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Synthesis and crystal structure of methyl 4,6-dideoxy-4-(3-deoxy-L-*glycero*-tetronamido)-2-*O*-methyl-α-D-mannopyranoside, the methyl α-glycoside of the terminal unit, and presumed antigenic determinant, of the O-specific polysaccharide of *Vibrio cholerae* O:1, serotype Ogawa ☆

Ping-sheng Lei [a], Yuji Ogawa [a,1], Judith L. Flippen-Anderson [b], Pavol Kováč [a,*]

[a] *NIDDK, National Institutes of Health, Bethesda, MD 20892, USA*

[b] *Laboratory for the Structure of Matter, Code 6030, Naval Research Laboratory, 4555 Overlook Ave., SW, Washington, DC 20375-5000, USA*

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Abstract

Methyl 4-azido-4,6-dideoxy-3-*O*-benzyl-α-D-mannopyranoside and its analogous 3-*O*-(4-methoxybenzyl) derivative were methylated and the 2-*O*-methyl derivatives formed were converted into methyl 4-amino-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranoside. Reaction of the latter with 3-deoxy-L-*glycero*-tetronolactone gave the methyl glycoside of 4,6-dideoxy-4-(3-deoxy-L-*glycero*-tetronamido)-2-*O*-methyl-α-D-mannopyranose, the monosaccharide that is reported to be the terminal moiety of the O-specific polysaccharide of *Vibrio cholerae* O:1, serotype Ogawa. The unit cell packing of the compound, which crystallized as a monohydrate, differs from that of the previously described crystalline compound lacking the 2-*O*-methyl group. The unmethylated sugar is the terminal moiety of the O-specific polysaccharide of *Vibrio cholerae* O:1, serotype

☆ Synthesis of ligands related to the *Vibrio cholerae* O-specific antigen. Part 5. For Part 4, see ref. [1].

* Corresponding author.

[1] On leave from Fuji Chemical Industries, Ltd., 530 Chokeiji, Takaoka, Toyama 933, Japan.

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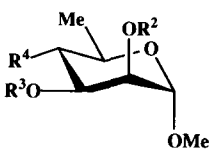
Inaba. The crystal structure of methyl 4,6-dideoxy-2-*O*-methyl-4-trifluoroacetamido-α-D-mannopyranoside is also described.

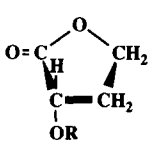
Keywords: O-Polysaccharide; *Vibrio cholerae* O:1, serotype Ogawa; α-D-Mannopyranoside, methyl 4,6-dideoxy-4-(3-deoxy-L-*glycero*-tetronamido)-2-*O*-methyl; Synthesis; Antigenic determinant

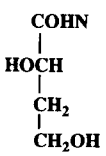
1. Introduction

The serological specificity of Gram-negative bacteria often resides in one of the structural constituents of their surface lipopolysaccharide (LPS), namely the O-specific polysaccharide (O-SP), or O-antigen. It appeared until recently [2] that the O-SPs of the two main strains of *Vibrio cholerae* O:1, Ogawa and Inaba, were the same. The polysaccharide was believed to consist of a relatively short [3] chain ($dp < 20$) of (1 → 2)-linked 4-amino-4,6-dideoxy-α-D-mannopyranose (D-perosamine) units, the amino groups of which were acylated with 3-deoxy-L-*glycero*-tetronic acid. Two more recent studies [4,5] showed, however, that the O-SPs of the two strains differ in that the terminal 4-*N*-tetronylated-D-perosaminyl group in the O-SP of the Ogawa strain is methylated at O-2.

In view of the continued occurrence of explosive epidemics and periodic pandemics of cholera [6], the almost century-long effort directed towards developing a safe, practical, and effective vaccine and new, reliable diagnostic tests for this disease has not diminished [7–9]. In this context, we [1,10–12] and others [13] have been interested in synthesizing ligands related to the O-SP of *V. cholerae* O:1 and evaluating their properties. Here, we describe the synthesis and crystal structure of the methyl α-glyco-







	R^2	R^3	R^4
1	H	Bn	N_3
2	Me	Bn	N_3
3	H	H	N_3
4	MBn	MBn	N_3
5	MBn	H	N_3
6	H	MBn	N_3
7	Me	MBn	N_3
8	Me	H	N_3
9	Me	H	NH_2
10	Me	Bn	NH_2
11	Me	H	A
12	H	H	A
13	Me	H	CF_3CONH

	R
14	H
15	Ac

A

MBn = 4-methoxybenzyl

side of the monosaccharide derivative which might be [4,5] the terminal antigenic determinant of the O-SP of *V. cholerae* O:1, serotype Ogawa.

2. Results and discussion

Synthesis.—The methyl group at position 2 in methyl α-D-perosaminide was introduced using methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside [14] (**1**) as the starting material, although in some preparations methyl 4-azido-4,6-dideoxy-3-*O*-(4-methoxybenzyl)-α-D-mannopyranoside (**6**) was used. The latter is formed during phase transfer-mediated [15] 4-methoxybenzylation of **3**, along with the 2-*O*-(4-methoxyben-

Table 1
Crystal and refinement data

	11	**13**
Formula	$C_{12}H_{23}NO_7 \cdot H_2O$	$C_{10}H_{16}NO_5F_3$
Formula weight	311.33	287.2
Crystal color, habit	Colorless, prism	Colorless, plate
Crystal size (mm)	0.12×0.26×0.48	0.10×0.20×0.40
Crystal system	Orthorhombic	Monoclinic
Space group	$P2_12_12_1$	$P2_1$
a (Å)	7.547(1)	10.882(11)
b (Å)	7.982(1)	5.271(4)
c (Å)	26.179(4)	12.155(10)
α (deg)	90	90
β (deg)	90	104.60(7)
γ (deg)	90	90
V (Å^3)	1577.0(4)	675(1)
Z	4	2
D_{calcd} (g cm^{-3})	1.311	1.404
μ, absorption coeff (cm^{-1})	0.09	0.12
$F(000)$	672	296
Temp (°C)	−50	22
Diffractometer	Siemens R3m/V	Siemens R3m/V
Reflections used for cell determination, 2θ range	25, 50–76	25, 43–55
λ, wavelength (Å)	Cu$K\alpha$, 1.54178	Cu$K\alpha$, 1.54178
2θ max (deg), scan mode	112, q/2q	112, q/2q
Total reflections measured	1402	1093
Unique data, R_{int}	1351, 0.014	999, 0.030
Refinement on F^2 using all data		
Observed data ($I > 2\sigma I$)	1304	713
Parameters refined	209	206
R [a], wR [b]	0.033, 0.090	0.070, 0.158
R [a], wR [b], S [c] (for all data)	0.035, 0.092, 1.04	0.101, 0.184, 1.16
Fourier excursions, e Å^{-3}	0.17, −0.16	0.24, −0.19

[a] $\Sigma||F_o| - |F_c||/\Sigma|F_o|$.
[b] $[\Sigma w(F_o^2 - F_c^2)^2/\Sigma(wF_o^2)^2]^{1/2}$.
[c] $[\Sigma w(F_o^2 - F_c^2)^2/\Sigma(N_o - N_p)]^{1/2}$.

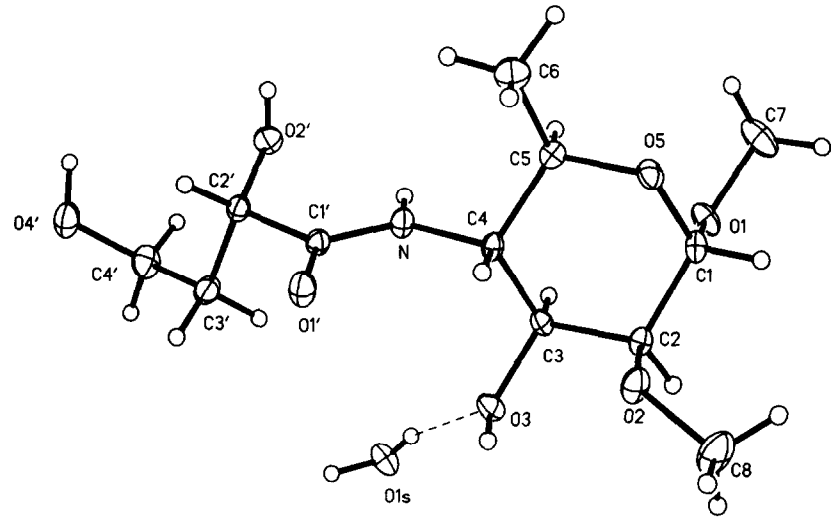


Fig. 1. Crystal structure of **11**. The figure is drawn using the experimentally determined coordinates with anisotropic thermal parameters shown at the 20% probability level. The cocrystallized water molecule is also shown, as is the one hydrogen bond within the asymmetric unit. All other hydrogen bonds involve symmetry related molecules.

zyl) isomer **5**, the preparation of which we carried out in connection with related syntheses. Methylation of **1** and **6** by the method of Fügedi and Nánási [16] gave the desired derivatives **2** and **7** in excellent (> 90%) yields. The azido → amino conversions

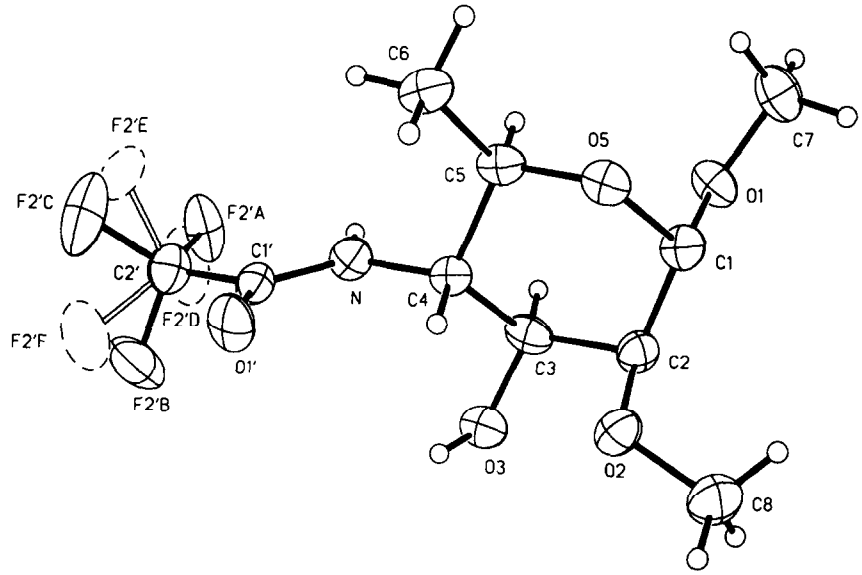


Fig. 2. Crystal structure of **13**. The figure is drawn using the experimentally determined coordinates with anisotropic thermal parameters shown at the 20% probability level. The disordered fluorine atoms are also shown.

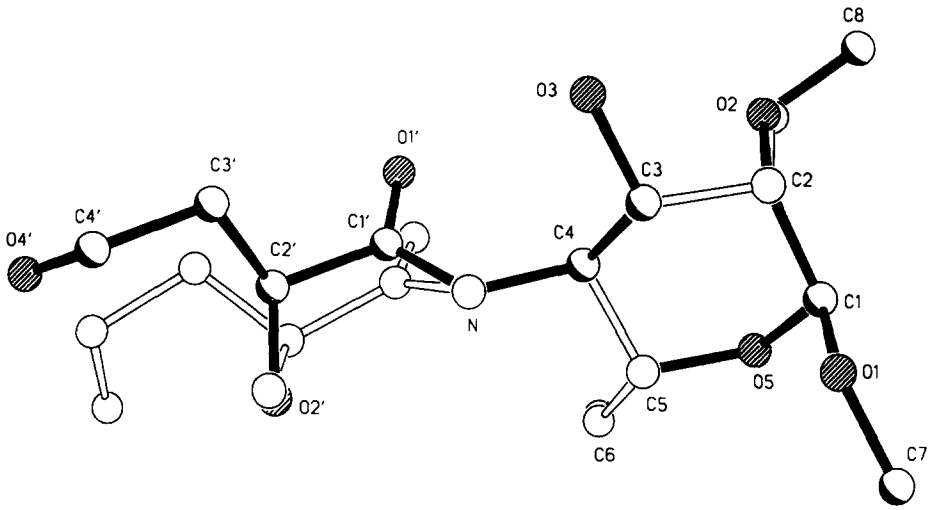


Fig. 3. Comparison of the conformations of **11** and **12**. The six ring atoms were used to perform the least-squares fit. Molecule **11** is shown with solid bonds.

were achieved either by catalytic hydrogenolysis (**2** → **9**) or by reduction with hydrogen sulfide (**8** → **9**).

Reaction of the amine **9** with the lactone [11] **14** consistently afforded the desired

Table 2
Atomic coordinates and equivalent isotropic displacement parameters for **11**

	x	y	z	U_{eq} (Å^2) [a]
C-1	10368(4)×10^{-4}	10738(4)×10^{-4}	4453(1)×10^{-4}	33(1)×10^{-3}
O-1	12066(3)	10523(3)	4239(1)	42(1)
C-2	9169(4)	9425(4)	4220(1)	31(1)
O-2	7488(3)	9462(3)	4463(1)	42(1)
C-3	8878(4)	9783(3)	3658(1)	28(1)
O-3	7785(3)	8561(3)	3421(1)	38(1)
C-4	8161(4)	11562(3)	3600(1)	28(1)
C-5	9376(4)	12814(3)	3859(1)	36(1)
O-5	9701(3)	12364(2)	4382(1)	37(1)
C-6	8613(7)	14562(4)	3878(2)	66(1)
C-7	13392(5)	11626(6)	4449(2)	68(1)
C-8	7327(6)	8448(6)	4902(1)	72(1)
N	7975(3)	12018(3)	3063(1)	34(1)
C-1′	6436(4)	12261(3)	2831(1)	30(1)
O-1′	4992(3)	12059(3)	3038(1)	43(1)
C-2′	6575(4)	12770(3)	2270(1)	31(1)
O-2′	8263(3)	13451(3)	2158(1)	40(1)
C-3′	6290(5)	11230(4)	1939(1)	41(1)
C-4′	6670(5)	11542(4)	1380(1)	49(1)
O-4′	5615(3)	12829(3)	1171(1)	53(1)
O-1s	8269(3)	6851(3)	2416(1)	47(1)

[a] U_{eq} is defined as one third of the trace of the orthogonalized U_{ij} tensor.

Table 3
Atomic coordinates and equivalent isotropic displacement parameters for **13**

	x	y	z	U_{eq} (Å^2) [a]
C-1	1965(10)×10^{-4}	5475(20)×10^{-4}	7403(8)×10^{-4}	65(3)×10^{-3}
O-1	1877(7)	8088(14)	7108(6)	72(2)
C-2	2888(10)	5301(24)	8549(9)	68(3)
O-2	2837(7)	2733(16)	8965(6)	75(2)
C-3	4239(9)	5558(21)	8468(8)	68(3)
O-3	5120(8)	5023(15)	9518(7)	79(2)
C-4	4506(8)	3847(20)	7554(7)	53(2)
C-5	3553(10)	4378(20)	6417(8)	64(3)
O-5	2298(6)	4000(14)	6531(5)	66(2)
C-6	3701(10)	2667(23)	5449(8)	77(3)
C-7	866(10)	8610(24)	6178(10)	89(4)
C-8	1891(11)	2359(31)	9541(10)	107(4)
N	5804(8)	4124(19)	7464(7)	66(2)
C-1′	6562(12)	2183(27)	7455(9)	70(3)
O-1′	6272(8)	−76(16)	7483(8)	90(3)
C-2′	7872(14)	2770(25)	7441(10)	89(4)
F-2′A	8066(37)	5248(27)	7365(47)	108(12)
F-2′B	8629(35)	2143(119)	8449(36)	153(18)
F-2′C	8345(55)	1656(99)	6683(49)	170(20)
F-2′D	8444(47)	4997(65)	7777(45)	170(22)
F-2′E	7955(40)	2588(110)	6349(19)	124(13)
F-2′F	8776(31)	1091(83)	7954(53)	160(16)

[a] U_{eq} is defined as one third of the trace of the orthogonalized U_{ij} tensor.

ligand **11** in over 80% yields. In this context, it seems worth emphasizing the importance of careful removal of trifluoroacetic acid (TFAA), the catalyst used during preparation [11] of **14** from **15**. This can be achieved by evaporation (at least three times) of water from crude **14**. When the acylation of **9** is performed with **14** that is contaminated with residual TFAA, a small amount of the trifluoroacetamido derivative (**13**) is formed along with **11**. The structure of **13**, indicated by mass spectroscopy and ^{19}F NMR spectroscopy (but not so clearly by ^{1}H or conventional ^{13}C NMR spectroscopy), was elucidated by X-ray crystallographic analysis. Due to the loss of NOE enhancement, signals for the quaternary carbons of the $COCF_3$ group were not observed in a routine (see Experimental) ^{13}C NMR spectrum of **13**. The requisite signals were recorded after acquiring 8000 transients.

Crystalline **11** was the monohydrate, as corroborated by analytical data and X-ray analysis. X-ray analysis also revealed other characteristics in which **11** differs from its non-methylated analog [10] **12**.

Crystallography.—The X-ray structure of **12**, the C-2-hydroxy analog of **11**, has been reported [10]. Crystallographic data for **11** and **13** are listed in Table 1, and the structures are illustrated in Figs 1 and 2. Coordinates for the non-hydrogen atoms are listed in Tables 2 and 3. The 6-membered ring is in a normal $^{4}C_1$ chair conformation in all three compounds, and the conformations (Fig. 3) of the extended side chains in **11** and **12** show general agreement (for torsion angles, see Table 4). In **12**, the amide bond

Table 4
Pertinent torsion angles

Angle	Magnitudes (deg)		
	11	**12** [a]	**13**
C-1–C-2–C-3–C-4	−55.3	−56.2	−48.5
C-2–C-3–C-4–C-5	54.5	56.6	55.1
C-3–C-4–C-5–O-5	−53.5	−57.1	−57.9
C-4–C-5–O-5–C-1	54.9	59.2	56.5
C-5–O-5–C-1–C-2	−56.2	−58.5	−51.4
O-5–C-1–C-2–C-3	56.1	56.1	45.8
C-2–C-1–O-1–C-7	−176.4	−176.4	−167.6
C-1–C-2–O-2–C-8	−88.3		−86.4
O-2–C-2–C-3–O-3	−61.3	−58.4	−55.7
C-1–O-5–C-5–C-6	178.0	−178.7	−179.6
N-C-4-C-5-C-6	65.2	63.7	58.6
C-5–C-4–N–C-1	−125.7	−96.6	−106.3
C-4–N–C-1′–C-2′	178.7	164.1	−175.8
C-4–N–C-1′–O-1′	−3.2	−12.5	2.5
N–C-1′–C-2′–C-3′	98.8	130.9	
O-1′–C-1′–C-2′–O-2′	160.9	−176.5	
C-1′–C-2′–C-3′–C-4′	−171.4	169.8	
C-2′–C-3′–C-4′–O-4′	−58.7	−60.5	
O-2′–C-2′–C-3′–C-4′	−50.0	−64.1	

[a] Data for compound **12** were taken from ref. [11].

is *trans* but not quite planar [10] (average deviation of atoms from the C-4–N–C-1′–O-1′ plane is 0.035 Å). In both **11** and **13** the amide bond is *trans* and planar (average deviation of atoms from the C-4–N–C-1′–O-1′ plane is 0.007 Å for **13** and 0.009 Å for **11**). In **13**, the CF_3 moiety is disordered, with two positions for each fluorine atom such that the two sets of fluorine atoms are rotated by approximately 60° from one another.

Table 5
Hydrogen bond parameters for **11** and **13**

Donor	Acceptor	Symmetry of acceptor	D–H (Å)	H ··· A (Å)	D–H ··· A (deg)	D ··· A (Å)	
11							
N	O–1s	$2-x, 0.5+y, 0.5-z$	0.802	2.326	163.2	3.102	3-centered
N	O–2′	Intramolecular	0.802	2.259	109.8	2.641	3-centered
O–3	O–4	$1-x, -0.5+y, 0.5-z$	0.862	1.995	166.0	2.839	
O–2′	O–1s	$x, 1+y, z$	0.825	1.975	174.0	2.797	
O–4′	O–1	$2-x, 0.5+y, 0.5-z$	0.897	2.098	164.9	2.974	
O–1s	O–3		0.723	2.305	157.9	2.988	
O–1s	O–1′	$1-x, -0.5+y, 0.5-z$	0.932	1.816	170.2	2.738	
13							
N	O–1′	$x, 1+y, z$	0.860	2.272	161.0	3.098	3-centered
N	F–2′A	Intramolecular	0.860	2.173	107.2	2.563	3-centered
O–3	O–3	$1-x, 0.5+y, 2-z$	0.850	2.381	122.6	2.922	3-centered
O–3	O–2	$1-x, 0.5+y, 2-z$	0.850	2.154	143.9	2.884	3-centered

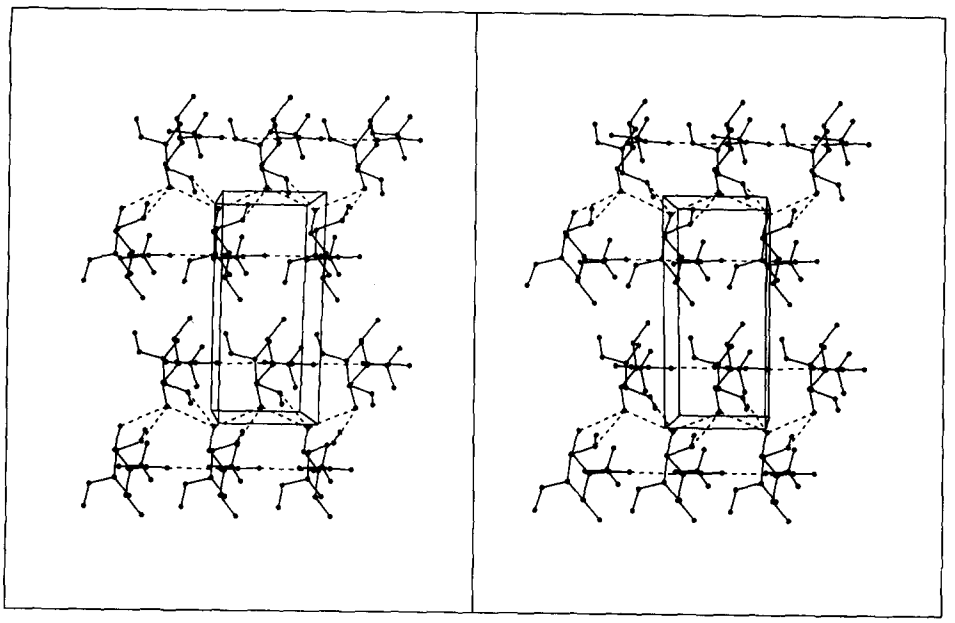


Fig. 4. Stereo drawing of the unit cell of **11**. The figure is drawn looking down the *a* axis.

The occupancy ratio is 1:1. The amide H in all three molecules is involved in a 3-centered hydrogen bond with two acceptor atoms. One of the acceptor · · · H linkages is intramolecular and the other is intermolecular (see Table 5 for a list of hydrogen bonds in **11** and **13**). The cocrystallized water molecule in **11** is heavily involved in

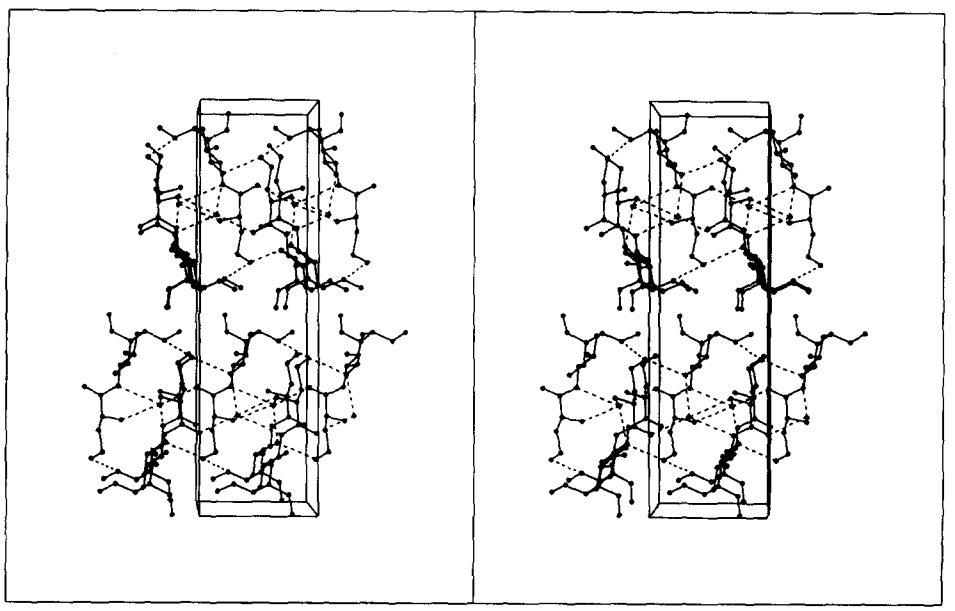


Fig. 5. Stereo drawing of the unit cell of **13**. The figure is drawn looking down the *b* axis.

hydrogen bonding, such that the unit-cell packing of **11** differs from that observed in **12**, with an O-4′ · · · O-1 interaction being the only one common to the two structures. The unit-cell packing of **11** is illustrated in Fig. 4. In **13** there is an additional 3-centered hydrogen bond linking the molecules in infinite columns that spiral around a crystallographic 2-fold screw axis. In this case, O-3 is the donor and both O-3 and O-2 act as acceptors (see Fig. 5).

3. Experimental

General.—Thin-layer chromatography (TLC) was performed with *A*, 8:1 toluene–EtOAc; *B*, 6:1 hexane–EtOAc; *C*, 8:1 CH_2Cl_2–MeOH. Detection was effected with iodine vapor and, where applicable, by charring with 5% sulfuric acid in ethanol or with UV light. 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, 75 MHz for ^{13}C, and 282.2 MHz for ^{19}F. The measurements were done at ambient temperature, using a Varian XL 300 or a Varian Gemini spectrometer. For spectra determined in organic solvents chemical shifts are reported in ppm downfield of the signal of Me_4Si (for ^1H and ^{13}C), or of C_6F_6 (for ^{19}F); ^1H shifts determined in D_2O were measured from the signal of HOD (δ 4.78). Routinely, the numbers of transients (scans) acquired were 4 and 128 for ^1H and ^{13}C spectra, respectively. The ^{13}C shifts were measured relative to the signal of $CDCl_3$ (δ 77.0), benzene (δ 128.0), or MeOH (δ 49.0). Assignments of NMR signals were made by first-order analysis of the spectra, and by comparison with spectra of related substances. When feasible, the assignments were supported by homonuclear decoupling experiments or homonuclear and heteronuclear 2-dimensional correlation spectroscopy, run with the software supplied with the spectrometers. Chemical ionization mass spectra (CIMS) were measured using ammonia as the reactive gas. Solutions in organic solvents were dried with anhydrous sodium sulfate, and concentrated at 40°/2 kPa.

The crystallographic data for **11** and **13** were collected on a computer-controlled automatic diffractometer and corrected for Lorentz and polarization effects but not for absorption effects. The structures were solved by direct methods with the aid of the program SHELXTL [17], which includes the graphics program used to generate Figs 1 and 2. The structures were refined by full-matrix least-squares on F^2 values using the program SHELXLS93 [18]. The parameters refined included the coordinates and anisotropic thermal parameters for all non-hydrogen atoms. For the most part, hydrogen atoms were included using a riding model, in which the coordinate shifts of their covalently bonded atoms were applied with C–H = 0.96 Å, N–H = 0.91 Å and O–H = 0.82 Å. Hydrogen angles were idealized and U_{iso}H values were set at fixed ratios to the U_{iso} values of the bonded atoms. In compound **11**, coordinates were refined for H atoms bonded to nitrogen and oxygen atoms. Additional experimental and structure analysis details are given in Table 1.[2]

[2] 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 1EW, UK.

Methyl 4-azido-4,6-dideoxy-2,3-di-O-(**4**), *-2*-O-(**5**), *and -3*-O-*(4-methoxybenzyl)-α*-D-*mannopyranoside* (**6**).—4-Methoxybenzyl chloride (22 mL, 162 mmol) was added to a mixture of aq 20% sodium hydroxide (300 mL) with the azide **3** [1,11,14] (15 g, 74 mmol) and tetrabutylammonium bromide (4.8 g, 14.8 mmol) in dichloromethane (300 mL). The mixture was stirred for 24 h, when TLC (solvent A) showed that all starting material was consumed. The phases were separated, and the aqueous phase was extracted with dichloromethane. The extracts were combined and washed with water. After drying and concentration, the residue was chromatographed to give first the di-*O*-(4-methoxybenzyl) derivative **4** (4.4 g, 13%), $[\alpha]_D$ +85° (*c* 1.2); ^{1}H NMR ($CDCl_3$): δ 4.66–4.57 (m, 3 H, H-1, CH_2Ph), 4.49 (s, 2 H, CH_2Ph), 3.81, 3.79 (2 s, 6 H, 2 PhOCH_3), 3.69 (m, partially overlapped, H-2), 3.68 (dd, partially overlapped, $J_{2,3}$ 3.2, $J_{3,4}$ 10.4 Hz, H-3), 3.55 (t, 1 H, H-4), 3.49–3.39 (m, 1 H, H-5), 3.28 (s, 3 H, OCH_3), and 1.32 (d, 3 H, $J_{5,6}$ 6.0 Hz, H-6); ^{13}C-NMR ($CDCl_3$): δ 99.18 (C-1), 78.00 (C-3), 72.66 (C-2), 72.39, 71.49 (2 *C*H_2Ph), 67.06 (C-5), 64.26 (C-4), 55.25 (2 C, 2 *C*H_3OPh), 54.81 (OCH_3), and 18.53 (C-6); ^{1}H NMR (C_6D_6): δ 4.65 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.57, 4.48, 4.41, 4.38 (4 d, 4 H, ^2J 11.6 Hz, 2 CH_2Ph), 3.87 (dd, partially overlapped $J_{2,3}$ 2.6, $J_{3,4}$ 9.8 Hz, H-3), 3.81 (t, partially overlapped, H-4), 3.74 (dd, 1 H, H-2), 3.56–3.36 (m, 1 H, H-5), 3.27, 3.28 (2 s, 6 H, 2 PhOCH_3), 2.98 (s, 3 H, OCH_3), and 1.26 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR (C_6D_6): δ 99.62 (C-1), 78.77 (C-3), 73.84 (C-2), 72.91, 71.59 (2 *C*H_2Ph), 67.57 (C-5), 64.93 (C-4), 54.74 (2 C, 2 PhO*C*H_3), 54.43 (OCH_3), 18.71 (C-6); CIMS: *m*/*z* 461 ([M + 18]$^+$). Anal. Calcd for $C_{23}H_{29}N_3O_6$: C, 62.29; H, 6.59; N, 9.47. Found: C, 62.40; H, 6.57; N, 9.44.

Next eluted was the 2-*O*-(4-methoxybenzyl) derivative **5** (16.7 g, 70%), $[\alpha]_D$ +5.8° (*c* 1.2); ^{1}H NMR ($CDCl_3$): δ 4.71 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1), 4.68 4.46 (2 d, 2 H ^2J 11.3 Hz, CH_2Ph), 3.80 (m, 4 H, H-3, PhOCH_3), 3.87 (dd, 1 H, $J_{2,3}$ 3.7 Hz, H-2), 3.47 (m, 1 H, H-5), 3.32 (s, 3 H, OCH_3), 3.22 (t, 1 H, *J* 9.9 Hz, H-4), 2.40 (d, 1 H, $J_{3,OH}$ 10.5 Hz, OH-3), and 1.31 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C-NMR ($CDCl_3$): δ 97.88 (C-1), 77.01 (C-2), 72.69 (*C*H_2Ph), 70.24 (C-3), 66.56 (C-4), 66.34 (C-5), 55.25 (PhO*C*H_3), 54.87 (OCH_3), 18.31 (C-6); CIMS: *m*/*z* 341 ([M + 18]$^+$). Anal. Calcd for $C_{15}H_{21}N_3O_5$: C, 55.72; H, 6.55; N, 13.00. Found: C, 55.83; H, 6.54; N, 12.85.

Eluted next was the 3-*O*-(4-methoxybenzyl) derivative **6** (3.8 g, 16%), $[\alpha]_D$ +145° (*c* 1.25); ^{1}H NMR ($CDCl_3$): δ 4.68 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.61, 4.56 (2 d, 2 H, ^2J 11.2 Hz, CH_2Ph), 3.91 (m, 1 H, H-2), 3.9 (s, 3 H, PhOCH_3), 3.67 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$, 9.5 Hz, H-3), 3.48 (m, 1 H, H-5), 3.32 (s, 3 H, OCH_3), 2.63 (d, 1 H, $J_{2,OH}$ 2.0 Hz, OH), and 1.31 (d, 3 H,$J_{5,6}$ 6.1 Hz, H-6); ^{13}C-NMR ($CDCl_3$): δ 100.07 (C-1), 77.88 (C-3), 71.55 ((*C*H_2Ph), 67.09 (C-2), 66.36 (C-5), 63.83 (C-4), 55.15 (PhO*C*H_3), 54.80 (OCH_3), and 18.30 (C-6); CIMS: *m*/*z* 341 ([M + 18]$^+$). Anal. Calcd for $C_{15}H_{21}N_3O_5$: C, 55.72; H, 6.55; N, 13.00. Found: C, 55.82; H, 6.53; N, 13.00.

Methyl 4-azido-4,6-dideoxy-3-O-*(4-methoxybenzyl)-2*-O-*methyl-α*-D-*mannopyranoside* (**7**).—Sodium hydroxide (powdered, 2 g, 35 mmol) followed by methyl iodide (1.1 mL, 18 mmol) was added to a solution of the foregoing 3-*O*-(4-methoxybenzyl) derivative **6** (3.8 g, 11.8 mmol) in Me_2SO (20 mL). The mixture was stirred at room temperature for 3 h, when TLC (solvent *B*) showed that the reaction was complete. After filtration, water (100 mL) was added to the filtrate and the pH was adjusted to 7 by the addition of acetic acid. The resulting solution was extracted with dichloromethane,

the extract was dried and concentrated, and the material in the residue was chromatographed to give **7** (3.5 g, 89%), $[\alpha]_D$ +129° (*c* 1.5); ^{1}H NMR ($CDCl_3$): δ 4.69 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.62 (s, 2 H, CH_2Ph), 3.81 (s, 3 H, PhOCH_3), 3.69 (dd, 1 H, $J_{2,3}$ 3.4, $J_{3,4}$ 10.0 Hz, H-3), 3.50–3.38 (m, 3 H, H-2,4,5, overlapping s at 3.47,3 H, OCH_3-2), 3.32 (s, 3 H, OCH_3-1), and 1.31 (d, 3 H, $J_{5,6}$ 5.6 Hz, H-6); ^{13}C-NMR ($CDCl_3$): δ 98.40 (C-1), 77.81 (C-3), 76.39 (C-2), 71.61 (*C*H_2Ph), 66.89 (C-5), 64.09 (C-4), 59.34 (OCH_3-2), 55.18 (PhO*C*H_3), 54.83 (OCH_3-1), and 18.46 (C-6); CIMS: *m*/*z* 355 ($[M + 18]^+$), and 338 ($[M + 1]^+$). Anal. Calcd for $C_{16}H_{23}N_3O_5$: C, 56.96; H, 6.87; N, 12.45. Found: C, 56.79; H, 6.91; N, 12.43.

*Methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-2-*O*-methyl-α-*D*-mannopyranoside* (**2**).—Methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-α-D-mannopyranoside [14] (**1**, 9.1 g) was methylated as described for the preparation of **7**. The product was isolated by chromatography to give **2** (9.4 g, 95%), $[\alpha]_D$ +117° (*c* 0.4); ^{1}H NMR ($CDCl_3$): δ 4.70 (d, partially overlapped, $J_{1,2}$ ~ 2.0 Hz, H-1), 4.68 (s, partially overlapped, CH_2Ph), 3.71 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 9.7 Hz, H-3), 3.52–3.40 (m, 3 H, H-2,4,5, overlapping s at 3.47, 3 H, OCH_3-2), 3.32 (s, 3 H, OCH_3-1), and 1.33 (d, 3 H, $J_{5,6}$ 5.9 Hz, H-6); ^{13}C-NMR ($CDCl_3$): δ 98.42 (C-1), 78.24 (C-3), 76.39 (C-2), 72.0 (*C*H_2Ph), 66.95 (C-5), 64.22 (C-4), 59.36 (OCH_3-2), 54.90 (OCH_3-1), and 18.51 (C-6); CIMS: *m*/*z* 325 ($[M + 18]^+$). Anal. Calcd for $C_{15}H_{21}N_3O_4$: C, 58.62; H, 6.89; N, 13.67. Found: C, 58.50; H, 6.92; N, 13.73.

*Methyl 4-azido-4,6-dideoxy-2-*O*-methyl-α-*D*-mannopyranoside* (**8**).—Solid 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 2.40 g, 10.4 mmol) was added with stirring at room temperature to a mixture of compound **7** (3.2 g, 9.5 mmol), dichloromethane (40 mL), and water (2 mL). The stirring was continued for 4 h, when TLC (solvent *B*) showed complete conversion of the starting material to a slower moving product. Dichloromethane (100 mL) was added, the resulting solution was washed with aq Na_2SO_3, dried, and concentrated, and the residue was chromatographed to give **8** (1.8 g, 87%), mp 54.5–55°C (from ethyl acetate–hexane or ether); $[\alpha]_D$ +77.4° (*c* 1.1); ^{1}H NMR ($CDCl_3$): δ 4.76 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 3.82 (dt, 1 H, $J_{2,3}$ 3.7, $J_{3,4} \cong J_{3,OH}$ 10 Hz, H-3), 3.54–3.46 (m, 1 H, H-5, overlapping s at 3.50, 3 H, OCH_3-2), 3.44 (dd, 1 H, H-2), 3.36 (OCH_3-1), 3.19 (t, 1 H, H-4), 2.49 (d, 1 H, OH), and 1.32 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C-NMR ($CDCl_3$): δ 96.98 (C-1), 79.26 (C-2), 70.22 (C-3), 66.45 (C-4), 66.26 (C-5), 58.73 (OCH_3-2), 54.89 (OCH_3-1), and 18.26 (C-6); CIMS: *m*/*z* 235 ($[M + 18]^+$). Anal. Calcd for $C_8H_{15}N_3O_4$: C, 44.23; H, 6.96; N, 19.34. Found: C, 44.29; H, 6.97; N, 19.42.

*Methyl 4,6-dideoxy-4-(3-deoxy-*L*-glycero-tetronamido)-2-*O*-methyl-α-*D*-manno-pyranoside* (**11**) *and methyl 4,6-dideoxy-2-*O*-methyl-4-trifluoroacetamido-α-*D*-manno-pyranoside* (**13**).—a. Hydrogen sulfide gas was passed for 30 min through a solution of the aforementioned azido derivative **8** (1.6 g) in 7:3 pyridine–Et_3N (90 mL). The mixture, contained in a loosely closed flask, was kept overnight at room temperature. TLC (solvent *C*) then showed that the reaction was complete, and that a single product was formed. After concentration, the residue was eluted from a column of silica gel to give methyl 4-amino-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranoside (**9**), ^{1}H NMR ($CDCl_3$): δ 4.75 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 3.57 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 10.0 Hz, H-3), 3.70–3.60 (m, 1 H, H-5), 3.49 (s, OCH_3-2), 3.41 (dd, 1 H, H-2), 3.36 (s, 3 H, OCH_3-1),

and 1.28 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C-NMR ($CDCl_3$): δ 97.64 (C-1), 79.24 (C-2), 69.75 (C-3), 67.71 (C-5), 58.90 (OCH_3-2), 55.63 (C-4), 54.78 (OCH_3-1), and 17.93 (C-6); CIMS: m/z 209 ($[M + 18]^+$) and 192 ($[M + 1]^+$).

b. A mixture of the benzyl derivative **2** (8.2 g) and 5% palladium-on-carbon catalyst (4 g) in ethanol (150 mL) was stirred in a hydrogen atmosphere at atmospheric pressure for 3 days. TLC (solvents *B* and *C*) showed that in addition to the desired **9** two faster-moving minor byproducts, presumably **8** and **10**, were present. After conventional processing and chromatography the amine **9** obtained (4.6 g, 90%) was indistinguishable from the material described in a. The minor byproducts collected during chromatography were treated as described in a to give a little more of **9**, isolated by chromatography.

A solution of lactone **14** (2 g, 20 mmol) and amine **9** (2.5 g, 13.1 mmol) in pyridine (12 mL), contained in a screw-capped flask, was heated overnight at 110°C. TLC (solvent *C*) showed that all the **9** was consumed. After concentration, chromatography gave the desired addition product **11** (3.3 g, 81%), mp 67–68°C (from ethyl acetate); $[\alpha]_D$ +22° (*c* 0.6, water); ^{1}H NMR ($CDCl_3$): δ 7.19 (d, 1 H, $J_{4,NH}$ 8.8 Hz, NH), 5.07 (d, 1 H, *J* 4.9 Hz, OH), 4.77 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 4.34–4.18 (m, 2 H, H-2′, OH), 3.94–3.76 (m, 4 H, H-3,4,4′ab), 3.73–3.62 (m, 1 H, H-5), 3.49 (s, 3 H, OMe-2), 3,44 (bs, 1 H, H-2), 3.37 (s, 3 H, OCH_3-1), 2.78 (s, 1 H, OH), 2.13–1.82 (2 m, 2 H, H-3′a,b), and 1.21 (d, 3 H, $J_{5,6}$ 6.1 Hz, H-6); ^{13}C-NMR ($CDCl_3$): δ 175.85 (CO), 97.59 (C-1), 79.42 (C-2), 70.62 (C-2′), 69.05 (C-3), 66.99 (C-5), 59.33 (C-4′), 58.90 (OCH_3-2), 54.90 (OCH_3-1), 53.97 (C-4), 36.07 (C-3′), and 17.86 (C-6); ^{1}H NMR (D_2O): δ 4.89 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.27 (dd, 1 H, $J_{2',3'a}$ 3.9, $J_{2',3'b}$ 8.5 Hz, H-2′), 3.92 (dd, 1 H,$J_{2,3}$ 3.4, $J_{3,4}$ 10.5 Hz, H-3), 3.87–3.76 (m, 2 H, H-4,5), 3.72 (m, 2 H, H-4′a,b), 3.56 (dd, 1 H, H-2), 3.48 (s, 3 H, OCH_3-2), 3.40 (s, 3 H, OCH_3-1), 2.19–1.79 (2 m, 2 H, H-3′a,b), and 1.17 (d, 3 H, $J_{5,6}$ 5.7 Hz, H-6); ^{13}C NMR (D_2O): δ 177.25 (CO), 97.85 (C-1), 79.13(C-2), 69.08 (C-2′), 67.77 (C-3), 67.16 (C-5), 58.93 (OCH_3-2), 57.93 (C-4′), 54.90 (OCH_3-1), 53.36 (C-4), 36.03 (C-3′), and 16.87 (C-6); CIMS: m/z 294 ($[M + 1]^+$) and 311 ($[M + 18]^+$). Anal. Calcd for $C_{12}H_{23}NO_7 \cdot H_2O$: C, 46.29; H, 8.09; N, 4.50. Found: C, 46.30; H, 8.08; N, 4.50.

When the above described coupling was carried out with synthon **14** containing residual TFAA (vide supra), a fast-moving byproduct was formed along with **11**, as shown by TLC. After isolation by chromatography the byproduct, **13**, showed mp 178–179°C (from ethyl acetate–hexane); $[\alpha]_D$ +42.1° (*c* 0.8); ^{1}H NMR ($CDCl_3$): δ 6.27 (d, 1 H, $J_{NH,4}$ 9.8 Hz, NH), 4.81 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1), 3.96–3.86 (m, 1 H, H-4), 3.75 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 10.2 Hz, H-3), 3.5 (s, 3 H, OCH_3-2), 3.47 (dd, 1 H, H-2), 3.38 (s, 3 H, OCH_3-1), 2.40 (bs, 1 H, OH), and 1.24 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C-NMR ($CDCl_3$): δ 158.92 (q, $J_{C,F}$ 36.9 Hz, CONH), 116.49 (q, $J_{C,F}$ 288.5 Hz, CF_3), 97.05 (C-1), 78.97 (C-2), 68.77 (C-3), 66.55 (C-5), 58.63 (OCH_3-2), 55.26 (C-4), 55.04 (OCH_3-1), and 17.72 (C-6); ^{19}F NMR ($CDCl_3$): δ −89.9; CIMS: m/z 288 ($[M + 1]^+$) and 305 ($[M + 18]^+$). Anal. Calcd for $C_{10}H_{16}F_3NO_5$: C, 41.82; H, 5.61; F, 19.84; N, 4.88. Found: C, 41.60; H, 5.64; F, 19.50; N, 4.80.

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References

[1] M. Gotoh and P. Kováč, *Carbohydr. Res.*, 268 (1995) 73–84.
[2] B. Lindberg, in P.J. Garegg, and A.A. Lindberg (Eds), *Carbohydrate Antigens*, ACS Symposium Series 519, American Chemical Society, Washington, DC, 1993, pp 64–71.
[3] L. Kenne, B. Lindberg, P. Unger, B. Gustafsson, and T. Holme, *Carbohydr. Res.*, 100 (1982) 341–349.
[4] K. Hisatsune, S. Kondo, Y. Isshiki, T. Iguchi, and Y. Haishima, *Biochem. Biophys. Res. Commun.*, 190 (1993) 302–307.
[5] T. Ito, T. Higuchi, M. Hirobe, K. Hiramatsu, and T. Yokota, *Carbohydr. Res.*, 256 (1994) 113–128.
[6] J.B. Kaper and M.M. Levine, in J. Cryz, Jr (Ed.), *Vaccines and Immunotherapy*, Pergamon Press, New York, 1991, pp 73–85.
[7] S.C. Szu, R. Gupta, and J.B. Robbins, in K.I. Wachsmuth, P.A. Blake, and Ø. Olsvik (Eds), *Vibrio cholerae and Cholera: Molecular to Global Perspectives*, American Society for Microbiology, Washington, DC, 1994, pp 381–394.
[8] R.K. Gupta, S.C. Szu, R.A. Finkelstein, and J.B. Robbins, *Infect. Immun.*, 60 (1992) 3201–3208.
[9] L. Carillo, R.H. Gilman, R.E. Mantle, N. Nunez, J. Watanabe, J. Moron, V. Quispe, A. Ramirez-Ramos, and the Loyaza Cholera Working Group in Peru, *J. Clin. Microbiol.*, 32 (1994) 856–857.
[10] M. Gotoh, C.L. Barnes, and P. Kováč, *Carbohydr. Res.*, 260 (1994) 203–218.
[11] M. Gotoh and P. Kováč, *J. Carbohydr. Chem.*, 13 (1994) 1193–1213.
[12] S. Bystrický, S.C. Szu, M. Gotoh, and P. Kováč, *Carbohydr. Res.*, 270 (1995) 115–122.
[13] A.A. Mohar, O.M. Alonso, A.A. Alvarez, and V.V. Bencomo, Abstracts, XVIIth International Carbohydrate Symposium, Ottawa, Canada, 1994, B2.110.
[14] M.J. Eis and B. Ganem, *Carbohydr. Res.*, 176 (1988) 316–323.
[15] T. Peters and D.R. Bundle, *Can. J. Chem.*, 67 (1989) 497–502.
[16] P. Fügedi and P. Nánási, *J. Carbohydr.*, 8 (1981) 547–555.
[17] G.M. Sheldrick, *SHELXTL-Plus*, Siemens Analytical X-ray Instruments, Inc., Madison, WI, USA, 1992.
[18] G.M. Sheldrick, personal communication.