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Carbohydrate Research 260 (1994) 203–218

CARBOHYDRATE RESEARCH

Improved synthesis and the crystal structure of methyl 4,6-dideoxy-4-(3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranoside, the methyl α-glycoside of the intracatenary repeating unit of the O-polysaccharide of *Vibrio cholerae* O:1 †

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(Received December 8th, 1993; accepted in revised form February 18th, 1994)

Abstract

The crude product of deamination of the commercially available L-homoserine was acetylated and the 2-*O*-acetyl-3-deoxy-L-*glycero*-tetronolactone (**18**) formed was used to *N*-acylate methyl perosaminide (methyl 4-amino-4,6-dideoxy-α-D-mannopyranoside, **12**) and its 2,3-*O*-isopropylidene derivative. The major product isolated from the reaction was the crystalline methyl 4-(4-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (**1**, 70–75%) resulting from acetyl group migration in the initially formed 2′-*O*-acetyl derivative. *O*-Deacetylation of **1** gave the title amide **2**. Compound **2**, obtained crystalline for the first time, was fully characterized, and its crystal structure was determined. Deoxytetronamido derivatives diastereomeric with **1** and **2**, respectively, were obtained by the acylation of **12** with 2-*O*-acetyl-3-deoxy-D-*glycero*-tetronolactone (prepared from D-homoserine), and subsequent deacetylation. Structures of several byproducts of the reaction of **12** with **18** have been deduced from their spectral characteristics. Since these byproducts were various *O*-acetyl derivatives of **2**, the title compound could be obtained in

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† Synthesis of ligands related to the *Vibrio cholerae* O-specific antigen. Part 2. For a preliminary report of some of this work, see Part 1 [1].

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Elsevier Science B.V.
SSDI 0008-6215(94)00073-0

~ 90% yield by deacetylating (Zemplén) the crude mixture of *N*-acylation products, followed by chromatography.

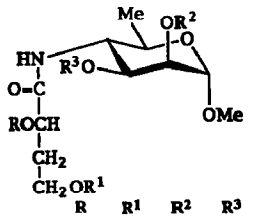
Key words: α-D-Mannopyranoside, methyl 4,6-dideoxy-4-(3-deoxy-L-*glycero*-tetronamido-); O-Polysaccharide; Perosamine; *Vibrio cholerae* O:1

1. Introduction

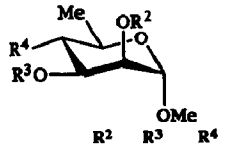
There are many serogroups of *Vibrio cholerae*, all causing disease with symptoms of Asian cholera. *Vibrio cholerae* O:1 Gram-negative bacteria occur as two immunologically distinct variants: Ogawa and Inaba. The O-polysaccharides of both consist [2,3] of 1 → 2-linked 4-amino-4,6-dideoxy-α-D-mannopyranosyl (perosaminyl) residues, the amino groups of which are acylated with 3-deoxy-L-*glycero*-tetronic acid. The two strains differ in that the terminal, nonreducing perosamine moiety of the O-polysaccharide of the Ogawa strain is methylated at O-2, as recently shown by Hisatsune et al. [4]. Systematic prevention of cholera by immunization has not yet been achieved because of lack of a protective vaccine, although there is wide interest, in our laboratory and elsewhere, in developing vaccines based on natural lipopolysaccharides or their constituents. To identify fragments of the O-antigen with good potential for eliciting the desired immunoresponse, we study the mode of binding with the homologous antibodies of ligands related to the O-polysaccharide. Here we describe an improved synthesis, as well as the crystal structure, of the methyl α-glycoside of the monomeric repeating unit common to both the Ogawa and Inaba strains.

2. Results and discussion

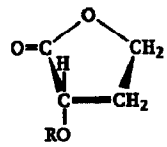
Synthesis.—Three independent syntheses of the methyl α-glycoside of perosamine, the key intermediate in the synthesis of the target methyl 4,6-dideoxy-4-(3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranoside (**2**), have recently been described [3,5,6]. Also, Kenne and co-workers [3] have reported an elegant preparation, from a commercially available precursor, of 2,4-dihydroxy-L-*glycero*-tetrono-γ-lactone (**17**), a useful *N*-acylating reagent for the construction of **2**. Treatment [3] of methyl α-D-perosaminide (**12**) with ~ 2.5 molar proportions of **17** gave the amorphous glycoside **2** in a yield of 45%. Possible side-reactions in that transformation, or other reasons for the moderate yield of the desired amide **2**, were not discussed. However, the availability of a procedure capable of converting the amine **12**, or its derivative, to the corresponding 3-deoxy-L-*glycero*-tetronamide is essential for an efficient synthesis of analogous oligosaccharides. That, in turn, might open avenues for the synthesis of a synthetic vaccine against the disease caused by *Vibrio cholerae*. The reported 45% yield of **2** was deemed unsatisfactory, especially if oligosaccharides in this series were to be prepared by a similar approach, leading us to seek a more efficacious *N*-acylation of the amine **12**.



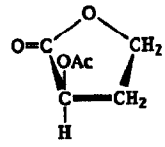
	R	R^1	R^2	R^3
1	H	Ac	H	H
2	H	H	H	H
3	Ac	Ac	Ac	Ac
4	H	Ac	Ac	H
5	H	Ac	H	Ac
6	Ac	Ac	H	H
7	Ac	H	H	H
8	H	Ac	—Ipd—	
9	Ac	Ac	—Ipd—	
10	Ac	H	—Ipd—	
11	H	H	—Ipd—	



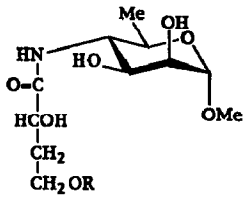
	R^2	R^3	R^4
12	H	H	NH_2
13	H	H	NHAc
14	—Ipd—		N_3
15	—Ipd—		NH_2
16	—Ipd—		NHAc



	R
17	H
18	Ac



19



	R
20	Ac
21	H

It occurred to us that an acylated lactone, such as **18**, could be easier to obtain in a pure state than the parent compound **17**, making it a superior reagent. Thus, the product of the deamination of L-homoserine [3,7] was acetylated. However, because of the volatility of **18**, the acetylation protocol could not involve its separation from high boiling solvents and/or reagents. Accordingly, the treatment with a cation-exchange resin [3] of the crude product of deamination was omitted leaving it, after dehydration, in an admixture with salts. Treatment of this material with acetic anhydride, without additional acidic or basic promoter, gave the acetylated lactone **18** in an acceptable yield (~ 60%, after distillation). The basicity of the salts carried over from the previous step was apparently sufficient to drive the acetylation reaction. In fact, the crude product formed in this way was cleaner and easier to purify than that obtained from acetylations catalyzed with either acid (H_2SO_4) or base (pyridine).

The amine **12** used in this work was prepared by combining the advantages of two previous syntheses [5,6]. Thus, we found the conversion of methyl 2,3-*O*-isopropylidene-α-D-mannopyranoside [8] to the corresponding 6-deoxy derivative as described by Bundle et al. [6] superior. On the other hand, the further conversion of the latter compound to the amine according to Eis and Ganem [5], was experimentally less demanding than the procedure of Bundle et al. [6]. When we treated the amine **12** with **18**, essentially as described [3] for the preparation of **2** but using only a 50% molar excess of the *N*-acylating reagent, we obtained the crystalline monoacetyl derivative **1** in 70–75% yield, following purification of the

crude product by chromatography. The 4′-position * of the acetyl group in **1**, suggesting the occurrence of acetyl group migration ** during this and similar condensations reported here, was strongly indicated by the ^{1}H NMR spectral data obtained for products of acetylation and deacetylation of **1**. Thus, the signal for H-2′ was shifted downfield following the conversion **1** → **3** and the signal for H-4′ was shifted upfield following the conversion **1** → **2**. Unambiguous proof of the structure **1** was obtained by X-ray analysis, and detailed data will be published elsewhere [9]. Conventional deacetylation of **1** readily furnished the known [3] **2**, which we now obtained crystalline. Compound **2** furnished the crystalline per-*O*-acetate **3**, and the NMR spectra of the latter were used as aids in the assignment of signals in the spectra of some related substances (*vide infra*). The diastereomers of **1** and **2**, compounds **20** and **21**, were similarly obtained using the lactone **19** to acylate **12**. Lactone **19** was prepared from D-homoserine by use of the procedure for the synthesis of **18**, and the values of $[\alpha]_D$ found for **18** and **19** were virtually identical in magnitude, but opposite in sign (see Experimental).

In addition to the desired *N*-acylation of the amine **12** by the lactone **18**, these two compounds can interact in many other ways, due to the presence of the acetate ester function in the lactone **18**, as well as free hydroxyl groups in **12**. Indeed, examination by TLC of the crude mixture from the *N*-acylation of **12** with **18** revealed that, in addition to the major product **1**, several other compounds were formed. Their amounts and ratios in successive preparations varied somewhat. It can be reasonably assumed that if the above described *N*-acylation protocol were to be utilized for the similar *N*-acylation of 1 → 2-linked perosamine-containing oligosaccharides, the number of products resulting from side reactions would increase. To undergird attempts at avoiding loss of yield of the desired product through unwanted side reactions, we considered it important to elucidate the nature of the byproducts.

Three of the byproducts having greater chromatographic mobilities than **1** were formed in relatively large amounts. These were isolated by chromatography and each showed peaks at m/z 364 ($[M + 1]^+$) and 381 ($[M + 18]^+$) in their ammonia CI mass spectra, indicating that they could be *O*-acetyl derivatives of **1**. Although the compounds were not obtained in an analytically pure state, the comparison of their NMR spectra with the unambiguously assigned (HETCOR) spectra of **1–3** strongly suggest that the three substances are products (**4–6**) of transacetylation. The ^{1}H NMR spectra show that the compounds are amides (doublets, δ_{NH} 6.98, 6.92, and 6.53, respectively for **4–6**), they show signals for two $COCH_3$ groups (2.16, 2.07; 6-proton singlet at 2.08; and 2.17, 2.06, respectively for **4–6**), and they contain features characteristic of both the methyl α-perosaminide (singlets, δ_{OCH_3}

* In descriptions of NMR data, and occasionally elsewhere in the text, atoms associated with the 3-deoxy-L-*glycero*-tetronamido group are denoted with a prime.

** In our earlier communication [1], the major product isolated from this condensation was erroneously reported as having the structure **7**.

3.37, 3.39, and 3.36; and doublets, δ_{H-6} 1.25, 1.25, and 1.21, respectively for **4–6**) and the 3-deoxy-*glycero*-tetronic acid moieties (high-field multiplets for H-3'a and H-3'b).

The location of the *O*-acetyl groups in **4–6** was deduced from the ^{13}C NMR data. For example, the ^{13}C NMR chemical shift for C-1 in **4** (δ 98.41) compared well with that observed for the acetyl derivative **3** (δ 98.28). On the other hand, the chemical shifts for C-1 in **5** and **6** compare well with that for C-1 in **1**. They are shifted downfield (δ 100.54 and 100.80, respectively), unlike those for **3** and **4**, due to the absence of the negative β-shift effect [10] of an acyl group at the vicinal C-2 position. Similarly, the signal for C-4 of **5**, bearing an acetyl group at the neighboring HO-3, appeared at δ 50.63 (δ_{C-4} 51.48 for **3**) whereas the signal for C-4 in **4** and **6** having HO-3 unsubstituted appears downfield at δ 53.90 and 53.67, respectively. That the most likely location of the two *O*-acetyl groups in **6** is at C-2' and C-4' is suggested by the similarity in chemical shifts found for C-2',3' and C-4' of **6** to those for C-2',3' and C-4' in **3**. Likewise, the signal for C-4' in **4** and **5**, having the HO-4' acetylated, appears downfield ($\delta_{C-4'}$ 61.03 and 61.04, respectively), as does that of **1** ($\delta_{C-4'}$ 61.16).

Two byproducts showing chromatographic mobility lower than that of **1** were also found in the crude mixture from the reaction of **12** with **18**. The slower of these two was indistinguishable from **2** by TLC. The material that showed only slightly lower mobility than **1** had the same R_f as an authentic sample of the *N*-acetyl compound **13**. The NMR spectra of this material, as well as the profile of the ion current during mass spectrometry, indicated that it was a mixture of two substances. The ammonia CI mass spectrum of one of the substances showed peaks at m/z 322 and 339 ($[M + 1]^+$ and $[M + 18]^+$, respectively), suggesting that it was isomeric with **1**, and the spectrum of the other substance showed peaks at m/z 220 and 237 ($[M + 1]^+$ and $[M + 18]^+$, respectively), indicating it to be *O*- or an *N*-acetyl derivative of **12**. The latter compound readily crystallized, and it was identified (mp, NMR) as methyl 4-acetamido-4,6-dideoxy-α-D-mannopyranoside (**13**). The plausible explanation we offer for the formation of **13** is the attack of the amino group of **12** at the carbonyl group of the acetoxy function in **18**, rather than at the C=O group of its lactone ring. This at the same time resulted in the formation of **17**, the presence of which in the mixture accounts for the formation of compound **2** during condensation, as well as for the formation of the amide **11** during condensation of **15** and **18** (see below). Comparison of the NMR spectra of the amorphous substance isomeric with **1**, present in the isolated material in admixture with **13**, with the spectra of related substances described above strongly pointed to its having structure **7**, being thus the expected primary product of the condensation of **12** and **18**.

The nature of the major byproducts formed along with **7** and **1** during the reaction of **12** and **18**, i.e., all being *O*-acetyl derivatives of the target amide **2**, suggested a protocol which could improve the overall yield of **2**. Thus, the crude product of the acylation of **12** by **18** was subjected to *O*-deacetylation (Zemplén). TLC of the mixture showed the presence of one major and one very minor product, indistinguishable from **2** and **13**, respectively. The material showing the

Table 1
Crystallographic data for the methyl glycoside **2**

Formula	$C_7H_{21}NO_7$
MW	279.29
Crystal system	Monoclinic
Space group	$P2_1$
a (Å)	10.870 (5)
b (Å)	6.875 (6)
c (Å)	10.636 (5)
β (°)	117.39 (1)
V (Å^3)	705.8 (8)
Z	2
D_c (g·cm^{-1})	1.31
M (cm^{-1})	1.0
F (000)	300
Radiation: Mo$K\alpha$, graphite monochromator	$\lambda = 0.71073$ Å
Diffractometer	Enraf–Nonius CAD4
Number of orienting reflections and range	25, $11 < \theta < 15$
Temperature (°C)	22
Scan method	$\omega - 2\theta$
Data collection range	$2.5 < 2\theta < 53$
No. of unique data	1587
No. of observed data ($I > 2.0\sigma(I)$), N	1140
No. of parameters, P	187
R^a	0.047
R_w^b	0.053
S, goodness of fit^c	1.28
Max. shift/error, final	0.003
Largest positive peak (e/Å^3)	0.21
Largest negative hole (e/Å^3)	−0.22

$^a R = \Sigma(|F_o| - |F_c|)/\Sigma|F_o|$. $^b R_w = \{\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2\}^{1/2}$; $w = 1/[(\sigma F_o)^2 + 0.0008^* F_o^2]$; $^c S = [\Sigma w(|F_o| - |F_c|)^2/(N-P)]^{1/2}$.

mobility of **2** was isolated by chromatography and it was shown by NMR spectroscopy to be pure **2**. In this way, the yield of the isolated **2** reached ~ 90%, based on **12**. The syntheses of the lactone **18** and the methyl α-D-perosaminide derivative **2** are thus simple, and suitable for large-scale preparations.

We anticipate the synthesis of oligosaccharides related to the O-polysaccharide of *Vibrio chlolerae* O:1 to be the next stage of our endeavor. From this standpoint, it was important to know if *N*-acylation with **18** of a 2,3-di-*O*-substituted derivative of **12** would give a less complex crude product than that from **12** itself, inasmuch as compounds analogous to **4** and **5** could not be formed from protected **12**. To explore this possibility the free amine **15** was prepared from the described [6] 4-azido-2,3-*O*-isopropylidene derivative **14** and the reaction of oily **15** with **18** was performed under conditions identical to those applied in the preparation of **1**. The reaction was also carried out in the absence of pyridine, using neat **18** as both solvent and reagent. Byproducts, both more and less polar than the amide **8** (major

Table 2
Atomic coordinates and equivalent isotropic thermal parameters for the methyl glycoside 2

Atom	x	y	z	B or B_{eq} [a] (Å^2)
C-1	0.7965 (4)	0.8902 (9)	0.4021 (5)	3.97 (24)
C-2	0.7699 (4)	0.9899 (8)	0.5147 (4)	3.50 (20)
C-3	0.6214 (4)	0.9517 (8)	0.4879 (4)	2.89 (19)
C-4	0.5220 (4)	1.0190 (8)	0.3391 (4)	3.12 (18)
C-5	0.5579 (4)	0.9141 (9)	0.2327 (4)	3.88 (22)
O-5	0.6998 (3)	0.9508 (7)	0.2659 (3)	4.21 (17)
O-1	0.7920 (3)	0.69074	0.4223 (3)	4.27 (16)
C-7	0.8241 (5)	0.5748 (11)	0.3282 (5)	5.3 (3)
O-2	0.7945 (3)	1.1915 (7)	0.5074 (4)	4.60 (18)
O-3	0.5932 (3)	1.0543 (7)	0.5887 (3)	3.63 (15)
C-6	0.4730 (6)	0.9853 (12)	0.0820 (5)	6.1 (3)
N	0.3788 (4)	0.9746 (8)	0.3036 (4)	3.44 (17)
C-1′	0.2743 (4)	1.0918 (9)	0.2260 (4)	3.18 (20)
O-1′	0.2915 (3)	1.2609 (7)	0.1979 (3)	3.93 (15)
C-2′	0.1287 (4)	1.0062 (8)	0.1640 (4)	3.34 (21)
O-2′	0.1267 (3)	0.8159 (7)	0.2139 (3)	4.12 (16)
C-3′	0.0281 (4)	1.1310 (8)	0.1900 (4)	3.62 (20)
C-4′	−0.1205 (5)	1.0635 (10)	0.1064 (5)	4.29 (23)
O-4′	−0.1623 (3)	1.0710 (8)	−0.0411 (3)	5.02 (17)
H-n	0.361 (5)	0.860 (8)	0.323 (5)	4.0

[a] B_{eq} is the mean of the principal axes of the thermal ellipsoid.

product, resulting from acetyl group migration), were formed and compound **8** was isolated in ~60% yield. The structures of side products, **9–11** and **16**, were analogous to those of some of the compounds derived from **12** and **18**. These structures were deduced from CIMS and NMR characteristics (see Experimental), in a way similar to that described above for the assigment of structures to **4–7**.

Crystallography.—Crystallographic data for **2** are listed in Table 1, and final coordinates for the crystal structure are given in Table 2. Bond lengths, bond angles, and conformational angles for the nonhydrogen atoms are given in Table 3 *. A perspective drawing of the molecule, with atom numbers [12], is given in Fig. 1 [11]. The pyranose ring is in a nearly ideal chair conformation, with puckering constants (Cremer and Pople [12]) of $Q = 0.576$ Å, $\theta = 2.98$ and $\phi = 208.01$. The L configuration of the *N*-acyl group is established by the known configuration of the other chiral centers. The conformation of the side chain is less than fully extended, as can be seen in Fig. 1 and from the conformational angles

* Lists of H-atom coordinates, anisotropic thermal parameters for the non-H atoms, and structure factors have been deposited with the Cambridge Crystallographic Data Centre. The data may be obtained on request from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

(Table 3). The amide bond is *trans*, but with a significant deviation from planarity, reflecting an alteration in the magnitude of the π overlap and changes in the hybridization of the C and N atoms involved in the bond. Using the parameters defined by Winkler and Dunitz [13] and those given in Table 3, the nonplanarity can be derived from both twisting of the C-1′ – N bond and from out-of-plane bending at the N atom. The N atom is involved in a weak intramolecular interaction with O-2′ and in an intermolecular H-bond with O-3 of an adjacent molecule. The hydrogen-bonding interactions, listed in Table 4, describe the

Table 3
Bond distances, bond angles, conformational angles, and amide-bond conformational parameters for the methyl glycoside **2**

Bond distances (Å)			
C-1–C-2	1.519(7)	C-5–C-6	1.516(7)
C-1–O-5	1.406(6)	O-1–C-7	1.442(6)
C-1–O-1	1.393(6)	N–C-1′	1.326(6)
C-2–C-3	1.527(6)	C-1′–O-1′	1.236(7)
C-2–O-2	1.421(8)	C-1′–C-2′	1.525(6)
C-3–C-4	1.521(6)	C-2′–O-2′	1.415(7)
C-3–O-3	1.429(5)	C-2′–C-3′	1.513(7)
C-4–C-5	1.536(6)	C-3′–C-4′	1.514(6)
C-4–N	1.455(5)	C-4′–O-4′	1.420(6)
C-5–O-5	1.438(5)	N–H-n	0.86(5)
Bond angles (°)			
C-2–C-1–O-5	110.8(4)	C-4–C-5–C-6	113.0(4)
C-2–C-1–O-1	106.8(4)	O-5–C-5–C-6	105.4(4)
O-5–C-1–O-1	112.8(4)	C-1–O-5–C-5	114.3(3)
C-1–C-2–C-3	110.1(4)	C-1–O-1–C-7	113.6(4)
C-1–C-2–O-2	106.7(4)	C-4–N–C-1′	122.8(5)
C-3–C-2–O-2	111.5(4)	N–C-1′–O-1′	122.7(4)
C-2–C-3–C-4	109.0(3)	N–C-1′–C-2′	117.2(5)
C-2–C-3–O-3	110.4(4)	O-1′–C-1′–C-2′	120.0(4)
C-4–C-3–O-3	108.9(4)	C-1′–C-2′–C-3′	113.3(4)
C-3–C-4–N	111.7(3)	O-2′–C-2′–C-3′	108.6(4)
C-5–C-4–N	108.3(4)	C-2′–C-3′–C-4′	112.6(4)
C-4–C-5–O-5	110.1(4)	C-3′–C-4′–O-4′	110.5(4)
C-4–N–H-n	117(3)	C-1′–N–H-n	118(3)
Conformational angles (°)			
O-5–C-1–C-2–C-3	56.1(3)	O-5–C-1–C-2–O-2	−65.1(3)
O-1–C-1–C-2–C-3	−67.1(3)	O-1–C-1–C-2–O-2	171.7(5)
C-2–C-1–O-5–C-5	−58.5(3)	O-1–C-1–O-5–C-5	61.2(3)
C-2–C-1–O-1–C-7	−176.4(4)	O-5–C-1–O-1–C-7	61.6(3)
C-1–C-2–C-3–C-4	−56.2(3)	C-1–C-2–C-3–O-3	−176.6(5)
O-2–C-2–C-3–C-4	62.1(3)	O-2–C-2–C-3–O-3	−58.4(3)
C-2–C-3–C-4–C-5	56.6(3)	C-2–C-3–C-4–N	176.2(5)
O-3–C-3–C-4–C-5	177.5(5)	O-3–C-3–C-4–N	−62.9(3)
C-3–C-4–C-5–O-5	−57.1(3)	C-3–C-4–C-5–C-6	−174.6(5)

Table 3 (continued)

Conformational angles (°)			
N–C-4–C-5–O-5	−78.8(5)	N–C-4–C-5–C-6	63.7(3)
C-3–C-4–N–C-1′	143.5(5)	C-5–C-4–N–C-1′	−96.6(4)
C-4–C-5–O-5–C-1	59.2(3)	C-6–C-5–O-5–C-1	−178.7(5)
C-4–N–C-1′–O-1′	−12.5(2)	C-4–N–C-1′–C-2′	164.1(5)
N–C-1′–C-2′–O-2′	7.1(2)	N–C-1′–C-2′–C-3′	130.9(5)
O-1′–C-1′–C-2′–O-2′	−176.2(5)	O-1′–C-1′–C-2′–C-3′	−52.4(3)
C-1′–C-2′–C-3′–C-4′	169.8(5)	O-2′–C-2′–C-3′–C-4′	−64.1(3)
C-2′–C-3′–C-4′–O-4′	−60.5(3)	O-1′–C-1′–N–H-n	178.0(3)
C-2′–C-1′–N–H-n	−6.0(3)		
Amide bond conformational parameters [a]			
$\tau_1 = (\omega_1 + \omega_2)/2, \lvert\omega_1 - \omega_2\rvert < \pi$	171(3)		
$\chi_c = \omega_1 - \omega_3 + \pi$, Mod 2π	−3.4 (7)		
$\chi_n = \omega_2 - \omega_3 + \pi$	+11(3)		

[a] ω_1 C-2′–C-1′–N–C-4; ω_2 O-1′–C-1′–N–H-n; ω_3 O-1′–C-1′–N–C-4; ω_4 C-2′–C-1′–N–H-n

crystal packing and no doubt strongly influence the conformation assumed by the molecule. Fig. 2 shows a stereoview of the packing interactions.

3. Experimental

General methods.—Thin-layer chromatography (TLC) was performed with solvent mixtures having a range of polarities, namely *A*, 1:1 hexane–EtOAc; *B*, 8:1 CH_2Cl_2–MeOH; *C*, 5:1 CH_2Cl_2–MeOH; *D*, 10:1 CH_2Cl_2–acetone; *E*, 10:1 toluene–acetone; *F*, 1:1 toluene–EtOAc; and *G*, 15:1 CH_2Cl_2–MeOH. Detec-

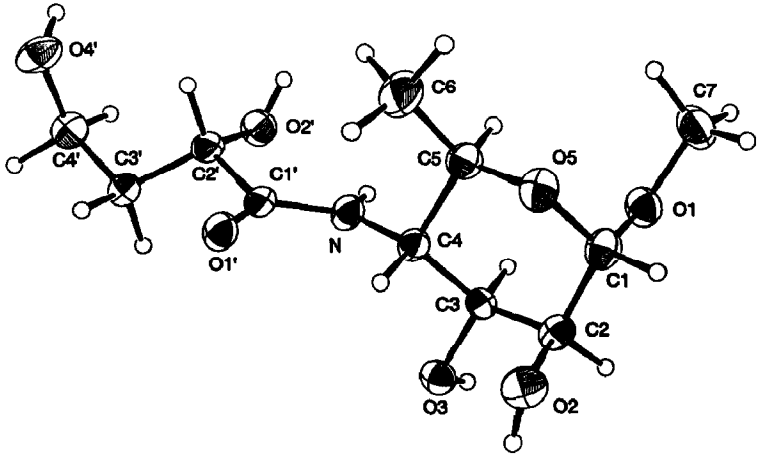


Fig. 1. Perspective ORTEP drawing of the methyl glycoside 2.

Table 4
Hydrogen-bonding network *

D–H···A*	D···A (Å)	D–H (Å)	H···A (Å)	<(D–H···A) (°)
O-2–H-O2···O-2′ [a]	2.813(8)	0.86(6)	1.97(6)	164(4)
O-3–H-O3···O-1′ [b]	2.859(7)	0.91(6)	1.99(6)	160(4)
N–H-n···O-3 [b]	3.072(6)	0.86(5)	2.27(6)	157(4)
N–H-n···O-2′ [c]	2.684(7)	0.86(5)	2.28(6)	109(4)
O-2′–H-O2′···O-4′ [d]	2.649(7)	0.87(6)	1.79(6)	175(4)
O-4′–H-O4′···O-1′ [d]	2.674(7)	0.89(6)	1.83(6)	158(4)

* Symmetry code: [a] $-x+1,\ y+1/2,\ -z+1$; [b] $-x+1,\ y-1/2,\ -z+1$; [c] $x,\ y,\ z$; [d] $-x,\ y-1/2,\ -z$.

tion was effected with iodine vapors and, where applicable, by charring with 5% H_2SO_4 in EtOH. Unless stated otherwise, optical rotations were measured at 25°C for solutions in $CHCl_3$, using a Perkin–Elmer, Model 241 MC polarimeter. NMR spectra were obtained at 300 MHz for ^1H and 75 MHz for ^{13}C. The measurements were done at ambient temperature, using a Varian XL 300 or a Varian Gemini spectrometer. The solvent used is listed for each compound. Chemical shifts are reported in ppm downfield of the signal of Me_4Si; ^1H shifts determined in D_2O were measured from the signal of HOD (δ 4.78), and ^{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 2-dimensional correlation spectroscopy, using commercial software supplied with the spectrometers. Chemical ionization mass spectra (CIMS) were measured using ammonia as the reactive gas. Experimental details of the crystallographic study are given in Table 1. The structure was solved using direct methods, and refined by full-matrix least squares on F_o, using the NRCVAX [14] suite of programs. Data were corrected for Lorentz and polarization effects and for extinction, but no absorption correction was applied. Nonhydrogen atoms were refined with anisotropic thermal parameters. Following initial refinement, hydrogen atoms were located in difference Fourier maps. In the final model, the OH

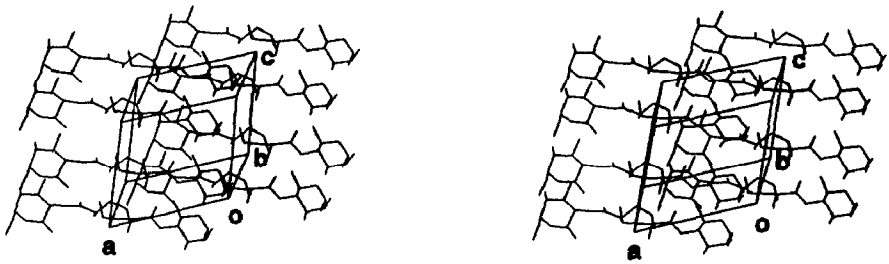


Fig. 2. Stereo drawing of the unit cell of 2.

and NH hydrogen atoms were refined with fixed isotropic thermal parameters while the remaining hydrogen atoms were included at calculated positions, also with isotropic thermal parameters. Atomic scattering factors and anomalous-dispersion corrections were taken from the *International Tables for X-ray Crystallography* (1974) [15]. L-Homoserine and its D enantiomer were purchased from Sigma Chemical Company, and used as supplied. Solutions in organic solvents were dried with anhyd Na_2SO_4, and concentrated at 40°C/2 kPa.

2-O-Acetyl-3-deoxy-L-glycero-*tetronolactone* (**18**).—A solution of sodium nitrite (5.1 g, 74 mmol) in water (30 mL) was added dropwise at room temperature, over a period of 30 min, to a stirred solution of L-homoserine (3 g, 25 mmol) in 50% AcOH (90 mL). After 16 h, the solution was concentrated with coevaporation of toluene, and the residue was dried at 50°C/2 kPa for 3 h. The suspension of the crude product in 2:1 $ClCH_2$-CH_2Cl–Ac_2O (90 mL) was stirred overnight at 60°C. The mixture was allowed to cool, and the temperature was kept ambient by external cooling with tap water while water (50 mL) was added to hydrolyze excess Ac_2O. The pH of the mixture was adjusted to ~6 with solid $NaHCO_3$, and the mixture was extracted with CH_2Cl_2. The organic phase was washed twice with aq $NaHCO_3$, dried, and concentrated. TLC (solvent *A*) shoved that one major product was present. The crude product was distilled at 95–100°C (bath) and 26 Pa to give virtually pure (TLC, NMR) **18** (2.17 g, 60%). The analytical sample, obtained by distillation of a sample purified by chromatography to remove some base-line material, showed $[\alpha]_D$ −20.7° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 5.43 (dd, 1 H, $J_{2,3a}$ 8.7, $J_{2,3b}$ 9.2 Hz, H-2), 4.48 (m, 1 H, H-4a), 4.31 (m, 1 H, H-4b), 2.72 (m, 1 H, H-3a), 2.31 (m, 1 H, H-3b), and 2.19 (s 3 H, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 172.53, 169.57 (2 CO), 67.60 (C-2), 65.01 (C-4), 28.90 (C-3), and 20.62 ($COCH_3$); CIMS: *m/z* 162 ($[M + 18]^+$). Anal. Calcd for $C_6H_8O_4$: C, 50.0; H, 5.59. Found: C, 49.92; H, 5.64.

Batches of up to 20 g of L-homoserine, when treated as just described, similarly gave **18** in ~60% yields.

2-O-Acetyl-3-deoxy-D-glycero-*tetronolactone* (**19**).—D-Homoserine, when treated as described for the preparation of **18**, gave the title lactone **19** in a yield comparable with that of **18**; $[\alpha]_D$ +20.2° (*c* 1.4, $CHCl_3$); the NMR data were identical with those of **18**; CIMS: *m/z* 162 ($[M + 18]^+$). Anal. Calcd for $C_6H_8O_4$: C, 50.0; H, 5.59. Found: C, 50.12; H, 5.55.

Methyl (*4-O-acetyl-3-deoxy*-L-glycero-*tetronamido*)*-4,6-dideoxy-4-α-D-manno-pyranoside* (**1**).—Methyl 6-deoxy-2,3-*O*-isopropylidene-α-D-mannopyranoside, prepared by the method of Bundle [6], was converted into **12** as described by Eis [5]. A solution of the lactone **18** (0.65 g, 4.5 mmol) and amine **12** (0.53 g, 3 mmol) in pyridine (1.5 mL), in a tightly closed screw-capped vial, was heated at 105–110°C for 16 h. During the first ~6 h, the intensity of the spot representing the unresolved mixture of **7** and **13** increased. Later, the intensity of that spot gradually decreased, and that of the spot representing **1** increased, due to the spontaneous conversion **7** → **1**. Eventually, one major and several minor products were formed (TLC, solvent *B*). After concentration followed by the addition and evaporation of toluene the residue was chromatographed.

Eluted first was material identified by NMR spectroscopy as methyl 2-*O*-acetyl-4-(4-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (**4**). The structurally important signals present in the ^{1}H NMR spectrum ($CDCl_3$) were at δ 6.98 (d, 1 H, $J_{4,NH}$ 8.9 Hz, NH), 4.68 (bs, H-1), 3.37 (s, 3 H, OCH_3), 2.16, 2.07 (2 s, partially overlapped with the signals of H-3′ab, ~ 3 H each, 2 × $COCH_3$), and 1.25 (d, $J_{5,6}$ 6.1 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 98.41 (C-1), 71.50 (C-2), 69.25 (C-2′), 67.92 (C-3), 66.91 (C-5), 61.03 (C-4′), 55.04 (OCH_3), 53.90 (C-4), 33.4 (C-3′), 20.92, 20.87 ($COCH_3$), and 17.76 (C-6); CIMS: *m/z* 364 ($[M + 1]^+$), and 381 ($[M + 18]^+$).

Eluted next was material identified by NMR spectroscopy as methyl 3-*O*-acetyl-4-(4-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-α-D-mannopyranoside (**5**). The structurally important signals present in the ^{1}H NMR spectrum ($CDCl_3$) were at δ 6.98 (d, 1 H, $J_{4,NH}$ 10.2 Hz, NH), 5.17 (dd, 1 H, $J_{2,3}$ 2.9, $J_{3,4}$ 10.9 Hz, H-3), 4.72 (s, 1 H, H-1), 3.39 (s, 3 H, OCH_3), 2.08 (s, 6 H, 2 × $COCH_3$), and 1.25 (d, 3 H, $J_{5,6}$ 6.2 Hz); ^{13}C NMR ($CDCl_3$): δ 100.54 (C-1), 71.48 (C-3), 69.13 (C-2′), 68.70 (C-2), 67.40 (C-5), 61.04 (C-4′), 54.95 (OCH_3), 33.55 (C-3′), 20.82 (2 × $COCH_3$), and 17.65 (C-6); CIMS: *m/z* 364 ($[M + 1]^+$) and 381 ($[M + 18]^+$).

Eluted next was material identified by NMR spectroscopy as methyl 4,6-dideoxy-4-(2,4-di-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranoside (**6**). The diagnostically important signals present in the ^{1}H NMR spectrum ($CDCl_3$) were at δ 6.53 (d, 1 H, $J_{4,NH}$ 9.0 Hz, NH), 4.71 (s, H-1), 3.36 (s, 3 H, OCH_3), 2.17, 2.06 (2 s, partially overlapped with the multiplets of H-3′, ~ 3 H each, 2 × $COCH_3$), and 1.21 (d, 3 H, $J_{5,6}$ 6.1 Hz); ^{13}C NMR ($CDCl_3$): δ 100.85 (C-1), 71.34 (C-2′), 69.87 (C-2), 69.38 (C-3), 66.82 (C-5), 60.08 (C-4′), 54.91 (OCH_3), 53.67 (C-4), 30.74 (C-3′), 20.78, 20.72 ($COCH_3$), and 17.67 (C-6); CIMS: *m/z* 364 ($[M + 1]^+$), 381 ($[M + 18]^+$).

Next eluted was the desired, major product **1** resulting from acetyl group migration (0.7 g, 73%); mp 129–130°C (from EtOAc); $[\alpha]_D$ +48.5° (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.26 (d, 1 H, $J_{4,NH}$ 8.8 Hz, NH), 4.81 (bd, 1 H, *J* 5.65 Hz, disappears on deuteration, OH), 4.70, (s, 1 H, H-1), 4.52, 4.38 (2 bs, disappear on deuteration, 2 OH), 4.33-4.17 (m, 3 H, H-2′,4′ab), 3.93–3.82 (m, 3 H, H-2,3,4), 3.74–3.82 (m, 1 H, H-5), 3.36 (s, 3 H, OCH_3), 2.28–2.17 (m, 1 H, H-3′a), 2.07 (s, 3 H, $COCH_3$), 1.85–1.39 (m, 1 H, H-3′b), and 1.21 (d, 1 H, $J_{5,6}$ 5.9 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 175.22, 171.65 (2 CO), 100.89 (C-1), 69.97 (C-2), 69.22 (2 C, C-2′,3), 66.74 (C-5), 61.16 (C-4′), 54.90 (OCH_3), 53.62 (C-4), 33.33 (C-3′), 21.05 ($COCH_3$), and 17.90 (C-6); CIMS: *m/z* 322 ($[M + 1]^+$) and 339 ($[M + 18]^+$). Anal. Calcd for $C_{13}H_{23}NO_8$: C, 48.59; H, 7.21; N, 4.36. Found: C, 48.67; H, 7.23; N, 4.36.

Eluted next was material whose ammonia CI mass spectrum showed peaks indicative of the presence of two substances. One of the components in the mixture crystallized in the NMR tube from a solution of the mixture in $CDCl_3$. Recrystallization from MeOH gave material melting at 187–190°C. The ^{1}H NMR characteristics agreed with those found for the independently synthesized **13** (*vide infra*); CIMS: *m/z* 220 and 237 ($[M + 1]^+$ and $[M + 18]^+$). The substance present in admixture with **13** was the expected methyl 4-(2-*O*-acetyl-3-deoxy-L-*glycero*-

tetronamido)-4,6-dideoxy-α-D-mannopyranoside (7), as shown by the diagnostically important spectral data: ^{1}H NMR ($CDCl_3$): δ 4.72 (bs, 1 H, H-1), 3.37 (s, 3 H, OCH_3), and 2.19 (s, partially overlapped with multiplets for H-3'a,b, ~3 H, $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 100.91 (C-1), 69.85, 69.71, 69.62 (C-2,3,2'), 66.86 (C-5), 57.56 (C-4'), 54.96 (OCH_3), 53.41 (C-4), 34.30 (C-3'), 20.87 ($COCH_3$), and 17.62 (C-6); CIMS: *m/z* 322 ($[M + 1]^+$ and 339 $[M + 18]^+$).

*Methyl 4,6-dideoxy-4-(3-deoxy-*L*-glycero-tetronamido)-α-*D*-mannopyranoside* (**2**). —(*a*) Methanolic M NaOMe was added to a solution of **1** (643 mg, 2 mmol) in MeOH (50 mL) until a strongly alkaline solution was obtained, and the solution was kept at room temperature overnight. TLC (solvent *C*) showed that one product was formed. After conventional processing, **2** was obtained in virtually theoretical yield; mp 136–138°C (from MeOH–acetone); $[\alpha]_D$ +34° (*c* 1.7, H_2O), lit. [3] $[\alpha]_D$ +34°; NMR data agreed with those reported [3]. Observed minor differences in chemical shifts resulted from the different conditions of measurement. Anal. Calcd for $C_{11}H_{21}NO_7$: C, 47.31; H, 7.58; N, 5.02. Found: C, 47.21; H, 7.62; N, 5.00.

(*b*) A solution of the amine **12** (532 mg, 3 mmol) in pyridine (1.5 mL) was treated with the lactone **18** (650 mg, 4.5 mmol), as described for the preparation of **1**. The mixture was concentrated and a solution of the residue in MeOH (70 mL) was made strongly alkaline by addition of M methanolic NaOMe. The solution was kept overnight at room temperature, when TLC (solvent *C*) showed complete conversion of the starting material. One major and one very minor product were formed, the faster of which (minor) showed the same chromatographic mobility as **13**. Conventional processing and chromatography gave **2** (750 mg, 89.5%). The NMR spectra of the material showed it to consist of pure **2**.

*Methyl 4-(4-*O*-acetyl-3-deoxy-*D*-glycero-tetronamido)-4,6-dideoxy-α-*D*-mannopyranoside* (**20**).—A solution of the lactone **19** (650 mg, 4.5 mmol) and amine **12** (532 mg, 3 mmol) was processed as described for the preparation of **1**. TLC (solvent *G*) showed products having mobilities similar to those seen during the preparation of **1**. Chromatography gave **20** (590 mg, 61%); mp 144–146°C (from EtOAc); $[\alpha]_D$ +84.8° (*c* 0.5, $CHCl_3$); ^{1}H NMR (6:1 $CDCl_3$–CD_3OD): δ 7.15 (δ, low intensity, $J_{4,NH}$ 9.8 Hz, residual NH signal due to incomplete deuteration), 4.69 (bd, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.31–4.21 (m, 2 H, H-4'ab), 4.15 (dd, 1 H, $J_{2',3'a}$ 3.6, $J_{2',3'b}$ 9.2 Hz, H-2'), 3.93–3.88 (m, 1 H, H-4), 3.86–3.85 (m, 1 H, H-2), 3.75–3.67 (m, 1 H, H-3), 3.67–3.61 (m, 1 H, H-5), 3.38 (s, 3 H, OCH_3), 2.24–2.13 (m, 1 H, H-3'a), 2.07 (s, 3 H, $COCH_3$), 1.89–1.77 (m, 1 H, H-3b), and 1.21 (d, 3 H, $J_{5,6}$ 6.4 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 176.23, 171.80 (2 CO), 100.95 (C-1), 69.84 (C-3), 69.79 (C-2), 69.29 (C-2'), 66.95 (C-5), 60.93 (C-4'), 54.95 (OCH_3), 52.88 (C-4), 33.38 (C-3'), 20.84 ($COCH_3$), and 17.64 (C-6). Anal. Calcd for $C_{13}H_{23}NO_8$: C, 48.59; H, 7.21; N, 4.36. Found: C, 48.69; H, 7.19; N, 4.33.

*Methyl 4,6-dideoxy-4-(3-deoxy-*D*-glycero-tetronamido)-α-*D*-manno pyranoside* (**21**).—Compound **20** was treated as described for the preparation of **2** (method *a*). After conventional processing and freeze drying the amorphous **21** was obtained in virtually theoretical yield; $[\alpha]_D$ +76.2° (*c* 0.8, H_2O); ^{1}H NMR (D_2O): 4.74 (s, 1 H, H-1), 4.28 (dd, 1 H, $J_{2',3'a}$ 3.9, $J_{2',3'b}$ 8.8 Hz, H-2'), 3.95-3.80 (m, 4 H, H-2,3,4,5),

3.73 (t, 2 H, *J* 6.0 Hz, H-4'a,b), 3.39 (s, 3 H, OCH_3), 2.10–1.98, 1.90–1.75 (2 m, 1 H each, H-3'a,b), and 1.18 (d, 3 H, $J_{5,6}$ 5.50 Hz, H-6); ^{13}C NMR (D_2O): δ 177.70 (CO), 101.13 (C-1), 69.30 (C-2), 69.15 (C-2'), 68.25 (C-3), 67.32 (C-5), 58.01 (C-4'), 54.87 (OCH_3), 52.87 (C-4), 36.06 (C-3'), and 16.87 (C-6). Anal. Calcd for $C_{11}H_{21}NO_7$: C, 47.31; H, 7.58; N, 5.02. Found: C, 47.12; H, 7.68; N, 4.95.

*Methyl 2,3-di-*O*-acetyl-4,6-dideoxy-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-α-*D*-mannopyranoside* (**3**).—A solution of compound **2** (100 mg) in pyridine (1 mL) was treated with Ac_2O (1 mL). After standing 3 h at room temperature the mixture was processed conventionally. Elution from a small column of silica gel (solvent *D*) gave **3** in virtually theoretical yield; mp 119–120°C (from EtOAc–hexane); $[\alpha]_D$ +45.4° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.09 (d, 1 H, $J_{4,NH}$ 9.2 Hz, NH), 5.26 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 10.9 Hz, H-3), 5.13 (bdd, 1 H, H-2), 5.10 (dd, 1 H, $J_{2',3'a}$ 4.8, $J_{2',3'b}$ 7.8 Hz, H-2'), 4.66 (d, 1 H, $J_{1,2}$ 2 Hz, H-1), 4.22 (dd, partially overlapped, 1 H, $J_{4,5}$ 10.1 Hz, H-4), 4.18–4.06 (m, 2 H, H-4'a,b), 3.68 (m, 1 H, H-5), 3.38 (s, 3 H, OCH_3), 2.05–2.20 (m, 14 H, 4 CH_3CO, H-3'a,b), and 1.25 (d, 3 H, $J_{5,6}$ 6.0 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 171.28, 170.60, 169.98, 169.60, 169.41 (5 CO), 98.28 (C-1), 70.91 (C-2'), 69.23 (C-2), 68.36 (C-3), 68.10 (C-5), 59.81 (C-4'), 55.08 (OCH_3), 51.48 (C-4), 30.60 (C-3'), 21.00, 20.82, 20.80, 20.78 (4 $COCH_3$), and 17.83 (C-6); CIMS: *m/z* 465 ($[M + 18]^+$). Anal. Calcd for $C_{19}H_{29}NO_{11}$: C, 51.00; H, 6.53; N, 3.13. Found: C, 50.74; H, 6.51; N, 3.10.

*Methyl 4-amino-4,6-dideoxy-2,3-*O*-isopropylidene-α-*D*-mannopyranoside* (**15**).—A solution of **14** [6] (2.5 g) in MeOH (150 mL) was stirred under H_2 in the presence of 5% Pd–C catalyst (1 g), at room temperature and normal pressure, for 18 h. TLC (solvent *E*) showed that the reaction was complete. After filtration and concentration, the residue was eluted from a small column of silica gel to give pure (TLC, NMR), oily **15** (1.9 g, 85%). A portion, when distilled at 90°C (bath) and 13 Pa, had $[\alpha]_D$ +26.3° (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 4.88 (s, 1 H, H-1), 4.05 (d, 1 H, $J_{2,3}$ 5.4 Hz, H-2), 3.86 (dd, 1 H, $J_{3,4}$ 8.5 Hz, H-3), 3.47 (m, 1 H, H-5), 3.37 (s, 3 H, OCH_3), 2.62 (dd, 1 H, $J_{4,5}$ 8.7 Hz, H-4), 1.52, 1.35 (2 s, 3 H each, Me_2C), and 1.26 (d, 3 H, $J_{5,6}$ 6.6 Hz, H-6). A signal due to NH_2 was not observed. ^{13}C NMR ($CDCl_3$): δ 109.12 (C-7), 98.12 (C-1), 78.79 (C-3), 74.90 (C-2), 66.75 (C-5), 56.64 (C-4), 54.73 (OCH_3), 28.16, 26.28 (2 CH_3C), 17.46 (C-6); CIMS: *m/z* 218 ($[M + 1^+]$) and 235 ($[M + 18]^+$). Anal. Calcd for $C_{10}H_{19}NO_4$: C, 55.28; H, 8.81; N, 6.45. Found: C, 55.04; H, 8.85; N, 6.40.

Methyl *4-(4-*O*-acetyl-3-deoxy-*L*-glycero-tetronamido)-4,6-dideoxy-2,3-*O*-isopropylidene-α-*D*-mannopyranoside* (**8**).—A solution of the amine **15** (390 mg, 1.8 mmol) and the lactone **18** (390 mg, 2.7 mmol) in pyridine (0.9 mL) was heated in a screw-capped flask at 100–110°C for 16 h. TLC (solvent *F*) showed that one major product was formed. One of the byproducts showed higher chromatographic mobility than the major product and three byproducts had lower mobility. The dark solution was concentrated, and the residue was chromatographed to give first methyl 4,6-dideoxy-4-(2,4-di-*O*-acetyl-3-deoxy-*L*-*glycero*-tetronamido)-2,3-*O*-isopropylidene-α-*D*-mannopyranoside (**9**). Its ^1H NMR spectrum ($CDCl_3$) showed definite, structurally significant signals at δ 6.32 (d, 1 H, $J_{4,NH}$ 9.3 Hz, NH), 5.25 (dd, 1 H, $J_{2',3'a}$ 4.7, $J_{2',3'b}$ 7.7 Hz, H-2'), 4.85 (s, 1 H, H-1), 3.40 (s, 3 H, OCH_3),

2.17, 3.06 (2 s, partially overlapped with the multiplet for H-3'ab, ~3 H each, 2 × $COCH_3$), 1.54, 1.34 (2 s, 3 H each, Me_2C), and 1.25 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 109.78 (Me_2C), 98.46 (C-1), 74.85, 74.42 (C-2,3), 71.16 (C-2'), 66.06 (C-5), 59.93 (C-4'), 55.10 (OCH_3), 52.80 (C-4), 30.68 (C-3'), 27.48, 25.93 (Me_2C), 20.75 (2 C, CH_3CO), 18.32 (C-6); CIMS: *m/z* 421 ($[M + 18]^+$), 404 ($[M + 1]^+$) and 421 ($[MH - 32]^+$).

Eluted next was the major product (**8**) resulting from acetyl group migration (390 g, 60%); $[\alpha]_D$ +3.2° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.88 (d, 1 H, $J_{4,NH}$ 8.6 Hz, NH), 4.88 (s, 1 H, H-1), 4.35–4.43 (m, 1 H, H-4'a), 4.08–4.21 (m, 4 H, H-2',4'b,2,3), 3.76–3.87 (m, 2 H, H-4,5), 3.73 (d, 1 H, $J_{2',OH}$ 5.1 Hz, OH), 3.34 (s, 3 H, OCH_3), 2.21–2.31 (m, 1 H, H-3'a), 2.08 (s, 3 H, $COCH_3$), 1.85–1.95 (m, 1 H, H-3'b), 1.55, 1.33 (2 s, 6 H, 2 Me_2C), and 1.23 (d, 3 H, $J_{5,6}$ 5.8, H-6); ^{13}C NMR ($CDCl_3$): δ 173.32, 172.06 (2 CO), 109.63 (Me_2C), 98.19 (C-1), 75.24 (C-3), 74.71 (C-2), 69.23 (C-2'), 65.50 (C-5), 61.08 (C-4'), 54.95 (OCH_3), 53.50 (C-4), 33.70 (C-3'), 27.67, 26.06 (Me_2C), 20.83 ($COCH_3$), and 17.85 (C-6). Anal. Calcd for $C_{16}H_{27}NO_8$: C, 53.18; H, 7.53; N, 3.88. Found: C, 52.92; H, 7.57; N, 3.90.

Eluted next was methyl 4-acetamido-4,6-dideoxy-2,3-*O*-isopropylidene-α-D-mannopyranoside (**16**), the ^1H and ^{13}C NMR spectral data for which agreed with those found for the authentic sample described below; *m/z* 277 ($[M + 18]^+$), 260 ($[M + 1]^+$), 228 ($[MH - 32]^+$).

Next eluted was methyl 4-(2-*O*-acetyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2,3-*O*-isopropylidene-α-D-mannopyranoside (**10**). Structurally significant signals in the ^1H NMR spectrum ($CDCl_3$) were at δ 6.60 (d, 1 H, $J_{4,NH}$ 7.8 Hz, NH), 4.86 (s, 1 H, H-1), 3.40 (s, 3 H, OCH_3), 2.17 (s, 3 H, $COCH_3$), 1.54, 1.37 (2 s, 3 H each, Me_2C), and 1.24 (d, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 109.77 (Me_2C), 98.30 (C-1), 74.86, 74.51 (C-2,3), 71.81 (C-2'), 65.70 (C-5), 58.15 (C-4'), 55.02 (OCH_3), 53.12 (C-4), 34.65 (C-3'), 27.49, 25.95 (Me_2C), 20.80 (CH_3CO), and 18.04 (C-6); CIMS: *m/z* 379 ($[M + 18]^+$), 362 ($[M + 1]^+$), and 330 ($[MH - 32]^+$).

Eluted last was methyl 4,6-dideoxy-4-(3-deoxy-L-*glycero*-tetronamido)-2,3-*O*-isopropylidene-α-D-mannopyranoside (**11**). Structurally significant signals in the ^1H NMR spectrum ($CDCl_3$) were at δ 7.21 (d, 1 H, $J_{4,NH}$ 9.01, NH), 4.89 (s, 1 H, H-1), 3.39 (s, 3 H, OCH_3), 1.55, 1.34 (2 s, 3 H each, (Me_2C), and 1.22 (d, 3 H, $J_{5,6}$ 5.9 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 109.60 (Me_2C), 98.06 (C-1), 75.34, 74.76 (C-2,3), 71.15 (C-2'), 65.33 (C-5), 59.90 (C-4'), 54.88 (OCH_3), 53.48 (C-4), 35.78 (C-3'), 27.64, 26.10 (Me_2C), and 17.58 (C-6); CIMS: *m/z* 379 ($[M + 18]^+$), 362 ($[M + 1]^+$), and 330 ($[MH - 32]^+$).

Essentially the same results were obtained when the acylation reaction was conducted in the absence of pyridine.

Methyl 4-acetamido-4,6-dideoxy-2,3-O-isopropylidene-α-D-mannopyranoside (**16**). —A solution of the amine **15** (0.4 g) was treated, overnight at room temperature, with 1:1 pyridine–Ac_2O reagent (2 mL). The mixture was processed conventionally, and the crude product was chromatographed (solvent *G*) to give pure **16** (0.4 g, 93%); mp 126–127°C (from diisopropyl ether); $[\alpha]_D$ +40° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.02 (d, 1 H, $J_{4,NH}$ 9.1 Hz, NH), 4.87 (s, 1 H, H-1), 4.12 (dd, 1 H, $J_{2,3}$ 5.3, $J_{3,4}$ 7.9 Hz, H-3), 4.07 (d, 1 H, H-2), 3.85 (m, 1 H, H-4), 3.72 (m, 1 H, H-5),

3.38 (s, 3 H, CH_3), 2.0 (s, 3 H, $NHCOCH_3$), 1.55, 1.34 (2 s, (Me_2C), and 1.24 (d, 3 H, $J_{5,6}$ 6.5 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 169.97 (CO), 109.40 (Me_2C), 98.06 (C-1), 75.33 (C-3), 74.68 (C-2), 65.72 (C-5), 54.95 (OCH_3), 53.57 (C-4), 27.76, 26.20 (Me_2C), and 23.50 ($NHCOCH_3$). Anal. Calcd for $C_{12}H_{21}NO_5$: C, 55.58; H, 8.16; N, 5.40. Found: C, 55.63; H, 8.18; N, 5.38.

*Methyl 4-acetamido-4,6-dideoxy-α-*D*-mannopyranoside* (**13**).—A solution of the amide **16** (0.3 g) in aq 80% AcOH (5 mL) was heated at 80°C until TLC (15:1 CH_2Cl_2–MeOH) showed that all starting material was consumed. After concentration and chromatography, crystallization from MeOH gave the title compound **13** (0.2 g, 80%); mp 184–186°C, lit. [6] mp 185–186°C. The ^1H NMR characteristics observed for **13** agreed with those reported [6].

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