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Syntheses of the L-*manno* and some other analogs of the terminal determinants of the O-PS of *Vibrio cholerae* O:1

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Abstract

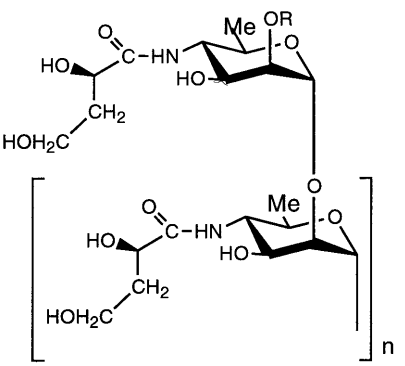
Analogs of the methyl α-glycosides of the terminal residues of the O-specific polysaccharides (O-PS) of *Vibrio cholerae* O:1, serotype Inaba and Ogawa, have been prepared as probes to study their interaction with anti *V. cholerae* O:1 antibodies. They differ from the termini of the respective O-PSs in anomeric or absolute configuration of perosamine, position of the *O*-methyl group in D-perosamine, and nature of the *N*-acyl side chain. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: *Vibrio cholerae* O:1; *Yersinia enterocolitica*; *Brucella abortus*; Perosamine

1. Introduction

Cholera is a serious, enteric infectious disease caused, inter alia, by *Vibrio cholerae* O:1 bacterial pathogens. A satisfactory vaccine for the disease is not available. One of the ongoing projects in this laboratory is aimed at developing a potent immunogen for anti *V. cholerae* O:1 antibodies that would provide long-lasting protection against cholera. Our approach involves conjugation of synthetic, linker-equipped fragments of the O-specific antigen (O-specific polysaccharide, O-PS) to a carrier protein.

V. cholerae O:1 is divided into two serotypes, Inaba and Ogawa. The internal parts of their O-PSs are identical and consist[1,2] 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)-α-D-perosamine]. The two O-PSs differ in that the position O-2 of the upstream, terminal perosamine group in the Ogawa strain is methylated (Fig. 1). We have



R

Inaba	H
Ogawa	Me

Fig. 1.

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studied[3] binding of some monoclonal antibodies for *V. cholerae* O:1 with synthetic fragments of the O-PS and some analogs of the terminal monosaccharide residues. We found, among other things, that some antibodies showed a remarkable tolerance to irregularities in the structure of ligands. For example, changing the L configuration of the tetronamido side chain for that of the D-series did not result in cessation of binding of the monoclonal antibodies specific to *V. cholerae* O:1, serotype Ogawa. Another interesting finding was that the monosaccharide ligand belonging to the Ogawa series that was deoxygenated at position 4′ showed higher affinity for the antibodies studied than the ligand reflecting the structure of the end-group in the natural Ogawa O-PS. To learn more about the structural requirements for binding, and perhaps reveal structural elements that would increase binding, we have now synthesized a series of new analogs of the upstream terminal monosaccharides of the two serotypes.

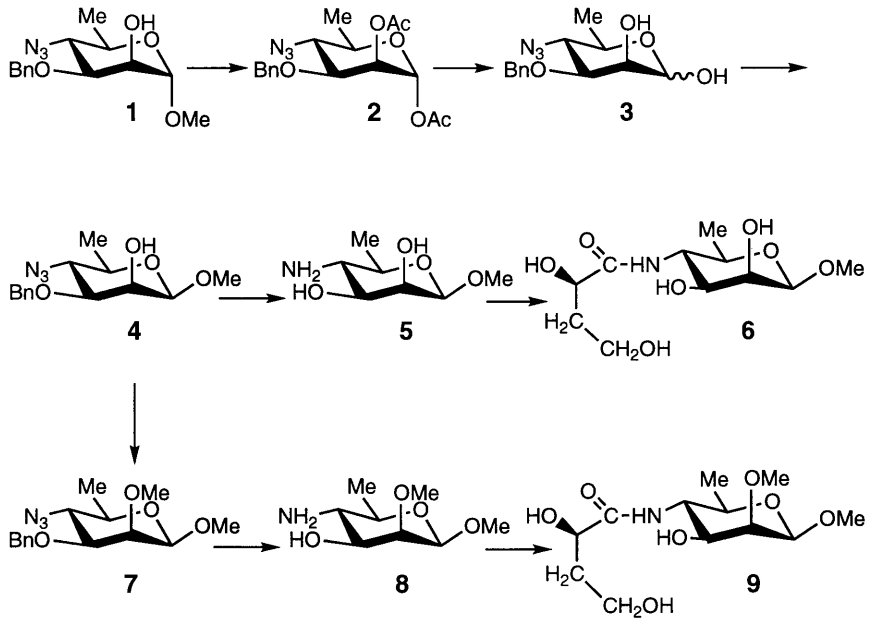
In addition to the O-PS of *V. cholerae* O:1, variously N-acylated perosamine is the key constituent of O-specific polysaccharides of many other bacterial pathogens. There, the O-PS differ from that of *V. cholerae* O:1 mainly in the nature of the *N*-acyl side chain. In this context, it is interesting to note that *N*-3-hydroxypropionyl-α-D-perosamine and *N*-3-hydroxypropionyl - 2 - *O* - methyl - α - D-perosamine were identified as constituents of the lipopolysaccharide from Vibrios serogroup 1875, referred to as Original and Variant, respectively.[4,5] On the other hand, the *O*-polysaccharide chain from *V. cholerae* O-76[6] and O-144[7] are homopolymers of, respectively, *N*-2-hydroxy-L- and D-propionyl-α-L-perosamine. Thus, some of the compounds herein described for the first time are related to structural features of important epitopes in the above *O*-polysaccharides and, as such, can be useful for immunochemical studies also in those antigen–antibody systems.

2. Results and discussion

The synthetic ligands reported here differ from the end-groups in the natural *V. cholerae* O:1 O-PSs in the anomeric or absolute configuration of perosamine, the nature of the amide group, or in the position of the methyl group in the D-perosamine moiety.

To prepare the β analogs of the monosaccharide determinants of the O-PS of *V. cholerae* O:1, serotype Inaba (**6**) and Ogawa (**9**), we applied our method of glycosylation via locked anomeric configuration,[8,9] which involves the use of 1,2-*O*-stannylene acetals as intermediates. We showed previously[10] that the undesirable epimerization that often accompanies the formation of such compounds from free sugars could be avoided by positioning an alkyl group at O-3 in the synthon. Thus, diol **3**, obtained from **1** through the acetate **2**,[11–13] was converted to the corresponding stannylene acetal, which was then treated[8,9] with methyl trifluoromethanesulfonate (methyltriflate), to give stereospecifically the corresponding β-glycoside (**4**) in 82% yield. Subsequent methylation of **4** gave **7**. The foregoing azides were converted to the corresponding amines (**5** and **8** respectively), which were treated with 3-deoxy-L-*glycero*-tetronolactone, to give the target glycosides **6** and **9** (Scheme 1). The intermediate compound **2**, previously obtained amorphous,[14] was now obtained crystalline.

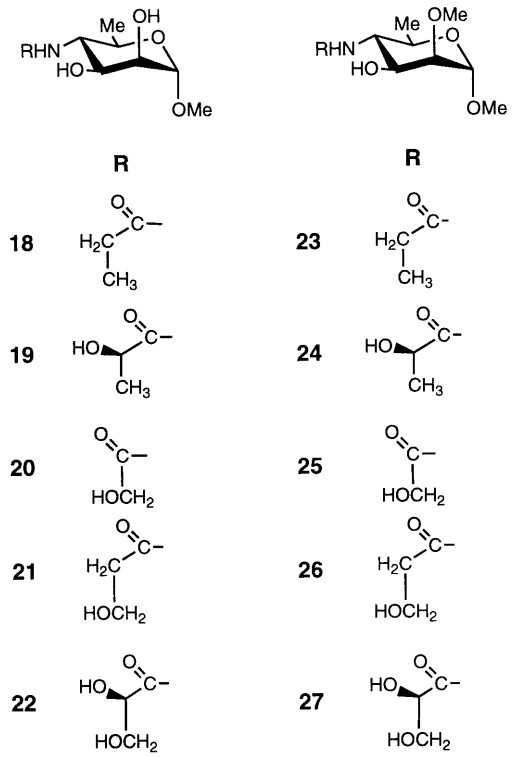
Probing the importance of the location of the methyl group at O-2 for binding of the monosaccharide determinant of the O-PS of *V. cholerae* O:1, serotype Ogawa, with their homologous antibodies required the 3-*O*-methyl derivative **15**. It was obtained from diol **10**[11–13] as starting material. Peters and Bundle[15] have shown that benzylation of **10** under phase-transfer catalysis gave 2-*O*-benzyl ether as the major product. When **10** was treated with iodomethane under the same conditions, similar regioselectivity of alkylation was observed, yielding the crystalline, hitherto unknown 2-methyl ether **12** as the main product. In addition, a small amount of the 2,3-di-*O*-methyl derivative **13** and the 3-*O*-methyl derivative **11** were also obtained (Scheme 2). The use of this method of methylation was preferred, compared to the one yielding preferentially the corresponding 3-alkyl derivative,[12] as compound **12** was



Scheme 1.

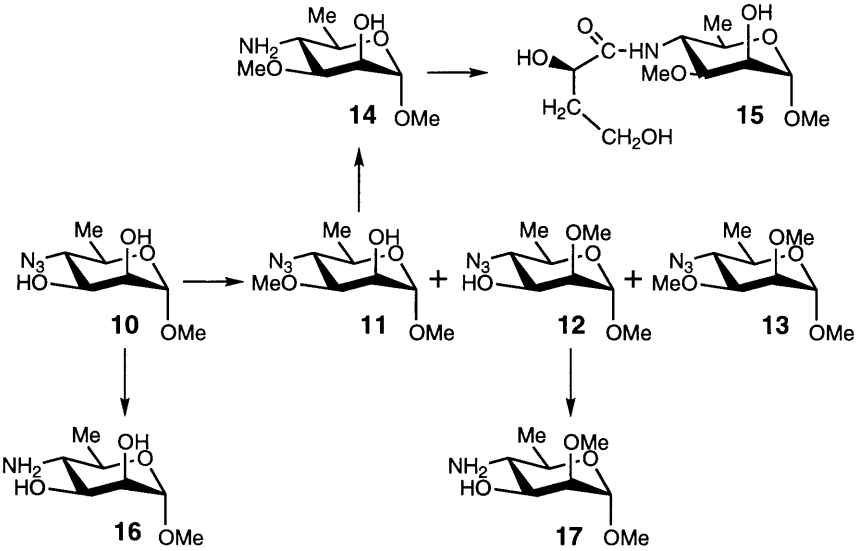
needed in much larger amount during this work. Compound **15** was then readily synthesized from **11** via amine **14**.

Many analogs here prepared (**18**–**27**) differ from the regular constituents of the O-PS of *V. cholerae* O:1 in the acylamido group.

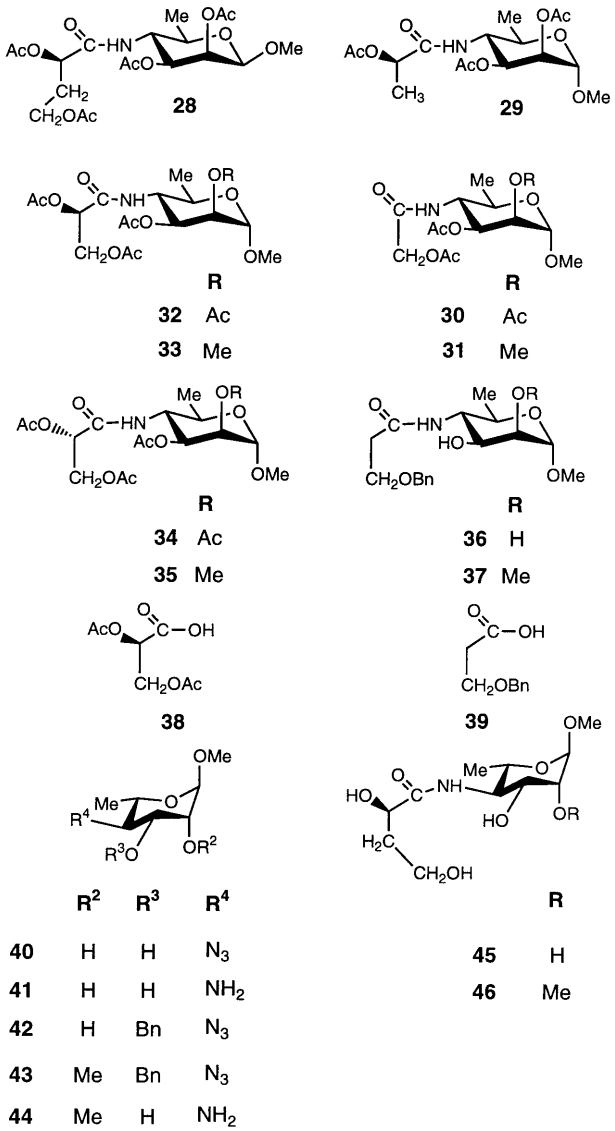


They were obtained from amines **16** and **17** by N-acylation with various acylating reagents, some of which were available commercially. The hitherto unknown acid **38** was prepared by acetylation of the hemicalcium salt of the parent, unprotected acid. In many cases, chromatography of crude products of N-acylation readily yielded pure materials. When purification of desired products was difficult, crude products of such couplings were acetylated with Ac_2O in pyridine, and the per-*O*-acetates thus obtained were purified by chromatography and crystallization. Subsequent deacetylation then gave the pure, desired substances (c.f., for example the sequence **5** → crude **6** → **28** → **6**).

Each of the reactions of **38** with amines **16** and **17** gave two products. To aid separation and purification, crude products of these reactions were processed as described above, and the fully acetylated derivatives were isolated by chromatography. The reaction of **16** yielded compounds **32** and **34**, the former largely predominating. When examined by CIMS, these compounds produced the same pseudomolecular ion peaks, and their NMR spectra were very similar, indicating that these materials were a pair of diastereoisomers.



Scheme 2.



Compounds **32** and **34** were fully characterized and identified as the L- and D-*glycero* isomers, respectively, based on the optical rotation data. The specific optical rotation we found for the commercial L-glyceric acid hemicalcium salt monohydrate suggested that the material contained a small proportion of the D enantiomeric form, which explains the formation of diastereoisomers described above. Similarly, the predominating product of the reaction of **17** with the same N-acylation reagent, followed by acetylation, was the L-compound **33**.

The title L analogs **45** and **46** were obtained by N-acylations with 3-deoxy-L-*glycero*-tetronolactone of amines **41**[16] and **44**, which were obtained from methyl α-L-rhamnopyranoside[17] following synthetic strategies developed,[11–13,18] for the corresponding D analogs.

3. Experimental

Optical rotations were measured at 25 °C with a Perkin–Elmer model 341 automatic polarimeter. All reactions were monitored by thin-layer chromatography (TLC) on silica gel-coated glass slides (Whatman or Analtech). Detection was effected by charring with 5% H_2SO_4 in EtOH and, when applicable, by UV light. Preparative chromatography was performed by gradient elution from columns

of Silica Gel 60 (E. Merck, particle size 0.035–0.070 mm) using at the onset of development a solvent mixture slightly less polar than that used for TLC. NMR spectra were obtained at 300 MHz for ^{1}H and 75 MHz for ^{13}C with a Varian Mercury spectrometer. Assignment of signals were made by first-order analysis, homonuclear decoupling and/or homo- and heteronuclear two-dimensional correlation spectroscopy. Chemical ionization mass spectra (CIMS, positive and negative ion) were obtained on a Finnigan 4000 quadrupole mass spectrometer with NH_3 reagent gas. Electron-impact ionization spectra were obtained with a VG 70E mass spectrometer. Palladium-on-charcoal (5%) catalyst (ESCAT 103) was purchased from Engelhart Industries. The use of this catalyst largely eliminated problems encountered when products of debenzylation and amines are formed at the same time.[19,20] L-Lactic (2-hydroxypropionic) acid was purchased from Sigma Chemical Co., and glycolic acid was purchased from Aldrich Chemical Co. L-Gyceric acid hemicalcium salt monohydrate {$[\alpha]_D$ −12.5° (*c* 4.6, H_2O), lit.[21], $[\alpha]_D$ −14.6° (*c* 5.0 H_2O)}, β-propiolactone and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC) were purchased from Fluka Chemical company. *O*-(7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) was purchased from PerSeptive Biosystems. The commercial products listed were used without further purification. Solutions in organic solvents were dried with anhyd Na_2SO_4 and concd at 40 °C/2 kPa.

1,2-Di-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranose (**2**).—This compound was prepared from the corresponding methyl glycoside (**1**) as described.[11,14] Crystallization from 2-Pr_2O–hexane gave pure **2**, mp 47.5–48.5 °C $[\alpha]_D$ +112.5° (*c* 0.7, $CHCl_3$); Ref.[11] $[\alpha]_D$ +89° (*c* 1, $CHCl_3$) for the material obtained in admixture with a small amount of the β isomer; Ref.[14], +111° (*c* 1.3, CH_2Cl_2) for the previously reported amorphous, pure α-acetate. Anal. Calcd for $C_{17}H_{21}N_3O_6$: C, 56.19; H, 5.83; N, 11.56. Found: C, 56.39; H, 5.86; N, 11.70.

4-Azido-3-O-benzyl-4,6-dideoxy-α,β-D-mannopyranose (**3**).—Compound **2**, or the unresolved mixture of anomeric acetates obtained as described above, was deacetylated (Zemplén) to give **3** in virtually theoretical yield, mp 135.5–136.5 °C (from EtOH–Et_2O), $[\alpha]_D$ +95° (*c* 0.75, MeOH). ^{1}H NMR ($CDCl_3$–CD_3OD): δ 5.11 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.72, 4.67 (2 d, 2 H, CH_2Ph), 4.00 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 3.82–3.74 (m, 2 H, H-3,5), 3.42 (t, 1 H, *J* 10.1 Hz, H-4), 1.30 (d, 3 H, $J_{5,6}$ 6.0 Hz, H-6). Before deuterium exchange, ^{1}H NMR ($CDCl_3$) showed the signal for H-1 as a doublet of doublets, and the signal for H-2 as a multiplet. The signals for HO-1 ($J_{1,OH}$ 3.7 Hz) and HO-2 ($J_{2,OH}$ 1.8 Hz) appear at δ 2.56 and 2.44, respectively. ^{13}C NMR ($CDCl_3$–CD_3OD): δ 93.54 (C-1), 77.74 (C-3), 71.62 (*C*H_2Ph), 67.34 (C-2), 66.21 (C-5), 64.00 (C-4), 18.25 (C-6). Anal. Calcd for $C_{13}H_{17}N_3O_4$: C, 55.91; H, 6.14; N, 15.05. Found: C, 55.74; H, 6.14; N, 15.15.

Methyl 4-azido-3-O-benzyl-4,6-dideoxy-β-D-mannopyranoside (**4**).—A mixture of **3** (279 mg, 1 mmol) and Bu_2SnO (149 mg, 1 mmol) in anhyd MeOH was stirred at 70 °C until a clear soln was obtained (~1.5 h). CsF (0.91 g, 6 mmol) and toluene (5 mL) was added, the mixture was concd, and the residue was kept at 50 °C/133 Pa for 3 h. Methyl triflate (136 μL, 1.2 mmol) was added at −10 °C to a suspension of the residue and molecular sieves 4 Å (0.5 g) in DMF (10 mL), which had been stirred at rt for 2 h. Stirring was continued at the same temperature until TLC (2:1 hexane–acetone) showed that the reaction was essentially complete (~30 min). Chromatography gave pure **4** (241 mg, 82%), mp 88.5–89 °C (from 2-Pr_2O), $[\alpha]_D$ +17° (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 4.76, 4.68 (2 d, 2 H, *J* 11.8 Hz, CH_2Ph), 4.25 (d, 1 H, $J_{1,2}$ 1.1 Hz, H-1), 4.07 (m, 1 H, H-2), 3.52 (s, partially overlapped, OCH_3), 3.49 (t, partially overlapped, H-4), 3.40 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 9.5 Hz, H-3), 3.14 (m, 1 H, H-5), 2.44 (bs, 1 H, OH), 1.45 (d, 3 H, $J_{5,6}$ 6.1 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 100.44 (C-1, $J_{C\text{-}1,H\text{-}1}$ 155.4 Hz), 79.81 (C-3), 71.31 (*C*H_2Ph), 70.79 (C-5), 67.19 (C-2), 63.57 (C-4), 56.95 (OCH_3), 18.41 (C-6); CIMS: *m*/*z* 293 ([M + 18]$^+$). Anal. Calcd for $C_{14}H_{19}N_3O_4$: C, 57.33; H, 6.53; N, 14.33. Found: C, 57.49; H, 6.53; N, 14.20.

Methyl 4-amino-4,6-dideoxy-β-D-mannopyranoside (**5**).—A soln of the azide **4** (1.43 g)

in EtOH (50 mL) was treated at rt and atmospheric pressure with H_2 in the presence of ESCAT 103 (1 g) until TLC (4:1 CH_2Cl_2–MeOH) showed that the reaction was complete (24–48 h). The mixture was worked up conventionally to give **5** in virtually theoretical yield, mp 124–125.5 °C (from MeOH–hexane), $[\alpha]_D$ −109° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 4.34 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 3.88 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 3.53 (s, 3 H, OCH_3), 3.29 (dd, 1 H, $J_{3,4}$ 10 Hz, H-3), 3.17 (m, 1 H, H-5), 2.70 (t, 1 H, *J* 9.7 Hz, H-4), 1.32 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 100.84 (C-1), 73.35 (C-3), 72.60 (C-5), 69.71 (C-2), 56.40 (OCH_3), 54.12 (C-4), 17.28 (C-6); CIMS: *m*/*z* 178 ($[M+1]^+$), 195 ($[M+18]^+$). Anal. Calcd for $C_7H_{15}NO_4$: C, 47.45; H, 8.53; N, 7.90. Found: C, 47.32; H, 8.46; N, 7.80.

Methyl 2,3-di-O-*acetyl-4-(2,4-di*-O-*acetyl-3-deoxy*-L-glycero-*tetronamido)-4,6-dideoxy-β*-D-*mannopyranoside* (**28**) *and methyl 4-(3-deoxy*-L-glycero-*tetronamido)-4,6-dideoxy-β*-D-*mannopyranoside* (**6**).—A mixture of amine **5** (356 mg, 2 mmol), 3-deoxy-L-*glycero*-tetronolactone (490 mg, 4.8 mmol) and pyridine (0.2 mL) was heated at 110 °C (bath) overnight. After cooling to rt, pyridine (2 mL) and Ac_2O (2 mL) was added, and the mixture was kept at rt overnight. After concentration, chromatography and crystallization gave the fully acetylated compound **28** (0.96 g, 77.5%), mp 139–139.5 °C (from EtOAc–2-Pr_2O), $[\alpha]_D$ +30° (*c* 0.75, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 6.01 (d, 1 H, $J_{4,NH}$ 9.3 Hz, NH), 5.43 (dd, 1 H, $J_{1,2}$ 0.9, $J_{2,3}$ 3.3 Hz, H-2), 5.07 (dd, partially overlapped, $J_{2',3a'}$ 5.07, $J_{2',3b'}$ 7.9 Hz, H-2′), 5.04 (dd, partially overlapped, $J_{3,4}$ 10.8 Hz, H-3), 4.48 (d, 1 H, H-1), 4.20–4.06 (m, 3 H, H-4,4′a,b), 3.51 (s, partially overlapped, OCH_3), 3.52–3.42 (m, partially overlapped, H-5), 2.22–2.03 (m, 14 H, H-3′a,b, including 4 s at 2.19, 2.16, 2.05, 2.04, 4 $COCH_3$), 1.32 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 171.07, 170.27, 169.66, 169.43, 162.53 (5 CO), 99.46 (C-1), 71.98 (C-5), 70.99 (C-2′), 70.31 (C-3), 68.37 (C-2), 59.77 (C-4′), 57.18 (OCH_3), 51.52 (C-4), 30.53 (C-3′), 20.90, 20.82, 20.72, 20.67 (4 COCH_3), 17.78 (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, 51.15; H, 6.69; N, 3.23.

Deacetylation of **28** (Zemplén) gave **6** in virtually theoretical yield, mp 179.5–180 °C (from EtOH), $[\alpha]_D$ −84.5° (*c* 1.2, MeOH), ^{1}H NMR (D_2O): δ 4.51 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 4.27 (dd, 1 H, $J_{2',3'a}$ 4.0, $J_{2',3'b}$ 8.8 Hz, H-2′), 3.98 (dd, 1 H, $J_{2,3}$ 2.6 Hz, H-2), 3.77–3.74 (m, partially overlapped, H-3,4), 3.72 (q, partially overlapped, H-4′a,b), 3.57–3.49 (m, 4 H, H-5, including s, 3.51, OCH_3), 2.08–1.77 (2 m, 2 H, H-3′a,b), 1.20 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR (D_2O): δ 177.27 (CO), 101.16 (C-1), 71.12 (C-5), 70.63 (C-3), 69.84 (C-2), 69.08 (C-2′), 57.93 (C-4′), 56.92 (OCH_3), 53.00 (C-4), 36.05 (C-3′), 16.93 (C-6); CIMS: *m*/*z* 280 ($[M+1]^+$), 297 ($[M+18]^+$). Anal. Calcd for $C_{11}H_{21}NO_8 \cdot 0.5\,H_2O$: C, 44.43; H, 7.67; N, 4.71. Found: C, 44.55; H, 7.58; N, 4.55.

Methyl 4-azido-3-O-*benzyl-2*-O-*methyl-4,6-dideoxy-β*-D-*mannopyranoside* (**7**).—Iodomethane (0.52 mL, 8.5 mmol) was added to a stirred mixture of the β-glycoside **4** (1.66 g, 5.66 mmol) and powdered KOH (1 g, ~20 mmol) in Me_2SO (20 mL), and stirring was continued for 1 h). TLC (2:1 hexane–acetone) showed that the reaction was complete and that one faster moving product was formed. After filtration through a sintered glass funnel (coarse porosity), water (100 mL) was added to the filtrate, pH was adjusted to 7.5 by addition of dilute AcOH, and the product was extracted into CH_2Cl_2. The organic phase was dried, concd, and the residue was passed through a short column of silica gel to give the title compound **7** (1.7 g, 98%) as an oil, $[\alpha]_D$ +18° (*c* 0.9, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 4.74, 4.68 (2 d, 1 H each, ^2J 11.9 Hz, CH_2Ph), 4.19 (d, 1 H, $J_{1,2}$ 0.7 Hz, H-1), 3.61 (s, partially overlapped, OCH_3-2), 3.60 (bd, partially overlapped, H-2), 3.48 (s, partially overlapped, OCH_3-1), 3.47 (t, partially overlapped, *J* 9.4 Hz, H-4), 3.35 (dd, 1 H, $J_{2,3}$ 2.9, $J_{3,4}$ 9.9 Hz, H-3), 3.14–3.05 (m, 1 H, H-5), 1.38 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 101.98 (C-1, $J_{C\text{-}1,H\text{-}1}$ 154.0 Hz), 80.52 (C-3), 76.65 (C-2), 71.75 (*C*H_2Ph), 71.05 (C-5), 64.00 (C-4), 61.29 (OCH_3-2), 57.03 (OCH_3-1), 18.41 (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, 58.57; H, 6.84; N, 13.77.

Methyl 4-amino-4,6-dideoxy-2-O-*methyl-β*-D-*mannopyranoside* (**8**).—The 2-*O*-methyl

derivative **7** (1.68 g) was treated with H_2, as described above for the preparation of **5**, to give the title compound **8** (0.89 g, 85%), mp 152.5–153 °C (from EtOH–hexane), $[\alpha]_D$ −124° (*c* 0.9, MeOH). ^{1}H NMR ($CDCl_3$–CD_3OD): δ 4.35 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1), 3.62 (s, 3 H, OCH_3-2), 3.53–3.51 (m, 4 H, including s at 3.52 for OCH_3-1, H-2), 3.27 (dd, 1 H, $J_{2,3}$ 3.5, $J_{3,4}$ 10.1 Hz, H-3), 3.18–3.09 (m, 1 H, H-5), 2.63 (t, 1 H, *J* 9.6 Hz, H-4), 1.32 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR ($CDCl_3$–CD_3OD): δ 102.31 (C-1), 79.58 (C-2), 73.29 (C-3), 72.70 (C-5), 61.48 (OCH_3-2), 56.61 (OCH_3-1), 54.76 (C-4), 17.29 (C-6); CIMS: *m*/*z* 192 ($[M+1]^+$), 209 ($[M+18]^+$). Anal. Calcd for $C_8H_{17}NO_4$: C, 50.25; H, 8.96; N, 7.32. Found: C, 50.20; H, 8.95; N, 7.40.

Methyl 4-(3-deoxy-L-glycero-*tetronamido)-4,6-dideoxy-2*-O-*methyl-β*-D-*mannopyranoside* (**9**).—A mixture of amine **8** (640 mg, 3.35 mmol), 3-deoxy-L-*glycero*-tetronolactone (684 mg, 6.7 mmol) and pyridine (0.2 mL) was stirred at 110 °C (bath) overnight. TLC (5:1 CH_2Cl_2–MeOH) showed that all starting amine was consumed. Elution from a small column of silica gel gave **9** (702 mg, 75%), which solidified on standing, mp 229–229.5 °C (from EtOH), $[\alpha]_D$ −108° (*c* 0.9, MeOH); ^{1}H NMR ($CDCl_3$–CD_3OD): δ 4.38 (d, 1 H, $J_{1,2}$ ~0.6 Hz, H-1), 4.21 (dd, 1 H, $J_{2',3a'}$ 4.0, $J_{2',3b'}$ 7.6 Hz, H-2′), 3.80–3.74 (m, 3 H, H-4,4′a,b), 3.62 (s, partially overlapped, OCH_3-2), 3.60 (dd, partially overlapped, $J_{2,3}$ 3.3, $J_{3,4}$ 10.3 Hz, H-3), 3.57 (dd, 1 H, H-2), 3.52 (s, 3 H, OCH_3-1), 3.49–3.40 (m, 1 H, H-5), 2.08–1.97, 1.91–1.78 (2 m, 1 H each, H-3′a,b), 1.26 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR ($CDCl_3$–CD_3OD): δ 101.86 (C-1), 79.56 (C-2), 70.88 (C-3), 70.51 (C-5), 69.38 (C-2′), 60.88 (OCH_3-2), 58.01 (C-4′), 56.16 (OCH_3-1), 53.26 (C-4), 35.94 (C-3′), 16.83 (C-6); CIMS: *m*/*z* 294 ($[M+1]^+$), 311 ($[M+18]^+$). Anal. Calcd for $C_{11}H_{23}NO_7$: C, 49.14; H, 7.90; N, 4.78. Found: C, 49.18; H, 8.02; N, 4.77.

Methyl 4-azido-2-O- (**12**), *3*-O- (**11**) *and 2,3-di*-O-*methyl-4,6-dideoxy*-α-D-*mannopyranoside* (**13**).—A mixture of compound **10**,[11–13] (5 g, 23.6 mmol) Bu_4NHSO_4 (1.28 g), 20% NaOH (120 mL) and MeI (5.9 mL, 94.8 mmol) in CH_2Cl_2 (120 mL) was stirred at rt overnight. TLC (6:1 toluene–acetone) showed that the starting diol was essentially consumed and that three products were formed. The phases were separated, the aq soln was extracted with CH_2Cl_2, and the combined solutions in CH_2Cl_2 were washed with satd aq NaCl. The organic phase was dried and concd, and the residue was chromatographed.

The 2,3-di-*O*-methyl derivative **13** eluted first was a colorless oil (0.7 g, 12.4%), $[\alpha]_D$ +125° (*c* 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 4.74 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 3.57 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 3.52, 3.50 (2 s, partially overlapped, OCH_3-2,3), 3.52–3.39 (m, 3 H, H-3,5 including t at 3.42, H-4), 3.35 (s, 3 H, OCH_3-1), 1.31 (d, 3 H, $J_{5,6}$ 5.7 Hz, H-6). ^{13}C NMR ($CDCl_3$): δ 98.16 (C-1), 79.96 (C-3), 75.69 (C-2), 66.85 (C-5), 63.98 (C-4), 59.15, 57.24 (2 OCH_3-2,3), 54.90 (OCH_3-1), 18.38 (C-6). CIMS: *m*/*z* 249 ($[M+18]^+$). Anal. Calcd for $C_9H_{17}N_3O_4$: C, 46.74; H, 7.41; N, 18.17. Found: C, 46.68; H, 7.35; N, 18.17.

Eluted next was the 2-*O*-methyl derivative **12** (2.66 g, 50.2%), mp 57–57.5 °C (from 2-Pr_2O), $[\alpha]_D$ +71° (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 4.76 (bd, 1 H, H-1), 3.82 (ddd, partially overlapped, 1 H, $J_{2,3}$ 3.8, $J_{3,4}$ 10.1, $J_{3,OH}$ 10.1 Hz, H-3), 3.53–3.43 (m, 5 H, H-5, including s, 3.48 for OCH_3-2 and dd, $J_{1,2}$ ~1.4 Hz for H-2), 3.36 (s, 3 H, OCH_3-1), 3.19 (t, 1 H, H-4), 1.32 (d, 3 H, $J_{5,6}$ 6.0 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 97.02 (C-1), 79.30 (C-2), 70.27 (C-3), 66.53 (C-4), 66.29 (C-5), 58.75 (OCH_3-2), 54.93 (OCH_3-1), 18.29 (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, 44.52; H, 7.07; N, 19.55.

Eluted last was the amorphous 3-*O*-methyl derivative **11** (1.18 g, 22.3%), $[\alpha]_D$ +156.5° (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 4.73 (d, $J_{1,2}$ 1.5 Hz, H-1), 4.02 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 3.56–3.44 (m, 5 H, H-3,5, including s at 3.50 for OCH_3-2), 3.36 (s, partially overlapped, OCH_3-1), 3.34 (t, partially overlapped, *J* 9.7 Hz, H-4), 1.32 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 100.90 (C-1), 80.55 (C-3), 66.52 (C-2), 66.35 (C-5), 63.69 (C-4), 57.22 (OCH_3-3), 54.97 (OCH_3-1), 18.32 (C-6); CIMS: *m*/*z* 235 ($[M+18]^+$). Found: C, 44.37; H, 7.01; N, 19.13.

Methyl 4-(3-deoxy-L-glycero-*tetronamido)-4,6-dideoxy-3*-O-*methyl*-α-D-*mannopyranoside*

(**15**).—Compound **11** (1.19 g) was treated with H_2 as described for the preparation of **8**. The crude product was purified by chromatography to give amorphous, hygroscopic methyl 4 - amino - 4,6 - dideoxy - 3 - *O* - methyl - α - D-mannopyranoside (**14**) (725 mg, 75%). ^{1}H NMR ($CDCl_3$–CD_3OD): δ 4.70 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.01 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 3.61–3.49 (m, 1 H, H-5), 3.43 (s, 3 H, OCH_3-3), 3.37 (s, 3 H, OCH_3-1), 3.24 (dd, 1 H, $J_{3,4}$ 9.9 Hz, H-3), 2.83 (t, 1 H, *J* 9.8 Hz, H-4), 1.28 (d, 3 H, $J_{5,6}$ 6.4 Hz, H-6); ^{13}C NMR ($CDCl_3$–CD_3OD): δ 100.89 (C-1), 80.42 (C-3), 68.35 (C-5), 64.68 (C-2), 55.96 (OCH_3-3), 54.47 (OCH_3-1), 52.65 (C-4), 17.41 (C-6), CIMS: *m*/*z* 192 ($[M+1]^+$), 209 ($[M+18]^+$).

Amine **14** (380 mg, 2 mmol) was treated with 3-deoxy-L-*glycero*-tetronolactone (858 mg, 8.4 mmol) as described for the preparation of **9**. Chromatography and crystallization from acetone gave **15** (381 mg, 65%), mp 137–137.5 °C (from acetone), $[\alpha]_D$ +43° (*c* 0.8, MeOH). ^{1}H NMR ($CDCl_3$–CD_3OD): δ 4.72 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.25 (dd, 1 H, $J_{2',3'a}$ 3.9, $J_{2',3'b}$ 8.2 Hz, H-2′), 4.05 (dd, 1 H, $J_{2,3}$ 2.9 Hz, H-2), 3.96 (t, 1 H, *J* 10.3 Hz, H-4), 3.80–3.69 (m, 3 H, H-5,4′a,b), 3.47 (dd, 1 H, $J_{3,4}$ 10.4 Hz, H-3), 3.39 (s, 3 H, OCH_3-1), 3.38 (s, 3 H, OCH_3-3), 2.09–1.98, 1.88–1.76 (2 m, 1 H each, H-3′a,b), 1.23 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$–CD_3OD): δ 100.52 (C-1), 78.16 (C-3), 69.87 (C-2′), 66.81 (C-5), 65.51 (C-2), 58.66 (C-4′), 56.35 (OCH_3-3), 54.51 (OCH_3-1), 51.14 (C-4), 36.09 (C-3′), 17.25 (C-6); CIMS: *m*/*z* 294 ($[M+1]^+$), 311 ($[M+18]^+$). Anal. Calcd for $C_{12}H_{23}NO_7$: C, 49.14; H, 7.90; N, 4.78. Found: C, 49.21; H, 7.78; N, 4.84.

Methyl 4,6-dideoxy-4-propanamido-α-D-mannopyranoside (**18**).—A mixture of azide **10**[11–13] (1.29 g, 5.9 mmol), propanoic anhydride (1.5 mL, 11 mmol) and ESCAT 103 (0.5 g) was stirred in a H_2 atmosphere for 2 h. After concentration, chromatography and crystallization gave the title compound **18** (1.28 g, 92%), mp 171–171.5 °C (from EtOH), $[\alpha]_D$ +82° (*c* 0.8, MeOH). ^{1}H NMR (4:1 $CDCl_3$–CD_3OD): δ 4.66 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 3.87 (t, partially overlapped, *J* 10.2, H-4), 3.82 (dd, partially overlapped, $J_{2,3}$ 3.4 Hz, H-2), 3.70–3.58 (m, 2 H, H-5, including dd at 3.67, $J_{3,4}$ 10.6 Hz, H-3), 2.25 (t, 2 H, *J* 7.6 Hz, H-2′), 1.22 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6), 1.15 (t, 3 H, H-3′); ^{13}C NMR (4:1 $CDCl_3$–CD_3OD): δ 100.78 (C-1), 69.52 (C-2), 69.30 (C-3), 54.43 (OCH_3), 52.93 (C-4), 29.21 (C-2′), 17.35 (C-6), 9.55 (C-3′); CIMS: *m*/*z* 234 ($[M+1]^+$), 252 ($[M+18]^+$). Anal. Calcd for $C_{10}H_{19}NO_5$: C, 51.49; H, 8.21; N, 6.00. Found: C, 51.34; H, 8.14; N, 5.92.

Methyl 4,6-dideoxy-2-O-methyl-4-propanamido-α-D-mannopyranoside (**23**).—A mixture of azide **12** (740 mg, 3.4 mmol), ESCAT 103 (0.7 g) and propanoic anhydride (0.87 mL, 6.8 mmol) was treated with H_2 as described for the preparation of **18** to give the title compound **23** (660 mg, 78%), mp 188.5–189 °C (from EtOH). $[\alpha]_D$ +65° (*c* 0.9, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 4.37 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 3.86 (t, 1 H, *J* 10.3 Hz, H-4), 3.67 (dd, 1 H, $J_{2,3}$ 3.4, $J_{3,4}$ 10.7 Hz, H-3), 3.59 (m, 1 H, H-5), 3.50 (s, 3 H, OCH_3-2), 3.42 (dd, 1 H, H-2), 3.37 (s, 3 H, OCH_3-1), 2.25 (q, 1 H, $J_{2',3'}$ 7.6 Hz, H-2′), 1.20 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6), 1.15 (t, 3 H, H-3′). Before deuterium exchange, the NH and H-3 signals appear at δ 5.46 (s) and 3.69 (dt). ^{13}C NMR ($CDCl_3$): δ 97.44 (C-1), 79.28 (C-2), 69.81 (C-3), 67.35 (C-5), 58.87 (OCH_3-2), 54.91 (OCH_3-1), 54.10 (C-4), 29.82 (C-2′), 17.93 (C-6), 9.82 (C-3′). CIMS: *m*/*z* 248 ($[M+1]^+$), 265 ($[M+18]^+$). Anal. Calcd for $C_{11}H_{21}NO_5$: C, 53.43; H, 8.56; N, 5.66. Found: C, 53.31; H, 8.45; N, 5.52.

*Methyl 4,6-dideoxy-4-(*L-glycero-*2-hydroxypropanamido)-α-D-mannopyranoside* (**19**) *and methyl 2,3-di-O-acetyl-4,6-dideoxy-4-(2-O-acetyl-L-glycero-propanamido)-α-D-mannopyranoside* (**29**).—A mixture of amine **16**[12] (558 mg, 3 mmol), L-lactic acid (270 mg, 9 mmol), EDAC (1.72 g, 9 mmol) and HOBT (1.28 g, 9 mmol) in 1:2 MeCN–CH_2Cl_2 (15 mL) was stirred at rt overnight. TLC (2:1 CH_2Cl_2–MeOH) then showed that all amine had been consumed. After concentration, the residue was chromatographed to give **19** that still contained some UV positive material. Acetylation, as described below for the preparation of **28**, gave **29** (0.97 g, 82.7%), mp 131.5–132 °C (from EtOAc–2-Pr_2O), $[\alpha]_D$ +63° (*c* 0.8, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 6.12 (d, 1 H, $J_{4,NH}$ 9.4 Hz, NH), 5.27 (dd, 1 H, $J_{2,3}$ 3.4, $J_{3,4}$ 11.1 Hz, H-3), 5.14 (dd, 1 H, $J_{1,2}$ 1.8 Hz, H-2), 5.07 (q, 1 H, $J_{2',3'}$ 6.9 Hz, H-2′),

4.66 (d, 1 H, H-1), 4.22 (2 t, partially overlapped, H-4), 3.75–3.65 (m, 1 H, H-5), 3.39 (s, 3 H, OCH_3), 2.17, 2.15, 2.04 (3 s, 3 H each, 3 $COCH_3$), 1.42 (d, 3 H, H-3′), 1.25 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 171.03, 170.72, 170.01, 169.48 (4 CO), 98.30 (C-1), 70.44 (C-2′), 69.17 (C-2), 68.36 (C-3), 67.95 (C-5), 55.02 (OCH_3), 51.21 (C-4), 20.92, 20.86, 20.70 (3 $CO\mathit{C}H_3$), 17.67 (C-6), 17.55 (C-3′); CIMS: m/z 250 ($[M+1]^+$), 267 ($[M+18]^+$). Anal. Calcd for $C_{16}H_{25}NO_9$: C, 51.19; H, 6.71; N, 3.73. Found: C, 51.40; H, 6.74; N, 3.76.

Compound **29** was deacetylated (Zemplén) to give the crystalline hemihydrate of **19** in virtually theoretical yield, mp 137.5–138 °C (from acetone–hexane), $[\alpha]_D$ +66° (*c* 0.6, MeOH). ^1H NMR ($CDCl_3$–CD_3OD): δ 7.25 (d, 1 H, $J_{4,NH}$ 9.4 Hz, NH), 4.68 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.17 (q, 1 H, $J_{2',3'}$ 6.8 Hz, H-3′), 3.88–3.81 (m, 2 H, H-2,4), 3.78–3.67 (m, 2 H, H-3,5), 3.37 (s, 3 H, OCH_3), 1.40 (d, 3 H, H-3′), 1.22 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 100.82 (C-1), 69.58 (C-2), 69.14 (C-3), 67.86 (C-2′), 66.78 (C-5), 54.54 (OCH_3), 53.06 (C-4), 20.17 (C-3′), 17.36 (C-6). Anal. Calcd for $C_{10}H_{19}NO_6 \cdot 0.5\ H_2O$: C, 46.50; H, 7.80; N, 5.42. Found: C, 46.68; H, 7.73; N, 5.40.

*Methyl 4,6-dideoxy-4-(*L-glycero-*2-hydroxypropanamido)-2-*O*-methyl-α-*D*-mannopyranoside* (**24**).—A mixture of amine **17**[18] (prepared from azide **12** as described below, 191 mg, 1 mmol), L-lactic acid (108 mg, 1.2 mmol), EDAC (574 mg, 3 mmol) and 1-hydroxybenzotriazole (406 mg, 3 mmol) in CH_2Cl_2 (10 mL) was stirred at rt overnight. TLC (6:1 CH_2Cl_2–MeOH) then showed that all amine was consumed and that one major, faster moving product was formed. The mixture was washed successively with 2 M HCl, aq $NaHCO_3$ and NaCl, dried, and concd, and the residue was chromatographed to give the pure title compound **24** (205 mg, 78%), mp 165.5–166 °C (from EtOH–hexane), $[\alpha]_D$ +50° (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$–CD_3OD): δ 7.13 (d, <1 H, due to incomplete H→D exchange, $J_{1,2}$ 9.3 Hz, NH), 4.77 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 4.15 (q, 1 H, $J_{2',3'}$ 6.9 Hz, H-2′), 3.82 (t, 1 H, *J* 10.3, H-4), 3.75 (dd, partially overlapped, $J_{2,3}$ 3.1, $J_{3,4}$ 10.3 Hz, H-3), 3.75–3.63 (m, partially overlapped, H-5), 3.51 (s, 3 H, OCH_3-2), 3.44 (dd, 1 H, H-2), 3.38 (s, 3 H, OCH_3-1), 1.40 (d, 3 H, H-3′), 1.21 (s, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$–CD_3OD): δ 97.57 (C-1), 79.29 (C-2), 68.91 (C-3), 67.95 (C-2′), 66.88 (C-5), 58.72 (OCH_3-2), 54.66 (OCH_3-1), 53.48 (C-4), 20.16 (C-3′), 17.41 (C-6). CIMS: m/z 264 ($[M+1]^+$), 281 ($[M+18]^+$). Anal. Calcd for $C_{11}H_{21}NO_6$: C, 50.18; H, 8.04; N, 5.32. Found: C, 50.28; H, 8.01; N, 5.24.

*Methyl 4,6-dideoxy-4-(hydroxyacetamido)-α-*D*-mannopyranoside* (**20**).—Methyl 4-amino-4,6-dideoxy-α-D-mannopyranoside (**16**) (224 mg, 1.26 mmol)[12] was treated with glycolic acid (123 mg, 1.62 mmol) as described below for the corresponding 2-*O*-methyl derivative, to give **20** (240 mg, 81%), mp 145.5–146 °C (from acetone), $[\alpha]_D$ +67° (*c* 0.8, H_2O). ^1H NMR ($CDCl_3$–CD_3OD): δ 4.68 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.07, 4.00 (2 d, 2 H, ^2J 16.3 Hz, H-2′a,b), 3.92 (t, 1 H, *J* 10.3 Hz, H-4), 3.85 (dd, 2 H, $J_{2,3}$ 3.3 Hz, H-2), 3.78–3.67 (m, 2 H, including dd for H-3 at 3.74, $J_{3,4}$ 10.5 Hz, H-5), 3.38 (s, 3 H, OCH_3), 1.23 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$–CD_3OD): δ 100.77 (C-1), 69.45 (C-2), 49.13 (C-3), 66.73 (C-5), 61.38 (C-2′), 54.47 (OCH_3), 52.73 (C-4), 17.30 (C-6); CIMS: m/z 236 ($[M+1]^+$), 253 ($[M+18]^+$). Anal. Calcd for $C_9H_{17}NO_6$: C, 45.95; H, 7.28; N, 5.95. Found: C, 46.01; H, 7.26; N, 6.02.

Compound **20** was further characterized as methyl 2,3-di-*O*-acetyl-4,6-dideoxy-4-(acetoxyacetamido)-α-D-mannopyranoside (**30**), mp 158–161 °C (from EtOH), $[\alpha]_D$ +77° (*c* 0.9, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 6.12 (d, 1 H, $J_{4,NH}$ 9.3 Hz, NH), 5.27 (dd, 1 H, $J_{3,4}$ 11.1, $J_{2,3}$ 3.4 Hz, H-3), 5.13 (dd, 1 H, $J_{1,2}$ 1.8 Hz, H-2), 4.67 (d, 1 H, H-1), 4.68 (d, 1 H, ^2J 15.5 Hz, H-2′a), 4.40 (d, 1 H, H-2′b), 4.25 (q, 1 H, *J* 10.1 Hz, H-4), 3.72 (m, 1 H, H-5), 3.39 (s, 3 H, OCH_3), 2.19, 2.16, 2.01 (3 s, 3 H each, 3 $COCH_3$), 1.28 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6). ^{13}C NMR ($CDCl_3$): δ 171.9, 170.57, 170.05, 167.96 (4 CO), 98.57 (C-1), 69.46 (C-2), 68.40 (C-3), 68.34 (C-5), 62.75 (C-2′), 55.22 (OCH_3), 51.64 (C-4), 21.01, 20.80, 20.68 (3 $C\mathit{H}_3CO$), 17.89 (C-6); CIMS: m/z 379 ($[M+18]^+$). Anal. Calcd for $C_{15}H_{23}NO_9$: C, 49.86; H, 6.42; N, 3.88; O, 39.85. Found: C, 49.94; H, 6.41; N, 3.88.

Methyl 4,6-dideoxy-4-(hydroxyacetamido)-2-O-methyl-α-D-mannopyranoside (**25**).—Azide **12** was treated with H_2 as described for the preparation of **5** to give methyl 4-amino-4,6-dideoxy-2-*O*-methyl-α-D-mannopyranoside (**17**), which was identical (NMR) with the previously described, independently synthesized compound.[18] To a soln of the foregoing amine **17** (450 mg, 2.35 mmol) and glycolic acid (358 mg, 4.7 mmol) in MeCN (25 mL) was added HATU (1.79 g, 4.7 mmol), and diisopropylethylamine (0.85 mL, 4.7 mmol), and the mixture was stirred for 1 h at rt. TLC (5:1 $CHCl_3$–MeOH) then showed that all starting amine had been consumed. After concentration and chromatography the material that was obtained, which still contained some impurities, was acetylated to give methyl 3-*O*-acetyl-4,6-dideoxy-4-(acetoxyacetamido)-2-*O*-methyl-α-D-mannopyranoside (**31**) (516 mg, 66%), mp 143.5–144.5 °C (from EtOAc), $[\alpha]_D$ +105° (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 6.04 (d, 1 H, $J_{4,NH}$ 9.2 Hz, NH), 5.20 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 11.3 Hz, H-3), 4.75 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 4.67, 4.38 (2 s, 1 H each, ^2J 15.5 Hz, H-2′a,b), 4.28 (q, 1 H, *J* 9.9 Hz, H-4), 3.70–3.60 (m, 1 H, H-5), 3.51 (s, 3 H, OCH_3-2), 3.49 (dd, 1 H, H-2), 3.38 (s, 3 H, OCH_3-1), 2.19, 2.08 (2 s, 3 H each, 2 $COCH_3$), 1.26 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 98.58 (C-1), 77.85 (C-2), 70.89 (C-3), 68.42 (C-5), 62.57 (C-2′), 59.57 (OCH_3-2), 54.92 (OCH_3-1), 51.57 (C-4), 20.94, 20.68 (2 CO*C*H_3), 17.92 (C-6); CIMS: *m*/*z* 334 ($[M+1]^+$), 351 ($[M+18]^+$). Anal. Calcd for $C_{14}H_{23}NO_8$: C, 50.44; H, 6.95; N, 4.20; O, 38.40. Found: C, 50.45; H, 7.01; N, 4.13.

Deacetylation of **31** gave the title substance **25**, $[\alpha]_D$ +40° (*c* 0.8, H_2O). ^{1}H NMR ($CDCl_3$): δ 7.03 (d, 1 H, $J_{4,NH}$ 9.7 Hz, NH), 4.78 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.06 (dd, 2 H, ^2J 16.4 Hz, H-2′a,b), 3.90 (dd, 1 H, $J_{3,4}$ 10.1, $J_{4,5}$ 10.1 Hz, H-4), 3.78 (bdd, 1 H, $J_{2,3}$ ~2.8 Hz, H-3), 3.67 (m, 1 H, H-5), 3.50 (s, 3 H, OCH_3-2), 3.45 (dd, 1 H, H-2), 3.38 (s, 3 H, OCH_3-1), 1.24 (d, 3 H, H-6); ^{13}C NMR ($CDCl_3$): δ 174.13 (CO), 97.39 (C-1), 79.27 (C-2), 69.29 (C-3), 66.91 (C-5), 62.21 (C-2′), 58.79 (OCH_3-2), 54.90 (OCH_3-1), 53.58 (C-4), 17.82 (C-6); CIMS: *m*/*z* 250 ($[M+1]^+$) and 267 ($[M+18]^+$). Anal. Calcd for $C_{10}H_{19}NO_6$: C, 48.19; H, 7.68; N, 5.62; O, 38.51. Found: C, 48.29; H, 7.71; N, 5.66.

Methyl 2,3-di-O-acetyl-4,6-dideoxy-4-(2,3-di-O-acetyl-D- (**34**) *and* L-glycero-*propanamido)-α-D-mannopyranoside* (**32**).—To a suspension of L-glyceric acid hemicalcium salt monohydrate (2.0 g, 13.97 mmol) in pyridine (100 mL) was added Ac_2O (15 mL), and the mixture was stirred at rt for 24 h. Water (75 mL) was added at 0 °C to the resulting clear solution, and the mixture was stirred for 2 h to decompose the excess Ac_2O. The soln was concd and co-evaporated with toluene (3 × 150 mL). A suspension of the residue in $CHCl_3$ (400 mL) was washed with 10% HCl satd with NaCl (80 mL), and the aq layer was extracted with $CHCl_3$ (3 × 100 mL). The organic layers were combined, washed with brine (100 mL), dried, and concd to give 2,3-di-*O*-acetyl-L-glyceric acid (**38**) (2.52 g, 97%), which was sufficiently pure for the next step. ^{1}H NMR ($CDCl_3$): δ 9.11 (bs, 1 H, COOH), 5.37 (dd, 1 H, $J_{2,3a}$ 3.2, $J_{2,3b}$ 5.1 Hz, H-2), 4.54 (dd, 1 H, ^2J 12.1 Hz, H-3a), 4.45 (dd, 1 H, H-3b), 2.19, 2.11 (2 s, 3 H each, 2 $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 171.85, 170.97, 170.42 (3 CO), 69.92 (C-2), 62.49 (C-3), 20.48, 20.30 (2 CH_3); CIMS: *m*/*z* 208 ($[M+18]^+$).

To a soln of the foregoing acid **38** (1.59 g, 8.38 mmol) and amine **16** (1.41 g, 6.44 mmol) in MeCN (80 mL) was added HATU (3.18 g, 8.38 mmol), and the mixture was stirred for 4 h at rt. *N*,*N*-Diisopropylamine (DIPEA, 1.43 mL, 8.38 mmol) was added dropwise to complete the conversion, and after 30 min when TLC (5:1 CH_2Cl_2–Et_2O) showed complete disappearance of the starting amine, 10 mL of pyridine and 10 mL of Ac_2O was added, and stirring was continued overnight. The mixture was concd, and a soln of the residue in CH_2Cl_2 was washed successively with 1 N HCl and aq $NaHCO_3$. After concentration, chromatography gave first the D-*glycero* isomer **34** (0.511 g, 18.3%), mp 98–100 °C (from Et_2O), $[\alpha]_D$ +77° (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 6.31 (d, 1 H, $J_{4,NH}$ 9.6 Hz, NH), 5.34 (dd, 1 H, $J_{2,3a}$ 2.8, $J_{2,3b}$ 4.3 Hz, H-2′), 5.28 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 11.0 Hz, H-3), 5.12 (dd, 1 H, $J_{1,2}$ 1.8 Hz, H-2), 4.67 (d, 1 H, H-1), 4.45 (dd, 1 H, ^2J 12.3 Hz, H-3′a), 4.38 (dd, 1 H, H-3′b), 4.23 (q,

1 H, *J* 10.0 Hz, H-4), 3.74 (m, 1 H, H-5), 3.39 (s, 3 H, OCH_3), 2.21, 2.17, 2.07, 1.99 (4 s, 3 H each, 4 $COCH_3$), 1.29 (d, 3 H, $J_{5,6}$ 6.1 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 98.25 (C-1), 71.39 (C-2′), 69.25 (C-2), 68.16 (C-5), 67.86 (C-3), 63.38 (C-3′), 54.99 (OCH_3), 51.70 (C-4), 20.81, 20.63, 20.54, 20.50 (4 $CO\mathit{C}H_3$), 17.46 (C-6); CIMS: m/z 432 ($[M-1]^-$), 451 ($[M+18]^+$). Anal. Calcd for $C_{18}H_{27}NO_{11}$: C, 49.88; H, 6.28; N, 3.23. Found: C, 49.81; H, 6.37; N, 3.13.

Eluted next was a mixture of the two diastereoisomers (0.81 g, 29%).

Eluted last was the L-*glycero* isomer **32** (1.18 g, 42.3%, total yield of N-acylation, 89.7%), $[\alpha]_D$ +43° (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.27 (d, 1 H, $J_{4,NH}$ 9.6 Hz, NH), 5.27 (dd, partially overlapped, $J_{2,3}$ 3.4, $J_{3,4}$ 10.9 Hz, H-3), 5.25 (dd, partially overlapped, $J_{2,3a}$ 3.7, $J_{2,3b}$ 5.6 Hz, H-2′), 5.15 (dd, 1 H, $J_{1,2}$ 1.8 Hz, H-2), 4.66 (d, 1 H, H-1), 4.53 (dd, 1 H, ^2J 12.0 Hz, H-3′a), 4.31 (dd, partially overlapped, 1 H, ^2J 12.0 Hz, H-3′b), 4.22 (q, partially overlapped, *J* 10.3 Hz, H-4), 3.72 (m, 1 H, H-5), 2.18, 2.17, 2.07, 2.05 (4 s, 3 H each, 4 $COCH_3$); ^{13}C NMR ($CDCl_3$): δ 98.29 (C-1), 71.56 (C-2′), 69.06 (C-2), 68.25 (C-3), 67.73 (C-5), 62.58 (C-3′), 55.01 (OCH_3), 51.42 (C-4), 20.85, 20.67, 20.56, 20.52 (4 $CO\mathit{C}H_3$), 17.65 (C-6); CIMS: m/z 432 ($[M-1]^+$), 451 ($[M+18]^+$). Found: C, 49.75, H, 6.35, N, 3.16.

Methyl 4,6-dideoxy-4-(2,3-dihydroxy-L-glycero-*propanamido)*-α-D-*mannopyranoside* (**22**).—Deacetylation of compound **32** (1.03 g, Zemplén) gave, after chromatography, deprotected substance **22** (531 mg, 84%), mp 155–156°C (from EtOH–EtOAc), $[\alpha]_D$ +41° (*c* 0.8, H_2O). ^1H NMR (D_2O): δ 4.72 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 4.24 (dd, 1 H, $J_{2,3'a}$ 4.0, $J_{2,3'b}$ 4.9 Hz, H-3′), 3.93–3.75 (m, 6 H, H-2,3,4,5,3′a,b), 3.37 (s, 3 H, OCH_3), 1.18 (d, 3 H, $J_{5,6}$ 5.8 Hz, H-6); ^{13}C NMR (D_2O): δ 175.14 (CO), 100.99 (C-1), 72.61 (C-2′), 69.23 (C-2), 67.90 (C-3), 67.34 (C-5), 63.41 (C-3′), 54.85 (OCH_3), 53.12 (C-4), 16.89 (C-6); CIMS: m/z 266 ($[M+1]^+$), 283 ($[M+18]^+$). Anal. Calcd for $C_{10}H_{19}NO_7$: C, 45.28; H, 7.22; N, 5.28. Found: C, 45.46; H, 7.28; N, 5.33.

Methyl 3-O-*acetyl-4,6-dideoxy-4-(2,3-di*-O-*acetyl*-D- (**35**) *and* L-glycero-*propanamido)-2*-O-*methyl*-α-D-*mannopyranoside* (**33**).—To a mixture of amine **17** (300 mg, 1.57 mmol), acid **38** (650 mg, 3.42 mmol) and HATU (775 mg, 2.04 mmol) in MeCN (20 mL) was stirred at rt until TLC (3:1 CH_2Cl_2–MeOH) showed that all starting amine was consumed (~ 3 h). Pyridine (4 mL) and Ac_2O (4 mL) were added, and stirring was continued overnight. After concentration, a soln of the residue in CH_2Cl_2 was washed successively with 1 N HCl and aq $NaHCO_3$. Chromatography of the material in the organic phase gave first the D-*glycero* isomer **35** (0.16 g, 25%), mp 170–171 °C (from EtOH–Et_2O), $[\alpha]_D$ +105° (*c* 0.75, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 6.20 (d, 1 H, $J_{4,NH}$ 11.0 Hz, NH), 5.36 (dd, 1 H, $J_{2',3'a}$ 2.8, $J_{2',3'b}$ 4.3 Hz, H-2′), 5.21 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 11.3 Hz, H-3), 4.75 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.45 (dd, 1 H, $J_{2',3a}$ 3.0, ^2J 12.1 Hz, H-3′a), 4.38 (dd, $J_{2',3b}$ 4.2 Hz, H-3′b), 4.28 (q, 1 H, *J* 10.4 Hz, H-4), 3.66 (m, 1 H, H-5), 3.52 (d, 3 H, OCH_3-2), 3.47 (dd, 1 H, H-2), 3.39 (s, 3 H, OCH_3-1), 2.22, 2.06, 2.05 (3 s, 3 H each, 3 $COCH_3$), 1.27 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 172.21, 170.19, 169.08 (4 CO), 98.67 (C-1), 77.95 (C-2), 71.43 (C-2′), 70.65 (C-3), 68.63 (C-5), 63.60 (C-3′), 59.59 (OCH_3-2), 54.95 (OCH_3-1), 51.87 (C-4), 20.87, 20.82, 20.64 (3 $CO\mathit{C}H_3$), 17.66 (C-6); CIMS: m/z 404 ($[M+1]^+$), 423 ($[M+18]^+$). Anal. Calcd for $C_{17}H_{27}NO_{10}$: C, 50.37; H, 6.71; N, 3.46. Found: C, 50.58; H, 6.71; N, 3.50.

Eluted next was a mixture of the two diastereoisomers (0.12 g, 19%).

Eluted last was the L-*glycero* isomer **33** (0.215 g, 34%, total yield of N-acylation, 78%), mp 110–112 °C (from Et_2O–hexane), $[\alpha]_D$ +66° (*c* 0.8, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 6.13 (d, 1 H, $J_{4,NH}$ 9.4 Hz, NH), 5.26 (dd, 1 H, $J_{2',3'a}$ 3.8, $J_{2',3'b}$ 5.3 Hz, H-2′), 5.21 (dd, 1 H, $J_{2,3}$ 2.8, $J_{3,4}$ 11.0 Hz), 4.74 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1), 4.53 (dd, 1 H, ^2J 12.0 Hz, H-3′a), 4.30 (dd, partially overlapped, H-3′b), 4.25 (q, partially overlapped, H-4), 3.66 (m, 1 H, H-5), 3.51 (m, 4 H, H-2, OCH_3-2), 3.38 (s, 3 H, OCH_3-1), 2.18, 2.13, 2.07 (3 s, 3 H each, 3 $COCH_3$), 1.23 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 171.59, 170.42, 169.49, 167.00 (4 CO), 98.52 (C-1), 77.70 (C-2), 71.56 (C-2′), 70.81 (C-3), 67.98 (C-5), 62.67 (C-3′), 59.54 (OCH_3-2), 54.91 (OCH_3-1), 51.64 (C-4), 17.76 (C-6); CIMS: m/z 404 ($[M+1]^+$), 423

($[M+18]^+$). Found: C, 50.34; H, 6.72; N, 3.49.

Methyl 4,6-dideoxy-4-(2,3-dihydroxy-L-*glycero-propanamido)-2*-O-*methyl-α*-D-*mannopyranoside* (**27**).—Deacetylation of **33** (Zemplén) gave amorphous, hygroscopic **27** in virtually theoretical yield, $[\alpha]_D$ +18° (*c* 0.7, H_2O). ^1H NMR ($CDCl_3$): δ 4.91 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.26 (dd, 1 H, $J_{2',3a}$ 4.0, $J_{2',3b}$ 5.2 Hz, H-2′), 3.97 (bdd, 1 H, $J_{2,3}$ 3.4, $J_{3,4}$ 10.5 Hz, H-3), 3.86–3.77 (m, 4 H, H-4,5,3′a,b), 3.59 (dd, 1 H, H-2), 3.50 (s, 3 H, OCH_3-2), 3.42 (s, 3 H, OCH_3-1), 1.19 (d, 3 H, $J_{5,6}$ 5.8 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 175.3 (CO), 97.82 (C-1), 79.07 (C-2), 72.56 (C-2′), 67.65 (C-3), 67.20 (C-5), 63.40 (C-3′), 58.92 (OCH_3-2), 54.92 (OCH_3-1), 53.54 (C-4), 16.87 (C-6). CIMS: *m*/*z* 280 ($[M+1]^+$), 297 ($[M+18]^+$).

3-Benzyloxypropionic acid (**39**).—This compound was prepared from β-propiolactone as described by Li et al.,[22] except that the amount of HCl added to the aq phase before extraction with CH_2Cl_2 was adjusted to make it strongly acidic. ^1H NMR data agreed with those reported. ^{13}C NMR ($CDCl_3$): δ 177.5 (CO), 137.7 (1 C, Ar), 128.3 (2 C, Ar), 127.66 (3 C, Ar), 72.9 (CH_2Ph), 65.0 (C-3), 34.6 (C-2).

Methyl 4,6-dideoxy-4-(3-benzyloxypropanamido)-α-D-*mannopyranoside* (**36**).—HATU (644 mg, 1.7 mmol), followed by DIPEA (0.3 mL, 1.7 mmol), was added to a soln of amine **16**[11–13] (250 mg, 1.41 mmol) and acid **39** (520 mg, 2.8 mmol) in MeCN (15 mL). The mixture was stirred for 1 h at rt, when TLC (2:1 CH_2Cl_2–MeOH) showed that all starting amine was consumed. After concentration, a soln of the residue in dichloromethane was processed as described above for the preparation of **33**, and chromatography gave **36** (434 mg, 90%), mp 122–124 °C (from EtOAc), $[\alpha]_D$ +89° (*c* 0.9, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 6.42 (d, 1 H, $J_{NH,4}$ 8.2 Hz, NH), 4.70 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 4.54 (s, 2 H, CH_2Ph), 3.88 (m, 1 H, H-4), 3.84 (dd, 1 H, $J_{2,3}$ 3.5 Hz, H-2), 3.75 (m, 2 H, H-3′a,b), 3.67 (dd, 1 H, $J_{3,4}$ 10.0 Hz, H-3), 3.50 (m, 1 H, H-5), 3.38 (s, 3 H, OMe), 2.54 (t, 2 H, *J* 6.2 Hz, H-2′a,b), 1.18 (d, 3 H, H-6); ^{13}C NMR ($CDCl_3$): δ 173.9 (CO), 100.4 (C-1), 73.4 (CH_2Ph), 71.1 (C-3), 69.6 (C-2), 66.2 (C-3′), 66.2 (C-5), 55.0 (OMe), 54.2 (C-4), 37.0 (C-2′), 17.9 (C-6); CIMS: *m*/*z* 340 ($[M+1]^+$) and 357 ($[M+18]^+$). Anal. Calcd for $C_{17}H_{25}NO_6$: C, 60.16; H, 7.42; N, 4.13. Found: C, 60.21; H, 7.45; N, 4.13.

Methyl 4,6-dideoxy-4-(3-hydroxypropanamido)-α-D-*mannopyranoside* (**21**).—A soln of compound **36** (315 mg) in EtOH (10 mL) was treated with H_2 at atmospheric pressure and rt in the presence of 5% Pd–C catalyst ($\sim$100 mg) to give **21** in virtually theoretical yield, mp 134–136 °C (from acetone), $[\alpha]_D$ +67° (*c* 1, H_2O). ^1H NMR (D_2O): 4.70 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 3.89 (dd, 1 H, $J_{2,3}$ 2.9 Hz, H-2), 3.85–3.66 (m, 5 H, H-3,4,5,3′a,b), 3.66 (s, 3 H, OCH_3), 2.48 (t, 2 H, *J* 6.0 Hz, H-2′a,b), 1.17 (d, 3 H, $J_{5,6}$ 6.0 Hz, H-6); ^{13}C NMR (D_2O): δ 174.9 (CO), 100.95 (C-1), 69.15 (C-2), 68.34 (C-3), 67.54 (C-5), 57.99 (C-3′), 54.86 (OCH_3), 53.02 (C-4), 38.90 (C-2′), 16.93 (C-6); CIMS: *m*/*z* 250 ($[M+1]^+$), 267 ($[M+18]^+$). Anal. Calcd for $C_{10}H_{19}NO_6$: C, 48.19; H, 7.68; N, 5.62. Found: C, 48.39; H, 7.77; N, 5.42.

Methyl 4,6-dideoxy-4-(3-benzyloxypropanamido)-2-O-*methyl-α*-D-*mannopyranoside* (**37**).—To a soln of compound **17** (192 mg, 1 mmol) and 3-benzyloxypropionic acid (**39**) (216 mg, 1.2 mmol) in MeCN (10 mL) was added HATU (456 mg, 1.2 mmol), and the mixture was stirred at rt for 30 min. After addition of DIPEA (0.22 mL, 1.2 mmol), stirring was continued and, after 2 h, the mixture was processed as described in the preparation of **33**. Chromatography (neat AcOEt) gave compound **37** (289 mg, 82%), mp 89–90 °C (from acetone–Et_2O), $[\alpha]_D$ +38° (*c* 0.5, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 6.47 (d, 1 H, $J_{NH,4}$ 9.4 Hz, NH), 4.75 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.51 (2 d, 2 H, *J* 11.8 Hz, CH_2Ph), 3.87 (m, 1 H, H-4), 3.73 (t, 2 H, *J* 5.7 Hz, H-3′a,b), 3.66 (dd, 1 H, $J_{2,3}$ 3.4, $J_{3,4}$ 10.5 Hz, H-3), 3.49 (m, 1 H, H-5), 3.45 (s, 3 H, OCH_3-2), 3.42 (dd, 1 H, H-2), 3.34 (s, 3 H, OCH_3-1), 2.51 (dd, 2 H, $J_{2,3'a}$ 6.9, $J_{2,3'b}$ 5.4 Hz, H-2′), 1.18 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 172.9 (CO), 97.2 (C-1), 79.2 (C-2), 73.0 (CH_2Ph), 69.3 (C-3), 66.9 (C-5), 66.2 (C-3′), 58.5 (OCH_3-2), 54.7 (OCH_3-1), 53.7 (C-4), 36.9 (C-2′), 17.7 (C-6). CIMS: *m*/*z* 352 ($[M-1]^-$), 354 ($[M+1]^+$). Anal. Calcd for $C_{18}H_{27}NO_6$: C, 61.17; H, 7.70; N, 3.96. Found: C, 60.88; H, 7.69; N, 4.03.

*Methyl 4,6-dideoxy-4-(3-hydroxypropanamido)-2-*O*-methyl-α-*D*-mannopyranoside* (**26**). —A soln of the benzyl ether **37** (175 mg) in EtOH was treated with H_2 as described for the preparation of **21** to give **26** in virtually theoretical yield, mp 145–146.5 °C (from acetone), $[\alpha]_D$ +47° (*c* 0.6, H_2O). ^{1}H NMR (D_2O): δ 4.90 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 3.87–3.70 (m, 5 H, H-3,4,5,3′a,b), 3.58 (dd, 1 H, $J_{2,3}$ 3.0 Hz, H-2), 3.48 (s, 3 H, OCH_3-2), 3.40 (s, 3 H, OCH_3-1), 2.5 (t, 2 H, *J* 6.3 Hz, H-2′a,b), 1.18 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 174.98 (CO), 97.82 (C-1), 79.03 (C-2), 68.10 (C-3), 67.42 (C-5), 58.91 (OCH_3-2), 57.97 (C-3′), 54.95 (OCH_3-1), 38.87 (C-2′), 16.86 (C-6); CIMS: *m*/*z* 264 ($[M+1]^+$), 281 ($[M+18]^+$). Anal. Calcd for $C_{11}H_{21}NO_6$: C, 50.18; H, 8.04; N, 5.32. Found: C, 50.25; H, 8.02; N, 5.30.

*Methyl 4,6-dideoxy-4-(3-deoxy-*L*-glycero-tetronamido)-α-*L*-mannopyranoside* (**45**).—L-Rhamnose was converted to the 4-azido derivative **40** essentially as described by Martin et al.,[23] which was then converted to the corresponding amine **41**[16] whose NMR spectra agreed with those reported for the D enantiomer.[11–13]

A mixture of the foregoing amine **41** (520 mg, 2.9 mmol), 3-deoxy-L-*glycero*-tetronolactone (450 mg, 4.4 mmol) and pyridine (0.05 mL) was stirred at 100 °C until TLC (6:1:0.2 CH_2Cl_2–MeOH–concd NH_4OH) showed that all starting material was consumed. The crude product was chromatographed (twice, first using 6:1:0.2 CH_2Cl_2–MeOH–concd NH_4OH, then 9:1 EtOAc–MeOH), to give the title compound **45** as a white foam in virtually theoretical yield, $[\alpha]_D$ −72° (*c* 1.3, H_2O). ^{1}H NMR (D_2O): δ 4.72 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 4.27 (dd, 1 H, $J_{2',3'a}$ 3.8, $J_{2',3'b}$ 5.8 Hz, H-2′), 3.92–3.27 (m, 4 H, H-2,3,4,5), 3.72 (q, 2 H, *J* 5.6, *J* 7.4 Hz, H-4′), 3.38 (s, 3 H, OCH_3), 2.02 (m, 1 H, H-3′a), 1.80 (m, 1 H, H-3′b), 1.17 (d, 3 H, $J_{5,6}$ 5.6 Hz, H-6); ^{13}C NMR ($CDCl_3$): δ 177.50 (CO), 101.0 (C-1), 69.2 (C-2), 69.1 (C-2′), 68.2 (C-3), 67.3 (C-5), 58.0 (C-4′), 54.9 (O CH_3), 52.9 (C-4), 36.0 (C-3′), 16.9 (C-6). CIMS: *m*/*z* 280 ($[M+1]^+$) and 297 ($[M+18]^+$). Anal. Calcd for $C_{11}H_{21}NO_7$: C, 47.31; H, 7.58; N, 5.02. Found: C, 47.05; H, 7.65; N, 5.00.

*Methyl 4-azido-3-*O*-benzyl-4,6-dideoxy-α-*L*-mannopyranoside* (**42**).—Azide **40** (12.2 g) was benzylated as described for the D enantiomer,[12] to give, after chromatography, the title 3-benzyl ether **42** (13.1 g, 74%), $[\alpha]_D$ −116° (*c* 1.2, MeOH). ^{1}H NMR data agreed with those reported for the D enantiomer;[12] CIMS: *m*/*z* 311 ($[M+18]^+$). Anal. Calcd for $C_{14}H_{19}N_3O_4$: C, 57.33; H, 6.53; N, 14.33. Found: C, 57.29; H, 6.46; N, 14.30.

*Methyl 4-azido-3-*O*-benzyl-2-*O*-methyl-4,6-dideoxy-α-*L*-mannopyranoside* (**43**).—The foregoing benzyl ether **42** (9.7 g) was methylated as described for the corresponding D enantiomer[18] to give, after chromatography, the title 2-methyl ether **43** (9.6 g, 94%) as an oil, $[\alpha]_D$ −128° (*c* 1.3, $CHCl_3$), lit.[18], +117° for the D enantiomer. The NMR data agreed with those reported for the D enantiomer;[18] 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, 58.61; H, 6.88; N, 13.64.

*Methyl 4-amino-4,6-dideoxy-2-*O*-methyl-α-*L*-mannopyranoside* (**44**).—The foregoing fully protected compound **43** (7.3 g) was treated with H_2 as described above for similar conversions to give amine **44** in virtually theoretical yield, $[\alpha]_D$ −50° (*c* 1.3, MeOH). The NMR data agreed with those reported for the D enantiomer;[18] CIMS: *m*/*z* 192 ($[M+1]^+$), 209 ($[M+18]^+$). Anal. Calcd for $C_8H_{17}NO_4$: C, 50.25; H, 8.96; N, 7.32. Found: C, 49.95; H, 8.96; N, 7.26.

*Methyl 4,6-dideoxy-4-(3-deoxy-*L*-glycero-tetronamido)-2-*O*-methyl-α-*L*-mannopyranoside* (**46**).—A melt of amine **44** (778 mg, 4.07 mmol) and 3-deoxy-L-*glycero*-tetronolactone (830 mg, 8.14 mmol) was stirred at 85 °C for 7 h. Chromatography (twice, first using 9:1 CH_2Cl_2–MeOH, then 1:1 CH_2Cl_2–acetone) gave **46** (980 mg, 82%), mp 127–129 °C (from acetone), $[\alpha]_D$ −61° (*c* 1, H_2O). ^{1}H NMR (D_2O): δ 4.88 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.26 (dd, 1 H, $J_{2',3'a}$, $J_{2',3'b}$ 8.7 Hz, H-2′), 3.91 (m, 1 H, H-3), 3.82–3.76 (m, 2 H, H-4,5), 3.72 (q, 2 H, *J* 5.8, *J* 7.3 Hz, H-4′a b), 3.55 (dd, 1 H, $J_{2,3}$ 3.4 Hz, H-2), 3.47 (s, 3 H, OCH_3-2), 3.39 (s, 3 H, OCH_3-1), 2.01 (m, 1 H, H-3′a), 1.80 (m, 1 H, H-3′b), 1.16 (d, 3 H, $J_{5,6}$ 5.6 Hz, H-6); ^{13}C NMR (D_2O): δ 177.4 (CO), 97.8 (C-1), 79.1 (C-2), 69.1 (C-2′), 67.9 (C-3), 67.1 (C-5), 58.9

(C-4′), 57.9 (OCH_3-2), 54.9 (OCH_3-1), 53.3 (C-4), 36.0 (C-3′), 16.9 (C-6). CIMS: m/z 294 ($[M+1]^+$), 311 ($[M+18]^+$). Anal. Calcd for $C_{12}H_{23}NO_7$: C, 49.14; H, 7.90; N, 4.78. Found: C, 49.21; H, 7.97; N, 4.76.

References

1. Hisatsune, K.; Kondo, S.; Isshiki, Y.; Iguchi, T.; Haishima, Y. *Biochem. Biophys. Res. Commun*. **1993**, *190*, 302–307.
2. Ito, T.; Higuchi, T.; Hirobe, M.; Hiramatsu, K.; Yokota, T. *Carbohydr. Res*. **1994**, *256*, 113–128.
3. Wang, J.; Zhang, J.; Miller, C. E.; Villeneuve, S.; Ogawa, Y.; Lei, P.-s.; Lafaye, P.; Nato, F.; Karpas, A.; Bystrický, S.; Szu, S. C.; Robbins, J. B.; Kováč, P.; Fournier, J.-M.; Glaudemans, C. P. J. *J. Biol. Chem*. **1998**, *273*, 2777–2783.
4. Isshiki, Y.; Kondo, S.; Haishima, Y.; Iguchi, T.; Hisatsune, K. *J. Endotoxin Res*. **1996**, *3*, 143–149.
5. Kondo, S.; Ishida, K.; Isshiki, Y.; Haishima, Y.; Iguchi, T.; Hisatsune, K. *Biochem. J*. **1993**, *292*, 531–535.
6. Kondo, S.; Sano, Y.; Isshiki, Y.; Hisatsune, K. *Microbiology* **1996**, *142*, 2879–2885.
7. Sano, Y.; Kondo, S.; Isshiki, T.; Shimada, T.; Hisatsune, K. *Microbiol. Immunol*. **1996**, *40*, 735–741.
8. Hodosi, G.; Kováč, P. *J. Am. Chem. Soc*. **1997**, *119*, 2335–2336.
9. Hodosi, G.; Kováč, P. *Carbohydr. Res*. **1998**, *308*, 63–75.
10. Hodosi, G.; Kováč, P. *J. Carbohydr. Chem*. **1998**, *17*, 557–565.
11. Bundle, D. R.; Gerken, M.; Peters, T. *Carbohydr. Res*. **1988**, *174*, 239–251.
12. Eis, M. J.; Ganem, B. *Carbohydr. Res*. **1988**, *176*, 316–323.
13. Gotoh, M.; Kováč, P. *J. Carbohydr. Chem*. **1994**, *13*, 1193–1213.
14. Zhang, J.; Kováč, P. *Carbohydr. Res*. **1997**, *300*, 329–339.
15. Peters, T.; Bundle, D. R. *Can. J. Chem*. **1989**, *67*, 497–502.
16. Brimacombe, J. S.; Ching, O. A.; Stacey, M. *J. Chem. Soc. C* **1969**, 1270–1274.
17. Rainer, H.; Scharf, H.-D.; Runsink, J. *Liebigs Ann. Chem*. **1992**, 103–107.
18. Lei, P.-s.; Ogawa, Y.; Flippen-Anderson, J. L.; Kováč, P. *Carbohydr. Res*. **1995**, *275*, 117–129.
19. Eklind, K.; Gustafsson, R.; Tidén, A.-K.; Norberg, T.; Aberg, P.-M. *J. Carbohydr. Chem*. **1996**, *15*, 1161–1178.
20. Ogawa, Y.; Lei, P.-s.; Kováč, P. *Carbohydr. Res*. **1995**, *277*, 327–331.
21. *The Merck Index*, 12th ed.; Budvari, S., Ed.; Merck Research Laboratories: Whitehouse Station, NJ, USA, 1996; Entry 4492, p. 763.
22. Li, A.-H.; Moro, S.; Forsyth, N.; Melman, N.; Ji, X.-d.; Jacobson, K. A. *J. Med. Chem*. **1999**, *42*, 706–721.
23. Martin, A.; Butters, T. D.; Fleet, G. W. J. *Tetrahedron: Asymmetry* **1999**, *10*, 2343–2360.