWO ASSEMBLY STRATEGIES[1]

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ABSTRACT

The two strategies engaged in the construction of the title disaccharide **17** comprise: 1. assembly of a diamino disaccharide and its *N*-acylation using chiral reagents to introduce the 4-(3-deoxy-L-*glycero*-tetronyl) group, followed by deprotection, and 2. preparation of a glycosyl acceptor and a glycosyl donor both having the chiral 3-deoxy-L-*glycero*-tetronamido group already in place, their condensation to give a fully substituted disaccharide, and deprotection. Accordingly, the crystalline diamino disaccharide methyl 2-*O*-(4-amino-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-4-amino-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside, (**14**), was prepared from the known [Bundle, D. R. *et al.*, *Carbohydr. Res.*, **174**, 239 (1988)] diazido disaccharide **12**, and treated with the lactone **30**, or its acetylated or benzylated analogs **31** and **32**, respectively, as the *N*-acylating reagents. Subsequent deprotection of the respective products applying standard chemistry gave **17**. Alternatively, the methyl α-glycoside of the monomeric intracatenary repeating unit of *Vibrio cholerae* O:1 (**2**) was converted to the fully benzoylated glycosyl chloride **26**, and the latter glycosyl donor was condensed with methyl 3-*O*-benzyl-4,6-dideoxy-4-(2,4-di-*O*-benzoyl-3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranoside (**24**), to give the corresponding, fully protected derivative **27**. Deprotection then readily gave **17**. It appears that the title disaccharide can be most efficiently synthesized using synthons **24** and **26**. The lactones **30** and **32** appear to be promising acylating reagents for the introduction of the 3-deoxy-L-*glycero*-tetronamido group when higher oligosaccharides in this series will be synthesized *via* their (poly)amino precursors.

INTRODUCTION

Vibrio cholerae O:1 occurs as two main immunologically distinct strains, Ogawa and Inaba. The third, Hikojima, is a rare, unstable intermediate form.[3] The O-antigens of serotypes Ogawa and Inaba contain the same intracatenary monosaccharide repeating unit, 4-amino-4,6-dideoxy-D-mannose (D-perosamine), *N*-acylated with 3-deoxy-L-*glycero*-tetronic acid. The O-polysaccharides of these two strains have been reported to differ[4] in the upstream[5] end-moiety of D-perosamine, which is methylated at *O*-2 in the O-antigen of the Ogawa strain but not in the other. The occurrence of 2-*O*-methylperosamine in the O-polysaccharide of the Ogawa serotype has been recently confirmed.[6] Systematic prevention of cholera by immunization has not been achieved because of lack of a protective vaccine. We have been generally interested in finding synthetic substitutes to vaccines based on natural lipopolysaccharides or fragments thereof. Such work requires studies of the mode of binding of ligands related to the O-polysaccharide with the homologous antibodies. Until recently, preparation of such substances related to the O-polysaccharide of *Vibrio cholerae* O:1 has been hampered by the lack of an efficient synthesis of their monomeric constituent. In the initial stage of our work aimed at identifying structural requirements for molecules expected to elicit protective antibodies when linked to a suitable carrier, we have already been able to improve the original synthesis[7] of the methyl α-glycoside **2** of the monomeric, intracatenary repeating unit of the O-polysaccharide of *Vibrio cholerae* O:1. We obtained it in the crystalline state for the first time and fully characterized it, including the description of its crystal structure.[8,9] We expect this to open avenues for the synthesis of more complex ligands in this series, a prerequisite for attempting the preparation of a synthetic vaccine against cholera.

The strategy for the assembly of aminosugar-containing oligosaccharides whose amino group is *N*-acylated with simple (acetyl or formyl) residues is usually rather straightforward, because the required *N*-acylation reagent is readily available. It normally involves the construction of the corresponding azido oligosaccharide, such as the diazido disaccharide **12** or **13**, which is subsequently converted, *via* the corresponding 4-amino derivative, into the oligosaccharide containing the requisite 4-acylamino group. Such chemistry was used, for example, by Bundle *et al.*[10-12] in the synthesis of oligosaccharides containing acetamido and formamido groups. For the synthesis of oligosaccharides related to the O-polysaccharide of *Vibrio cholerae* O:1, another strategy of assembly can be proposed. It would involve the assembly of oligosaccharides using, as synthons, glycosyl donors and glycosyl acceptors which have the required *N*-3-deoxy-L-*glycero*-tetronamido groups already in place. Here, we report on the results obtained by applying the two aforementioned approaches.

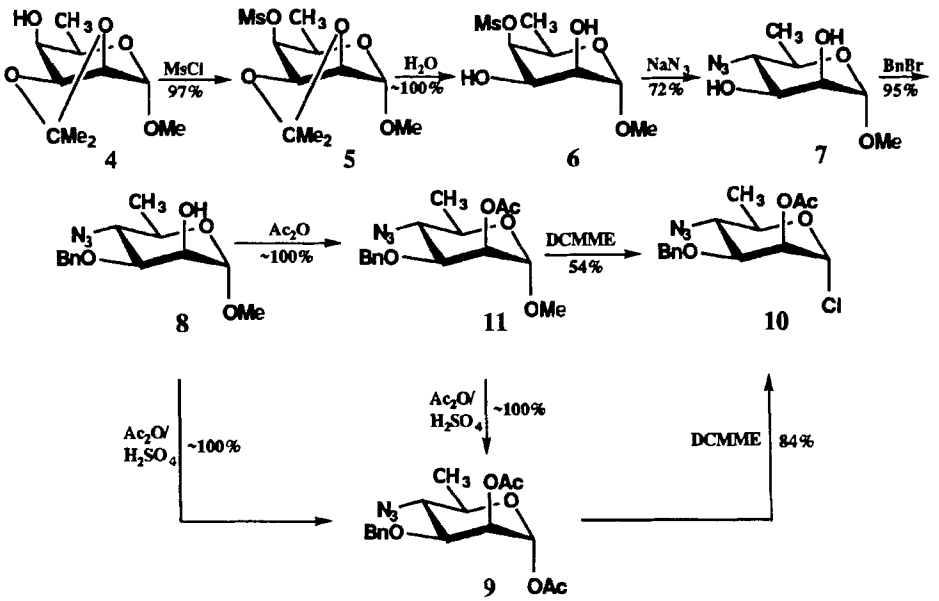
RESULTS AND DISCUSSION

The *N*-acylation of the amine **1** with ~3 molar equivalents of a crude preparation of the lactone **30** gave[7] the methyl glycoside of the monomeric, intracatenary repeating unit of the O-polysaccharide of *Vibrio cholerae* O:1, **2**, in 45% yield. When we performed[9] a similar *N*-acylation using 50% molar excess of the pure acetylated lactone **31**, the major product isolated (~72%) was the mono-*O*-acetyl derivative **3**, resulting from *O*-acetyl group migration. *O*-Deacetylation then gave[9] the desired glycoside **2** in virtually theoretical yield. Minor products of transacetylation and *N*-acetylation were also formed during the *N*-acylation with **31**. The nature of these by products, as well as of those formed[9] during the *N*-acylation of a 2,3-*O*-protected derivative of **1** with **31**, suggests that the *N*-acylation with lactone **31** of a diaminodisaccharide such as **14** or of higher members in this series might result in even more complex reaction mixtures.

	R
30	**H**
31	**Ac**
32	**Bn**

To test the feasibility of the approach in which the azido disaccharide is assembled first, we have used the known[11] disaccharide **12** as the starting material, but have changed somewhat the protocols described for the preparation of some of its precursors. We have previously noted[9] that, for the large scale preparation of **7**, the 4-*O*-trifluoromethanesulfonyl derivative of **4** used by Bundle *et al.*,[11] can be conveniently replaced with the mesyl derivatives **5** and **6** (Scheme 1), as described by Eis *et al.*.[13] The original[13] preparation of the important intermediate **5** has now been improved by conducting the mesylation of **4** with pyridine as base, rather then triethylamine.[13] In this way, no by-products[13] were formed, and the desired derivative **5** was obtained in virtually theoretical yield. Further conversion of **5** to **8** *via* **6** and **7** was carried out following the protocol of Eis *et al.*[13] Acetolysis of **8** then furnished the known[11] derivative **9** which was converted to the glycosyl chloride **10**, using dichloromethyl methyl ether (DCMME) and zinc chloride[14] (Scheme 1). The same glycosyl chloride could be obtained also by the cleavage of the glycoside **11** with the same reagent, but side reactions[15] were more extensive than during the conversion of **9** → **10**. Instead of using[11] a crude preparation of **10**, we have purified this glycosyl donor by chromatography. When the product **10** thus obtained was used to make (Scheme 2) the

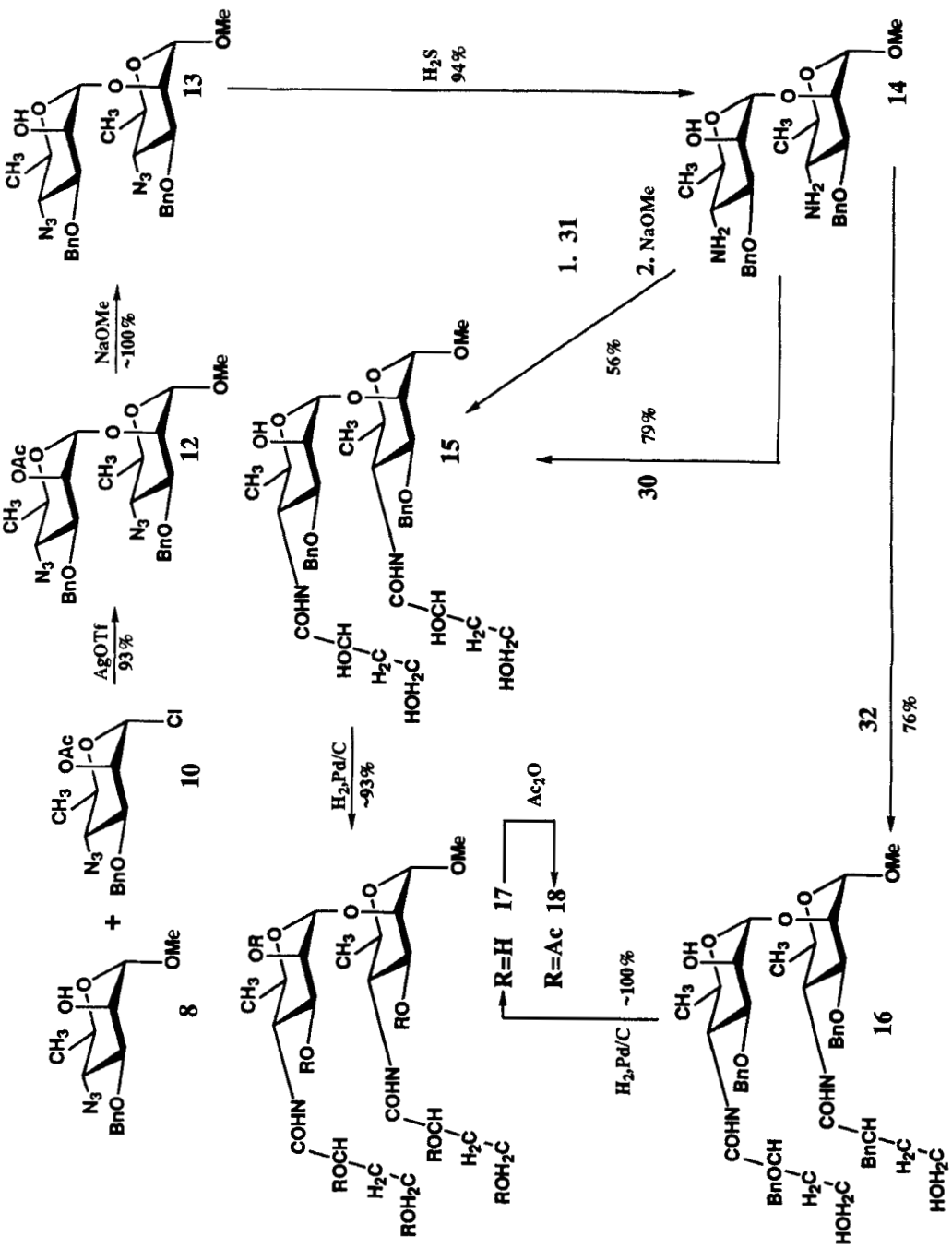
	R^2	R^3	R^4
1	**H**	**H**	NH_2
3	**H**	**H**	CONH
			HOCH
			CH_2
			CH_2OAc
21	**MBn**	**Bn**	**NHAc**



Scheme 1

disaccharide **12**, under the base-deficient conditions[16] we routinely use for making 1,2-*trans*-linked oligosaccharides, the glycosylation reaction was complete within 10 min (*cf.* 18 h, ref. 11). The desired product **12** was obtained in 93% yield after a single chromatography when toluene-ethyl acetate mixtures were used as eluant. The difficulties experienced previously[11] during this stage of the synthesis of **12** were not encountered. After deacetylation of **12**, the known[11] disaccharide **13** was now obtained crystalline.

Subsequent reduction of the azido groups gave the crystalline diamino disaccharide **14** (Scheme 2) which was converted to the corresponding tetronamido derivative **17** *via* the pentahydroxy derivative **15** (Scheme 2). Essentially the same mixture of products was formed from **14** and the acetylated lactone **31** when the reaction was conducted with neat reagent or in the presence of pyridine.[7,8] Consistent with the results of similar reactions conducted with the acetylated lactone **31** and perosamine derivatives,[8] numerous products were formed, with one of them only slightly predominating. The number of components originally present decreased dramatically upon deacetylation (Zemplén) of such a crude product, and now one component clearly predominated. Three of the by-products were isolated during resolution of the mixture by chromatography. Although they were not obtained in an analytically pure state, their ammonium CI MS indicated that they could be



Scheme 2

the products of partial tetronamidation and of partial tetronamidation *and* *N*-acetylation (see Experimental). The major product was isolated by chromatography in 56% yield, and it was shown by spectral characteristics to be the disaccharide **15**. Subsequent debenzylation of **15** gave the target disaccharide **17**. The ^{1}H NMR spectrum of **17**, showing the purity of the amorphous material obtained, is shown in Fig. 1. The compound was fully characterized by means of the per-*O*- acetylated derivative **18**.

In an attempt to avoid losses of the desired product of the *N*-3-deoxy-L-*glycero*-tetronylation described above, due to *N*-acetylation of **14** by the *O*-acetylated acylating reagent **31**, the *N*-tetronylation of **14** was carried out with the lactone **30**, obtained from pure **31** by deacetylation, and also with the benzylated lactone **32**, obtained by benzylation of **30**. The conversion **31**→**30** with aqueous trifluoroacetic acid gave virtually pure deacetylated lactone **30**, as shown by NMR. It is worth mentioning that the ^{1}H NMR spectrum of the crude product of deacetylation of **31** taken in D_2O revealed, in agreement with the findings of Kenne *et al.*[7], that the sample contained, in addition to **30**, a large proportion of the corresponding tetronic acid. Upon concentration of the solution in D_2O, and successive evaporation of acetone-toluene and carbon tetrachloride from the residue, the NMR spectra (^{1}H and ^{13}C) of the resulting material taken in $CDCl_3$ showed that the acid formerly present reverted virtually completely to the corresponding lactone **30**. The ^{1}H NMR spectrum of such material, practically pure **30**, taken subsequently in D_2O again showed the presence of a mixture of **30** and the corresponding acid, the lactone predominating. Although products of *N*-acetylation could not be formed when **30** was used for *N*-acylation of **14**, examination of the crude product thus obtained showed the presence of some minor byproducts. After the usual processing (see Experimental), compound **15** (Scheme 2) was obtained in 79% yield, after chromatography. This high yield in the *N*-3-deoxy-L-*glycero*-tetronylation, achieved using only 50% molar excess of the acylation reagent (*cf.*, 45% yield, using[7] ~300% molar excess of the reagent) suggests that the lactone **30**, when prepared from L-homoserine[7] *via* the acetate **31**, can be obtained in a higher degree of purity and is, therefore, a more efficacious *N*-acylation reagent than that originally described.

To undergird attempts at increasing the yield in the *N*-3-deoxy-L-*glycero*-tetronylation of derivatives of perosamine, we prepared the benzylated lactone **32**. In its reaction with the diamine **14**, lactone **32** was a less reactive *N*-acylating reagent then either **30** or **31**. When 100% molar excess of the reagent was used, the coupling product **16** was isolated in 75% yield, after 72 h of reaction time. Several minor by-products were formed, but their nature was not examined. Debenzylation by catalytic hydrogenolysis afforded **17** identical with the substance described above. The conversion **14** + **32** → **17** could also be carried out without the isolation of the intermediate **16** (see Experimental).

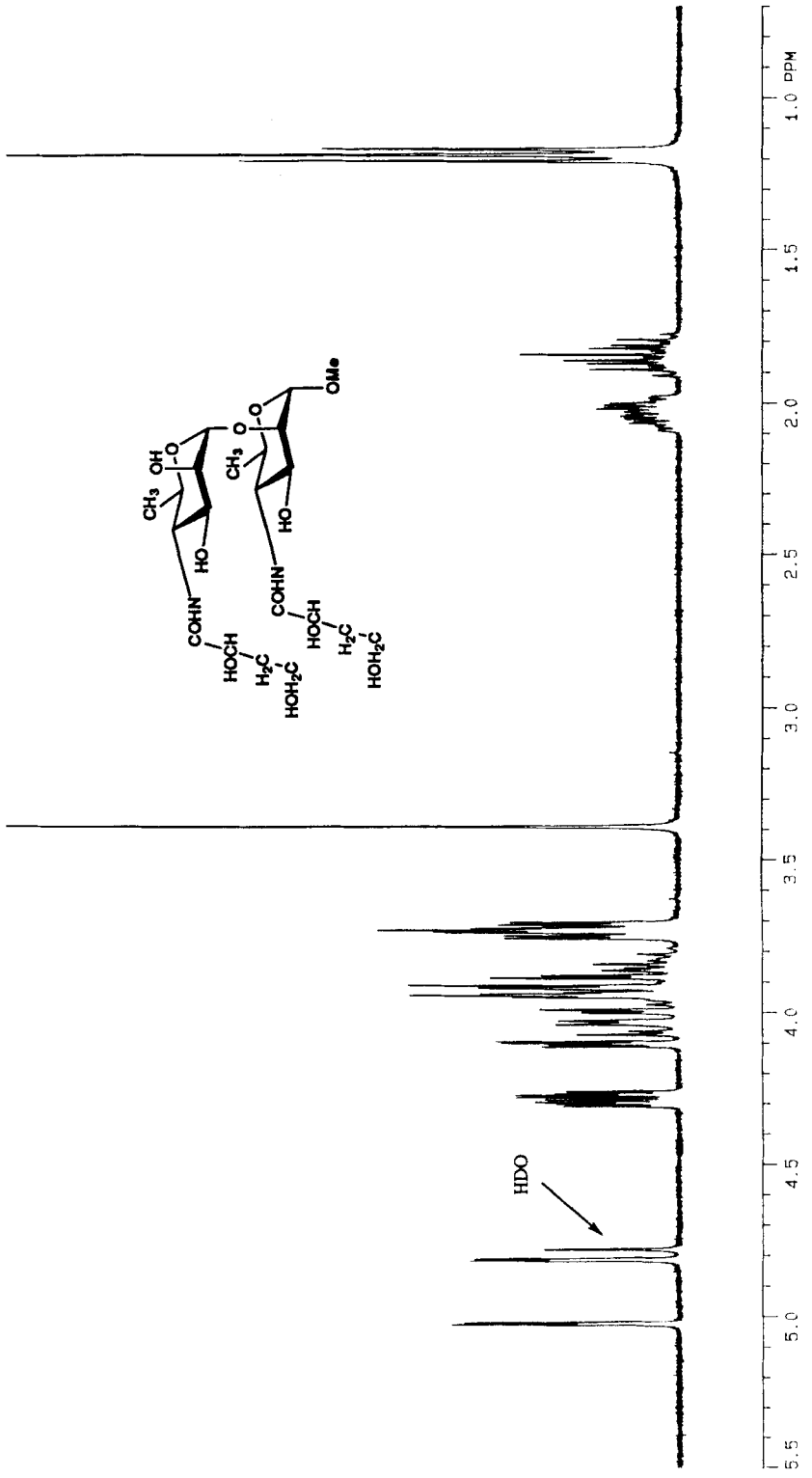
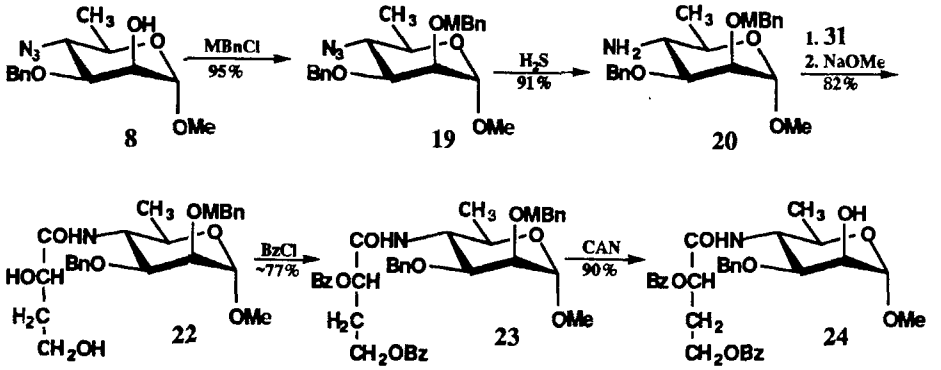
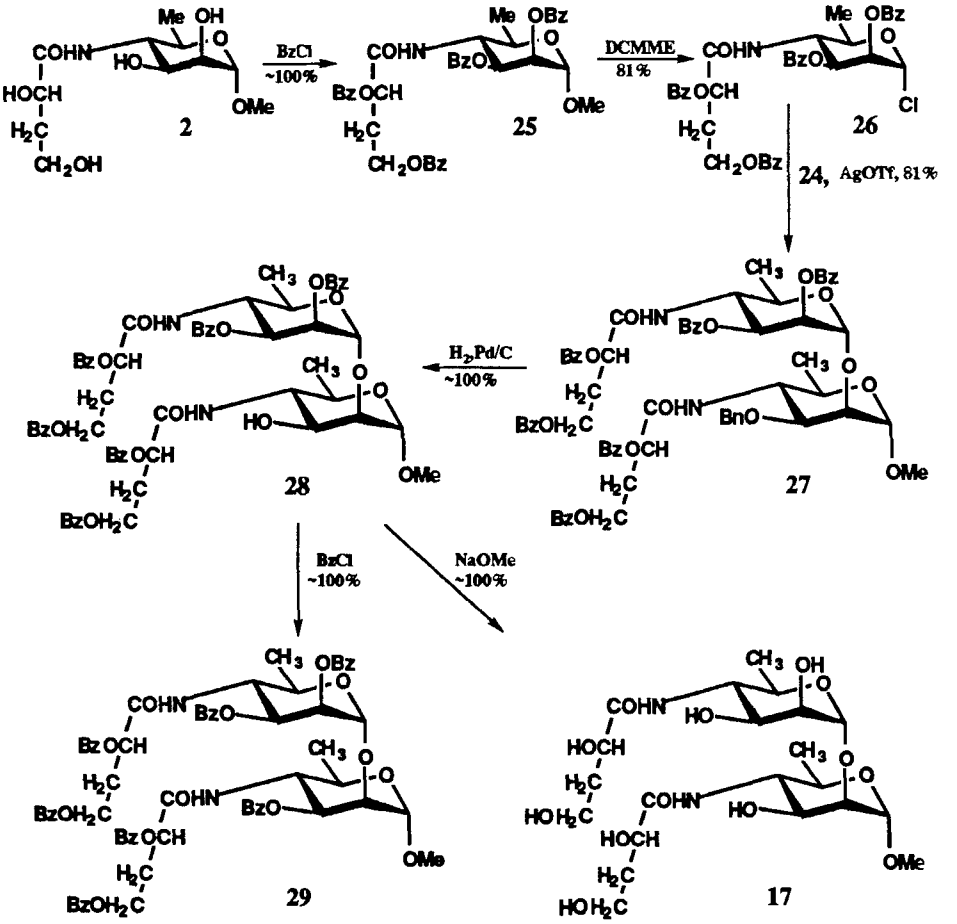


Fig. 1. The ^{1}H NMR spectrum of **17**. For the assignments and the conditions of the measurement, see the text.



Scheme 3



Scheme 4

To investigate the preparation of the disaccharide **17** following a different strategy, the requisite glycosyl acceptor **24** was obtained (Scheme 3) from the benzyl derivative[13] **8** by successive *p*-methoxybenzylation[17] (→ **19**), reduction of the azido group with hydrogen sulfide (→ **20**), and *N*-acylation of the resulting amine with the lactone **31**. The major product of the condensation was not isolated. Instead, the crude product of the foregoing reaction was de-*O*-acetylated (Zemplén), and the resulting dihydroxy derivative **22**, characterized by NMR spectral data, was benzoylated at *O*-2' and *O*-4' (→ **23**). Subsequent removal of the *p*-methoxybenzyl group by treatment of **23** with ceric ammonium nitrate (CAN) gave the crystalline substance **24**. To obtain the required glycosyl donor, methyl perosaminide[9] **2** was benzoylated, and the crystalline, fully benzoylated methyl glycoside **25** was treated[14] with the DCMME-$ZnCl_2$ reagent, to give the glycosyl chloride **26** (Scheme 4).

Condensation of the glycosyl donor **26** with the glycosyl acceptor **24** (Scheme 4) was performed under base deficient conditions[16] using silver trifluoromethanesulfonate (triflate) as the promoter and 2,4,6-trimethylpyridine as the acid scavenger, as described above for the preparation of **12**. The product **27**, obtained in 81% yield, was subjected to hydrogenolysis to give the crystalline disaccharide **28**. Debenzoylation then gave the target disaccharide **17**. For further characterization, the fully benzoylated disaccharide **29** was readily obtained by conventional benzoylation of **28**.

In conclusion, the intracatenary disaccharide repeating unit can be most conveniently prepared by the condensation of **24** with **26**, both of which already have the 3-deoxy-L-*glycero*-tetronamido group in place. Preparation of the two synthons involves simple, high yielding steps. In addition, the chloride **26** can be conveniently used as a reagent to elongate oligosaccharides in the described series by one unit. Since this glycosyl donor has no selectively removable blocking group at C-2, its use does not allow further extension of the oligosaccharide chain. For that purpose, more complex glycosyl donors, *e.g.* those described by Bundle *et al.*,[10-12] have to be used. *N*-Acylations performed during this work with lactones **30** and **32** were not single-product reactions. Best results were obtained with the lactone **30**, which can be conveniently obtained[7] from commercially available L-homoserine, and purified[9] *via* the acetyl derivative **31**. The latter *N*-acylation reagent, as well as its benzylated analog **32**, are promising reagents for use in the synthesis of higher oligosaccharides in this series.

EXPERIMENTAL

General methods. Unless stated otherwise, optical rotations were measured at 25 °C for solutions in $CHCl_3$ with a Perkin Elmer automatic polarimeter, Model 241 MC.

Thin-layer chromatography (TLC) was performed with solvent mixtures of appropriately adjusted polarity consisting of *A*, dichloromethane-acetone; *B*, hexane-ethyl acetate; *C*, toluene-ethyl acetate; *D*, dichloromethane-methanol-25% aqueous ammonia; *E*, dichloromethane-isopropyl alcohol-25% aqueous ammonia ; *F*, dichloromethane-methanol, and *G*, toluene-acetone. The detection was effected by charring with 5% sulfuric acid in ethanol and, when applicable, by UV light. For preparative chromatography of glycosyl chlorides, the silica gel was dried at 160 °C for 16 h. Assignments of NMR signals, obtained at 300 MHz for ^{1}H 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 heteronuclear 2-dimensional correlation spectroscopy, using commercial software supplied with the spectrometer. Some assignments were aided by mutual comparison of the spectra, and by comparison with spectra of related[9,11] substances. When reporting NMR data, and occasionally elsewhere in the text, atoms associated with the 3-deoxy-L-*glycero*-tetronamido group are denoted with a prime. Sugar residues in oligosaccharides are serially numbered, beginning with the one bearing the aglycone, and identified by a superscript in listings of signal assignments. Chemical ionization mass spectra (CIMS) were measured using ammonia as the reactive gas. Reactions requiring anhydrous conditions were performed under Ar, and reagents and solvents were handled with gas-tight syringes. Silver trifluoromethanesulfonate (AgOTf), purchased from Aldrich Chemical Co., was dried at 70 °C/133 Pa for 2 h. DCMME was purchased from Fluka Chemical Co., and used as supplied. *N*-Acylations with lactones **30-32** were most conveniently performed in screw-capped V-vials (Wheaton Glass Company). Dry, alcohol-free chloroform was obtained by passing commercial, reagent grade solvent (500 mL) through a column of activated alumina (100 g). Solutions in organic solvents were dried with anhydrous sodium sulfate, and concentrated at 40 °C/2 kPa.

Methyl 6-deoxy-2,3-*O*-isopropylidene-4-*O*-methanesulfonyl-α-D-mannopyranoside (**5**). Mesyl chloride (7.1 mL, 90 mmol) was added dropwise with stirring at -5 °C to a solution of methyl 6-deoxy-2,3-*O*-isopropylidene-α-D-mannopyranoside[11] (**4**, 6.5 g, 30 mmol) in dichloromethane (70 mL) containing pyridine (9 mL, 110 mmol). The mixture was stirred at room temperature for 24 h, when TLC (solvent *A*) showed that all starting material was consumed and that a single, faster moving product was formed. The mixture was partitioned between dichloromethane and aqueous sodium hydrogen carbonate solution, and the organic phase was concentrated with coevaporation of toluene to remove pyridine, to give **5** (8.56 g, 97%) identical with that previously described.[13] The product was directly used for further conversions[13] to obtain **8**.

2-*O*-Benzyl-3-deoxy-L-*glycero*-tetronolactone (32). A solution of the lactone **31** (1.44 g, 10 mmol) in M trifluoroacetic acid (30 mL) was kept at 60 °C for 3 h and then concentrated, successively, with acetone-toluene, and CCl_4 (see Results and Discussion). NMR spectra taken in $CDCl_3$ showed that the deacetylation was complete, and that the product thus obtained was almost pure **30**. ^1H NMR ($CDCl_3$) δ 4.56-4.41 (m, 2 H, H-4a,b), 4.28 (m, 1 H, H-2), 2.67-2.57 (m, 1 H, H-3a), 2.38-2.21 (m, 1 H, H-3b); ^{13}C NMR ($CDCl_3$) δ 179.00 (CO), 67.22 (C-2), 65.19 (C-4), 30.78 (C-3).

A mixture of the foregoing product, benzyl bromide (6 mL, 50 mmol) and silver oxide (4.6 g, 20 mmoL) in *N,N*-dimethylformamide (10 mL) was stirred in the dark at room temperature for 3 h. After filtration and concentration of the filtrate, the residue was chromatographed (solvent *B*), to give the major product **32** (1.7 g, 88%): $[\alpha]_D$ -83.4° (*c* 0.8); CIMS: *m/z* 210 ($[M + 18]^+$); ^1H NMR ($CDCl_3$) δ 4.93, 4.72 (2 d, 1 H each, ^2J 11.7 Hz, CH_2Ph), 4.44-4.37 (m, 1 H, H-2), 4.24-4.14 (m, 2 H, H-4a,b), 2.50-2.39, 2.33-2.21 (2 m, 1 H each, H-3ab); ^{13}C NMR ($CDCl_3$) δ 72.37 (C-2), 72.07 (CH_2Ph), 65.43 (C-4), 29.82 (C-3).

Anal.Calcd for $C_{11}H_{12}O_3$: C, 68.74; H, 6.29. Found: C, 68.49; H, 6.23.

2-*O*-Acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl chloride (10). a. Methyl 4-azido-4,6-dideoxy-3-*O*-benzyl-α-D-mannopyranoside[13] (**8**, 1 g) was acetylated conventionally with acetic anhydride-pyridine, to give methyl 2-*O*-acetyl-4-azido-4,6-dideoxy-3-*O*-benzyl-α-D-mannopyranoside (**11**, 1.1 g, ~100%). ^1H NMR data agreed with those reported;[11] ^{13}C NMR ($CDCl_3$) δ 98.75 (C-1), 76.03 (C-3), 71.54 (CH_2Ph), 67.27 (C-5), 66.71 (C-2), 63.93 (C-4), 54.98 (OCH_3), 20.83 ($COCH_3$), 18.33 (C-6).

Freshly fused $ZnCl_2$ (20 mg) was added to a solution of the foregoing product **11** in alcohol-free chloroform (5 mL) containing DCMME (1 mL), and the solution was stirred with the exclusion of atmospheric moisture at 55 °C until TLC (solvent *B*) showed almost complete conversion of the starting material into a faster moving product (~6 h). One faster (major), and two slower moving products, not detected by UV light, were formed. The mixture was diluted with dry toluene, filtered through a medium porosity sintered-glass funnel, and concentrated with coevaporation of toluene. The residue was eluted from a short column of silica gel (~25 g), to give the fastest moving component (**10**) as a colorless mass, 0.55 g (54%, based on **8**): ^1H NMR ($CDCl_3$) δ 5.96 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.62 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-3), 4.68, 4.56 (2 d, 1 H each, ^2J 11.2 Hz, CH_2Ph), 4.09 (dd, 1 H, $J_{3,4}$ 10.0 Hz, H-3), 3.82 (m, 1 H, H-5), 3.48 (t, *J* 10 Hz, H-4), 2.13 (s, 3 H, $COCH_3$), 1.36 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$) δ 169.92 (CO), 89.85 (C-1), 74.60 (C-3), 71.94 (CH_2Ph), 70.05, 69.55 (C-2,5), 63.37 (C-4), 20.72 ($COCH_3$), 18.02 (C-6).

b. Freshly fused $ZnCl_2$ (~200 mg) was added to a solution of the di-*O*-acetyl derivative[11] (**9**, 6.5 g) in dry dichloromethane (65 mL) containing DCMME (6.5 mL), and the solution was stirred at 35 °C for 2 h. TLC (solvent *B*) showed that almost all starting material had been consumed and that one major product was formed. The mixture was processed as described above and chromatography gave **10** (5.1 g, 84%).

Methyl 2-*O*-(2-*O*-acetyl-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (12). A solution of the nucleophile **8** (0.37 g, 1.25 mmol), 2,4,6-trimethylpyridine (165 μL, 1.25 mmol) and the glycosyl chloride **10** (0.55 g, 1.6 mmol) in dichloromethane (5 mL) was added at -25 °C to a stirred suspension of AgOTf (0.46 g, 1.8 mmol) in dichloromethane. The mixture became acidic after 10 min, and TLC (solvent *C*) showed that both starting materials were consumed. After filtration, the filtrate was washed with an aqueous mixture of sodium hydrogen carbonate and sodium thiosulfate, the organic phase was dried, concentrated, and the residue was chromatographed, to give amorphous **12** (0.7 g, 93%). The ^1H NMR data agreed with those reported;[11] ^{13}C NMR ($CDCl_3$) δ 169.87 (CO), 99.69 (C-1^1), 99.36 (C-1^2), 77.70 (C-3^1), 75.30 (C-3^2), 73.63 (C-2^1), 71.96, 71.50 (2 CH_2Ph), 67.50 (C-2^2), 67.10 (C-5^2), 66.84 (C-5^1), 64.03 (C-4^1), 63.75 (C-4^2), 54.81 (OCH_3), 20.79 ($COCH_3$), 18.38, 18.34 (C-6^1,6^2).

Methyl 4-azido-2-*O*-(4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (13). The foregoing diazido disaccharide **12** (7.88 g) was deacetylated (Zemplén), to give **13** in a virtually theoretical yield. A portion, when crystallized from ethanol, had mp 102-103 °C and $[\alpha]_D$ +108° (*c* 0.7). Its ^1H NMR characteristics agreed with those reported,[11] minor differences observed resulting from different conditions of measurements. CIMS: *m/z* 572 ($[M + 18]^+$); ^{13}C NMR ($CDCl_3$) δ 100.83 (C-1^2), 99.82 (C-1^1), 77.82, 77.58 (C-3^1,3^2), 73.74 (C-2^1), 72.13, 72.07 (2 CH_2Ph), 67.26, 67.19 (C-2^2,5^2), 66.92 (C-5^1), 64.34 (C-4^1), 63.84 (C-4^2), 54.87 (OCH_3), 18.54, 18.38 (C-6^1,6^2).

Anal. Calcd for $C_{27}H_{34}N_6O_7$: C, 58.47; H, 6.17; N, 15.15. Found: C, 58.55; H, 6.20; N, 15.23.

Methyl 2-*O*-(4-amino-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranosyl)-4-amino-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside (14). Compound **13** (1 g) was treated with hydrogen sulfide, as described below for the preparation of **20**. Chromatography (solvent *D*) gave pure **14** (0.82 g, 90%), mp 122-123 °C (after crystallization from dichloromethane-ether and recrystallization from toluene at 5 °C): $[\alpha]_D$ +6.6°(*c* 0.7); CIMS: *m/z* 503 ($[M + 1]^+$); ^1H NMR ($CDCl_3$): δ 5.04 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1^2), 4.69 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1^1), 4.68 (d, 2 H, ^2J 11.5 Hz, CH_2Ph), 4.51, 4.47 (2 d, 1 H each, ^2J 11.3 Hz, CH_2Ph), 4.08 (dd, 1 H, $J_{2,3}$ 3.1 Hz, H-2^2), 3.99 (bdd, 1 H,

$J_{2,3}$ ~2.7 Hz, H-2^1), 3.70-3.61 (m, 1 H, H-5^2), 3.57-3.44 (m, 3 H, H-3^1,5^1,5^2), 3.33 (s, 3 H, OCH_3), 2.86, 2.85 (2 t, 1 H each, J ~9.8, H-4^1,4^2), 1.40-1.10 (m, 10 H, H-6^1,6^2,NH_2^1,NH_2^2); ^{13}C NMR ($CDCl_3$) δ 101.12 (C-1^2), 100.38 (C-1^1), 79.83, 79.69 (C-3^1,3^2), 72.41 (C-2^1), 71.55, 71.25 (2 CH_2Ph), 69.62 (C-5^2), 69.55 (C-5^1), 66.48 (C-2^2), 54.64 (OCH_3), 53.66, 53.29 (C-4^1,4^2), 18.21, 18.03 (C-6^1,6^2).

Anal. Calcd for $C_{27}H_{38}N_2O_7$: C, 64.51; H, 7.62; N, 5.57. Found: C, 64.34; H, 7.65; N, 5.55.

Methyl 2-*O*-[4,6-dideoxy-4-(3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranosyl]-4,6-dideoxy-4-(3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranoside (17) and methyl 3-*O*-acetyl-2-*O*-[4,6-dideoxy-3-*O*-acetyl-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranosyl]-4,6-dideoxy-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranoside (18). a. In a screw-capped vial, a solution of the diamine **14** (1 g, 2 mmol) and the acetylated lactone **31** (432 mg, 3 mmol) in pyridine (3 mL) was heated at 105-110 °C for 16 h. Several products were formed, one slightly predominating, as shown by TLC (solvent *D*). The solution was concentrated and a solution of the crude product in methanol (50 mL) was treated for 3 h with M methanolic sodium methoxide (2 mL). TLC showed that one major and, essentially, three faster moving minor products were formed. After neutralization with Amberlite IR 120 (H^+) resin, chromatography gave first the three by-products, which showed peaks in their CI mass spectra at *m/z* 605 ([M + 1]$^+$), 664 ([M + 18]$^+$), and 664 ([M + 18]$^+$), respectively (*cf.*, Results and Discussion).

Eluted next was the amorphous methyl 2-*O*-[3-*O*-benzyl-4,6-dideoxy-4-(3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranosyl]-3-*O*-benzyl-4,6-dideoxy-4-(3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranoside (**15**, 0.79g, 56%), which was sufficiently pure for the next step, CIMS: *m/z* 707 ([M + 1]$^+$), 724 ([M + 18]$^+$); ^{13}C NMR (acetone-d_6) δ 175.22 (CO), 102.51 (C-1^2), 101.12 (C-1^1), 77.32, 77.20 (C-3^1,3^2), 73.95 (C-2^1), 71.38 (2 C, C-$2'^1$,$2'^2$), 69.31, 68.72 (C-5^1,5^2), 67.55 (C-2^2), 59.97 (2 C, C-$4'^1$,$4'^2$), 54.79 (OCH_3), 52.88, 52.07 (C-4^1,4^2), 38.15 (2 C, C-$3'^1$.$3'^2$), 18.47 (2 C, C-6^1,6^2).

The products of the condensation of **14** and **31** were formed in essentially the same ratio when the reaction was carried out in the absence of pyridine.

A solution of the foregoing compound **15** (0.54 g) in ethanol (25 mL) was stirred in a hydrogen atmosphere for 16 h, at room temperature and normal pressure, in the presence of 5% palladium-on-charcoal catalyst. After filtration and concentration of the filtrate, chromatography (solvent *D*) gave amorphous **17** as a hygroscopic white foam, (0.37 g, 93%): FABMS, *m/z* 527 ([M + 1]$^+$), 549 ([M + Na]$^+$); $[\alpha]_D$ 0° (*c* 1.4, H_2O), $[\alpha]_D$ +3.7° (*c* 0.9, 2,2,2-trifluoroethanol); ^1H NMR (D_2O) δ 5.02 (d, 1 H, $J_{1,2}$ 1.7 Hz,

H-1^2), 4.81 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1^1), 4.32-4.27 (m, 2 H, H-2'^{1,2}), 4.10 (dd, 1 H, $J_{2,3}$ 3.1 Hz, H-2^2), 4.05 (dd, partially overlapped, 1 H, $J_{3,4}$ 10.4 Hz, H-3^2), 4.01 (dd, partially overlapped, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ ~10 Hz, H-3^1), 3.97-3.94 (dd, partially overlapped, $J_{2,3}$ 3.1 Hz, H-2^1), 3.97-3.80 (m, H-2^1,4^1,4^2,5^1,5^2), 3.77-3.70 (m, 4 H, H-4'^{1,2}a,b), 3.39 (s, 3 H, OCH_3), 2.10-2.00 (m, 2 H, H-3'^{1,2}a), 1.91-1.79 (m, 2 H, H-3'^{1,2}b), 1.19, 1.18 (2 d, partially overlapped, J 5.9 Hz, H-6^{1,2}); ^{13}C NMR (D_2O) δ 102.27 (C-1^2), 99.72 (C-1), 77.84 (C-2^1), 69.28 (C-2^2), 69.15 (2 C, 2'^{1,2}), 68.12, 67.54 (5^1,5^2), 67.86 (C-3^2), 67.71 (C-3^1), 57.98 (2 C, 4'^{1,2}), 55.02 (OCH_3), 53.12, 52.87 (C-4^{1,2}), 36.12 (2 C, C-3'^{1,2}), 16.97 (C-6^{1,2}).

For characterization, a portion of the disaccharide **17** was acetylated with pyridine and acetic anhydride, to give **18** as a colorless foam, CIMS: *m/z* 838 ([M + 18]$^+$); $[\alpha]_D$ +37° (*c* 0.7); ^{1}H NMR ($CDCl_3$) δ 6.31 (d, 1 H, $J_{4,NH}$ 9.1 Hz, NH^2), 6.16 (d, 1 H, $J_{4,NH}$ 9.0 Hz, NH^1), 5.31 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 11.1 Hz, H-3^2), 5.22-5.20 (m, partially overlapped, H-2^2), 5.20 (dd, partially overlapped, $J_{2,3}$ 3.2 Hz, H-3^1), 5.14-5.09 (m, 2 H, H-2'^{1,2}), 4.92 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1^2), 4.67 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1^1), 4.31-4.22 (m, 1 H, H-4^2), 4.21-4.03 (m, 5 H, H-4^1, 4'^{1}ab,4'^{2}ab), 3.89 (dd, 1 H, H-2^1), 3.84-3.74 (m, 1 H, H-5^2), 3.69-3.59 (m, 1 H, H-5^1), 3.37 (s, 3 H, OCH_3), 2.23-2.00 (27 H, 7 s, 3 H each, of $COCH_3$ overlapped with multiplets of H-3'^{1}ab,3'^{2}ab), 1.24 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6^1), 1.20 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6^2); ^{13}C NMR ($CDCl_3$) δ 99.59 (C-1^1), 99.33 (C-1^2), 76.21 (C-2^1), 70.91 (2 C, C-2'^{1,2}), 69.78 (C-2^2), 69.59 (C-5^2), 69.22 (C-3^1), 68.26 (C-5^1), 68.02 (C-3^2), 59.82, 59.77 (C-4^{1,2}), 54.99 (OCH_3), 30.66, 30.52 (C-3'^{1}ab,3'^{2}ab), 17.83, 17.64 (C-6^{1,2}).

Anal. Calcd for $C_{35}H_{52}N_2O_{20}$: C, 51.21; H, 6.38; N, 3.41. Found: C, 50.97; H, 6.32; N, 3.36.

b. The crystalline diamine **14** (0.5 g, 1 mmol) was added to a solution of the lactone **30** [prepared from **31** (0.44 g, 3 mmol), as described above in the preparation of its benzylated analog **32**], in pyridine (1.5 mL), and the solution was kept at 105-110 °C overnight. TLC (solvent *D*) showed that one major product was formed, and chromatography gave **15** (0.56 g, 79%), indistinguishable from the above described substance. Subsequent hydrogenolysis, as described above, gave **17** in ~95% yield.

c. Compound **17** was obtained similarly as in b., following hydrogenolysis of **16**.

d. The diamine **14** was treated with the lactone **32** as described below for the preparation of **16**. After concentration, the crude product was treated with hydrogen gas, as described above. TLC (solvent *D*) showed that the products of hydrogenolysis of the by-products, formed in addition to **16** during the above *N*-acylation, were well separated from **17**. Chromatography, as described above, readily gave **17**.

e. Compound **28**, when debenzoylated with sodium methoxide in methanol, gave **17** in virtually theoretical yield, following elution from a column of silica gel.

Methyl 2-*O*-[3-*O*-benzyl-4,6-dideoxy-4-(2-*O*-benzyl-3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranosyl]-3-*O*-benzyl-4,6-dideoxy-4-(2-*O*-benzyl-3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranoside (16). A melt from the lactone **32** (0.576 g, 3 mmol) and the diamine **14** (0.5 g, 1 mmol) was stirred at 105-110 °C for 24 h. TLC (*E*) showed that all starting material was consumed and that, essentially, three products were formed, all showing greater chromatographic mobility than **14**. The slowest product, later shown to be the desired **16**, slightly predominated. More **32** (0.2 g, ~1 mmol) was added, and the melt was stirred for a further 48 h. The proportion of **16** largely increased, at the expense of the two faster moving products, presumably products of incomplete *N*-acylation. A number of other, minor by-products were also present, together with unchanged lactone **32**, as shown by TLC (solvent *E* and *B*). Chromatography (solvent *E*) gave the tetra-*O*-benzyl derivative **16** as a colorless foam (0.68 g, 76%), $[\alpha]_D$ -17° (*c* 0.6). CIMS: *m/z* 887 ($[M + 1]^+$), 904 ($[M + 18]^+$); ^{1}H NMR ($CDCl_3$) δ 6.47, 6.46 (2 d, partially overlapped with each other, 1 H each, $J_{4,NH}$ 9.7 and 9.3 Hz, respectively, NH^1, NH^2), 5.00 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1^2), 4.67-4.41 (m, 9 H, H-1^1, 4 CH_2Ph), 4.18 (bs, 1 H, H-2^2), 4.13-3.94 (m, 5 H, H-2^1,$2'^{1,2}$,4^1,4^2), 3.77-3.35 (m, 8 H, H-$3^{1,2}$,$5^{1,2}$,$4'^{1,2}$a,b), 3.35 (s, 3 H, OCH_3), 2.82 (bs, 3 H, 3 OH), 1.99-1.91 (m, 4 H, $3'^{1,2}$a,b), 1.20, 1.14 (2 d, $J_{5,6}$ 6.1 and 6.4 Hz, respectively, H-$6^{1,2}$); ^{13}C NMR ($CDCl_3$) δ 101.10 (C-1^2), 100.07 (C-1^1),78.50, 78.23 (C-$2'^{1,2}$), 75.79, 75.68 (C-$3^{1,2}$), 73.41 (C-2^1), 72.89, 71.34, 70.86 (C, C, 2C, 4 CH_2Ph), 68.10, 67.73 (C-$5^{1,2}$), 66.47 (C-2^2), 59.11 (2 C, C-$4'^{1,2}$), 54.91 (OCH_3), 52.15, 51.21 (C-$4^{1,2}$), 35.47 (2 C, C-$3'^{1,2}$), 18.23, 17.99 (C-$6^{1,2}$).

Anal.Calcd for $C_{49}H_{62}N_2O_{13}$: C, 66.34; H, 7.04; N, 3.15. Found: C, 66.08; H, 6.99; N, 3.17.

Methyl 4-azido-4,6-dideoxy-3-*O*-benzyl-2-*O*-p-methoxybenzyl-α-D-mannopyranoside (19). *p*-Methoxybenzyl chloride (0.6 mL, 4.5 mmol) was added to a mixture of the benzyl derivative[13] **8** (1.05 g, 3.58 mmol) and powdered KOH (1 g) in DMSO (4 mL). The mixture was stirred at room temperature for 1 h. Water (50 mL) was added, and the pH was adjusted to 7.5 by addition of acetic acid. The mixture was partitioned between water and dichloromethane and, after concentration of the organic phase, the crude product was chromatographed, to give pure, amorphous **19** (1.4 g, 94.5%): $[\alpha]_D$ +81° (*c* 0.7); ^{1}H NMR ($CDCl_3$) δ 4.63 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.61 (s, 2 H, CH_2Ph), 4.55 (dd, 2 H, ^2J 11.7 Hz, CH_2Ph), 3.78 (s, 3 H, CH_3OPh), 3.73-3.67 (m, 2 H, H-2,3), 3.58 (t, 1 H, *J* 9.8 Hz, H-4), 3.50-3.42 (m, 1 H, H-5), 3.29 (s, 3 H, OCH_3), 1.33 (d, 1 H, $J_{5,6}$ 6.1 Hz, H-6); ^{13}C NMR ($CDCl_3$) δ 99.09 (C-1), 78.28 (C-

3), 72.51 (C-2), 72.32, 71.73 (2 CH_2Ph), 67.03 (C-5), 64.28 (C-4), 55.19 (CH_3OPh), 54.79 (OCH_3), 18.49 (C-6).

Anal. Calcd for $C_{22}H_{27}N_3O_5$: C, 63.90; H, 6.58; N, 10.16. Found: C, 63.70; H, 6.57; N, 10.14.

Methyl 3-*O*-benzyl-4,6-dideoxy-4-(2,4-di-*O*-benzoyl-3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranoside (24). A fine stream of hydrogen sulfide gas was bubbled for 30 min through a solution of the azide **19** (0.7 g) in pyridine-triethylamine (7:3, 20 mL). The solution, in a flask closed with a rubber septum was left at room temperature overnight. TLC (solvent *C* and *E*) showed that the reaction was complete, and that one largely predominating product was formed. After concentration with coevaporation of toluene, the residue was chromatographed, to give chromatographically pure methyl 4-amino-4,6-dideoxy-3-*O*-benzyl-2-*O*-*p*-methoxybenzyl-α-D-mannopyranoside (**20**, 0.6 g, 91.5%): CIMS: *m/z* 388 ($[M + 1]^+$; ^1H NMR ($CDCl_3$) δ 4.71, (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.64, 4.57 (2 d, 1 H each, ^2J 12.1 Hz, CH_2Ph), 4.50, 4.34 (2 d, 1 H each, ^2J 11.6 Hz, CH_2Ph), 3.76 (s, 3 H, CH_3OPh), 3.74 (dd, 1 H, $J_{2,3}$ 2.9 Hz, H-2), 3.53-3.45 (m, 2 H, H-3,5), 3.31 (s, 3 H, OCH_3), 3.02 (t, 1 H, *J* 9.9 Hz, H-4), 1.28 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$) δ 99.18 (C-1), 79.76 (C-3), 72.03 (CH_2Ph), 71.93 (C-2), 71.03 (CH_2Ph), 69.62 (C-5), 55.14 (CH_3OPh), 54.51 (OCH_3), 53.57 (C-4), 18.02 (C-6).

A solution of the amine **20** (0.6 g, 1.66 mmol) and the lactone **31** (0.33 g, 2.32 mmol) in pyridine (2 mL) was heated at 105-110 °C for 16 h. TLC (solvent *C* and *E*) showed that the synthon **20** was almost completely consumed, and that one major and several minor products were formed. After concentration, a solution of the crude product in methanol (20 mL) was treated, overnight at room temperature, with M methanolic sodium methoxide (1 mL). TLC (solvent *A*) showed that one major and one very minor product were formed. After conventional processing, chromatography gave first the faster moving minor product, the NMR data of which showed that it was methyl 4-acetamido-3-benzyl-2-*O*-(4-methoxybenzyl)-α-D-mannopyranoside (**21**), contaminated with unidentified material, CIMS: *m/z* 430 ($[M + 1]^+$), 447 $(M + 18]^+$). Definite, structurally significant signals in the ^1H NMR spectrum ($CDCl_3$) were at δ 5.52 (δ, 1 H, $J_{4,NH}$ 8.7 Hz, NH), 4.67 (bd, 1 H, $J_{1,2}$ 1.3 Hz, H-1), 3.97 (m, 1 H, H-4), 3.78 (s, partially overlapped, $PhOCH_3$), 3.30 (s, 3 H, OCH_3), 1.92 (s, 3 H, $NHCOCH_3$), 1.22 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR ($CDCl_3$) δ 99.17 (C-1), 75.82 (C-3), 72.87 (C-2), 72.29, 70.99 (2 CH_2Ph), 67.63 (C-5), 55.16, 54.71 (OCH_3, $PhOCH_3$), 52.94 (C-4), 23.30 ($NHCOCH_3$), 17.95 (C-6).

Eluted next was the expected methyl 3-*O*-benzyl-4,6-dideoxy-2-*O*-(4-methoxybenzyl)-4-(3-deoxy-L-glycero-tetronamido)-α-D-mannopyranoside (**22**, 0.62 g,

82%), CIMS: *m/z* 490 ([M + 1]$^+$), 507 ([M + 18]$^+$) which was identified from the following NMR data: ^{1}H NMR ($CDCl_3$, after deuteration with a drop of D_2O) δ 4.66 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.62 (s, 2 H, C*H*$_2$Ph), 4.48, 4.35 (2 d, 1 H each, C*H*$_2$Ph), 4.23 (dd, 1 H, $J_{2',3'a}$ 3.7, $J_{2',3'b}$ 8.0 Hz, H-2'), 4.13 (m, 1 H, H-4), 3.76 (s, partially overlapped, PhOC*H*$_3$), 3.78-3.63 (m, 5 H, H-2,3,5,4'a,b), 3.30 (s, 3 H, OCH_3), 2.0 (m, 1 H, H-3'a), 1.75 (m, 1 H, H-3'b), 1.22 (d, 3 H, $J_{5,6}$ 6.1 Hz, H-6); ^{13}C NMR ($CDCl_3$, after deuteration with a drop of D_2O) δ 99.19 (C-1), 76.57 (C-3), 72.58 (C-2), 72.27 (*C*H$_2$Ph), 71.41 (C-2'), 71.23 (*C*H$_2$Ph), 67.82 (C-5), 60.25 (C-4'), 55.20 (PhO*C*H$_3$), 54.81 (OCH_3), 52.29 (C-4), 35.52 (C-3'), 18.00 (C-6).

To a solution of compound **22** (0.3 g, 0.61 mmol) in pyridine (3 mL) was added benzoyl chloride (0.3 mL, ~2.5 mmol) and the mixture was stirred overnight at room temperature. TLC (*G*) showed that one major and a minor, faster moving product were formed. Use of a larger excess of the benzoylating reagent caused more pronounced formation of this unidentified byproduct. Conventional processing and chromatography gave amorphous methyl 3-*O*-benzyl-4,6-dideoxy-2-*O*-(4-methoxybenzyl)-4-(2,4-di-*O*-benzoyl-3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranoside (**23**, 0.33 g, 77%), CIMS: *m/z* 715 ([M + 18]$^+$); ^{1}H NMR ($CDCl_3$): δ 6.12 (d, 1 H, $J_{4,NH}$ 8.5 Hz, NH), 5.57 (dd, 1 H, $J_{2',3'a}$ 4.4, $J_{2',3'b}$ 8.1 Hz, H-2'), 4.67 (bs, 1 H, H-1), 4.61 (s, 2 H, C*H*$_2$Ph), 4.49, 4.36 (2 d, partially overlapped, C*H*$_2$Ph), 4.48-4.34 (m, partially overlapped, H-4'a,b), 4.16-4.06 (m, 1 H, H-4), 3.87 (dd, 1 H, $J_{3,4}$ 10.6, $J_{2,3}$ 2.9 Hz, H-3), 3.82-3.75 (m, 2 H, H-2,5), 3.74 (s, 3 H, PhO*C*H$_3$), 3.28 (s, 3 H, OCH_3), 2.57-2.47 (m, 1 H, H-3'a), 2.44-2.31 (m, 1 H, H-3b), 1.26 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 99.14 (C-1), 75.87 (C-3), 72.40 (C-2), 72.11 (*C*H$_2$Ph), 71.96 (C-2'), 70.89 (*C*H$_2$Ph), 67.31 (C-5), 60.83 (C-4'), 55.04 (PhO*C*H$_3$), 54.71 (OCH_3), 53.10 (C-4), 31.01 (C-3'), 17.95 (C-6).

A solution of the foregoing, fully protected compound **23** (0.4 g, 0.57 mmol) in acetonitrile-water (10:1, 5.5 mL) was treated, with stirring at room temperature, with CAN (650 mg, 1.18 mmol). After 30 min, TLC (solvent *G*) showed that the starting material was no longer present, and that one major product was formed. The mixture was partitioned between dichloromethane and a mixture of saturated, aqueous solution of sodium chloride and sodium hydrogen carbonate, dried, and concentrated. The residue was chromatographed, to give **24** (0.3 g, 91%), mp 159-160 °C, $[\alpha]_D$ -3° (*c* 0.9), CIMS: *m/z* 595 ([M + 18]$^+$); ^{1}H NMR ($CDCl_3$) δ 6.00 (d, 1 H, $J_{4,NH}$ 9.2 Hz, NH), 5.55 (dd, 1 H, $J_{2',3'a}$4.4, $J_{2',3'b}$ 7.9 Hz, H-2'), 4.72 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.62 (d, 1 H, ^2J 11.8 Hz, C*H*Ph), 4.51-4.40 (m, 3 H, C*H*Ph, H-4'a,b), 4.06-3.97 (m, 2 H, H-2,4), 3.77-3.69 (m, 2 H, H-3,5), 3.32 (s, 3 H, OCH_3), 2.55 (d, partially overlapped, $J_{2,OH}$ 2.1 Hz, OH), 2.57-2.45 (m, partially overlapped, H-3'a), 2.43-2.33 (m, 1 H, H-3'b), 1.23 (d, 3

H, $J_{5,6}$ 6.3 Hz, H-6); ^{1}H NMR ($CDCl_3$): δ 100.14 (C-1), 75.98 (C-3), 72.03 (C-2'), 71.03 (CH_2Ph), 66.84 (C-5), 66.77 (C-2), 60.89 (C-4'), 54.99 (OCH_3), 52.17 (C-4), 31.07 (C-3'), 17.87 (C-6); ^{13}C NMR (C_6D_6-$CDCl_3$ 4:1) δ 6.05 (δ, 1 H, $J_{4,NH}$ 8.8 Hz, NH), 5.53 (dd, 1 H, $J_{2',3'a}$ 5.1, $J_{2',3'b}$ 7.2 Hz, H-2'), 4.67 (bd, $J_{1,2}$ ~0.7 Hz, H-1), 4.41, 4.30 (2 d overlapped, CH_2Ph), 4.43-4.26 (m, overlapped, H-4'a,b), 4.11 (m, 1 H, H-4), 3.92 (bd, 1 H, H-2), 3.79-3.70 (m, 2 H, H-3,5), 3.06 (s, 3 H, OCH_3), 2.74 (d, 1 H, $J_{2,OH}$ 2.3 Hz, OH), 2.40-2.29 (m, 2 H, H-3'a,b), 1.29 (d, 3 H, 6.2 Hz, H-6).

Anal. Calcd for $C_{32}H_{35}NO_9$: C, 66.53: H, 6.10; N, 2.42. Found: C, 66.44; H, 6.15; N, 2.41.

Methyl 2,3-di-*O*-benzoyl-4,6-dideoxy-4-(2',4'-di-*O*-benzoyl-3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranoside (25). Benzoyl chloride (2 mL, 17 mmol) was added to a solution of compound **10** (ref. 9, 0.56 g, 2 mmol) in pyridine (7 mL) and the mixture was stirred at room temperature overnight. TLC (solvent *G*) showed that the reaction was complete and that one product was formed. Conventional processing and chromatography gave **15** (1.25 g, 90%), mp 141-142 °C (from ether-isopropyl ether), $[\alpha]_D$ -125° (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.38 (d, 1 H, $J_{1,2}$ 9.4 Hz, H-1), 5.63 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 11 Hz, H-3), 5.54-5.50 (m, 2 H, H-2,2'), 4.86 (bd, $J_{1,2}$ 1.6 Hz, H-1), 4.62-4.52 (m, 1 H, H-4), 4.27-4.17 (m, 2 H, H-4'a,b), 3.85-3.76 (m, 1 H, H-5), 3.41 (s, 3 H, OCH_3), 2.29-2.18 (m, 1 H, H-3'a), 2.08-2.00 (m, 1 H, H-3b), 1.34 (d, 3 H, $J_{5,6}$ 6.5 Hz, H-6); ^{13}C NMR ($CDCl_3$) δ 98.49 (C-1), 71.69 (C-2'), 70.04 (C-2), 69.35 (C-3), 68.07 (C-5), 60.47 (C-4,4'), 55.16 (OCH_3), 51.93 (C-4), 30.83 (C-3'), 17.79 (C-6).

Anal. Calcd for $C_{39}H_{37}NO_{11}$: C, 67.33; H, 5.36; N, 2.01. Found: C, 67.05; H, 5.31; N, 1.95.

Methyl 3-*O*-benzyl-2-*O*-[2,3-di-*O*-benzoyl-4,6-dideoxy-4-(2,4-di-*O*-benzoyl-3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranosyl]-4,6-dideoxy-4-(2,4-di-*O*-benzoyl-3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranoside (27). A solution of the foregoing fully benzoylated compound **25** (0.7 g), DCMME (1 mL) in dry, alcohol-free chloroform (3 mL) was treated, in a round-bottomed flask equipped with a drying tube, with freshly fused zinc chloride (~50 mg) for 2 h at 50 °C. TLC (solvent *G*) then showed that almost all starting material was consumed. One major product was formed. The mixture was filtered through a medium porosity sintered-glass funnel, the solids were washed with dry toluene, and the filtrate, combined with washings, was concentrated. Chromatography of the residue gave chromatographically pure, foamy 2,3-di-*O*-benzoyl-4,6-dideoxy-4-(2,4-di-*O*-benzoyl-3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranosyl chloride (**26**), 0.57 g (81.5 %): ^{1}H NMR ($CDCl_3$) δ 6.44 (d, 1 H, $J_{4,NH}$ 9.5 Hz, NH), 6.19 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 5.92

(dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 11.1 Hz, H-3), 5.66 (dd, 1 H, H-2), 5.51 (dd, 1 H, $J_{2',3'a}$ 4.3, $J_{2',3'b}$ 8.3 Hz, H-2'), 4.72-4.61 (m, 1 H, H-4), 4.28-4.19 (m, 2 H, H-4'a,b), 4.19-4.08 (m, 1 H, H-5), 2.20-2.01 (m, 2 H, H-3'ab), 1.37 (d, 3 H, $J_{5,6}$ 6.1 Hz, H-6); ^{13}C NMR ($CDCl_3$) δ 89.50 (C-1), 72.11 (C-2), 71.72 (C-2'), 71.29 (C-5), 67.99 (C-3), 60.47 (C-4'), 51.52 (C-4), 30.50 (C-3'), 17.54 (C-6).

A solution of the nucleophile **24** (270 mg, 0.46 mmol), glycosyl chloride **26** (490 mg, 0.7 mmol) and 2,4,6-trimethylpyridine (85 μL, 0.65 mmol) in dichloromethane (~3 mL) was added at 0 °C to a stirred suspension of AgOTf (257 mg, 1 mmol) in dichloromethane (~3 mL). The cooling bath was removed and, after 1 h, TLC (solvent *G*) showed that the glycosyl donor was consumed and that only a small amount of the glycosyl acceptor was present. One major product was formed. The mixture was filtered, the filtrate was washed with a mixture of aqueous solutions of sodium hydrogen carbonate and sodium thiosulfate, dried and concentrated. Chromatography gave the title, fully protected disaccharide **27** (0.47 g, 81%): $[\alpha]_D$ -87° (*c* 1.3); CIMS: *m/z* 1258 ($[M + 1]^+$); ^{1}H NMR ($CDCl_3$) δ 6.60 (d, 1 H, $J_{4,NH}$ 9.4 Hz, NH^2), 6.10 (d, 1 H, $J_{4,NH}$ 7.9 Hz, NH^1), 5.67 (dd, partially overlapped, $J_{2,3}$ 3.2 Hz, H-3^2), 5.66-5.63 (m, overlapped, H-2^2), 5.55-5.48 (m, 2 H, H-$2'^{1,2}$), 5.04 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1^2), 4.67 (d, 1 H, $J_{1,2}$ 1,5 Hz, H-1^1), 4.63-4.40 (m, 5 H, CH_2Ph, H-4^2, $4'^1$a,b), 4.28-4.13 (m, 2 H, H-$4'^2$a,b), 4.02 (dd, 1 H, $J_{2,3}$ 2.9, $J_{3,4}$ 10.1 Hz, H-3^1), 4.00-3.80 (m, 4 H, H-2^1,4^1,5,$1^5$$2^2$), 3.29 (s, 3 H, OCH_3), 2.55-2.28 (m, 1 H, H-$3'^1$a,b), 2.26-2.00 (m, 1 H, H-$3'^2$a,b), 1.29, 1.18 (2 d, 3 H each, $J_{5,6}$, respectively 6.3 and 5.9 Hz, H $6^{1,2}$); ^{13}C NMR ($CDCl_3$) δ 99.91 (C-1^1), 99.40 (C-1^2), 74.95 (C-3^1), 74.59 (C-2^1), 72.06 (C-$2'^2$), 71.76 (*C*H_2Ph), 71.68 (C-$2'^1$), 69.95 (C-2^2), 69.40 (C-3^2), 68.82 (C-5^2), 66.80 (C-5^1), 60.91 (C-$4'^1$), 60.53 (C-$4'^2$), 54.88 (OCH_3), 53.87 (C-4^1), 52.03 (C-4^2), 31.09, 31.01 (C-$3'^1$,$3'^2$), 17.95, 17.83 (C-6^1,6^2).

Anal. Calcd for $C_{70}H_{68}N_2O_{19}$: C, 67.72: H, 5.52; N, 2.25. Found: C, 67.59; H, 5.56; N, 2.23.

Methyl 2-*O*-[2,3-di-*O*-benzoyl-4,6-dideoxy-4-(2,4-di-*O*-benzoyl-3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranosyl]-4,6-dideoxy-4-(2,4-di-*O*-benzoyl-3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranoside (28). A solution of portion of the foregoing, fully protected disaccharide **27** in acetone-ethanol, was hydrogenolyzed conventionally in the presence of 5% palladium-on-charcoal catalyst, to give the title disaccharide **28** in virtually theoretical yield, mp 104-108 °C (from dichloromethane-ether, twice): $[\alpha]_D$ -31° (*c* 0.6); CIMS: *m/z* 1169 ($[M + 18]^+$); ^{1}H NMR ($CDCl_3$) δ 6.78 (d, 1 H, $J_{4,NH}$ 9.5 Hz, NH^2), 6.42 (d, 1 H, $J_{4,NH}$ 8.1 Hz, NH^1), 5.75-5.68 (m, 2 H, H-2^2,3^2), 5.60 (dd, 1 H, $J_{2',3'a}$ 4.8, $J_{2',3'b}$ 6.8 Hz, H-$2'^2$), 5.46 (dd, 1 H, $J_{2',3'a}$ 4.3, $J_{2',3'b}$ 8.5 Hz, H-$2'^1$), 5.23 (s, 1 H, H-1^2), 4.73 (s, 1 H, H-1^1), 4.68-4.62 (m, 1 H, H-4^2), 4.58-4.44 (m, 2 H, H-$4'^2$a,b), 4.28-4.14 (m, 2 H, H-$4'^1$a,b), 4.09-4.00

(m, 1 H, H-5^1), 3.96-3.80 (m, 3 H, H-2^1,3^1,4^1), 3.69 (m, 1 H, 5^1), 3.50 (d, 1 H, $J_{3,OH}$ 8.2 Hz, OH), 3.19 (s, 3 H, OCH_3), 2.56-2.43 (m, 2 H, H-$3'^1$a,b), 2.28-2.17, 2.12-2.02 (2 m, 1 H each, H-$3'^2$a,b), 1.32 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6^2), 1.03 (d, 3 $J_{5,6}$ 6.2 Hz, H-6^1); ^{13}C NMR ($CDCl_3$) δ 100.05 (C-1^2), 99.67 (C-1^1), 72.29 (C-$2'^2$), 71.74 (C-$2'^1$), 69.97, 69.65 (C-2^2,3^2), 68.73 (2 C, C-3^1, 5^2), 66.77 (C-5^1), 61.02 (C-$4'^2$), 60.60 (C-$4'^1$), 54.97 (OCH_3), 54.71 (C-4^1), 51.76 (C-4^2), 31.06 (2 C, C-$3'^{1,2}$), 17.78 (2 C, C-$6^{1,2}$).

Anal. Calcd for $C_{63}H_{62}N_2O_{19}$: C, 65.72; H, 5.42; N, 2.43. Found: C, 65.44; H, 5.45; N, 2.38.

Methyl 3-*O*-benzoyl-2-*O*-[2,3-di-O-benzoyl-4,6-dideoxy-4-(2,4-di-*O*-benzoyl-3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranosyl]-4,6-dideoxy-4-(2,4-di-*O*-benzoyl-3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranoside (29). A small amount of the disaccharide **28** was benzoylated conventionally (pyridine-benzoyl chloride) to give, after processing and chromatography, the fully benzoylated, amorphous disaccharide **29** in virtually theoretical yield: $[\alpha]_D$ -100.3° (*c* 1), CIMS: *m/z* 1272 ($[M + 18]^+$); ^1H NMR ($CDCl_3$) δ 6.68 (d, 1 H, $J_{4,NH}$ 9.5 Hz, NH^2), 6.35 (d, 1 H, $J_{4,NH}$ 9.5 Hz, NH^1), 5.83 (dd, 1 H, $J_{1,2}$ 1.7, $J_{2,3}$ 3.2 Hz, H-2^2), 5.77 (dd, $J_{3,4}$ 11.1 Hz, H-3^2), 5.56-5.49 (m, 3 H, H-3^1,$2'^{1,2}$), 5.20 (d, 1 H, H-1^2), 4.71 (d, partially overlapped, H-1^1), 4.71-4.60 (m, partially overlapped, H-4^2), 4.47-4.35 (m, 1 H, H-4^1), 4.30-4.12 (m, 4 H, H-$4'^{1,2}$a,b), 4.06 (dd, 1 H, $J_{1,2}$ 1.8, $J_{2,3}$ 2.9 Hz, H-2^1), 4.03-3.95 (m, 1 H, H-5^2), 3.73-3.63 (m, 1 H, H-5^1), 3.34 (s, 3 H, OCH_3), 2.28-1.91 (m, 4 H, H-$3'^{1,2}$a,b), 1.31 (d, 3 H, $J_{5,6}$ 6.4 Hz, H-6^2), 1.23 (d, 3 H, $J_{5,6}$ 6.4 Hz, H-6^1); ^{13}C NMR ($CDCl_3$) δ 99.67 (C-1^2), 99.62 (C-1^1), 76.13 (C-2^1), 71.66 (2 C, C-$2'^{1,2}$), 70.33 (C-3^1), 70.03 (C-2^2), 69.17 (C-2, C-5^2,3^2), 68.22 (C-5^1), 60.56 (2 C, C-$4'^{1,2}$), 54.94 (OCH_3), 52.09 (C-4^2), 51.93 (C-4^1), 31.05, 30.97 (C-$3'^{1,2}$), 17.73 (2 C, C-$6^{1,2}$).

Anal. Calcd for $C_{70}H_{66}N_2O_{20}$: C, 66.97; H, 5.30; N, 2.23. Found: C, 66.83; H, 5.37; N, 2.16.

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5. When describing an O-polysaccharide antigen still attached to the core region, where the distinction between the two saccharide end-groups can not be made by referring to them as reducing or non-reducing ends, because both ends are non-reducing, we call the *upstream end-group* that sugar unit which is furthermost from the core, and the *downstream end group* the sugar unit which is directly attached to the core region.

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