J. CARBOHYDRATE CHEMISTRY, 17(3), 341-357 (1998)

SYNTHESIS OF SOME ANALOGS OF THE METHYL α-GLYCOSIDE OF THE PRESUMED ANTIGENIC DETERMINANT OF THE O-SPECIFIC POLYSACCHARIDE OF *VIBRIO CHOLERAE* O:1, SEROTYPE OGAWA[1]

Jian Zhang and Pavol Kováč

NIDDK, National Institutes of Health, 8 Center Drive, Bethesda, MD 20892-0815 (U.S.A.)

Received July 1, 1997 - Final Form December 4, 1997

ABSTRACT

The following analogs of the title determinant, methyl 4,6-dideoxy-4-(3-deoxy-L-*glycero*-tetronamido)-2-*O*-methyl-α-D-mannopyranoside, have been prepared: methyl 3,4,6-trideoxy-4-(3-deoxy-L-*glycero*-tetronamido)-2-*O*-methyl-α-D-mannopyranoside, methyl 4,6-dideoxy-4-(4-hydroxybutyramido)-2-*O*-methyl-α-D-mannopyranoside, methyl 4,6-dideoxy-4-(3,4-dideoxy-L-*glycero*-tetronamido)-2-*O*-methyl-α-D-mannopyranoside, methyl 4,6-dideoxy-4-(3-deoxy-D-*glycero*-tetronamido)-2-*O*-methyl-α-D-mannopyranoside, methyl 4,6-dideoxy-4-(2-deoxy-L-*glycero*-tetronamido)-2-*O*-methyl-α-D-mannopyranoside, methyl 4-acetamido-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranoside, methyl 4,6-dideoxy-4-(3-deoxy-L-*glycero*-tetronamido)-2-*O*-ethyl-α-D-mannopyranoside, and methyl 4,6-dideoxy-4-(3-deoxy-L-*glycero*-tetronamido)-2-*O*-propyl-α-D-mannopyranoside.

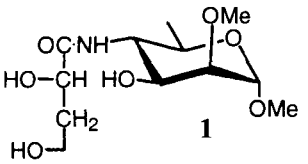
INTRODUCTION

This laboratory has studied the interaction of carbohydrate antigens and antibodies for a number of years.[2] One of the aspects we investigated (*e.g.* ref. 3,4) was

the involvement of hydrogen bonding in the binding process. Using deoxy and deoxyfluoro sugars, we were able to map, in great detail, the combining area of many carbohydrate antigens and antibodies,[2,4,5] including those for *Shigella dysenteria* type 1,[6] a bacterial pathogen causing severe health problems worldwide. Efforts to develop a synthetic immunogen to generate anti *Vibrio cholerae* O1 antibodies, prompted a study of the mode of binding of fragments of the O-antigen (O-PS) of *V. cholerae* O:1 with antibodies raised against the corresponding lipopolysaccharide.

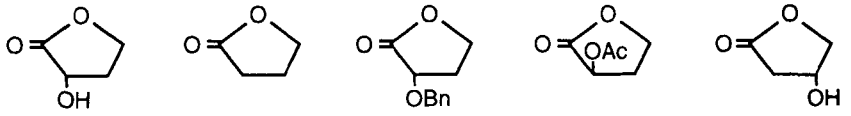
The O-PS of *V. cholerae* O:1, serotype Ogawa consists[7,8] of a chain of α-(1→2)-linked 4,6-dideoxy-4-(3-deoxy-L-*glycero*-tetronamido)-α-D-mannopyranose [4-(3-deoxy-L-*glycero*-tetronamido)-perosamine], in which the position *O*-2 of the upstream, terminal perosamine group is methylated. Our study[9] involving synthetic[10-15] methyl α-glycosides of mono- through hexasaccharide fragments of the O-PS found the terminal α-(1→2)-linked 4,6-dideoxy-4-(3-deoxy-L-*glycero*-tetronamido)-2-*O*-methyl-α-D-manno-pyranosyl group to be the immunologically dominant epitope. Studies aimed at obtaining detailed information on binding in this antigen-antibody system required analogs of this determinant. This paper describes the synthesis of a series of such substances.

RESULTS AND DISCUSSION



1

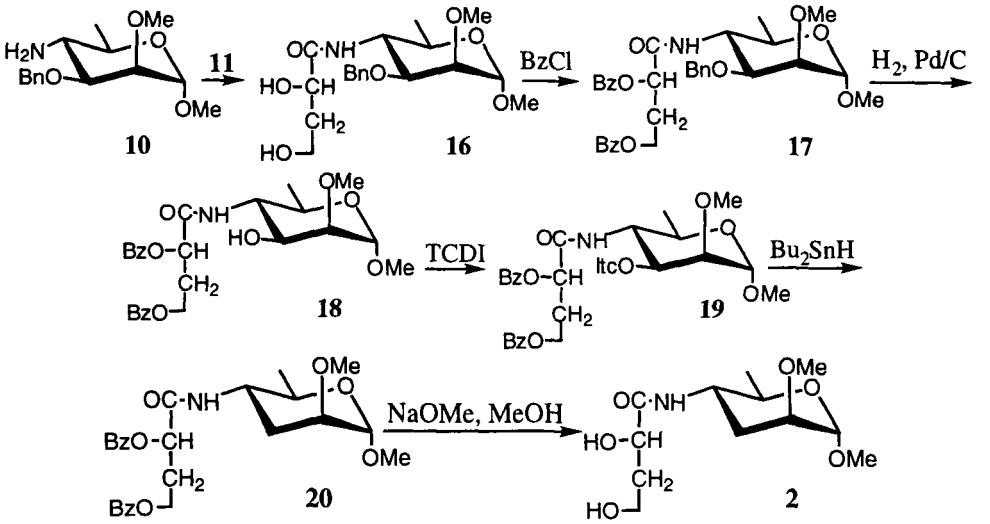
The synthesis of the 2-deoxy analog of the methyl α-glycoside of the determinant epitope (**1**), has been previously reported from this laboratory.[16] To be able to probe the involvement of hydrogen bonding in binding of anti *V. cholerae* O1, serotype Ogawa antibodies, we have now synthesized the three remaining, theoretically possible monodeoxy analogs of **1**, compounds specifically deoxygenated at position 3, 2' or 4' (**2-4**, respectively). Also, to reveal some essential structural requirements for binding, as well as tolerance of the antibody of structural irregularities in the antigen, we have synthesized analogs of **1** in which either the *N*-acyl group was different from that in the natural antigen (**5-7**), or in which the methyl group at position *O*-2 in perosamine was replaced with a larger alkyl group (compounds **8** and **9**).



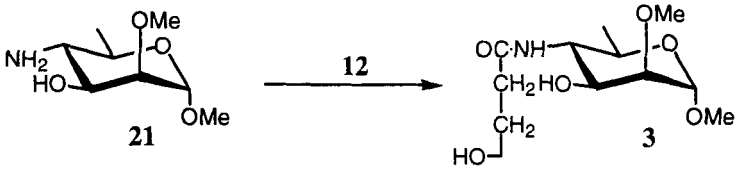
11 **12** **13** **14** **15**

Starting material for the preparation of the 3-deoxy derivative **2** was amine **10**.[16] Treatment of the latter with lactone **11**[14] (→**16**) followed by benzoylation (→**17**) and

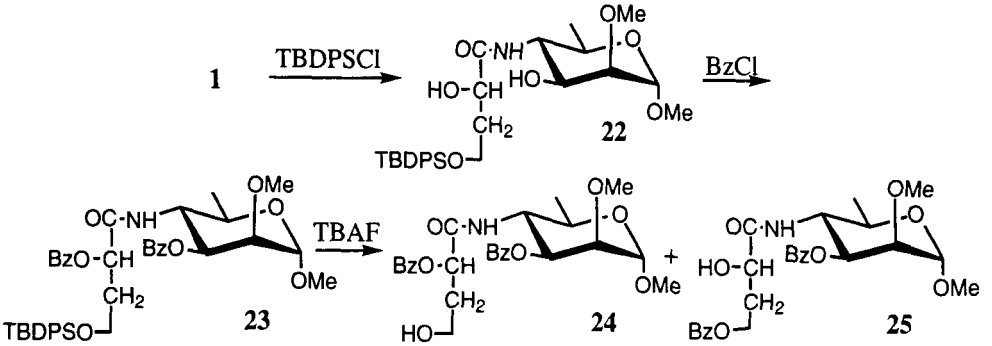
hydrogenolysis gave **18** having only HO-3 unsubstituted. Deoxygenation[17,18] through the imidazoylthiocarbonyl intermediate **19** gave, after debenzoylation, the target derivative **2**.



To obtain the 2'-deoxy- analog **3** of **1**, the known[14] amine **21** was treated with the commercially available γ-butyrolactone (**12**), to give the desired, crystalline compound.



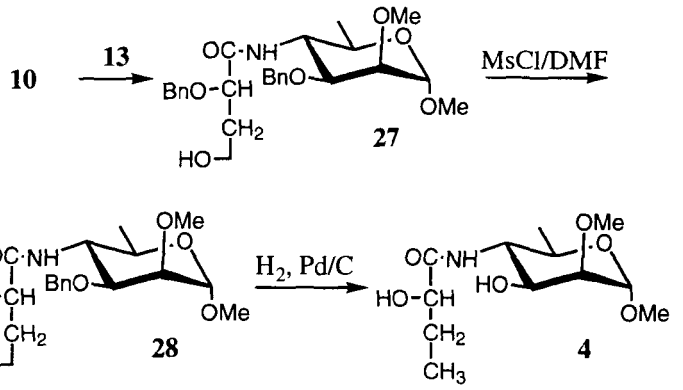
In our first approach to obtaining analog of **1** having position 4' deoxygenated, compound **4**, we wanted to utilize as starting material glycoside **1**, available from our previous work.[14] The primary hydroxyl group in this compound was temporarily protected with *t*-butyldiphenylsilyl (TBDPS) group (**1** → **22**), and subsequent benzoylation gave the expected fully protected compound **23**. Next, in an attempt to



obtain substance **24** having only the primary position unsubstituted, compound **23** was

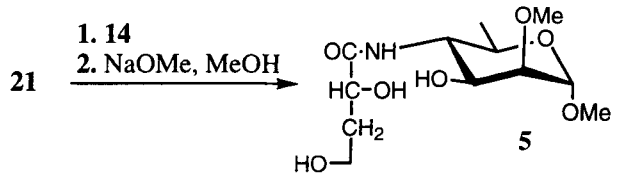
treated with *t*-butylammonium fluoride. Unexpectedly, the NMR spectra of the major product of desilylation of **23** were inconsistent with the expected structure **24**. While in the spectrum of **23** the ^{1}H signals for H-2' and H-3 appeared downfield (δ 5.48 and 5.37, respectively) as expected due to the presence of *O*-benzoyl groups at those positions, the spectrum of the major product of de-*O*-*t*-butyldiphenylsilylation contained in that region the signal of one proton only, that for H-3. Also, signals for the two H-4' protons, which in the spectrum of **23** appeared upfield (δ 3.68–3.53), were shifted downfield ($\delta_{H\text{-}4a}$ 4.37 and $\delta_{H\text{-}4b}$ 4.16), as would be expected for signals of protons which are part of an electron-withdrawing $COOCH_2$ group. At the same time, the signal for H-2' shifted upfield to δ 4.17, from δ 5.48 in the spectrum of **23**. This indicated that the cleavage of silyl ether was accompanied by benzoyl group migration, yielding substance **25** with position HO-2' unsubstituted as the major product. This was confirmed when acetylation of **25** gave **26** (see Experimental), whose ^{1}H NMR spectrum showed the signal of H-2' shifted downfield to δ 5.21. On the other hand, the NMR spectra of the minor product of de-*O*-*t*-butyldiphenylsilylation were consistent with the structure **24**. While we have previously observed AcO-2'→AcO-4' migration in a 3-deoxy-L-*glycero*-tetronic acid derivative,[10] the observed benzoyl group migration under the conditions of desilylation was unexpected. Consequently a different route to **4** was followed.

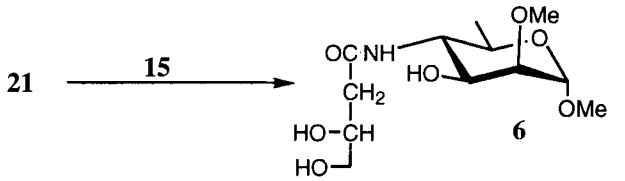
Amine **10** was treated with lactone **13**,[11] to give **27** having only the primary posi-



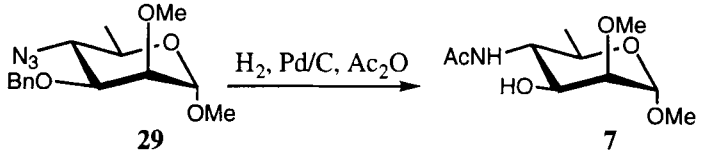
tion in the tetronamido group unsubstituted. One-step chlorination[19] of the latter with mesyl chloride in DMF gave **28**. Simultaneous cleavage of the benzyl groups and chlorine by catalytic hydrogenolysis gave the target compound **4**, together with a small amount of a byproduct whose spectral analysis showed it to be **1**, resulting from hydrolytic cleavage of the chlorine atom in **28**.

The D-*glycero* (**5**) and 2'-deoxy-3'-hydroxy (**6**) analogs of **1** were obtained using lactones **14**[10] and **15**, respectively, to *N*-acylate **21**. In the case of preparation of **5** the partially *O*-acetylated derivatives obtained[10] as intermediates were deacetylated (Zemplén).

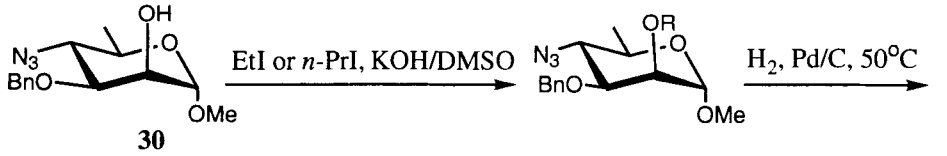




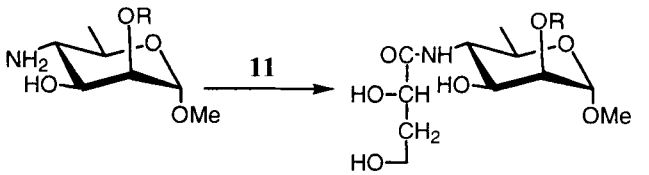
When the benzylated, azido derivative **29**[14] was subjected to catalytic hydrogenolysis in the presence of acetic anhydride, the acetamido derivative **7** was formed in high yield, and obtained crystalline.



Alkylation[20] with ethyl and propyl iodide at *O*-2 in the azide **30**[21] gave, respectively, the ethyl and propyl derivatives **31** and **32**. These were converted to the corresponding amines (by catalytic hydrogenation/hydrogenolysis, **31** → **33**, **32** → **34**), which were treated with lactone **11**[14] to give, respectively, the desired substances **8** and **9**.



R	
31	Et
32	*n*-Pr



R	
33	Et
34	*n*-Pr

R	
8	Et
9	*n*-Pr

EXPERIMENTAL

General methods. Unless stated otherwise, optical rotations were measured at 25 °C for solutions in $CHCl_3$ (*c* 1) with a Perkin Elmer automatic polarimeter, Model 241 MC. All reactions were monitored by thin-layer chromatography (TLC) on silica gel-coated glass slides (Whatman), using for development solvent mixtures of appropriately adjusted polarity consisting of *A*, hexane–acetone; *B*, toluene–acetone; *C*, hexane–EtOAc; *D*, toluene–EtOAc; *E*, EtOAc–MeOH. The detection was effected by charring with 5% sulfuric acid in EtOH. In cases when the material did not char with the former reagent the substances were visualized by dipping developed plates into 5% phosphomolybdic acid in ethanol and heating until permanent spots were visible. When applicable, detection with UV light was also used. Preparative chromatography was performed by gradient elution from columns of Silica Gel 60 (Fluka, particle size 0.035-0.070 mm) using, at the onset of development, a solvent mixture slightly less polar than that used for TLC. Assignments of NMR signals, obtained at 300 MHz for ^1H and 75 MHz for ^{13}C at 25 °C, were made by first-order analysis of spectra and, when feasible, the assignments were supported by APT and/or DEPT experiments, homonuclear decoupling and/or homo- and heteronuclear 2-dimensional correlation spectroscopy. The commercial software supplied with the spectrometers (Varian Gemini or Varian XL 300) was used. 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 °C/2 kPa. Palladium-on-charcoal catalyst (5%, ESCAT 103) was a product of Engelhard Industries. The use of this catalyst largely avoided problems encountered[16,22] when products of debenzylation *and* amines are simultaneously formed. γ-Butyrolactone (**12**) and 2-deoxy-L-*glycero*-tetronolactone (**15**) were purchased from Aldrich Chemical Co. ^1H NMR data found for the latter agreed with those reported,[23] and the spectra did not reveal presence of impurities.

Methyl 4-Amino-3-*O*-benzyl-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranoside (**10**). This compound was prepared as previously described.[16] ^1H NMR ($CDCl_3$): δ 4.75 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 4.71, 4.52 (2 d, 1 H each, ^2J 11.5 Hz, CH_2Ph), 3.54–3.46 (m, 6 H, H-2,3,5, incl s at 3.48 for OCH_3-2), 3.34 (s, 3 H, OCH_3-1), 2.93 (m, 1 H, H-4), 1.39 (bs, 2 H, NH_2), 1.27 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 98.49 (C-1), 79.67 (C-3), 75.68 (C-2), 71.26 (CH_2Ph), 69.40 (C-5), 59.00 (OCH_3-2), 54.51 (OCH_3-1), 53.42 (C-4), 17.90 (C-6); CIMS: *m/z* 282 ($[M + 1]^+$), 299 ($[M + 18]^+$).

Methyl 3-*O*-Benzyl-4,6-dideoxy-4-(2,4-di-*O*-benzoyl-3-deoxy-L-*glycero*-tetronamido)-2-*O*-methyl-α-D-mannopyranoside (**17**). A solution of **10** (990 mg, 3.52 mmol) and lactone (**11**, 720 mg, 7 mmol) in pyridine (3 mL) was heated at 100 °C

overnight. TLC (solvent *A*) showed that the starting amine was consumed, and that one major product was formed. After concentration, chromatography gave methyl 3-*O*-benzyl-4,6-dideoxy-4-(3-deoxy-L-*glycero*-tetronamido)-2-*O*-methyl-α-D-mannopyranoside (**16**, 990 mg, 73%). ^{1}H NMR ($CDCl_3$): δ 6.84 (d, 1 H, $J_{4,NH}$ 9.6 Hz, NH), 4.74 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.66, 4.50 (2 d, 1 H each, ^2J 11.8 Hz, CH_2Ph), 4.25 (dd, 1 H, $J_{2',3'a}$ 3.8, $J_{2',3'b}$ 8.0 Hz, H-2'), 4.08 (ddd, 1 H, partially overlapped, J ~10 Hz, H-4), 3.83–3.64 (m, 4 H, H-5, 4'ab, incl dd, at 7.75 for H-3), 3.56 (bt, 1 H, H-2), 3.49 (s, 3 H, OCH_3-2), 3.34 (s, 3 H, OCH_3-1), 2.07–1.97, 1.83–1.72 (2 m, 1 H each, H-3'a,b), 1.20 (d, 3 H, $J_{5,6}$ 6.0 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 98.63 (C-1), 76.57 (C-2), 76.17 (C-3), 71.58 (H-2'), 71.31 (*C*H_2Ph), 67.74 (C-5), 60.44 (C-4'), 59.25 (OCH_3-2), 54.83 (OCH_3-1), 52.02 (C-4), 35.45 (C-3'), 17.89 (C-6); CIMS: *m/z* 384 ([M + 1]$^+$), 401 ([M + 18]$^+$).

Pyridine (1.3 mL, 16 mmol) followed by benzoyl chloride (0.91 mL, 7.8 mmol) was added to a solution of the foregoing compound **16** (990 mg, 2.58 mmol) in CH_2Cl_2 (15 mL), and the mixture was stirred at room temperature for 2 h. TLC (solvent *C*) then showed that the reaction was complete and that one product was formed. Cold (0 °C) aqueous $NaHCO_3$ was added, to destroy excess benzoylation reagent, the mixture was partitioned between water and CH_2Cl_2, and the organic phase was concentrated. Chromatography of the material in the residue gave **17**, (1.45 g, 95%), $[\alpha]_D$ -17.4°; ^{1}H NMR ($CDCl_3$): δ 6.25 (d, 1 H, $J_{4,NH}$ 8.8 Hz, NH), 5.57 (dd, $J_{2',3'a}$ 4.4, $J_{2',3'b}$ 8.2 Hz, H-2'), 4.73 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1), 4.66, 4.49 (2 d, partially overlapped, ^2J 11.8 Hz, CH_2Ph), 4.49–4.38 (m, partially overlapped, H-4'a,b), 4.03 (ddd, 1 H, partially overlapped, H-4), 3.88 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 10.7 Hz, H-3), 3.84–3.74 (m, 1 H, H-5), 3.58 (dd, 1 H, H-2), 3.48 (s, 3 H, OCH_3-2), 3.32 (s, 3 H, OCH_3-1), 2.56–2.45, 2.41–2.29 (2 m, 1 H each, H-3'a,b), 1.24 (d, $J_{5,6}$, 3 H, H-6); ^{13}C NMR ($CDCl_3$): δ 98.60 (C-1), 76.06 (C-2), 75.77 (C-3), 71.92 (C-2'), 71.04 (*C*H_2Ph), 67.23 (C-5), 60.80 (C-4'), 59.20 (OCH_3-2), 54.78 (OCH_3-1), 52.91 (C-4), 30.95 (C-3'), 17.85 (C-6); CIMS: *m/z* 592 ([M + 1]$^+$), 609 ([M + 18]$^+$).

Anal. Calcd for $C_{33}H_{37}NO_9$: C, 67.01; H, 6.26; N, 2.37. Found: C, 66.89; H, 6.26; N, 2.36.

Methyl 4,6-Dideoxy-4-(2,4-di-*O*-benzoyl-3-deoxy-L-*glycero*-tetronamido)-2-*O*-methyl-α-D-mannopyranoside (18). A mixture of **17** (1.4 g) and 5% palladium-on-charcoal catalyst (0.3 g) in MeOH was stirred in a hydrogen atmosphere overnight, when TLC (solvent *C*) showed that the reaction was complete. After conventional processing, product **18** (1.2 g, ~100%) solidified on standing. Crystallization from methanol gave pure **18**, mp 63-64°, $[\alpha]_D$ +4° (*c* 0.8). ^{1}H NMR ($CDCl_3$): δ 6.06 (d, 1 H, $J_{4,NH}$ 9.3, NH), 3.70 (ddd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ ~10.2, $J_{3,OH}$ ~10.2 Hz, H-3), 2.60–2.45 (m, 3 H, OH, H-3'a,b); ^{1}H NMR ($CDCl_3$ + D_2O): δ 5.61 (dd, 1 H, $J_{2',3'a}$ 4.7, $J_{2',3'b}$ 6.9 Hz, H-2'), 4.76 (d, 1 H,

$J_{1,2}$ 1.7 Hz, H-1), 4.62–4.48 (m, 2 H, H-4'a,b), 3.98 (dt, 1 H, J ~10.2 Hz, H-4), 3.70 (dd, 1 H, $J_{2,3}$ 3.5, $J_{3,4}$ 10.6 Hz, H-3), 3.58–3.46 (m, 4 H, H-5, incl s at 3.47, OCH_3-2), 3.43 (m, 1 H, H-2), 3.33 (s, 3 H, OCH_3-1), 2.58–2.46 (m, 2 H, H-3'a,b), 1.21 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 97.46 (C-1), 79.20 (C-2), 72.23 (C-2'), 69.34 (C-3), 67.15 (C-5), 60.80 (C-4'), 58.83 (OCH_3-2), 54.90 (OCH_3-1), 54.13 (C-4), 30.99 (C-3'), 17.73 (C-6); CIMS: *m/z* 502 ($[M + 1]^+$), 519 ($[M + 18]^+$).

Anal. Calcd for $C_{26}H_{31}NO_9$: C, 62.28; H, 6.19; N, 2.79. Found: C, 62.11; H, 6.24; N, 2.84.

Methyl 3,4,6-Trideoxy-4-(2,4-di-*O*-benzoyl-3-deoxy-L-*glycero*-tetronamido)-2-*O*-methyl-α-D-mannopyranoside (**20**). A solution of **18** (919 mg, 1.82 mmol) and *N,N'*-thiocarbonyldiimidazole (4.84 g, 2.72 mmol) in toluene (40 mL) was heated under reflux for 6 h. TLC (solvent *D*) showed that the reaction was complete, and that one faster moving product was formed. After concentration, the product was chromatographed to give amorphous methyl 3-*O*-imidazoylthiocarbonyl-4-(2,4-di-*O*-benzoyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranoside (**19**, 1.01 g, 91%). ^1H NMR ($CDCl_3$): δ 8.29 (bs, 1 H, imidazoyl group), 7.95–7.83 (m, 4 H, aromatic protons), 7.58–7.49 (m, 4 H, 3 aromatic protons, incl 1 H, imidazoyl group), 7.39–7.33 (m, 4 H, aromatic protons), 6.89 (dd, 1 H, J 0.8 and 1.7 Hz, imidazoyl group), 6.51 (d, 1 H, $J_{4,NH}$ 9.9 Hz, NH), 5.82 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 10.8 Hz, H-3), 5.39 (dd, 1 H, $J_{2',3'a}$ 4.7, $J_{2',3'b}$ 8.5 Hz, H-2'), 4.80 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.61 (dt, 1 H, J ~10.5 Hz, H-4), 4.42–4.31 (m, 2 H, H-4'a,b), 3.86 (dd, 1 H, H-2), 3.82–3.73 (m, 1 H, H-5), 3.42 (s, 3 H, OCH_3-2), 3.38 (s, 3 H, OCH_3-1), 2.43–2.16 (m, 2 H, H-3'a,b), 1.30 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 98.26 (C-1), 80.44 (C-3), 76.09 (C-2), 71.73 (C-2'), 67.15 (C-5), 60.47 (C-4'), 59.24 (OCH_3-2), 55.00 (OCH_3-1), 50.61 (C-4), 30.45 (C-3'), 17.56 (C-6); CIMS: *m/z* 612 ($[M + 1]^+$).

To a solution of the foregoing compound **19** (1.01 g, 1.65 mmol) and 2,2'-azobis(2-methylpropionitrile (27 mg) in toluene (30 mL) was added, dropwise and with stirring at 110 °C, a solution of tributyltin hydride (722 mg, 2.48 mmol in toluene (10 mL). When TLC (solvent *C*) showed that the reaction was complete (~10 min after addition of the reagent), the mixture was concentrated, and chromatography gave **20** (0.74 g, 92%), $[\alpha]_D$ +45° (*c* 1.4). ^1H NMR ($CDCl_3$): δ 6.12 (d, 1 H, $J_{4,NH}$ 9.3 Hz, NH), 5.57 (dd, 1 H, $J_{2',3'a}$ 5.2, $J_{2',3'b}$ 6.9 Hz, H-2'), 4.56 (bs, 1 H, H-1), 4.55–4.44 (m, 2 H, H-4'a,b), 4.12–4.00 (m, 1 H, H-4), 3.60–3.50 (m, 1 H, H-5), 3.34 (s, 3 H, OCH_3-2), 3.32 (s, 3 H, OCH_3-1), 3.28 (m, 1 H, H-2), 2.60–2.42 (m, 2 H, H-3'a,b), 2.07–2.00 (2 t, 1 H, $J_{2,3a} \simeq J_{3a,4} \simeq 3.6$, ^2J 13.2 Hz, H-3a), 1.67 (ddd, 1 H, $J_{3b,4}$ 3, $J_{2,3b}$ 12.4 Hz, H-3b), 1.19 (d, 3 H, $J_{5,6}$ 6.0 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 97.73 (C-1), 75.62 (C-2), 71.72 (C-2'),

67.85 (C-5), 60.61 (C-4'), 56.61 (OCH_3-2), 54.51 (OCH_3-1), 46.28 (C-4), 30.72 (C-3'), 29.03 (C-3), 17.87 (C-6); CIMS: *m/z* 486 ($[M + 1]^+$), 503 ($[M + 18]^+$).

Anal. Calcd for $C_{26}H_{31}NO_8$: C, 64.33; H, 6.39; N, 2.89. Found: C, 64.25; H, 6.46; N, 2.90.

Methyl 4-(3-Deoxy-L-*glycero*-tetronamido)-2-*O*-methyl-3,4,6-trideoxy-α-D-mannopyranoside (2). Debenzoylation (Zemplén) of **20** (640 mg) gave, after chromatography, the deoxy derivative **2** (351 mg, 96%) which crystallized on standing, but crystallization from common solvents could not be effected, $[\alpha]_D$ +74° (*c* 0.8). ^1H NMR ($CDCl_3$): δ 7.06 (d, 1 H, $J_{4,NH}$ 9.6 Hz, NH), 5.34 (d, 1 H, *J* 4.5 Hz, OH), 4.34 (bs, 1 H, OH), 3.4, 3.39 (2 s, 3 H each, 2 OCH_3), 3.34 (m, 1 H, H-2), 2.12–1.95, 1.82–1.70 (2 m, 2 H, each, H-3a,b, H-3'a,b), 1.17 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^1H NMR (D_2O): δ 4.68 (bm due to H-1–H-3 interaction, 1 H, H-1), 4.21 (dd, 1 H, $J_{2',3'a}$ 3.9, $J_{2',3'b}$ 8.6 Hz, H-2'), 3.87–3.73 (m, 2 H, H-4,5), 3.71–3.67 (m, 2 H, H-4'a,b), 3.51–3.48 (m, 1 H, H-2), 2.06–1.92 (m, 2 H, H-3a,3'a), 1.84–1.72 (m, 2 H, H-3b,3'b), 1.13 (d, 1 H, $J_{5,6}$ 5.8 Hz, H-6); ^{13}C NMR (D_2O): δ 176.32 (CO), 97.51 (C-1), 75.60 (C-2), 68.91 (C-2'), 67.85 (C-5), 57.88 (C-4'), 56.42 (OCH_3-2), 54.70 (OCH_3-1), 46.37 (C-4), 36.02 (C-3'), 28.33 (C-3), 17.15 (C-6); CIMS: *m/z* 278 ($[M + 18]^+$), 295 ($[M + 18]^+$).

Anal. Calcd for $C_{12}H_{23}NO_6$: C, 51.99; H, 8.30; N, 5.05. Found: C, 51.81; H, 8.26; N, 4.93.

Methyl 4,6-Dideoxy-4-(4-hydroxybutyramido)-2-*O*-methyl-α-D-mannopyranoside (3). A solution of **21**[14] (400 mg) in γ-butyrolactone (2 mL) was heated at 100 °C until TLC (solvent *E*) showed that all starting amine was consumed (~48 h). After concentration (50 °C/133 Pa), to remove excess of the reagent, chromatography (solvent *C*) and crystallization gave **3** (510 mg, 88%), mp 79–80 °C, $[\alpha]_D$ +56°. ^1H NMR (D_2O): δ 4.88 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 3.80 (m, partially overlapped, H-3), 3.77 (t, partially overlapped, *J* ~10 Hz, H-4), 3.73 (m, partially overlapped, H-5), 3.59 (t, 2 H, *J* 6.6 Hz, H-4'a,b), 3.55 (dd, 1 H, $J_{2,3}$ 3.1 Hz, H-2), 3.47 (s, 3 H, OCH_3-2), 3.39 (s, 3 H, OCH_3-1), 2.32 (t, 2 H, *J* 7.1, H-2'a,b), 1.87–1.77 (m, 2 H, H-3'a,b), 1.17 (d, 3 H, $J_{5,6}$ 6.0 Hz, H-6); ^{13}C NMR (D_2O): d 97.82 (C-1), 79.07 (C-2), 68.13 (C-3), 67.37 (C-5), 60.98 (C-4'), 58.92 (OCH_3-2), 54.92 (OCH_3-1), 53.39 (C-4), 32.69 (C-2'), 27.87 (C-3'), 16.92 (C-6); CIMS: *m/z* 278 ($[M + 1]^+$), 295 ($[M + 18]^+$).

Anal. Calcd for $C_{12}H_{23}NO_6$: C, 51.99; H, 8.30; N, 5.05. Found: C, 52.07; H, 8.39; N, 5.06.

Methyl 4,6-Dideoxy-4-(4-*t*-butyldiphenylsilyl-3-deoxy-L-*glycero*-tetronamido)-2-*O*-methyl-α-D-mannopyranoside (22). A mixture of **1** (1.2 g, 4.1 mmol), imidazole (418 mg, 6.15 mmol) and *t*-butylchlorodiphenylsilane (1.68g, 6.1 mmol) in pyridine (7 mL) was stirred at room temperature for 1 h. TLC (solvent *A*) showed that

all starting material was consumed and that a major and a very minor, faster moving products were formed. Cold (0 °C) aqueous $NaHCO_3$ was added and, after 1 h, the mixture was partitioned between CH_2Cl_2 and water. After concentration of the organic phase, chromatography gave **22** (1.7 g, 79%) as a crystalline solid. The compound is dimorphous, mp 85–86 °C (from 2-propanol–hexane, silky crystals), mp 159–160 °C (from acetone-hexane, needles), $[\alpha]_D$ +24°. ^1H NMR ($CDCl_3$): δ 6.85 (d, 1 H, $J_{4,NH}$ 9.4 Hz, NH), 4.80 (d, $J_{1,2}$ 1.4 Hz, H-1), 4.77 (d, 1 H, $J_{2,OH}$ 2.8 Hz, OH), 4.42 (dd, 1 H, $J_{2',3'a}$ 3.0, $J_{2',3'b}$ 8.7 Hz, H-2'), 4.02–3.85 (m, 3 H, H4,4'a,b), 3.73 (dd, partially overlapped, $J_{2,3}$ 3.6, $J_{3,4}$ 10.5 Hz, H-3), 3.73–3.65 (m, partially overlapped, H-5), 3.49 (s, 3 H, OCH_3-2), 3.45 (dd, 1 H, H-2), 3.38 (s, 3 H, OCH_3-1), 2.66 (bs, 1 H, OH), 2.19–2.09, 2.03–1.96 (2 m, 1 H each, H-3'a,b), 1.25 (d, 3 H, $J_{5,6}$ 6.1 Hz, H-6), 1.05 (s, 9 H, 3 CH_3); ^{13}C NMR ($CDCl_3$): δ 174.5 (CO), 97.54 (C-1), 79.37 (C-2), 73.03 (C-2'), 69.61 (C-3), 66.97 (C-5), 63.70 (C-4'), 58.89 (OCH_3-2), 54.84 (OCH_3-1), 53.85 (C-4), 35.04 (C-3'), 26.71 (3 C, 3 CH_3), 18.90 [$C(CH_3)_3$], 17.86 (C-6); CIMS: *m/z* 532 ($[M + 1]^+$), 549 ($[M + 18]^+$).

Anal. Calcd for $C_{28}H_{41}NO_7Si$: C, 63.28; H, 7.72; N, 2.64. Found: C, 63.42; H, 7.82; N, 2.59.

Methyl 3-*O*-Benzoyl-4-(4-*O*-benzoyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranoside (23). A solution of the foregoing compound **22** (1.4 g, 3.1 mmol) in CH_2Cl_2 (20 mL) was treated with benzoyl chloride (1.3 g, 9.3 mmol) and pyridine (1.25 mL) to give, after chromatography, the fully protected compound **23** (2.28 g, ~100%), $[\alpha]_D$ -0.6° (*c* 0.8). ^1H NMR ($CDCl_3$): δ 6.05 (d, 1 H, $J_{4,NH}$ 9.8 Hz, NH), 5.48 (dd, 1 H, $J_{2',3'a}$ 3.9, $J_{2',3b}$ 10.1 Hz, H-2'), 5.37 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 11.1 Hz, H-3), 4.75 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.48 (ddd, partially overlapped, H-4), 3.68–3.53 (m, 4 H, H-2,5,4'a,b), 3.54 (s, 3 H, OCH_3-2), 3.60 (s, 3 H, OCH_3-1), 2.15–2.02, 1.78–1.66 (2 m, 1 H each, H-3'a,b), 1.26 (d, 3 H, $J_{5,6}$ 6.9 Hz, H-6), 0.95 (s, 9 H, 3 CH_3); ^{13}C NMR ($CDCl_3$): δ 98.93 (C-1), 77.79 (C-2), 71.99 (C-3), 71.52 (C-2'), 68.17 (C-5), 59.78 (OCH_3-2), 59.26 (C-4'), 54.86 (OCH_3-1), 51.17 (C-4), 34.33 (C-3'), 26.61 (3 C, 3 CH_3), 18.95 (3 CH_3), 17.74 (C-6); CIMS: *m/z* 740 ($[M + 1]^+$), 757 ($[M + 18]^+$).

Anal. Calcd for $C_{42}H_{49}NO_9Si$: C, 68.20; H, 6.63; N, 1.89. Found: C, 68.33; H, 6.73; N, 1.88.

Methyl 3-*O*-Benzoyl-4-(2-*O*-benzoyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranoside (24) and Methyl 3-*O*-Benzoyl-4-(4-*O*-benzoyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranoside (25). A solution of compound **23** (1.28 g, 1.7 mmol) in THF was treated, for 4 h at room temperature, with TBAF (70 mg, 0.27 mmol). TLC (solvent *A*) showed the

presence of a large amount of unchanged **12**, and that two products were formed. A fresh portion of TBAF (70 mg) was added, but the situation did not change after 12 h. The mixture was concentrated, and the residue was chromatographed to give first the unchanged starting material (400 mg, 31%).

Eluted next was the major product, spectral characteristics of which showed it was the unwanted, title compound **25** mg, 60%), $[\alpha]_D$ -0.5° (*c* 1.1). ^{1}H NMR ($CDCl_3$): δ 7.05 (d, 1 H, $J_{4,NH}$ 10.3 Hz, NH), 5.48 (dd, 1 H, $J_{2,3}$ 2.9, $J_{3,4}$ 10.9 Hz, H-3), 4.78 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.53–4.42 (m, 1 H, H-4), 4.36 (ddd, 1 H, $J_{3'a,4'a}$ 5.42, $J_{3'b,4'a}$ 9.0 Hz, $J_{4'a,4'b}$ 14.5 Hz, H-4'a), 4.21–4.12 m, 3 H, H-2',4'b,OH), 3.86–3.76 (m, 1 H, H-5), 3.69 (dd, 1 H, H-2), 3.52 (s, 3 H, OCH_3-2), 3.36 (s, 3 H, OCH_3-1), 2.16–2.03 (m, 1 H, H-3'a), 1.53–1.41 (m, 1 H, H-3b), 1.27 (d, 3 H, $J_{5,6}$ 6.1 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 98.97 (C-1), 77.68 (C-2), 72.43 (C-3), 69.07 (C-2'), 67.71 (C-5), 61.32 (C-4'), 59.81 (OCH_3-2), 54.79 (OCH_3-1), 50.89 (C-4), 33.78 (C-3'), 17.83 (C-6); CIMS: *m/z* 502 ([M + 1]$^+$), 519 ([M + 18]$^+$).

Anal. Calcd for $C_{26}H_{31}NO_9$: C, 62.28; H, 6.19; N, 2.79. Found: C, 62.22; H, 6.21; N, 2.77.

Eluted next was the expected compound **24** (43 mg, 5%), $[\alpha]_D$ -2.1°. ^{1}H NMR ($CDCl_3$): δ 6.34 (d, 1 H, $J_{4,NH}$ 9.8 Hz, NH), 5.45 (dd, partially overlapped, $J_{2,3}$ 3.0, $J_{3,4}$ 11 Hz, H-3), ~5.46 (dd, partially overlapped, $J_{2',3'a}$ ~5.9, $J_{2',3'b}$ ~7.0 Hz, H-2'), 4.79 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.54–4.44 (m, 1 H, H-4), 3.72–3.61 (m, partially overlapped, H-5), ~3.64 (dd, partially overlapped, H-2), 3.57 (s, 3 H, OCH_3-2), 3.55–3.38 (m, 2 H, H-4'a,b), 3.37 (s, 3 H, OCH_3-1), 2.26 (bt, 1 H, OH), 1.98–1.76 (m, 2 H, H-3'a,b), 1.26 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 98.91 (C-1), 77.90 (C-2), 72.04 (C-3), 71.80 (C-2'), 68.19 (C-5), 59.85 (OCH_3-2), 58.24 (C-4'), 54.92 (OCH_3-1), 51.50 (C-4), 34.44 (C-3'), 17.75 (C-6); CIMS: *m/z* 502 ([M + 1]$^+$), 519 ([M + 18]$^+$).

Anal. Calcd for $C_{26}H_{31}NO_9$: C, 62.28; H, 6.19; N, 2.79. Found: C, 62.27; H, 6.18; N, 2.75.

A portion (~5 mg) of **25** was treated for 1 h at room temperature with acetic anhydride-pyridine (0.5 mL). After concentration and coevaporation with toluene, to remove all volatiles, the ^{1}H NMR spectrum of the product showed it to be methyl 3-*O*-benzoyl-4-(2-*O*-acetyl-4-*O*-benzoyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranoside (**26**, see Discussion). ^{1}H NMR ($CDCl_3$): δ 6.09 (d, 1 H, $J_{4,NH}$ 9.5 Hz, NH), 5.44 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 11.1 Hz, H-3), 5.21 (dd, 1 H, $J_{2',3'a}$ 4.7, $J_{2',3'b}$ 8.2 Hz, H-2'), 4.79 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.52–4.42 (m, 1 H, H-4), 4.17–4.08 (m, 2 H, H-4'a,b), 3.75–3.66 (m, H-5), 3.65 (bt, 1 H, H-2), 3.56 (s, 3 H, OCH_3-2), 3.42 (s, 3 H, OCH_3-1), 2.14–2.03 (m, 4 H, H-3'a, include s, 2.04, $COCH_3$), 1.82 (m, 1 H, H-3b), 1.27 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6). ^{13}C NMR ($CDCl_3$): δ 99.00 (C-1), 77.91 (C-2),

72.00 (C-3), 71.18 (C-2'), 68.32 (C-5), 60.27 (C-4'), 59.89 (OCH_3-2), 55.02 (OCH_3-1), 51.45 (C-4), 30.68 (C-3'), 20.65 ($COCH_3$), 17.91 (C-6).

Methyl 3-*O*-Benzyl-4-(2-*O*-benzyl-3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranoside (**27**). Amine **21**[14] (350 mg, 1.24 mmol) was treated with lactone **13** (358 mg, 1.86 mmol) as described for the preparation of **16**. Chromatography gave **27** (470 mg, 80%), mp 136–137 °C (from ether), $[\alpha]_D$ -0.3°. ^1H NMR ($CDCl_3$): δ 6.49 (d, 1 H, $J_{4,NH}$ 8.5 Hz, NH), 4.74 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1), 4.67 (d, 1 H, ^2J 11.7 Hz, C*H*Ph), 4.50 (s, 2 H, CH_2Ph), 4.48 (d, 1 H, ^2J 11.7 Hz, C*H*Ph), 4.00 (t, partially overlapped, J 6.3 Hz, H-2'), 3.94 (bt, partially overlapped, J ~9.7 Hz, H-4), 3.89 (dd, partially overlapped, $J_{2,3}$ 2.88, $J_{3,4}$ 10.5 Hz, H-3), 3.79–3.69 (m, 1 H, H-5), 3.69–3.62 (m, 2 H, H-4'a,b), 3.57 (bt, 1 H, H-2), 3.49 (s, 3 H, OCH_3-2), 3.36 (s, 3 H, OCH_3-1), 2.65 (bs, 1 H, OH), 1.96–1.90 (m, 2 H, H-3'a,b), 1.21 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 98.58 (C-1), 78.59 (C-2'), 76.01 (C-2), 75.94 (C-3), 72.78, 71.10 (2 CH_2Ph), 67.27 (C-5), 59.23 (C-4'), 59.16 (OCH_3-2), 54.87 (OCH_3-1), 52.65 (C-4), 35.39 (C-3'), 18.12 (C-6). CIMS: *m/z* 474 ($[M + 1]^+$), 491 ($[M + 18]^+$).

Anal. Calcd for $C_{26}H_{35}NO_7$: C, 65.96; H, 7.40; N, 2.96. Found: C, 66.00; H, 7.39; N, 2.95.

Methyl 3-*O*-Benzyl-4-(2-*O*-benzyl-4-chloro-3,4-dideoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranoside (**28**). Mesyl chloride (0.68 mL, 8.7 mmol) was added dropwise, at room temperature, to a stirred solution of **27** (415 mg, 0.88 mmol) in DMF (10 mL), and the solution was stirred at 65 °C for 1 h. TLC (solvent *C*) then showed that the reaction was complete and that one product was formed. The mixture was partitioned between CH_2Cl_2 and cold (0 °C) aqueous $NaHCO_3$, the organic phase was concentrated, and chromatography of the material in the residue gave **28** (354 mg, 82%), mp 155–156 °C (from EtOAc–hexane), $[\alpha]_D$ -6° (*c* 1.1). ^1H NMR ($CDCl_3$): δ 6.36 (d, 1 H, $J_{4,NH}$ 8.8 Hz, NH), 4.74 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.67, 4.52, 4.48, 4.47 (4 d, 4 H, ^2J ~11.8 Hz, 2 CH_2Ph), 4.02 (dd, $J_{2',3'a}$ 4.5, $J_{2',3'b}$ 8.1 Hz, H-2'), 3.94 (bt, 1 H, J ~9.7 Hz, H-4), 3.85 (dd, $J_{2,3}$ 2.8, $J_{3,4}$ 10.5 Hz, H-3), 3.75–3.65 (m, 1 H, H-5), 3,60–3.53 (m, 3 H, H-2,4'a,b), 3.49 (OCH_3-2), 3.36 (OCH_3-1), 2.25–2.13, 2.06–1.91 (2 m, 2 H, H-3'a,b), 1.19 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 98.62 (C-1), 77.42 (C-2'), 76.06 (C-3), 76.01 (C-2), 73.18, 71.07 (2 CH_2Ph), 67.36 (C-5), 59.18 (OCH_3-2), 54.85 (OCH_3-1), 52.51 (C-4), 40.59 (CH_2Cl), 35.82 (C-3'), 18.10 (C-6), CIMS: *m/z* 492 ($[M + 1]^+$), 509 ($[M + 18]^+$).

Anal. Calcd for $C_{26}H_{34}ClNO_6$: C, 63.46; H, 6.96; N, 2.86. Found: C, 63.64; H, 6.99; N, 2.85.

Methyl 4-(3,4-Dideoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranoside (**4**). A solution of **28** (290 mg) in methanol (17 mL) containing

triethylamine (~0.1 mL) was stirred at 50 °C for 3 days in the presence of 5% palladium-on-charcoal catalyst (180 mg). Initially, several products were formed, as shown by TLC (solvent EtOAc-MeOH 6:1) and eventually two products were formed, the one showing faster chromatographic mobility predominating. After filtration and concentration of the filtrate, chromatography of the material in the residue gave first the desired product **4** 120 mg (73%), mp 151–152 °C (from EtOAc–hexane), $[\alpha]_D$ +31° (*c* 0.8). ^{1}H NMR (D_2O): δ 4.87 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.10 (dd, 1 H, $J_{2',3'a}$ 4.5, $J_{2',3'b}$ 7.1 Hz, H-2'), 3.89 (dd, 1 H, $J_{2,3}$ 3.6, $J_{3,4}$ 11.1 Hz, H-3), 3.82–3.73 (m 2 H, H-4,5), 3.54 (dd, 1 H, H-2), 3.47 (s, 3 H, OCH_3-2), 3.38 (s, 3 H, OCH_3-1), 1.85–1.58 (m, 2 H, H-3'a,b), 1.15 (d, 3 H, $J_{5,6}$ 5.8 Hz, H-6), 0.91 (t, 3 H, *J* 7.4, H-4'); ^{13}C NMR (D_2O): δ 97.87 (C-1), 79.22 (C-2'), 72.82 (C-2'), 67.73 (C-3), 67.24 (C-5), 58.99 (OCH_3-2), 54.92 (OCH_3-1), 53.34 (C-4), 26.96 (C-3'), 16.95 (C-6), 8.58 (C-4'); CIMS: *m/z* 278 ($[M + 1]^+$), 295 ($[M + 18]^+$).

Anal. Calcd for $C_{12}H_{23}NO_6$: C, 51.99; H, 8.30; N 5.05. Found: C, 51.98; H, 8.29; N, 5.01.

The NMR and mass spectral data obtained for the material eluted next were identical with those reported[14] for methyl 4-(3-deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranoside (**1**). Thus, this minor byproduct of hydrogenolysis resulted from hydrolytic cleavage of chlorine in **28**.

Methyl 4-(3-Deoxy-D-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranoside (5). A solution of amine **21**[14] (400 mg, 2.1 mmol) and lactone **14**[10] (454 mg, 3.15 mmol) in pyridine (~1 mL) was heated at 100 °C for 16 h. Several products were formed,[10] as shown by TLC (solvent *E*). After concentration and deacetylation of partially acetylated derivatives of **5** formed,[10] chromatography gave the major product **5** (510 mg, 83%), mp 129–130 °C, $[\alpha]_D$ +79° (*c* 0.7). 1H NMR ($CDCl_3$): δ 7.20 (d, 1 H, $J_{4,NH}$ 9.6 Hz, NH), 4.77 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.29 (dd, overlapped with an OH signal, $J_{2',3'a}$ 3.4, $J_{2',3'b}$ 9.3, H-2'), 3.89 (2 t, partially overlapped, *J* 10.1 Hz, H-4), 3.8–3.77 (m, 3 H, H-3,4'a,b), 3.68–3.59 (m, 1 H, H-5), 3.50 (s, 3 H, OCH_2-2), 3.44 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-2), 3.37 (s, 3 H, OCH_3-1), 2.11–2.00, 1.73–1.65 (2 m, 1 H each, H-3'a,b), 1.19 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 176.85 (CO), 97.62 (C-1), 79.39 (C-2), 69.45 (C-2'), 69.09 (C-3), 67.12 (C-5), 58.85 (2 C, C-4', OCH_3-2), 54.80 (OCH_3-1), 53.36 (C-4), 36.59 (C-3'), 17.64 (C-6); CIMS: *m/z* 294 ($[M + 1]^+$), 311 ($[M + 18]^+$).

Anal. Calcd for $C_{12}H_{23}NO_7$: C, 49.15; H, 7.85; N, 4.78. Found: C, 49.19; H, 7.91; N, 4.76.

Methyl 4-(2-Deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranoside (6). Amine **21**[14] (150 mg, 0.78 mmol) was treated with lactone **15** (120 mg, 1.17 mmol), as described for the preparation of **5**. Chromatography gave **6** (200 mg, 87%), mp 103.5–104.5 °C (from EtOAc-hexane), $[\alpha]_D$ +30° (*c* 1, H_2O). ^{1}H NMR

(D_2O): δ 4.86 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.11–4.03 (m, 1 H, H-3'), 3.85–3.68 (m, 3 H, H-3,4,5), 3.59 (dd, partially overlapped, $J_{3',4'a}$ 4.1, $J_{4'a,4'b}$ 11.8 Hz, H-4'a), 3.54 (dd, partially overlapped, $J_{2,3}$ 3.2 Hz, H-2), 3.48 (dd, partially overlapped, $J_{3',4'b}$ 6.2, H-4'b), 3.46 (s, partially overlapped, OCH_3-2), 3.37 (s, 3 H, OCH_3-1), 2.47 (dd, 1 H, $J_{2',3'a}$ 4.8, $J_{2',3'b}$ 14.6 Hz, H-2'a), 2.34 (dd, 1 H, $J_{2',3'b}$ 8.4 Hz, H-2'b), 1.17 (d, 3 H, $J_{5,6}$ 5.8 Hz, H-6); ^{13}C NMR (D_2O): δ 174.1 (CO), 97.80 (C-1), 79.00 (C-2), 68.91 (C-3'), 68.04 (C-3), 67.35 (C-5), 65.09 (C-4'), 58.92 (OCH_3-2), 54.94 (OCH_3-1), 54.53 (C-4), 40.10 (C-2'), 16.92 (C-6); CIMS: *m/z* 294 ($[M + 1]^+$), 311 ($[M + 18]^+$).

Anal. Calcd for $C_{12}H_{23}NO_7$: C, 49.15; H, 7.85; N, 4.78. Found: C, 49.07; H, 7.88; N, 4.73.

Methyl 4-Acetamido-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranoside (7). A solution of **29**[14] (500 mg, 1.63 mmol) in methanol (20 mL) containing acetic anhydride (1 mL) was stirred under hydrogen atmosphere in the presence of 5% palladium-on-charcoal catalyst until essentially all starting material and intermediate products converted to one material, as shown by TLC (solvent *A*). After filtration and concentration of the filtrate, chromatography gave pure **7** (314 mg, 83%), mp 108.5–109 °C (from ether), $[\alpha]_D$ -87° (*c* 0.9). ^1H NMR ($CDCl_3$): δ 5.86 (d, 1 H, $J_{4,NH}$ 9.1 Hz, NH), 4.78 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 3.91 (dt, 1 H, partially overlapped, *J* 10.3 Hz, H-4), 3.74–3.66 (m, 1 H, H-3), 3.64–3.50 (m, 1 H, H-5), 3.49 (s, 3 H, OCH_3-2), 3.44 (dd, 1 H, $J_{2,3}$ 3.6 Hz, H-2), 3.37 (s, 3 H, OCH_3-1), 2.03 (s, 3 H, $COCH_3$), 1.24 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 97.45 (C-1), 79.30, (C-2), 69.66 (C-3), 67.34 (C-5), 58.78 (OCH_3-2), 54.86 (OCH_3-1), 54.23 (C-4), 23.38 ($COCH_3$), 17.90 (C-6). CIMS: *m/z* 234 ($[M + 1]^+$), 251 ($[M + 18]^+$).

Anal. Calcd for $C_{10}H_{19}NO_5$: C, 51.50; H, 8.15; N, 6.01. Found: C, 51.23; H, 8.26; N, 5.82.

Methyl 4-Azido-3-*O*-benzyl-4,6-dideoxy-2-*O*-ethyl-α-D-mannopyranoside (**31**). Powdered potassium hydroxide (1.1 g, ~20 mmol) followed by ethyl iodide (0.8 mL, ~10 mmol) was added to a solution of the benzylated azido compound **30**[21] (1.88 g, 6.4 mmol) in $(CH_3)_2SO$, and the mixture was stirred at room temperature for 2 h, when TLC (solvent *C*) showed complete conversion of the starting material to one product. Water (20 mL) was added, and the resulting clear solution was neutralized with aqueous acetic acid. The mixture was partitioned between water and CH_2Cl_2, the organic phase was dried, concentrated, and chromatography gave pure, amorphous **31** (1.94 g, 94%), $[\alpha]_D$ +112°. ^1H NMR ($CDCl_3$): δ 4.67 (m, 3 H, H-1, C*H*$_2$Ph), 3.70 (dd, partially overlapped, $J_{2,3}$ 3.3, $J_{3,4}$ 10.0 Hz, H-3), 3.70–3.59 (m, partially overlapped, C*H*$_2$CH$_3$), 3.51 (t, partially overlapped, H-4), 3.45 (m, 1 H, H-5), 3.32 (s, 3 H, OCH_3), 1.32 (d, 3 H, $J_{5,6}$ 5.8 Hz, H-6), 1.20 (t, 3 H, *J* 6.9 Hz, CH$_2$C*H*$_3$); ^{13}C NMR ($CDCl_3$): δ 99.32 (C-1),

78.24 (C-3), 74.42 (C-2), 71.83 (CH_2Ph), 67.02 (C-5), 66.97 (CH_2CH_3), 64.22 (C-4), 54.77 (OCH_3), 18.48 (C-6), 15.48 (CH_2CH_3); CIMS: *m/z* 339 ([M + 18]$^+$).

Anal. Calcd for $C_{16}H_{23}N_3O_4$: C, 59.81; H, 7.17; N, 13.08. Found: C, 59.77; H, 7.22; N, 12.96.

Methyl 4-(3-Deoxy-L-*glycero*-tetronamido)-4,6-dideoxy-2-*O*-ethyl-α-D-mannopyranoside (8). A mixture of compound **31** (480 mg) and 5% palladium-on-charcoal catalyst (500 mg) in MeOH (15 mL) was stirred in a hydrogen atmosphere at room temperature and at atmospheric pressure, until TLC (solvent *E*) showed that only one product was present (5-7 days). After conventional processing, chromatography gave amorphous methyl 4-amino-4,6-dideoxy-2-*O*-ethyl-α-D-mannopyranoside (**33**, 300 mg, ~97%). ^{1}H NMR ($CDCl_3$): δ 4.72 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 3.74 (ddd, 1 H, ^2J 9.6, ^3J 7.2 Hz, CH_aCH$_3$), 3.59–3.41 (m, 4 H, H-2,3,5, CH_bCH$_3$), 3.35 (OCH_3), 2.66 (t, 1 H, *J* 9.9 Hz, H-4), 1.27 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6), 1.23 (t, 3 H, CH_2CH_3); ^{13}C NMR ($CDCl_3$): δ 98.22 (C-1), 77.41 (C-2), 70.84 (C-3), 68.73 (C-5), 66.27 (CH_2CH_3), 55.66 (C-4), 54.35 (OCH_3), 17.60 (C-6), 15.10 (CH_2CH_3); CIMS: *m/z* 206 ([M + 1]$^+$), 223 ([M + 18]$^+$).

The foregoing amine **33** (300 mg, 1.46 mmol) was treated with lactone **11** (194 mg, 1.9 mmol), as described for the preparation of **16**, to give **8** (404 mg, 90%), mp 121.5–122 °C (from EtOAc), $[\alpha]_D$ +5.4°. ^{1}H NMR ($CDCl_3$-D_2O): δ 4.72 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 4.26 (dd, 1 H, $J_{2',3'a}$ 3.9, $J_{2',3'b}$ 8.1 Hz, H-2'), 3.89–3.62 (m, 6 H, partially overlapped, H-3,4,5,4'a,b, incl dd at ~3.72 for CHaCH$_3$, J_{CHa,CH_3} ~7.1, $J_{CHa,CHb}$ ~9.5 Hz), 3.61–3.53 (m, partially overlapped, incl dd for CHbCH$_3$, and dd at ~3.53 for H-2, $J_{2,3}$ ~3.0 Hz), 3.36 (s, 3 H, OCH_3), 2.10–2.00, 1.92–1.80 (2 m, 1 H each, H-3'), 1.23 (t, partially overlapped, *J* 7.1 Hz, CH_2CH_3), 1.20 (d, partially overlapped, $J_{5,6}$ 6.4 Hz, H-6); ^{13}C NMR ($CDCl_3$+D_2O): d 98.53 (C-1), 77.59 (C-2), 70.20 (C-2'), 68.70 (C-3), 67.00 (C-5), 66.77 (CH_2CH_3), 58.93 (C-4'), 54.82 (OCH_3), 53.78 (C-4), 36.06 (C-3'), 17.57 (C-6), 15.26 (CH_2CH_3); CIMS: *m/z* 308 ([M + 1]$^+$), 325 ([M + 18]$^+$).

Anal. Calcd for $C_{13}H_{25}NO_7$: C, 50.81; H, 8.14; N, 4.56. Found: C, 50.85; H, 8.14; N, 4.51.

Methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-2-*O*-propyl-α-D-mannopyranoside (32). The azido derivative **30** (300 mg) was treated with *n*-propyl iodide, as described above for the preparation of **31**, to give **32** (335 mg, 97%), $[\alpha]_D$ +90° (*c* 0.8). ^{1}H NMR ($CDCl_3$): δ 4.68, 4.64 (2 d, partially overlapped, ^2J ~11.6 Hz, CH_2Ph), ~4.67 (d, partially overlapped, H-1), 3.70 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 9.4 Hz, H-3), 3.63 (dd, 1 H, $J_{1,2}$ 1.8 Hz, H-2), 3.55–3.40 (m, 4 H, H-4,5,OCH_2), 3.32 (s, 3 H, OCH_3), 1.66–1.54 (m, 2 H, CH_2CH$_3$), 1.32 (d, 3 H, $J_{5,6}$ 6.0 Hz, H-6), 0.89 (t, 3 H, *J* 7.5 Hz, CH_2CH_3); ^{13}C NMR ($CDCl_3$): δ 99.21 (C-1), 78.28 (C-3), 74.60 (C-2), 73.33 (OCH_2), 71.73 (CH_2Ph), 67.03

(C-5), 64.21 (C-4), 54.80 (OCH_3), 23.13 (CH_2CH_3), 18.53 (C-6), 10.41 (CH_2CH_3); CIMS: *m/z* 336 ($[M + 1]^+$), 353 ($[M + 18]^+$).

Anal. Calcd for $C_{17}H_{25}N_3O_4$: C, 60.90; H, 7.46; N, 12.54. Found: C, 61.00; H, 7.58; N, 12.45 .

Methyl 4,6-Dideoxy-4-(3-deoxy-L-*glycero*-tetronamido)-2-*O*-propyl-α-D-mannopyranoside (9). Compound **32** was converted to the corresponding amine, methyl 4-amino-4,6-dideoxy-2-*O*-propyl-α-D-mannopyranoside (**34**), as described for the preparation of **32**. ^1H NMR ($CDCl_3$): δ 4.74 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 3.67–3.40 (m, 5 H, H-2,3,5, OCH_2), 3.35 (s, 3 H, OCH_3), 2.65 (t, 1 H, *J* 9.6 Hz, H-4), 1.95–1.83 (bs, 3 H, OH, NH_2), 1.69–1.57 (m, 2 H, CH_2CH_3), 1.27 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6), 0.92 (t, 3 H, *J* 6.9 Hz, CH_2CH_3); ^{13}C NMR ($CDCl_3$): δ 98.11 (C-1), 77.60 (C-2), 72.58 (CH_2O), 71.17 (C-3), 68.82 (C-5), 55.84 (C-4), 54.41 (OCH_3), 22.82 (CH_2CH_3), 17.64 (C-6), 10.13 (CH_2CH_3), CIMS: *m/z* 220 ($[M + 1]^+$), 237 ($[M + 18]^+$).

Reaction of **34** (170 mg, 0.78 mmol) with lactone[14] **11** (103 mg, 1 mmol), as described for the preparation of **8**, gave **9** (226 mg, 91%), after isolation by chromatography, mp 85.5–86.5 °C (from EtOAc–hexane), $[\alpha]_D$ -0.8° (*c* 1.2, H_2O). ^1H NMR ($CDCl_3$): d 7.22 (δ, 1 H, $J_{4,NH}$ 9.1 Hz, NH). ^1H NMR ($CDCl_3$ + D_2O): δ 4.71 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1), 4.25 (dd, 1 H, $J_{2',3'a}$ 3.5, $J_{2',3'b}$ 8.2 Hz, H-2'), 3.88–3.41 (m, 8 H, H-2,3,4,5,4'a,b, OCH_2), 3.36 (s, 3 H, OCH_3), 2.09–1.98, 1.88–1.77 (2 m, 1 H each, H-3'a,b), 1.68–1.56 (m, 2 H, CH_2CH_3), 1.19 (d, $J_{5,6}$ 6.2 Hz, 3 H, C-6), 0.91 (t, 3 H, CH_2CH_3); ^{13}C NMR ($CDCl_3$ + D_2O): δ 98.37 (C-1), 77.72 (C-2), 77.03 (OCH_2), 69.78 (C-2'), 68.66 (C-3), 66.92 (C-5), 58.62 (C-4'), 54.77 (OCH_3), 53.70 (C-4), 36.14 (C-3'), 22.91 (CH_2CH_3), 17.71 (C-6), 10.25 (CH_2CH_3); CIMS: *m/z* 322 ($[M + 1]^+$).

Anal. Calcd for $C_{14}H_{27}NO_7$: C, 52.34; H, 8.41; N, 4.36. Found: C, 52.20; H, 8.32; N, 4.30.

REFERENCES

1. Synthesis of ligands related to the *Vibrio cholerae* O-specific antigen. Part 15. For Part. 14, see ref. 15.
2. C. P. J. Glaudemans, *Chem. Rev.*, **91**, 25 (1991).
3. E. M. Nashed, G. R. Perdomo, E. A. Padlan, P. Kováč, E. A. Kabat, and C. P. J. Glaudemans, *J. Biol. Chem.*, **265**, 20699 (1990).
4. C. P. J. Glaudemans, and P. Kováč in *Fluorinated Carbohydrates: Chemical and Biochemical Aspects, ACS Symp. Ser.* Vol. 374; N.F. Taylor, Ed.; American Chemical Society: Washington, D.C., 1988, p 78.

5. C. P. J. Glaudemans, P. Kováč, and E. M. Nashed in *Methods in Enzymology* Y.C. Lee, & R.T. Lee Eds.; Academic Press: New York, 1994, p 305.
6. V. Pavliak, V. Pozsgay, P. Kováč, A. Karpas, C. Chu, R. Schneerson, J. Robbins, and C. P. J. Glaudemans, *J. Biol. Chem.*, **268**, 25797 (1993).
7. K. Hisatsune, S. Kondo, Y. Isshiki, T. Iguchi, and Y. Haishima, *Biochem. Biophys. Res. Commun.*, **190**, 302 (1993).
8. T. Ito, T. Higuchi, M. Hirobe, K. Hiramatsu, and T. Yokota, *Carbohydr. Res.*, **256**, 113 (1994).
9. J. Wang, J. Zhang, C. E. Miller, S. Villenuve, Y. Ogawa, P.-s. Lei, P. Lafaye, F. Nato, S. Bystrický, A. Karpas, S. C. Szu, J. B. Robbins, P. Kováč, J.-M. Fournier, and C. P. J. Glaudemans, *J. Biol. Chem.*, in press.
10. M. Gotoh, C. L. Barnes, and P. Kováč, *Carbohydr. Res.*, **260**, 203 (1994).
11. M. Gotoh, and P. Kováč, *J. Carbohydr. Chem.*, **13**, 1193 (1994).
12. P.-s. Lei, Y. Ogawa, and P. Kováč, *Carbohydr. Res.*, **279**, 117 (1995).
13. P.-s. Lei, Y. Ogawa, and P. Kováč, *Carbohydr. Res.*, **281**, 47 (1996).
14. P.-s. Lei, Y. Ogawa, J. L. Flippen-Anderson, and P. Kováč, *Carbohydr. Res.*, **275**, 117 (1995).
15. J. Zhang, and P. Kováč, *Carbohydr. Res.*, **300**, 329 (1997).
16. Y. Ogawa, P.-s. Lei, and P. Kováč, *Carbohydr. Res.*, **277**, 327 (1995).
17. J. R. Rasmussen, C. J. Slinger, R. J. Kordish, and D. D. Newman-Evans, *J. Org. Chem.*, **46**, 4843 (1981).
18. J. R. Rasmussen, *J. Org. Chem.*, **45**, 2725 (1980).
19. M. E. Evans, L. Long, and F. P. Parrish, *J. Org. Chem.*, **33**, 1074 (1968).
20. P. Kováč in *Handbook of Derivatives for Chromatography*; K. Blau, & J.M. Halket, Eds.; John Wiley & Sons, Ltd.: Chichester, 1993, p 109.
21. M. J. Eis, and B. Ganem, *Carbohydr. Res.*, **176**, 316 (1988).
22. K. Eklind, R. Gustafsson, A.-K. Tidén, T. Norberg, and P.-M. Aberg, *J. Carbohydr. Chem.*, **15**, 1161 (1996).
23. Y. Hamada, F. Yokokawa, M. Kabeya, K. Hatano, Y. Kurono, and T. Shioiri, *Tetrahedron*, **52**, 8297 (1996).