Conclusions

Only one type of TO_4 site was found in SAPO-44 and MnAPSO-44. The Si substitutes mostly for P and in hydrated samples part of the Al assumes octahedral coordination. The highly crystalline structures of SAPO-44 and MnAPSO-44 break down upon dehydration of calcined samples which underwent prolonged exposure to water vapor.

Mn(II) in MnAPSO-44 with low Mn content (0.07 atom %) assumes octahedral coordination in the calcined hydrated samples. Dehydration to 400 °C changes the Mn(II) coordination to tetrahedral as evident by the considerable decrease in the hyperfine coupling constant from ~90 to 65 G. These results are consistent with Mn(II) situated in the framework. In the hydrated sample the Mn(II) is coordinated to four framework oxygens and two water molecules whereas in the dehydrated sample it is coordinated only to four framework oxygens one of which is part of an OH group. The Mn(II) framework substitution for Al is supported by relatively weak ^{27}Al modulation and ^{31}P modulation rather invariant to calcination and hydration.

The distribution of the Mn(II) throughout the sample in both as-synthesized and calcined samples in MnAPSO-44 (0.07 atom %) was found to be inhomogeneous. Only about 15% of the Mn(II) is distributed homogeneously and the rest is situated in areas "rich" in Mn(II).

Acknowledgment. This study was supported by funds granted to D.G. through a fellowship program sponsored by the Charles H. Revson Foundation. J.B. gratefully acknowledges the financial support from the National Slovene Board for Science and Technology (RSRDT), Grant No. C2-0538-104. We thank Professor L. Kevan for making refs 8 and 9 available to us prior to publication.

A Novel Approach for Stereochemical Analysis of 1-Carboxyethyl Sugar Ethers by NMR Spectroscopy†

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Abstract: A general strategy for the stereochemical analysis of 1-carboxyethyl sugar ethers is introduced in which the chirality of the lactyl group is related to that of the sugar moiety in a conformationally rigid lactone derivative. With the aid of molecular modeling, the stereochemistry is determined through the use of NOE measurements. This procedure was evaluated using synthetic diastereomeric samples of (*R*)- and (*S*)-methyl 3-*O*-(1-carboxyethyl)-α-L-rhamnopyranoside, and it was successfully applied to the stereochemical analysis of *N*-acetylmuramic acid. The generality of this approach is illustrated here with a 4-O-substituted 1-carboxyethyl sugar ether. It was found that the lactyl group of 4-*O*-(1-carboxyethyl)-D-mannopyranose, which was obtained from the capsular polysaccharide of *Rhodococcus equi* serotype 3, has the (*S*)-configuration.

Introduction

Bacterial cell surface polysaccharides frequently carry acidic non-carbohydrate components, which have been shown to be dominant antigenic determinants contributing to the polysaccharides' serological specificities.[1] Pyruvic acid, linked to a sugar residue as a cyclic acetal, is among the most frequently encountered acidic substituents.[1a,b]

Although less commonly encountered than pyruvic acid acetals, lactic acid ethers, which share a common biosynthetic precursor, are found as substituents (1-carboxyethyl) of a wide range of sugars.[1c] The 3-O-substituted (*R*)-1-carboxyethyl derivative of 2-amino-2-deoxy-D-glucose (muramic acid) and the related manno isomer are components of bacterial cell wall peptidoglycan.[2,3] Lactic acid ether-substituted sugars are also components of bacterial cell wall lipopolysaccharides and of extracellular polysaccharides. Both the (*R*)- and (*S*)-enantiomers of the lactyl group have been reported as substituents of D-glucose (substituted at the 4-position)[4-9] and L-rhamnose (substituted at the 3-position),[10-13] whereas only the (*S*)-enantiomer has been found as a substituent (at the 4-position) of D-mannose[14-16] and D-glucuronic acid.[17,18] Lactic acid ethers have also been found linked to the O-4 positions of D-galactose[19] and L-rhamnose,[20] although their chiralities have not been determined.

The structural diversity of these antigenic determinants is influenced by (i) the nature and configuration of the substituted monosaccharide residue, (ii) the position of the hydroxyl group that is substituted, and (iii) the chirality of the substituent.

The stereochemistry of the substituted monosaccharide can be readily determined after removal of the lactic acid moiety.

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‡ NRCC Research Associate 1988-1990.

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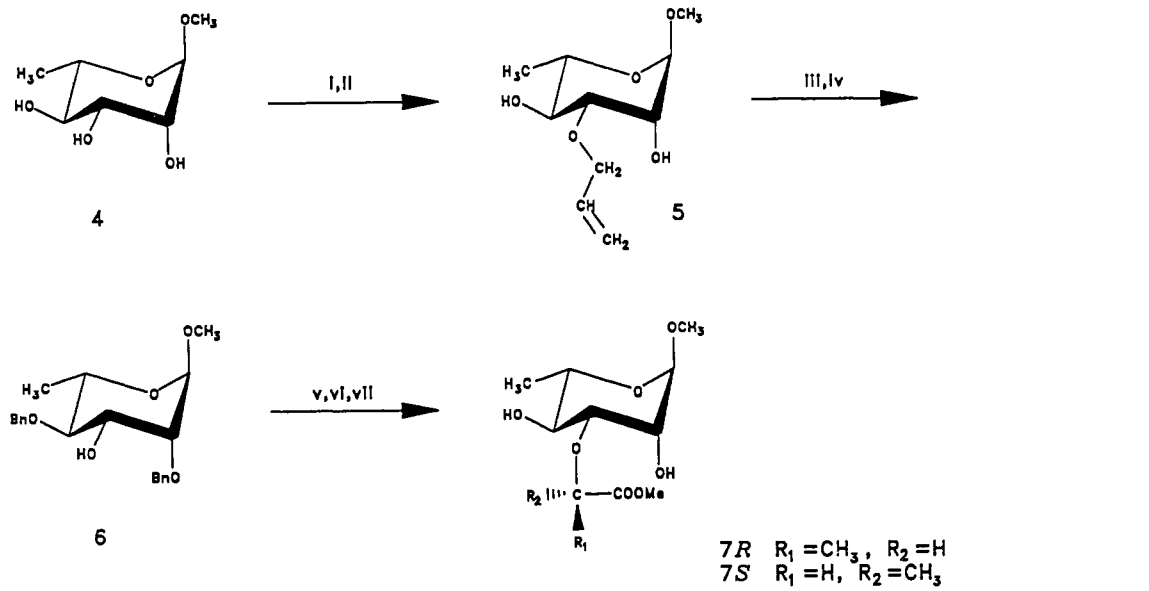
Scheme I[a]



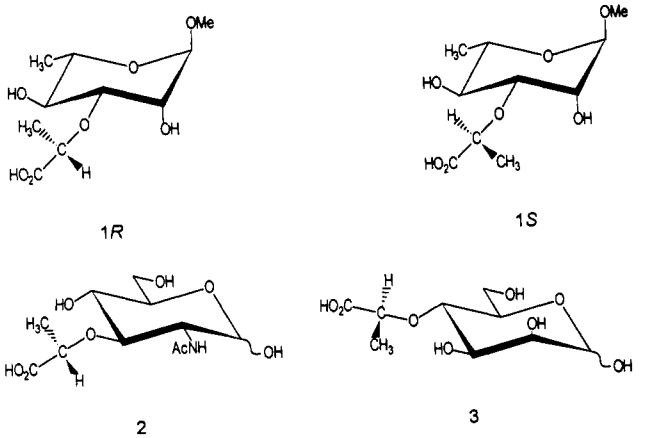
[a] (i) Dibutyl tin oxide, (ii) allyl bromide/tetrabutylammonium iodide, (iii) benzyl bromide, (iv) Pd/C AcOH, (v) NaH, (vi) (*S*)- or (*R*)-2-chloropropionic acid, (vii) CH_3N_2, (viii) Pd/H_2 EtOH.

Cleavage of the ether linkage of 1-carboxyethyl-substituted glycoses can be achieved by treatment with boron trihalide reagents[21,22] or aqueous hydrogen bromide,[21] while dealkylation of *N*-acetylmuramic acid is reported to occur under mild alkaline conditions.[23] Elimination of the carboxyethyl group has also been effected by the Hoffmann reaction,[24] a two-step procedure requiring the initial amide formation.[16] Determination of the absolute configuration of the resultant unsubstituted glycose is then determined by the measurement of optical rotation,[16] the application of specific enzymes,[5,8] or the comparison of chromatographic properties of diastereomeric derivatives with standards of known chirality.[7,10,18-20]

The position of substitution of the 1-carboxyethyl moiety within the glycose ring can be readily determined by mass spectral analysis of a carboxyl-reduced alditol derivative[9,10,15,19,20] or by high-resolution NMR techniques involving the analysis of ^{13}C NMR shielding effects[10,25] and/or long-range carbon–proton correlation experiments (HMBC).[26]

Determination of the absolute configuration of the 1-carboxyethyl group cannot be effected by the degradative methods since the chirality of the substituent may be altered during ether bond cleavage. Present methods used to obtain this information are of limited general use since they rely on the stereochemical correlation of the 1-carboxyethyl sugar ethers with synthetic samples of defined stereochemistry.

We have recently reported the occurrence of 3-*O*-[(*S*)-1-carboxyethyl]-L-rhamnose as a component of a serotype-specific capsular polysaccharide of *Rhodococcus equi*.[10] The stereochemistry of this 1-carboxyethyl sugar was determined chromatographically by correlation with standards of known absolute configuration. In the present investigation, a general proton NOE-based method for the determination of the relative stereochemistry of 1-carboxyethyl sugar ethers is described in which the chirality of the lactyl α-carbon is related to that of the attached sugar moiety in a conformationally rigid lactone derivative. This approach was successfully tested on synthetic samples of (*R*)- and (*S*)-diastereoisomers of methyl 3-*O*-(1-carboxyethyl)-α-L-rhamnopyranoside (**1***R* and **1***S*) and applied to the stereochemical analysis of *N*-acetylmuramic acid (**2**) and 4-*O*-(1-carboxyethyl)-D-mannopyranose (**3**). The 4-*O*-(1-carboxyethyl) derivative of D-mannose was isolated from the *R. equi* serotype 3 capsular polysaccharide.[15]



Results and Discussion

The determination of the relative and absolute configuration of the 1-carboxyethyl ether substituents of antigentic bacterial polysaccharides is of major importance in understanding their immunological reactivities.[1,27] NMR spectroscopy has proved to be an effective tool for simultaneous determination of the conformation and configuration of the sugar ring systems.[25,28] For stereochemical analysis, the combined application of NOE measurements and molecular mechanics calculations can provide the basis of a strategy for evaluating the configurations of several chiral centres within these molecules. 1-Carboxyethyl sugar ethers exhibit a high degree of molecular flexibility, and, as a conse-

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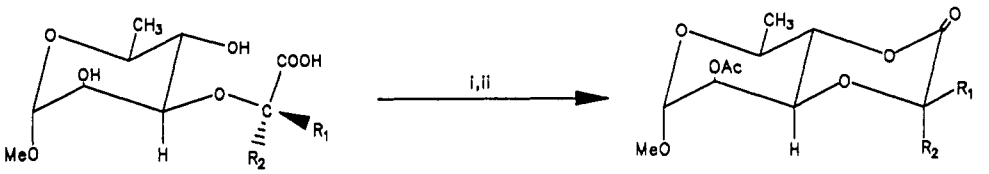
Scheme II[a]



1*R* $R_1 = CH_3$, $R_2 = H$ 8*R* $R_1 = CH_3$, $R_2 = H$

1*S* $R_1 = H$, $R_2 = CH_3$ 8*S* $R_1 =$, $R_2 = CH_3$

[a] (i) Acetic anhydride, (ii) pyridine.

Table I. ^1H Chemical Shifts and Coupling Constants[a] (Hz) for the Acetylated Lactone Derivatives of 1-Carboxyethyl-Containing Sugars

											lactic acid residue	
sugar	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5	H-6 ($J_{5,6}$)	H-6′ ($J_{5,6'}$ $J_{6,6'}$)	$OCOCH_3$	OCH_3	NH	CH_3 (J_{CH,CH_3})	CH
methyl 3-*O*-[(*R*)-1-carboxyethyl]-α-L-rhamnopyranoside (**1*R***)	4.67 (≤1)	5.24 (2.5)	3.97 (9.6)	4.26 (9.6)	3.82	1.38 (6.2)		2.04	3.40		1.55 (6.6)	4.48
methyl 3-*O*-[(*S*)-1-carboxyethyl]-α-L-rhamnopyranoside (**1*S***)	4.71 (≤1)	5.22 (2.7)	4.07 (9.5)	4.30 (9.7)	3.85	1.40 (6.2)		2.14	3.41		1.54 (6.6)	4.67
2-acetamido-2-deoxy-3-*O*-[(*R*)-1-carboxyethyl]-α-D-glucopyranose (**2**)	6.22 (2.9)	4.51 (9.5)	3.91 (9.6)	4.48 (9.6)	4.03	4.24 (1.7)	4.42 (5.1,12.1)	2.00–2.14		5.44	1.54 (6.6)	4.76
4-*O*-[(*S*)-1-carboxyethyl]-α-D-mannopyranose (**3**)	6.04 (2.0)	5.08 (2.3)	3.08 (9.7)	5.30 (9.7)	4.02	4.36 (1.7)	4.30 (5.8,12.0)	2.00–2.14			1.38 (6.8)	4.20

[a] Observed first-order chemical shifts and coupling constants (Hz) measured at 27 °C in $CDCl_3$.

Table II. ^{13}C Chemical Shifts[a] for the Acetylated Lactone Derivatives of 1-Carboxyethyl-Containing Sugars

										lactic acid residue		
sugar	C-1	C-2	C-3	C-4	C-5	C-6	$OCOCH_3$	$OCOCH_3$[b]	OCH_3	CH_3	CH	COOH[b]
methyl 3-*O*-[(*R*)-1-carboxyethyl]-α-L-rhamnopyranoside (**1*R***)	98.6	69.6	70.5	78.1	65.9	16.5	20.6	178.7	55.4	18.3	73.9	169.4
methyl 3-*O*-[(*S*)-1-carboxyethyl]-α-L-rhamnopyranoside (**1*S***)	98.6	69.8	67.7	77.7	65.7	16.4	20.7	178.7	55.3	17.6	71.3	169.9
2-acetamido-2-deoxy-3-*O*-[(*R*)-1-carboxyethyl]-α-D-glucopyranose (**2**)	90.7	49.9	69.1	74.3	68.8	60.8	20.7 (22.9)[c]	174.5 (175.5)[c]		17.6	70.8	168.8
4-*O*-[(*S*)-1-carboxyethyl]-α-D-mannopyranose (**3**)	90.8	73.4	73.6	71.6	71.5	62.7	20.9	174.3		19.1	77.14	169.1

[a] Observed first-order chemical shifts and coupling constants (Hz) measured at 27 °C in $CDCl_3$. [b] Assignments may be reversed. [c] Chemical shifts in parentheses are for the acetamido substituent.

quence, only limited stereochemical information can be obtained by NMR spectroscopy. However, it is to be expected that lactonization of the hydroxy acid would constrain the carboxyethyl group within a relatively rigid 1,3-dioxane-4-dione ring system, thereby permitting stereochemical assignment by an NOE-based method similar to that which has proved successful for polysaccharides containing cyclic pyruvic acid acetal substituents.[15,29]

The lactones derived from diastereomeric samples of (*R*)- and (*S*)-methyl 3-*O*-(1-carboxyethyl)-α-L-rhamnopyranoside (**1*R*** and **1*S***) served as model compounds for evaluating this strategy. The synthesis of the two diastereoisomers is outlined in Scheme I. Treatment of methyl α-L-rhamnopyranoside (**4**) first with dibutyltin oxide and subsequently with allyl bromide and tetrabutylammonium iodide regioselectively afforded the 3-*O*-allyl derivative **5**.[30] After benzylation, the allyl group was removed and the exposed hydroxyl group of methyl 2,4-di-*O*-benzyl-α-L-rhamnopyranoside (**6**) was alkylated with (*S*)-2-chloropropionic acid. This reaction proceeds with inversion of configuration at the asymmetric center of the 2-chloropropionic acid to give the (*R*)-1-carboxyethyl ether.[31] The crude product was esterified with diazomethane, and methyl 3-*O*-[(*R*)-1-methoxycarbonyl]-α-L-rhamnopyranoside (**7*R***) was isolated upon removal of the benzyl protecting groups. Examination of the ^1H and ^{13}C NMR spectra of this compound unambiguously confirmed its structure.

In a parallel series of reactions, the common intermediate **6** was alkylated with (*R*)-2-chloropropionic acid to give the corresponding methyl 3-*O*-[(*S*)-1-methoxycarbonyl]-α-L-rhamnopyranoside (**7*S***) after esterification and deprotection. This compound differed from the diastereoisomer **7*R*** in chromatographic mobility, specific optical rotation, and ^1H and ^{13}C NMR spectra (see Experimental Section).

The (*R*)- and (*S*)-diastereoisomers of methyl 3-*O*-(1-carboxyethyl)-α-L-rhamnopyranoside (**1*R*** and **1*S***) were obtained, respectively, by treating **7*R*** and **7*S*** with aqueous sodium hydroxide.

Lactonization was facilitated by stirring the free acid **1*R*** or **1*S*** with acetic anhydride, followed by addition of pyridine to effect acetylation of the remaining free hydroxyl group (Scheme II). After chromatographic isolation, the lactonized glycoses **8*R*** and **8*S*** were identified from their ^1H and ^{13}C NMR spectra (Tables I and II): the substantial downfield shifts of the H-4 and C-4 resonances were indicative of lactonization involving the O-4 position.

Assignment of the ^1H and ^{13}C resonances was made from homo- and heteronuclear chemical shift correlation techniques COSY[32] and HMQC.[33] The chemical shifts and vicinal proton coupling constant values were consistent with the $^1C_4$ ring conformation for the α-L-rhamnopyranose in the bicyclic system.[25,28] No significant difference was observed for ^1H or ^{13}C lactyl α-methyl

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Table III. Calculated Interproton Distances for Selected Protons in the Lactone Derivatives of 1-Carboxyethyl-Containing Sugars[a]

sugar	interproton distance in lactone (Å)			
	H-α,H-3	H-α,H-4	CH_3,H-3[b]	CH_3,H-4[b]
methyl 3-*O*-[(*R*)-1-carboxyethyl]-α-L-rhamnopyranoside (**1***R*)	2.39	4.08	4.29	3.83
methyl 3-*O*-[(*S*)-1-carboxyethyl]-α-L-rhamnopyranoside (**1***S*)	3.62	3.59	2.27	4.71
2-acetamido-2-deoxy-3-*O*-[(*R*)-1-carboxyethyl]-α-D-glucopyranose (**2**)	3.66	3.59	2.24	4.68
4-*O*-[(*S*)-1-carboxyethyl]-α-D-mannopyranose (**3**)	4.07	2.38	3.80	4.29

[a] Measured on the MM2-minimized structures of the respective nonacetylated analogues of **8***R*, **8***S*, **9**, and **11**. [b] Minimum interproton distance.

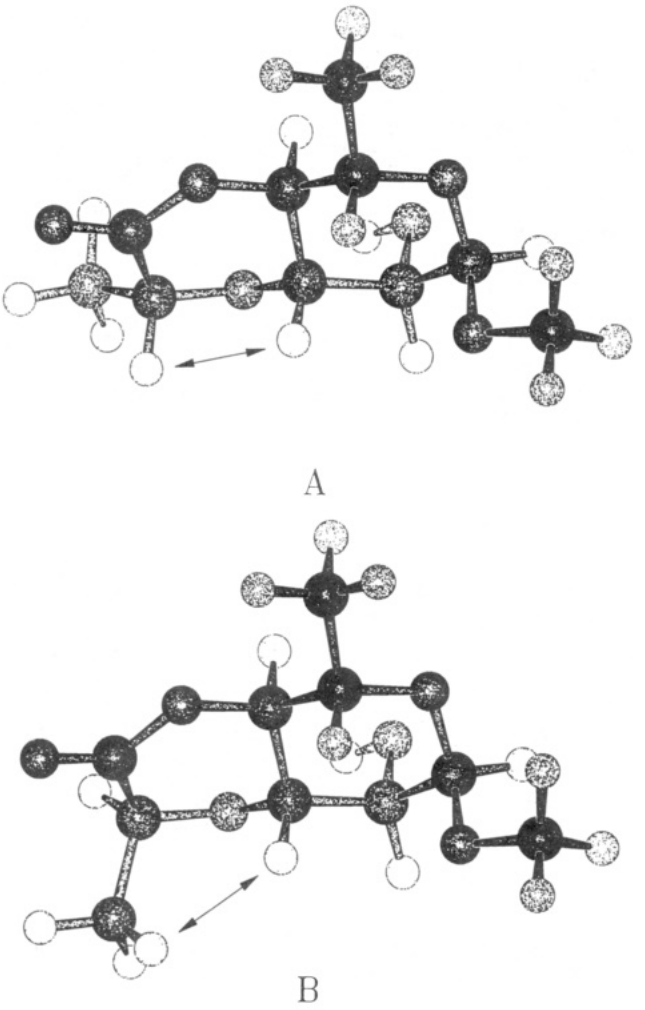

Figure 1. Computer generated models for the (A) (*R*)- and (B) (*S*)-3,4-lactone derivatives of methyl 3-*O*-(1-carboxyethyl)-α-L-rhamnopyranoside.

chemical shifts in the diastereomeric products (Tables I and II). However, the occurrence of specific ^{1}H NOEs between protons of the lactone and glycose ring systems provided a diagnostic indication of the orientation of the lactyl methyl group in the (*R*)- and (*S*)-lactones, and this was confirmed by molecular calculations.

Molecular modeling (minimized by MM2) clearly revealed that the methyl group in the (*R*)-lactone **8***R* derived from methyl 3-*O*-[(*R*)-1-carboxyethyl]-α-L-rhamnopyranoside (**1***R*), occupies the equatorial orientation (Figure 1A), whereas in the corresponding (*S*)-lactone **8***S* the methyl group has an axial disposition (Figure 1B). Proton NOEs are generally observed for interproton distances of less than ca. 3 Å.[34] Thus, the interproton distances

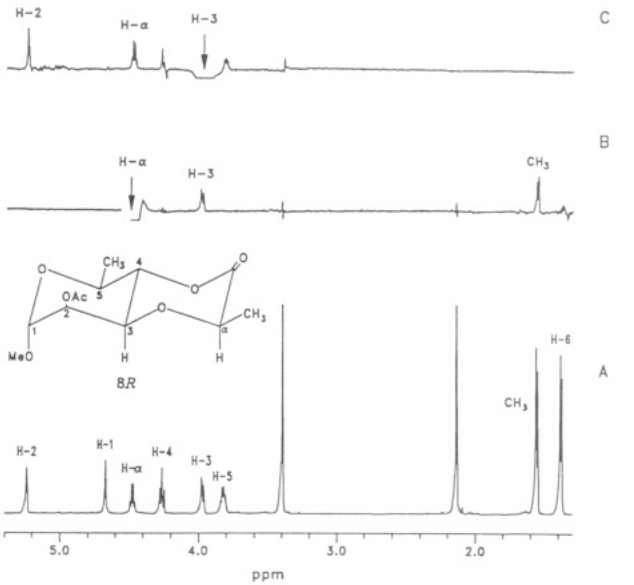

Figure 2. ^{1}H NMR spectrum of the acetylated lactone derivative of methyl 3-*O*-[(*R*)-1-carboxyethyl]-α-L-rhamnopyranoside (**8***R*) (A), NOE difference spectra obtained by irradiation of the lactyl α-proton resonance (B), and the H-3 resonance (C).

calculated from the minimum energy conformations of the bicyclic ring systems (Table III) would predict the occurrence of an NOE between H-3 of the rhamnopyranosyl ring system and the lactate α-proton in the (*R*)-lactone **8***R* but not in the (*S*)-lactone **8***S*; in the (*S*)-lactone, an NOE is predicted between H-3 and protons of the lactyl methyl group.

One-dimensional ^{1}H-^{1}H NOE measurements were made using difference spectroscopy.[35] As expected, irradiation of the α-proton of the (*R*)-lactone **8***R* resulted in an NOE at H-3 of the rhamnopyranosyl ring (Figure 2B). Correspondingly, in the reciprocal experiment, irradiation of H-3 resulted in an NOE at the α-proton (Figure 2C). No detectable NOE was observed at the methyl resonance, indicating that the methyl group occupies the equatorial position upon lactonization, and this is in full agreement with the lactyl moiety having the (*R*)-configuration.

In the (*S*)-lactone **8***S*, saturation of the H-3 resonance of the rhamnopyranosyl ring resulted in an NOE at the methyl resonance (Figure 3B), a result consistent only with the lactyl group having the (*S*)-configuration in which the methyl group occupies a position on the same face of the L-rhamnopyranosyl ring system as the axial H-3[36] (Figure 1B).

From an interpretation of NOE and molecular modeling data, assignment of the relative stereochemistry of 1-carboxyethyl-substituted glycoses can, in general, be made without the need for reference compounds. The general procedure involves lactonization of the free glycose in the presence of acetic anhydride to give the acetylated lactone derivative. Thus, stereochemical

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(36) It is noteworthy that these results provide direct, irrefutable evidence for a S_N2-like mechanism proceeding with Walden inversion at the asymmetric center of the substrate during the condensation of methyl 2,4-di-*O*-benzyl-α-L-rhamnopyranoside (**6**) and 2-chloropropionic acid (Scheme I).

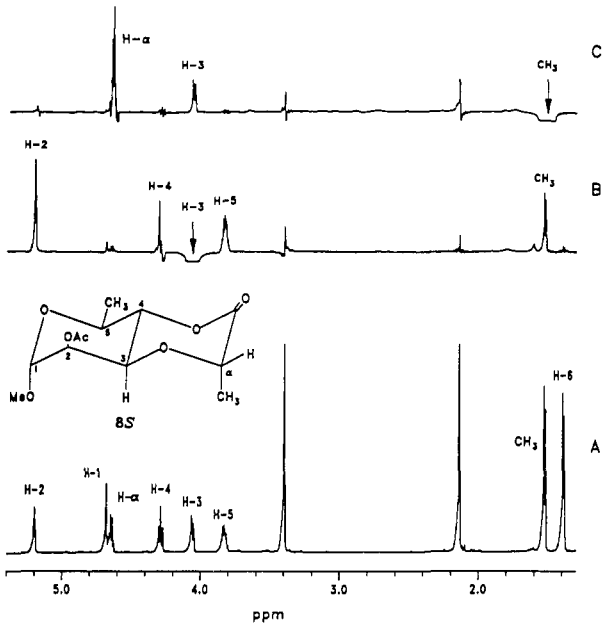


Figure 3. ^{1}H NMR spectrum of the acetylated lactone derivative of methyl 3-*O*-[(*S*)-1-carboxyethyl]-α-L-rhamnopyranoside (**8***S*) (A), NOE difference spectra obtained by irradiation of the H-3 resonance (B), and the lactyl methyl proton resonance (C).

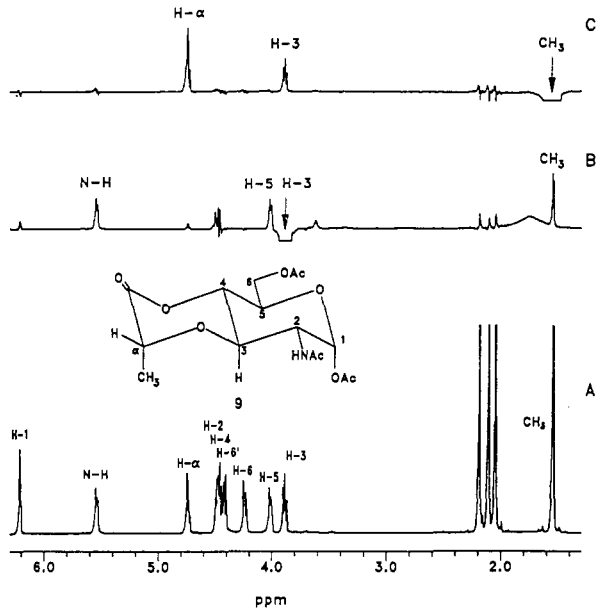
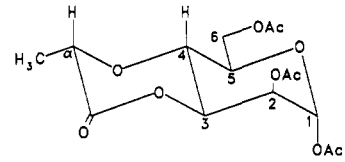


Figure 4. ^{1}H NMR spectrum of the acetylated lactone derivative of 2-amino-2-deoxy-3-*O*-[(*R*)-1-carboxyethyl]-α-D-glucose (**9**) (A), NOE difference spectra obtained by irradiation of the H-3 resonance (B), and the lactyl methyl proton resonance (C).

analysis of *N*-acetylmuramic acid (**2**) showed the occurrence of NOEs between the lactate methyl protons and H-3 of the 2-acetamido-2-deoxy-D-glucopyranosyl ring in the derived acetylated lactone **9** (Figure 4). This result is consistent with the interproton distances calculated from the minimum energy conformation (Table III) and is in full agreement with the lactyl moiety having the (*R*)-configuration. The absolute configuration of muramic acid has been previously determined by using the synthetic approach.[31a]

We have established that 4-*O*-(1-carboxyethyl)-β-D-mannopyranose is a component of the capsular polysaccharide of *R. equi* serotype 3 (**10**). The 1-carboxyethyl-substituted mannose **3** was isolated from the polysaccharide **10**, following removal of the cyclic pyruvate acetal groups, by the periodate oxidation/acid hydrolysis sequence shown in Scheme III. The absolute stereochemistry of the mannopyranosyl residues was deduced from the data obtained by molecular modeling and proton NOE measurements on the Glc*p*-(1→3)-Man*p* disaccharide unit of the intact polysaccharide.[15] The chirality of the lactyl α-carbon was correlated to that of the D-mannose residue from the observed proton NOEs in the acetylated lactone derivative **11**. The occurrence of NOEs between the α-proton and H-4 of the D-mannopyranose ring system, as predicted by molecular modeling (Table III), clearly indicated that the 1-carboxyethyl moiety has the (*S*)-chirality (as in **11**).



11

In summary, this study illustrates that the relative stereochemistry of 1-carboxyethyl glycose ethers can be readily determined by NMR spectroscopy by using a strategy which involves the combined application of NOE measurements and molecular modeling on a conformationally rigid lactone derivative. The absolute configuration of the glycose moiety can be readily achieved using conventional approaches following the dealkylation of the 1-carboxyethyl substituent.

As further examples of this class of immunologically significant acidic sugars are reported, this approach will provide a general method for configurational assignment which circumvents the necessity of chemical synthesis as proof of stereochemistry.

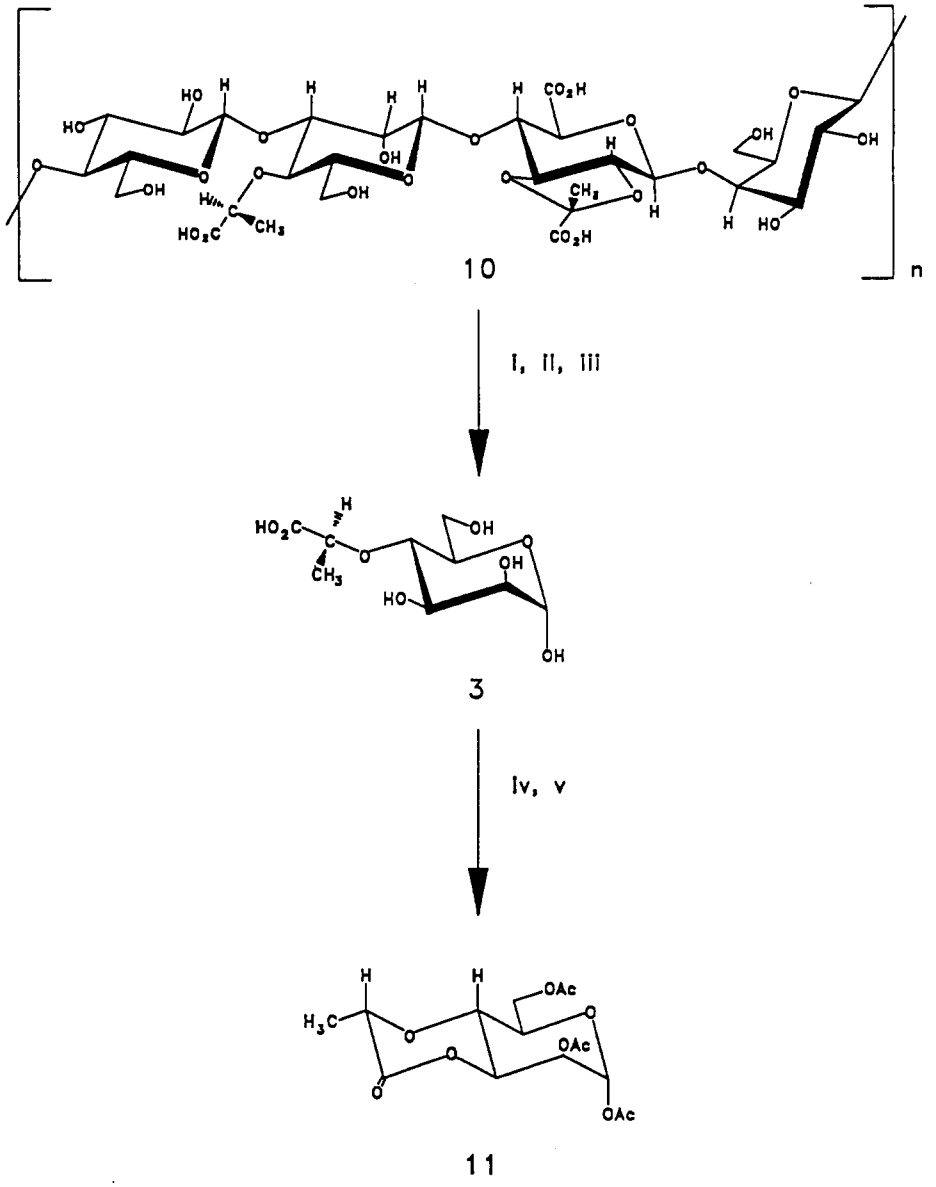
Experimental Section

Methyl 3-*O*-Allyl-α-L-rhamnopyranoside (5). A mixture of methyl α-L-rhamnopyranoside (**4**) (2.6 g, 14 mmol) and dibutyltin oxide (3.6 g, 14 mmol) in benzene (100 mL) was heated for 20 h at reflux temperature, with azeotropic distillation of water. The solution was concentrated to 40 mL, whereupon 1 equiv each of tetrabutylammonium iodide (5.2 g) and allyl bromide (1.2 mL) were added. After the solution was stirred for 7 h at 60 °C, the solvent was removed under vacuo and the residue was chromatographed over silica gel (eluted with 5% acetone in EtOAc) to give 1.44 g (45%) of **5** as an oil: $[\alpha]^{23}_D$ = −39.18° (*c* 1.1, H_2O); ^{1}H NMR (CD_3OD) δ 1.29 (d, 3 H, *J* = 6.0 Hz, H-6), 3.41 (s, 3 H, OCH_3), 3.48 (dd ≈ t, 1 H, *J* = 9.6 Hz, H-4), 3.54 (dd, 1 H, *J* = 3.5, 9.6 Hz, H-3), 3.67 (dq, 1 H, *J* = 6.0, 9.6 Hz, H-5), 4.09 (dd, 1 H, *J* = 1.8, 3.5 Hz, H-2), 4.15–4.29 (m, 2 H, allyl —CH_2—), 4.70 (d, 1 H, *J* = 1.8 Hz, H-1), 5.25–5.45 (m, 2 H, allyl =CH_2), 5.91–6.11 (m, 1 H, allyl —CH=); ^{13}C NMR δ 17.6 (C-6), 55.5 (OCH_3), 67.5 (C-4), 69.2 (C-2), 71.1 (allyl —CH_2—), 71.7 (C-5), 78.4 (C-3), 101.6 (C-1), 119.0 (allyl =CH_2), 135.0 (allyl —CH=). Anal. Calcd for $C_{10}H_{18}O_5$: C, 54.66; H, 8.24. Found: C, 55.05; H, 8.25.

Methyl 3-*O*-Allyl-2,4-di-*O*-benzyl-α-L-rhamnopyranoside (12). Over a period of 0.5 h, sodium hydride (80% in mineral oil, 0.75 g) was added to a cold (0 °C) stirred solution of **5** (1.0 g, 4.6 mmol) in *N*,*N*-dimethylformamide (12 mL). After the mixture was allowed to stand 1.5 h at room temperature, benzyl chloride (8.9 mL) was added, and the reaction was left for 22 h. Methanol was added cautiously to decompose the excess sodium hydride, and the solvent was evaporated. The residue was dissolved in chloroform, washed with water, and concentrated before purification over silica gel (eluted with 10% EtOAc in hexanes) to give 1.7 g (94%) of **12** as an oil: $[\alpha]^{23}_D$ = −40.64° (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.33 (d, 3 H, *J* = 6.0 Hz, H-6), 3.28 (s, 3 H, OCH_3), 3.57 (dd, 1 H, *J* = 9.6, 10.0 Hz, H-4), 3.65 (dq, 1 H, *J* = 6.0, 10.0 Hz, H-5), 3.72 (dd, 1 H, *J* = 3.5, 9.6 Hz, H-3), 3.74 (dd, 1 H, *J* = 1.8, 3.5 Hz, H-2), 4.00–4.10 (m, 2 H, allyl —CH_2—), 4.60 (d, 1 H, *J* = 1.8 Hz, H-1), 4.65–4.95 (m, 4 H, benzyl CH_2), 5.08–5.38 (m, 2 H, allyl =CH_2), 5.75–6.00 (m, 1 H, allyl —CH=), 7.15–7.43 (m, 10 H, aromatic); ^{13}C NMR δ 18.0 (C-6), 54.5 (OCH_3), 67.8 (C-5), 70.9 and 75.3 (benzyl CH_2), 72.8 (allyl, —CH_2—), 74.2 (C-3), 79.9 (C-2), 80.4 (C-4), 99.2 (C-1), 116.5 (allyl =CH_2), 127.5–128.4 (aromatic), 135.0 (allyl —CH=). Anal. Calcd for $C_{24}H_{30}O_5$: C, 72.36; H, 7.54. Found: C, 72.04; H, 7.50.

Methyl 2,4-Di-*O*-benzyl-α-L-rhamnopyranoside (6). A suspension of **12** (1.6 g, 4 mmol) and 10% palladium-on-charcoal (0.2 g) in ethanol (10 mL), glacial acetic acid (5 mL), and water (5 mL) was stirred for 48 h at 75 °C. The solid material was filtered off (Celite bed), and the filtrate was concentrated before being applied to a column of silica gel (eluted with 25% EtOAc in hexanes). Compound **6** was recovered, 1.1 g (77%),

Scheme III[a]



[a] (i) Mild acid hydrolysis, (ii) periodate oxidation, (iii) acid hydrolysis, (iv) acetic anhydride, (v) pyridine.

as a colorless oil: $[\alpha]^{23}_D = -15.42°$ (*c* 1.1, $CHCl_3$) (lit.[37] $[\alpha]^{23}_D$ −15.4°); ^1H NMR ($CDCl_3$) δ 1.30 (d, 3 H, *J* = 6.2 Hz, H-6), 3.21 (s, 3 H, OCH_3), 3.32 (dd, 1 H, *J* = 9.6, 9.8 Hz, H-4), 3.63 (dq, 1 H, *J* = 6.2, 9.8 Hz, H-5), 3.66 (dd, 1 H, *J* = 1.8, 3.5 Hz, H-2), 3.93 (dd, 1 H, *J* = 3.5, 9.6 Hz, H-3), 4.45–4.88 (m, 4 H, benzyl CH_2), 4.68 (d, 1 H, *J* = 1.8 Hz, H-1), 7.14–7.36 (m, 10 H, aromatic); ^{13}C NMR δ 18.0 (C-6), 54.6 (OCH_3), 67.0 (C-5), 71.6 (C-3), 73.0 and 74.9 (benzyl CH_2), 76.6 (C-2), 82.2 (C-4), 97.9 (C-1), 127.5–128.4 (aromatic). Anal. Calcd for $C_{21}H_{26}O_5$: C, 70.39; H, 7.26. Found: C, 70.12; H, 7.17.

Methyl 3-*O*-[(*R*)-1-(Methoxycarbonyl)ethyl]-2,4-di-*O*-benzyl-α-L-rhamnopyranoside (13*R*). Compound **6** (0.5 g, 1.4 mmol) was dissolved in 1,4-dioxane (13 mL), and after the addition of sodium hydride (0.33 g, 80% suspension in paraffin oil) the mixture was stirred at 95 °C for 1 h. A solution of (*S*)-2-chloropropionic acid ($[\alpha]^{23}_D = -17.12°$ (neat), 0.3 g, 2.8 mmol) in 1,4-dioxane (3 mL) was added when the temperature cooled to 65 °C. After the addition of NaH (1.3 g) in 1,4-dioxane (2 mL), the stirring was continued for 14 h at 65 °C. Upon cooling, the reaction was quenched by the addition of water (5 mL), and then the mixture was concentrated in vacuo to a thick syrup, which was dissolved in water (10 mL) and washed with chloroform (2 × 10 mL). The aqueous solution was acidified (pH 2.5–3.0) with hydrochloric acid (6 M) and then extracted with chloroform (2 × 10 mL). Following removal of the solvent, the residue was treated with ethereal diazomethane and chromatographed over silica gel (eluted with 25% EtOAc in hexane) to give 0.41 g (66%) of **13*R*** as an oil: $[\alpha]^{23}_D = +28.26°$ (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.30 (d, 3 H, *J* = 6.1 Hz, H-6), 1.38 (d, 3 H, *J* = 7.0 Hz, lactyl CH_3), 3.27 (s, 3 H, OCH_3), 3.55 (dd ≈ t, 1 H, *J* = 9.6 Hz, H-4), 3.60 (s, 3 H, $COOCH_3$), 3.62 (m, 1 H, *J* = 6.1, 9.6 Hz, H-5), 3.72 (dd, 1 H, *J* = 1.8, 2.6 Hz, H-2), 3.78 (dd, 1 H, *J* = 2.6, 9.6 Hz, H-3), 4.40–5.20 (m, 4 H, benzyl CH_2), 4.15 (q, 1 H, *J* = 7.0 Hz, lactyl CH), 4.65 (d, 1 H, *J* = 1.8 Hz, H-1), 7.18–7.44 (m, 10 H, aromatic); ^{13}C NMR δ 18.1 (C-6), 19.0 (lactyl CH_3), 51.8 ($COOCH_3$), 54.6 (OCH_3), 67.8 (C-5), 75.0 (C-2), 72.8 and 75.0 (benzyl CH_2), 73.4 (lactyl CH), 79.0 (C-3), 79.9 (C-4), 98.9 (C-1), 127.5–128.2 (aromatic). Anal. Calcd for $C_{25}H_{32}O_7$: C, 67.55; H, 7.22. Found: C, 67.28; H, 7.25.

Methyl 3-*O*-[(*S*)-1-(Methoxycarbonyl)ethyl]-2,4-di-*O*-benzyl-α-L-rhamnopyranoside (13*S*). The condensation of compound **6** (0.33 g) and (*R*)-chloropropionic acid ($[\alpha]^{23}_D = +17.39°$ (neat)) by the above method, gave **13*S*** (0.40 g, 65%): $[\alpha]^{23}_D = -73.19°$ (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.31 (d, 3 H, *J* = 6.1 Hz, H-6), 1.43 (d, 3 H, *J* = 6.9 Hz, lactyl CH_3), 3.25 (s, 3 H, OCH_3), 3.59 (dd, 1 H, *J* = 9.8, 10.2 Hz, H-4), 3.62 (m, 1 H, *J* = 6.1, 10.2 Hz, H-5), 3.66 (dd, 1 H, *J* = 1.7, 4.1 Hz, H-2), 3.69 (s, 3 H, $COOCH_3$), 3.96 (dd, 1 H, *J* = 4.1, 9.8 Hz, H-3), 4.34 (q, 1 H, *J* = 6.9 Hz, lactyl CH), 4.60–4.99 (m, 4 H, benzyl CH_2), 4.53 (d, 1 H, *J* = 1.7 Hz, H-1), 7.22–7.38 (m, 10 H, aromatic); ^{13}C NMR δ 17.9 (C-6), 19.2 (lactyl CH_3), 51.8 ($COOCH_3$), 54.5 (OCH_3), 67.9 (C-5), 73.4 and 75.3 (benzyl CH_2), 76.4 (lactyl CH), 77.0 (C-3), 80.4 (C-4), 81.4 (C-2), 99.7 (C-1), 127.4–128.4 (aromatic). Anal. Calcd for $C_{25}H_{32}O_7$: C, 67.55; H, 7.22. Found: C, 67.33; H, 7.17.

Methyl 3-*O*-[(*R*)-1-(Methoxycarbonyl)ethyl]-α-L-rhamnopyranoside (7*R*). A solution of **13*R*** (0.32 g) in absolute ethanol (5 mL) was hy-

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drogenated over 10% palladium-on-charcoal at room temperature for 16 h. Following removal of the solid material (Celite bed), the filtrate was concentrated and chromatographed over silica gel (eluted with 30% hexanes in EtOAc) to give **7R**, (0.15 g, 86%), as a colorless oil: $[\alpha]^{23}_D = -1.2°$ (*c* 0.5, MeOH); ^{1}H NMR (CD_3OD) δ 1.26 (d, 3 H, J = 6.9 Hz, H-6), 1.41 (d, 3 H, J = 7.0 Hz, lactyl CH_3), 3.33 (s, 3 H, OCH_3), 3.44 (dd, 1 H, J = 3.1, 9.0 Hz, H-3), 3.50 (dd, 1 H, J = 9.0, 9.1 Hz, H-4), 3.52 (m, 1 H, J = 9.1, 6.9 Hz, H-5), 3.75 (s, 1 H, $COOCH_3$), 3.89 (dd, 1 H, J = 1.7, 3.1 Hz, H-2), 4.29 (q, 1 H, J = 7.0 Hz, lactyl CH), 4.58 (d, 1 H, J = 1.7 Hz, H-1); ^{13}C NMR δ 19.0 (C-6), 20.4 (lactyl CH_3), 53.8 ($COOCH_3$), 56.2 (OCH_3), 70.3 (C-2), 70.8 (C-5), 73.6 (C-4), 75.4 (lactyl CH), 81.7 (C-3), 103.6 (C-1). Anal. Calcd for $C_{11}H_{20}O_7$: C, 49.99; H, 7.62. Found: C, 49.72; H, 7.66.

Methyl 3-*O*-[(*S*)-1-(Methoxycarbonyl)ethyl]-α-L-rhamnopyranoside (7*S*). Hydrogenolysis of **13S** (1.4 g), according to the procedure described above, gave **7S** (0.74 g, 89%): $[\alpha]^{23}_D = -95.7°$ (*c* 0.9, MeOH); ^{1}H NMR (CD_3OD) δ 1.26 (d, 3 H, J = 6.2 Hz, H-6), 1.41 (d, 3 H, J = 7.1 Hz, lactyl CH_3), 3.33 (s, 3 H, OCH_3), 3.42 (dd, 1 H, J = 3.1, 10.7 Hz, H-3), 3.48 (dd, 1 H, J = 9.1, 10.7 Hz, H-4), 3.53 (m, 1 H, J = 6.2, 10.7 Hz H-5), 3.73 (s, 1 H, $COOCH_3$), 3.95 (dd, 1 H, J = 1.7, 3.1 Hz, H-2), 4.39 (q, 1 H, lactyl CH), 4.56 (d, 1 H, J = 1.7 Hz, H-1); ^{13}C NMR δ 19.2 (C-6), 20.3 (lactyl CH_3), 53.8 ($COOCH_3$), 56.3 (OCH_3), 70.8 (C-5), 71.8 (C-2), 74.6 (C-4), 77.9 (lactyl CH), 82.5 (C-3), 103.6 (C-1). Anal. Calcd for $C_{11}H_{20}O_7$: C, 49.99; H, 7.62. Found: C, 49.77; H, 7.58.

1-Carboxyethyl-Substituted Sugars. The diastereoisomers of methyl 3-*O*-(1-carboxyethyl)-α-L-rhamnopyranoside (**1R** and **1S**) were obtained by saponification of the corresponding methyl esters. In separate experiments, the methyl esters **7R** and **7S** (200 mg) were heated in aqueous sodium hydroxide (0.1 M, 10 mL) at 100 °C for 6 h, followed by neutralization with hydrochloric acid (0.2 M, 5 mL), concentration under vacuo, and deionization by passing the aqueous solution (2 mL) through a column containing Rexyne 101 (H^+ form). The eluants were concentrated to dryness to give the acids **1R** and **1S**, respectively.

2-Acetamido-2-deoxy-3-*O*-[(*R*)-1-carboxyethyl]-D-glucose (**2**) was purchased from Aldrich and used as received.

4-*O*-(1-Carboxyethyl)-D-mannopyranoside (**3**) was obtained from the capsular polysaccharide of *R. equi* serotype 3 (ATCC 33703) as previously described.[15]

General Procedure for the Preparation of the Acetylated Lactone Derivatives of 1-Carboxyethyl-Substituted Sugars 8*R*, 8*S*, 9, and 11. Acetic anhydride (2 mL) was added to a suspension of **1R**, **1S**, **2**, or **3** in THF (10 mL) and stirred for 13 h, after which pyridine (2 mL) was added and the mixture was stirred for an additional 8 h. The solutions were concentration under vacuo, and the residues were either recrystallized from EtOH or chromatographed over silica gel (eluted with 20% hexane in EtOAc) to give the acetylated lactone derivatives **8R**, **8S**, **9**, or **11**, respectively. ^{1}H and ^{13}C NMR data are given in Tables I and II.

Lactones **8R** and **8S** each eluted as single peaks on GLC ($t_{R(GA)}$ 0.35 and $t_{R(GA)}$ 0.36, respectively); MS (CI) m/z 275 $(M + H)^+$, (EI) 243 (M^+ – MeOH), 214 (M^+ – AcOH), 185 (M^+ – lactic acid).[38]

Samples (0.2 mg) of lactones **9** and **11** were de-*O*-acetylated by being stirred in a solution of triethylamine:methanol:water (1:2:8) for 15 h at 22 °C and then evaporated to dryness in vacuo. The residues were converted to the corresponding peracetylated alditol acetate derivatives.[39,40] 1,4,5,6-Tetra-*O*-acetyl-3-*O*-(1-acetoxypropyl)-2-deoxy-2-acetamido-D-glucitol: GLC, $t_{R(GA)}$ 1.9, MS (CI) m/z 492 $(M + H)^+$ 1,2,3,5,6-Penta-*O*-acetyl-3-*O*-(1-acetoxypropyl)-D-mannitol: GLC, $t_{R(GA)}$ 1.5, MS (CI) m/z 493 $(M + H)^+$.

Gas Liquid Chromatography. GLC was performed with a Hewlett-Packard Model 5710A chromatograph fitted with a hydrogen flame detector and a Model 3380A electronic integrator using a fused-silica capillary column (0.3 mm × 25 m) containing 3% DB17 with the following temperature program: 2 min at 180 °C and then 2 °C/min to 240 °C. Retention times are quoted relative to D-glucitol hexaacetate ($t_{R(GA)}$). GLC-MS measurements were made using a JEOL AX505H system employing the stated GLC program conditions by electron impact (EI) with an ionization potential of 70 eV or by chemical ionization (CI) with ammonia as the reagent gas.

NMR Spectroscopy. ^{1}H and ^{13}C NMR spectra were obtained on samples in $CDCl_3$ at 600 MHz and 150 MHz, respectively, using a Bruker AMX-600 spectrometer with standard Bruker software. Chemical shifts are expressed relative to internal TMS (0.000 ppm).

^{1}H NOE spectra were obtained in the difference mode by selective irradiation of each line of the multiplet resonance for a total irradiation time of 1 s.[41]

Two-dimensional homonuclear proton correlation experiments (COSY[32]) were measured employing the conventional pulse sequences as previously described.[10]

Heteronuclear ^{1}H–^{13}C chemical shift correlations were obtained by ^{1}H detection via multiple quantum coherence[33] in the phase sensitive mode. ^{13}C decoupling was achieved using the GARP-1 composite pulse sequence during ^{1}H acquisition.[42]

Molecular Modeling. Starting geometries were generated on the PC-based program Alchemy (Tripos Associates Inc.). Atomic coordinates for α-L-rhamnopyranoside[43] and α-D-mannopyranoside[44] were taken from neutron diffraction data. The coordinates for 2-acetamido-2-deoxy-α-D-glucopyranose were generated from neutron diffraction data for 2-acetamido-2-deoxy-α-D-galactopyranose.[45] Structures were then refined by molecular mechanics using the MM2 algorithm (Allinger, QCPE, Indiana University)[46] optimized for carbohydrates[47] and viewed using Schakal (Keller, Kristallographisches Institut, Universitaet Freiburg). Calculations were performed on the MicroVax 3500.

General Methods. Commercial reagents and solvents were analytical grade. Concentrations were made under reduced pressure at bath temperatures below 40 °C.

Optical rotations were determined at 22 °C in 10-cm microtubes using a Perkin-Elmer Model 243 polarimeter.

Acknowledgment. We thank Mr. F. P. Cooper for the GLC-MS analyses.

(38) Except for relative intensities, the MS of **8R** and **8S** were identical.

(39) Gunner, S. W.; Jones, J. K. N.; Perry, M. B. *Can. J. Chem.* **1961**, *39*, 1892.

(40) The acetylated bicyclic lactone derivatives of the methyl glycosides of rhamnose gave the expected molecular ions (M + H) in their mass spectra, whereas the mass spectra of the related acetylated glycoses **9** and **11** were not structurally informative. The two latter lactones gave the expected mass spectral data[15] following prior conversion into the respective alditol acetate derivatives.[39]

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(42) Shaka, A. J.; Keeler, J. *Prog. Nucl. Magn. Reson. Spectrosc.* **1987**, *19*, 47.

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