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Lactones of methyl 3-*O*-[(*R*)- and (*S*)-1-carboxyethyl]-α-D-gluco-, galacto-, and manno-pyranosides

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Abstract

Lactones of methyl 3-*O*-[(*R*)- and (*S*)-1-carboxyethyl]-α-D-gluco-, galacto- and manno-pyranoside were prepared by treatment of the sugar derivatives in acetic acid. The lactones were formed between the 1-carboxyethyl substituent and 2-OH or 4-OH in different proportions depending on the stereochemistry of the parent compounds. Relative formation rates in acetic acid-d_4 and hydrolysis rates in buffered D_2O solutions at pD 2.4, 4.6 and 7.4 were estimated. Hydrolysis of the formed lactones is relatively slow in D_2O at pD 4.6, which permitted characterization of the lactones by ^1H and ^{13}C NMR spectroscopy in buffered D_2O solutions. Hydrolysis of the lactones in 1 M aqueous NaOH at 80 °C gave no detectable isomerization of the α-carbon. The set of lactones formed from the 1-carboxyethyl substituted methyl glycosides used in this study showed large similarities in the NMR shifts ($\Delta\delta$ values). Deviations from the observed shift pattern were found for two lactones. Our findings strongly suggest that those two lactones differ from the rest by adopting a boat-like conformation, whereas the others adopt pseudo-chair conformations.

Keywords: Lactones; Hydrolysis; Pyranosides

1. Introduction

Sugar residues etherified with lactic acid are important components in several bacterial polysaccharides. Both the (*R*)- and (*S*)-forms of the 1-carboxyethyl groups substituting C-3, C-4 or C-6 of different monosaccharides have been found [1–4]. The capsular polysaccharides from several strains of *Butyrivibrio fibrisolvens* are under investigation in our department and since 1-carboxyethyl substituted sugars are common components of these polysaccharides [3] they are of special interest. Degradation of 1-carboxyethyl substituted polysaccharides by acid hydrolysis (TFA) sometimes results in the formation of new sugar derivatives when the acid is removed by evaporation. These new compounds yield 1-carboxyethyl sugars upon treatment with an aqueous base, which indicates that they are esters. Downfield shifts in the ^1H NMR spectra indicated that lactones had been formed between the 1-carboxyethyl group and an adjacent hydroxyl group in the released monosaccharides.

Lactones have been observed in polysaccharides and gangliosides containing sialic acid [5–7], which suggests that lactones might also

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be found in polysaccharides containing 1-carboxyethyl sugars. NMR spectroscopy can be used to determine whether lactones occur in native polysaccharides [5]. To facilitate the detection of lactones it would be advantageous to know their NMR properties.

A problem with the identification of lactones in sialic acid containing saccharides is that lactones can easily be formed and hydrolyzed during the isolation and preparation of analytical samples [8] leading to uncertainty about the native material. It would thus be valuable to study the formation and hydrolysis reactions of lactones formed by 1-carboxyethyl sugars in order to determine if they are formed or hydrolyzed during analytical procedures.

To study the lactone formation and hydrolysis reactions we used, as model substances, the methyl 3-*O*-[(*R*)- and (*S*)-1-carboxyethyl]-α-D-glycopyranosides of glucose, galactose and mannose. Formation of lactones was studied in acetic acid-d_4, while the stability of the formed lactones towards hydrolysis was monitored at different pD in D_2O solutions.

2. Results and discussion

All 1-carboxyethyl substituted monosaccharide derivatives used in this study were available from previous work [9]. The substances were chosen so that the 3-*O*-(1-carboxyethyl) substituent had either two equatorial hydroxyl groups or one axial and one equatorial hydroxyl group in adjacent positions. When the 3-*O*-(1-carboxyethyl) substituted monosaccharide derivatives were treated with HOAc, conditions used for lactone formation [10], compounds with higher mobility on TLC were formed. The ^{1}H NMR spectra of the products indicated that the carboxyl group had formed lactones with the adjacent hydroxyl groups, 2-OH and 4-OH, as either the H-2 or H-4 signal was shifted downfield ~ 0.9 ppm and the FABMS gave an $[M+H]^+$ ion at m/z 249 corresponding to the 1-carboxyethyl sugar–water. ^{1}H NMR and TLC analysis indicated that the yield of lactones was $>90\%$ under the reaction conditions used, but the isolated yields were reduced due to side reactions during the work-up procedure and the removal of solvent by freeze-drying. Analysis of the crude freeze-dried product by FABMS indicated, from peaks at m/z 291 and 497, O-acetylation of lactones as well as ester formation between two sugar residues, respectively. The occurrence of these products was also supported by TLC (5:1 EtOAc–MeCN) showing the presence of spots with $R_f \sim 0.8$ and spots near the baseline. The lactones were separated on silica gel and identified by ^{1}H and ^{13}C NMR spectroscopy (Table 1). We could not completely separate some of the 2- and 4-lactones from each other by chromatography, but the components could be characterized by NMR spectroscopy on the mixtures. In the ^{1}H NMR spectra all spin systems, except that for the 2-lactone of methyl 3-*O*-[(*S*)-1-carboxyethyl]-α-D-Glc*p* [2-lactone-(*S*)-Glc*p*], were completely assigned and the 2- and 4-lactones were identified by the downfield shift of the signals for H-2 and H-4, respectively.

The relative rates of lactone formation in acetic acid-d_4 varied depending on the monosaccharide and absolute configuration of the 1-carboxyethyl group (Fig. 1). Formation rates seem to be correlated to the steric interactions in a product-like transition state. The lowest energy is achieved if the six-membered ring transition state has a chair conformation and the C-3′ methyl group adopts an 'equatorial' position (compounds **1**, **4**, **5** and **12**). If the C-3′ methyl group adopts an 'axial' position (compounds **2**, **3**, **7** and **10**) the energy will be higher due to the increased steric interactions of the large axial group. The C-3′ methyl group occurs in an 'equatorial' position for methyl 3-*O*-[(*R*)-1-carboxyethyl]-α-D-glucopyranoside when the 2-lactone is formed, whereas the C-3′ methyl group adopts an axial position when the 4-lactone is formed (Fig. 2). As a result the formation rate for the 2-lactone is higher than that for the 4-lactone. For the (*S*)-form similar interactions lead to the opposite situation with a higher formation rate for the 4-lactone instead of the 2-lactone. For the mannose and galactose derivatives the same type of interactions in the transition states occur when the lactone is formed with the equatorial hydroxyl group (compounds **5** and **12**). However, the energy of the transition

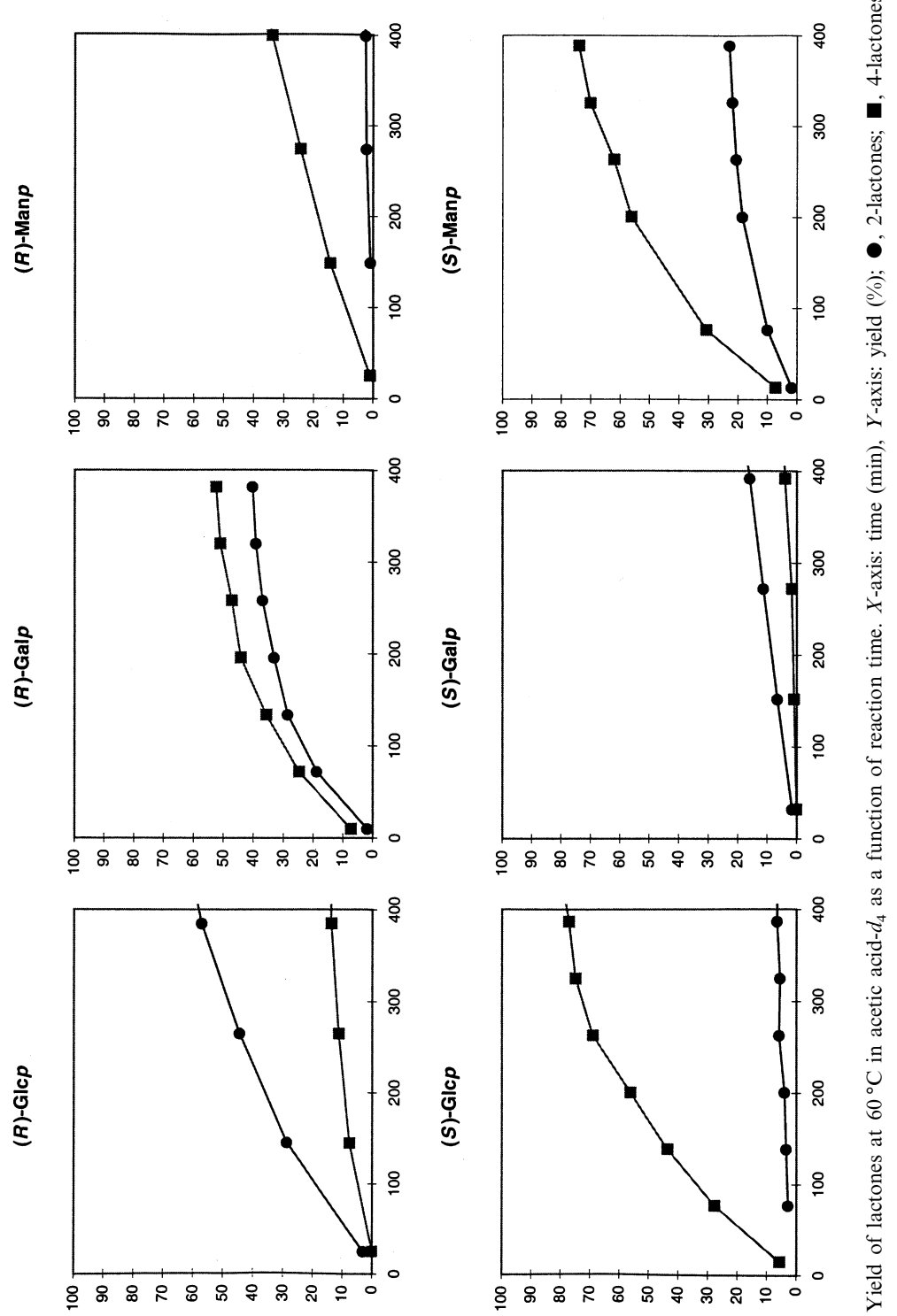
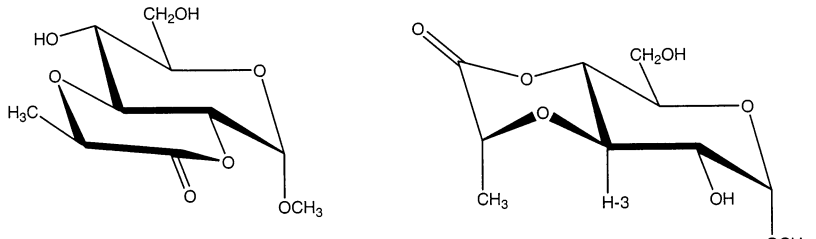


Fig. 1. Yield of lactones at 60 °C in acetic acid-d_4 as a function of reaction time. X-axis: time (min), Y-axis: yield (%); ●, 2-lactones; ■, 4-lactones.

Table 1
^{1}H and ^{13}C NMR chemical shifts of lactones formed from methyl 3-*O*-[(*R*)- and (*S*)-1-carboxyethyl]-α-D-gluco-, galacto-, and manno-pyranosides, in D_2O solutions

Compound	H-1 C-1	H-2 C-2	H-3 C-3	H-4 C-4	H-5 C-5	H-6a/H-6b C-6	OCH_3 OCH_3	C-1′	H-2′ C-2′	H-3′ C-3′
(*R*)-Glc*p* [a]	4.83 99.62	3.63 71.46	3.52 82.79	3.48 70.56	3.62 72.54	3.86/3.75 61.24	3.41 55.64	182.70	4.26 79.62	1.40 19.58
2-Lactone-(*R*)-Glc*p* [b] (**1**)	5.03 97.03	4.56 77.75	4.02 74.52	3.71 67.78	3.74 73.09	3.90/3.80 60.80	3.47 55.91	172.55	4.63 74.36	1.55 17.94
4-Lactone-(*R*)-Glc*p* (**2**)	4.89 100.10	3.82 69.82	4.09 70.98	4.41 75.48	3.93 69.63	3.88/3.82 59.86	3.45 56.11	173.70	4.84 72.17	1.52 17.86
(*S*)-Glc*p*	4.79 100.29	3.64 72.31	3.52 82.66	3.46 69.54	3.64 72.65	3.85/3.73 61.42	3.41 55.85	183.00	4.23 79.48	1.39 19.55
2-Lactone-(*S*)-Glc*p* (**3**)	5.05 96.9 [d]	4.56 77.5 [d]	4.05 70.4 [d]	3.70 –	– [c] –	–/– –	3.48 56.0 [d]	–	4.83 71.9 [d]	1.52 17.6 [d]
4-Lactone-(*S*)-Glc*p* (**4**)	4.89 100.07	3.83 69.76	4.07 74.53	4.40 75.73	3.93 69.72	3.88/3.81 59.8	3.45 56.08	173.00	4.67 74.53	1.55 18.07
(*R*)-Gal*p*	4.87 99.79	3.92 67.96	3.60 78.06	4.12 67.30	3.85 71.47	3.75/3.74 62.09	3.40 55.66	182.41	4.11 76.42	1.37 19.70
2-Lactone-(*R*)-Gal*p* (**5**)	5.08 97.23	4.82 75.57	4.14 71.44	4.23 67.73	4.00 72.22	3.77/3.77 61.66	3.47 55.92	173.33	4.68 74.46	1.54 17.96
4-Lactone-(*R*)-Gal*p* (**6**)	4.96 100.16	4.23 63.73	4.21 69.79	5.04 77.24	4.12 69.67	3.83/3.83 60.38	3.45 56.18	172.81	4.64 67.31	1.53 18.42
(*S*)-Gal*p*	4.84 100.18	3.91 68.12	3.63 78.95	4.04 67.62	3.89 71.09	3.74/3.74 62.14	3.41 55.79	182.79	4.06 77.45	1.37 19.40
2-Lactone-(*S*)-Gal*p* (**7**)	5.11 97.04	4.83 74.80	4.18 68.21	4.18 68.07	3.99 72.14	3.78/3.78 61.71	3.48 55.88	173.54	4.80 71.93	1.51 17.76
4-Lactone-(*S*)-Gal*p* (**8**)	4.87 100.10	3.78 68.89	4.33 71.40	5.06 73.89	4.16 68.30	3.84/3.84 60.77	3.44 56.18	175.18	4.68 67.76	1.41 16.28
(*R*)-Man*p*	4.79 101.21	4.01 68.50	3.58 79.97	3.71 66.70	3.63 73.26	3.90/3.76 61.83	3.40 55.51	182.52	4.08 77.61	1.38 19.35
2-Lactone-(*R*)-Man*p* (**9**)	5.04 98.34	4.96 74.55	4.24 73.01	3.62 67.30	3.72 72.31	3.90/3.77 61.12	3.45 55.85	174.44	4.70 68.36	1.43 16.57
4-Lactone-(*R*)-Man*p* (**10**)	4.84 101.89	4.13 68.97	4.15 70.01	4.69 73.06	3.90 70.70	3.92/3.83 60.33	3.43 55.79	173.90	4.85 72.18	1.51 17.79
(*S*)-Man*p*	4.78 101.57	4.05 67.97	3.54 79.40	3.73 66.34	3.61 73.61	3.88/3.74 61.80	3.39 55.49	182.22	4.10 76.39	1.38 19.64
2-Lactone-(*S*)-Man*p* (**11**)	4.92 99.04	4.91 77.34	4.12 71.66	4.05 62.14	3.74 72.69	3.93/3.79 61.14	3.45 55.82	-	4.68 67.88	1.54 18.47
4-Lactone-(*S*)-Man*p* (**12**)	4.83 101.91	4.16 68.73	4.12 72.65	4.65 73.67	3.89 70.80	3.90/3.82 60.30	3.43 55.77	173.27	4.73 74.73	1.54 18.10

[a] (*R*)-Glc*p* = methyl 3-*O*-[(*R*)-1-carboxyethyl]-α-D-glucopyranoside (Na^+ form) etc.
[b] 2-Lactone-(*R*)-Glc*p* = methyl 3-*O*-[(*R*)-1-carboxyethyl]-α-D-glucopyranosid-1′,2-lactone etc.
[c] Missing values due to small amount of sample or overlapping signals.
[d] Chemical shift extracted from cross peak in the HSQC spectrum.



2-Lactone-(*R*)-Glc*p* (**1**)

4-Lactone-(*R*)-Glc*p* (**2**)

Fig. 2. The 2-lactone of methyl 3-*O*-[(*R*)-1-carboxyethyl]-α-D-glucopyranoside with an equatorial C-3′ methyl group (left) and the 4-lactone with an axial C-3′ methyl group (right) with the lactone rings in pseudo-chair conformation.

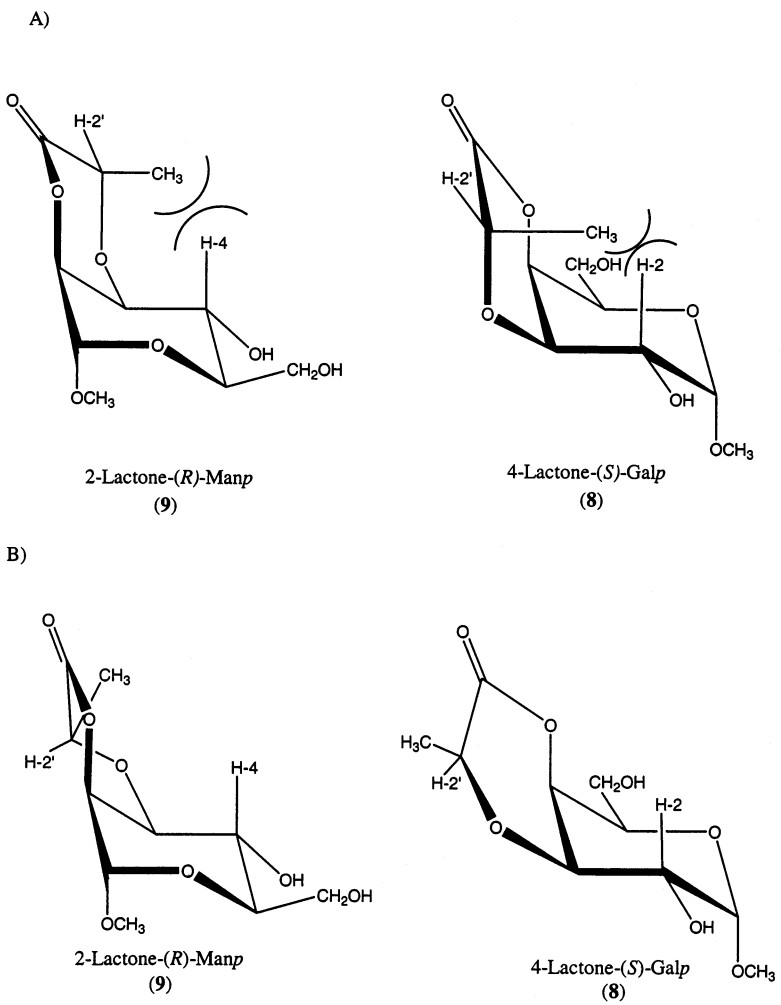


Fig. 3. (A) Steric interactions between the C-3′ methyl group and the adjacent axial ring proton in **9** and **8** with the lactone ring in pseudo-chair conformation. (B) Compounds **9** and **8** with the lactone ring in boat-like conformation.

state for lactone formation involving an axial hydroxyl group is increased (compare *cis*- and *trans*-decalin) with either an 'axially' positioned H-2′ (compounds **6** and **11**) or a C-3′ methyl group (compounds **8** and **9**) very close to either H-4 in mannose or H-2 in galactose. In the transition state where the C-3′ methyl group would be in an 'axial' position, the severe steric interactions could be decreased to some extent by forming a boat-like conformation in the transition state (Fig. 3).

When 2- or 4-lactones were treated with acetic acid-d_4 under similar conditions as those used for the formation of lactones, no

change in the proportion of the 2- and 4-lactones was observed by ^{1}H NMR spectroscopy. However, ~3% of the free acid was formed which indicates that a very slow equilibration between the lactones can occur during their formation in HOAc. The slow hydrolysis does not alter the kinetic measurements on the time scale used, thus the product ratio is expected to be under kinetic control.

When comparing the differences in chemical shift ($\Delta\delta$ values) observed for the ^{1}H and ^{13}C NMR signals of the lactones and their parent 3-*O*-(1-carboxyethyl) glycosides, it is evident that the 2- and 4-lactones show distinct similarities. The largest $\Delta\delta$ values were seen for the signals from the atoms involved in the lactone ring (Table 2). For all the lactones the signals from H-2 of the 2-lactones and H-4 of the 4-lactones showed downfield shifts of ~0.9 ppm, while the signals from H-3 and H-2′ shifted downfield ~0.6 and 0.4–0.8 ppm, respectively. The other proton signals showed only small downfield shifts or were unaffected. The ^{13}C signals for C-2 of the 2-lactones and C-4 of the 4-lactones were shifted downfield 5–10 ppm, whereas the signals from the adjacent C-1 and C-5, respectively, shifted upfield 2–3 ppm. The signals from C-3, C-1′ and C-2′, which are common for both 2- and 4-lactones, shifted upfield by 7–12, 8–10 and 2–10 ppm, respectively.

The large downfield shifts (~0.9 ppm) of the signals for the ring proton at the position of lactone formation (H-2 and H-4, respectively) are very useful for the identification of lactones since the signals appear in the less crowded anomeric region of the spectrum.

For the glucose derivatives with lactones involving one of two equatorial hydroxyl groups there are two pairs of pseudo-mirror images, 2-lactone-(*R*)-Glc*p*/4-lactone-(*S*)-Glc*p* (Fig. 4) and 2-lactone-(*S*)-Glc*p*/4-lactone-(*R*)-Glc*p*. In the galacto and manno derivatives, which form lactones with either an equatorial or an axial hydroxyl group, the four pairs of pseudo-mirror images are 2-lactone-(*S*)-Man*p*/4-lactone-(*R*)-Gal*p* (Fig. 4), 2-lactone-(*R*)-Man*p*/4-lactone-(*S*)-Gal*p*, 2-lactone-(*R*)-Gal*p*/4-lactone-(*S*)-Man*p* and 2-lactone-(*S*)-Gal*p*/4-lactone-(*R*)-Man*p*. The pairs of pseudo-mirror image compounds show similarities in ^{1}H and ^{13}C $\Delta\delta$ values as can be seen in Table 2 and Fig. 5.

For compounds 2-lactone-(*S*)-Glc*p*, 2-lactone-(*S*)-Gal*p*, 4-lactone-(*R*)-Glc*p* and 4-lactone-(*R*)-Man*p* the C-3 and the C-2′ signals occur at 3–4 and 2–4 ppm, respectively, upfield in comparison with those of the compounds with opposite configuration of the 1-carboxyethyl group, i.e., 2-lactone-(*R*)-Glc*p*, 2-lactone-(*R*)-Gal*p*, 4-lactone-(*S*)-Glc*p* and 4-lactone-(*S*)-Man*p*. These upfield shifts are in accordance with a pseudo-chair conformation of the lactone ring with an axial C-3′ methyl group for the first four compounds and an equatorial C-3′ methyl group for the latter compounds. The upfield shifts are caused by an axial group at C-2′ that has a steric interaction with H-3 (γ-gauche effect) [11]. Other chemical shift differences are observed for compounds 2-lactone-(*S*)-Man*p* and 4-lactone-(*R*)-Gal*p* and their isomers with opposite configuration of the 1-carboxyethyl group, i.e., 2-lactone-(*R*)-Man*p* and 4-lactone-(*S*)-Gal*p*. For these compounds only minor differences are observed for the C-3 and the C-2′ signals, whereas large differences occur for the C-2, C-4, C-1′ and C-3′ signals. These effects could be explained by a boat-like conformation of the lactone ring in 2-lactone-(*R*)-Man*p* and 4-lactone-(*S*)-Gal*p* as there is an extreme steric repulsion between H-4 or H-2 and the axial C-3′ methyl group in the chair conformation (Fig. 3).

Other observations supported these conformational differences (i) large differences in formation rates, (ii) $J_{\text{H-2′, H-3′}}$ is 0.5 Hz smaller for 4-lactone-(*S*)-Gal*p* and 2-lactone-(*R*)-Man*p* than that observed for the other lactones and (iii) molecular mechanics calculations [12] indicated that 2-lactone-(*R*)-Man*p* and 4-lactone-(*S*)-Gal*p* are the only lactones in which a boat conformation has lower energy than the pseudo-chair conformation. To verify the proposed conformational differences NOE difference spectroscopy was run on a mixture of 2- and 4-lactone-(*S*)-Gal*p* as well as on the separated 2- and 4-lactones of (*R*)-Glc*p* (Table 3). Irradiation of the H-3′ signal in 2-lactone-(*S*)-Gal*p* resulted in an NOE between H-3′ and H-3 and H-2′, which is in agreement with a pseudo-chair conformation. The fact that irradiation of the H-3′

Table 2
Chemical shift differences ($\Delta\delta$) [a] between lactones and their respective parent methyl 3-*O*-[1-carboxyethyl]-α-D-glycopyranosides

Compound	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	OCH_3	H-2′	H-3′
2-Lactone-(*R*)-Glc*p* [b] (**1**)	0.20	0.93	0.50	0.23	0.12	0.04	0.05	0.06	0.37	0.15
2-Lactone-(*S*)-Glc*p* (**3**)	0.26	0.92	0.53	0.24	– [c]	–	–	0.07	0.60	0.13
2-Lactone-(*R*)-Gal*p* (**5**)	0.21	0.90	0.54	0.11	0.15	0.02	0.03	0.07	0.57	0.17
2-Lactone-(*S*)-Gal*p* (**7**)	0.27	0.92	0.55	0.14	0.10	0.04	0.04	0.07	0.74	0.14
2-Lactone-(*R*)-Man*p* (**9**)	0.25	0.95	0.66	−0.09	0.09	0.00	0.01	0.05	0.62	0.05
2-Lactone-(*S*)-Man*p* (**11**)	0.14	0.86	0.58	0.32	0.13	0.05	0.05	0.06	0.58	0.16
4-Lactone-(*R*)-Glc*p* (**2**)	0.06	0.19	0.57	0.93	0.31	0.02	0.07	0.04	0.58	0.12
4-Lactone-(*S*)-Glc*p* (**4**)	0.10	0.19	0.55	0.94	0.29	0.03	0.08	0.04	0.44	0.16
4-Lactone-(*R*)-Gal*p* (**6**)	0.09	0.31	0.61	0.92	0.27	0.08	0.09	0.05	0.53	0.16
4-Lactone-(*S*)-Gal*p* (**8**)	0.03	−0.13	0.70	1.02	0.27	0.10	0.10	0.03	0.62	0.04
4-Lactone-(*R*)-Man*p* (**10**)	0.05	0.12	0.57	0.98	0.27	0.02	0.07	0.03	0.77	0.13
4-Lactone-(*S*)-Man*p* (**12**)	0.05	0.11	0.58	0.92	0.28	0.02	0.08	0.04	0.63	0.16

	C-1	C-2	C-3	C-4	C-5	C-6	OCH_3	C-1′	C-2′	C-3′
2-Lactone-(*R*)-Glc*p* (**1**)	−2.59	6.29	−8.27	−2.78	0.55	−0.44	0.27	−10.15	−5.26	−1.64
2-Lactone-(*S*)-Glc*p* (**3**)	−3.39	5.19	−12.26	–	–	–	0.15		−7.58	−1.95
2-Lactone-(*R*)-Gal*p* (**5**)	−2.56	7.61	−6.62	0.43	0.75	−0.43	0.26	−9.08	−1.96	−1.74
2-Lactone-(*S*)-Gal*p* (**7**)	−3.14	6.68	−10.74	0.45	1.05	−0.43	0.09	−9.25	−5.52	−1.64
2-Lactone-(*R*)-Man*p* (**9**)	−2.87	6.05	−6.96	0.60	−0.95	−0.71	0.34	−8.08	−9.25	−2.78
2-Lactone-(*S*)-Man*p* (**11**)	−2.53	9.37	−7.74	−4.20	−0.92	−0.66	0.33		−8.51	−1.17
4-Lactone-(*R*)-Glc*p* (**2**)	0.48	−1.64	−11.81	4.92	−2.91	−1.38	0.47	−9.00	−7.45	−1.72
4-Lactone-(*S*)-Glc*p* (**4**)	−0.22	−2.55	−8.13	6.19	−2.93	−1.62	0.23	−10.00	−4.95	−1.48
4-Lactone-(*R*)-Gal*p* (**6**)	0.37	−4.23	−8.27	9.94	−1.80	−1.71	0.52	−9.60	−9.11	−1.28
4-Lactone-(*S*)-Gal*p* (**8**)	−0.08	0.77	−7.55	6.27	−2.79	−1.37	0.39	−7.61	−9.69	−3.12
4-Lactone-(*R*)-Man*p* (**10**)	0.68	0.47	−9.96	6.36	−2.56	−1.50	0.28	−8.62	−5.43	−1.56
4-Lactone-(*S*)-Man*p* (**12**)	0.34	0.76	−6.75	7.33	−2.81	−1.50	0.28	−8.95	−1.66	−1.54

[a] The $\Delta\delta$ values (ppm) were obtained by subtracting the chemical shifts of the parent methyl 3-*O*-[1-carboxyethyl] substituted compound from those of the lactone. A positive $\Delta\delta$ value means a downfield shift.

[b] See Table 1.

[c] Missing values due to small amounts of sample and overlap (mixture).

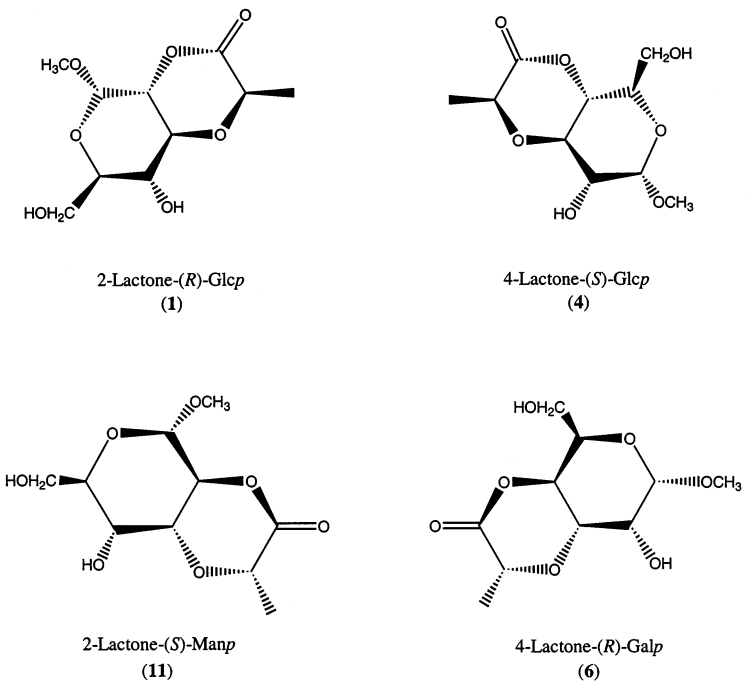


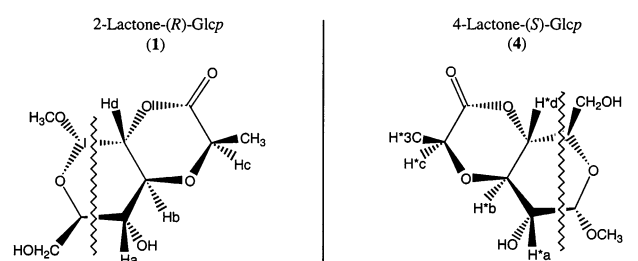
Fig. 4. Stereochemical similarities between pairs of lactones shown for **1** and **4** and **11** and **6**, respectively.

signal of 4-lactone-(*S*)-Gal*p* only showed an NOE between H-3′ and H-2′ and irradiation of the H-2′ signal showed an NOE between H-2′ and H-4 is consistent with a boat conformation for this lactone. For the 2-lactone-(*R*)-Glc*p* a strong NOE between H-3 and H-2′ and the absence of NOE between H-3′ and H-2 indicate a highly populated chair-like conformation for this lactone. For the 4-lactone-(*R*)-Glc*p* a strong NOE between H-3′ and H-3 and the absence of an NOE between H-2′ and H-4 indicate a highly populated chair-like conformation for this lactone also.

The rates of the lactone formation support a product-like transition state as they correspond to the amounts of steric interaction for the different lactones described above.

The stability of the lactones in water solution is of interest since the presence of lactones can be expected in 1-carboxyethyl containing polysaccharides after treatment with acid or activation of the carboxyl group with a carbodiimide derivative [5]. At both high and low pH the lactones were easily hydrolyzed. However, under slightly acidic conditions (pD ~ 4.5) in D_2O solutions the rate of the hydrolysis was fairly slow (half-life > 4 h) (Table 4). This relatively high stability permitted NMR characterization of the lactones in D_2O solutions, which means that the chemical shifts obtained are likely to be relevant in comparison with those from biological material. The lactones were not stable in prolonged contact with water and hydrolysis of some of the lactones was also observed after storage (~ 3 weeks) at -20 °C. Storage is therefore recommended in aprotic solvents such as MeCN or acetone.

The hydrolytic opening of the lactones was monitored by dissolving mixtures of the 2- and 4-lactone of each monosaccharide derivative in buffered D_2O at pD 2.4, 4.6 and 7.4, respectively, and the reactions were followed by ^{1}H NMR spectroscopy. The rates of hy-



	Ha/H*a	Hb/H*b	Hc/H*c	Hd/H*d	CH_3/CH*$_3$
2-Lactone-(*R*)-Glc*p*	0.23	0.50	0.37	0.93	0.15
4-Lactone-(*S*)-Glc*p*	0.19	0.55	0.44	0.94	0.16

Fig. 5. Example of the similarities in the NMR $\Delta\delta$ values. The ^{1}H $\Delta\delta$ values are shown for the pseudo-mirror image compounds 2-lactone (*R*)-Glc*p* (**1**) and 4-lactone-(*S*)-Glc*p* (**4**).

Table 3
Observed NOEs [a] of the 2- and 4-lactones from methyl 3-*O*-[(*R*)-1-carboxyethyl]-α-D-glucopyranoside (**1** and **2**) and from methyl 3-*O*-[(*S*)-1-carboxyethyl]-α-D-galactopyranoside (**7** and **8**) after presaturation of selected ^{1}H signals

Compound	Irradiated nuclei	Saturation (%)	Observed nuclei	Observed NOE (%)
2-Lactone-(*R*)-Glc*p* [b] (**1**)	H-2	94	H-1	12
			H-4	9.8
	H-3	87	H-2′	10.2
	H-2′	90	H-3	12.8
			H-3′	8.6
	H-3′	82	H-2′	9.7
4-Lactone-(*R*)-Glc*p* (**2**)	H-4	93	H-2+H-6	11.3
			H-2′	3.6
	H-3	89	H-3′	~5
	H-3′	83	H-3	7.5
			H-2′	12
2-Lactone-(*S*)-Gal*p* (**7**)	H-3′	85	H-2′	3.8
			H-3	2.0
4-Lactone-(*S*)-Gal*p* (**8**)	H-3′	87	H-2′	7.4
	H-2′	93	H-4	10.4
			H-3′	7.5
	H-4	100	H-2′	13.3
			H-3	9.5
			H-5	8.3

[a] Data were obtained by ^{1}H NOE-difference experiments at 600 MHz. The intensities of the irradiated signals were set to −100% except for the signal of H-3′, which was set to −300%. The observed NOEs are reported as % of the irradiated signal.
[b] See Table 1.

drolysis varied, but the half-life was larger than 50 min in all examples under the conditions used (Table 4), and three interesting features were seen (i) the pseudo-mirror image lactone pairs showed almost the same sensitivity to hydrolysis, (ii) the lactones that were formed faster were also hydrolyzed faster and (iii) the hydrolysis of the 4-lactones was faster or equal to that of the pseudo-mirror image 2-lactones.

Hydrolysis of lactones was also performed at higher pH, but the reaction was then too fast to follow by ^{1}H NMR spectroscopy. It is important to study the products formed in the hydrolysis reactions at higher pH since there is a risk of isomerization of the α-carbon in the lactones if strong basic conditions are used. Possible isomerization was analyzed on separated 2- and 4-lactone-(*S*)-Gal*p* since the open form of (*R*)-Gal*p* would be easily detected by ^{1}H NMR spectroscopy. No isomerization could be detected.

Table 4
Estimated rates of lactone hydrolysis [a]

Compound	A	B	C
2-Lactone-(*R*)-Glc*p* [b] (**1**)	0.77	0.16	0.76
4-Lactone-(*R*)-Glc*p* (**2**)	0.07	0.02	0.14
2-Lactone-(*S*)-Glc*p* (**3**)	0.06	<0.01	0.16
4-Lactone-(*S*)-Glc*p* (**4**)	1.0	0.19	0.85
2-Lactone-(*R*)-Gal*p* (**5**)	0.44	0.09	0.41
4-Lactone-(*R*)-Gal*p* (**6**)	0.53	0.15	0.65
2-Lactone-(*S*)-Gal*p* (**7**)	0.17	0.02	0.30
4-Lactone-(*S*)-Gal*p* (**8**)	0.05	0.01	0.02
2-Lactone-(*R*)-Man*p* (**9**)	<0.01	<0.01	<0.01
4-Lactone-(*R*)-Man*p* (**10**)	0.16	0.03	0.36
2-Lactone-(*S*)-Man*p* (**11**)	0.24	0.09	0.23
4-Lactone-(*S*)-Man*p* (**12**)	0.78	0.11	0.67

[a] Entries A, B and C: initial rate of hydrolysis (%/min) in buffered D_2O solutions at pD 2.4 (A), 4.6 (B) and 7.4 (C), respectively. Reaction rates are estimated from adapted linear functions.
[b] See Table 1.

3. Experimental

All the 3-*O*-(1-carboxyethyl) substituted monosaccharide derivatives were available from a previous study [9].

General methods.—Column chromatography was performed on Matrex silica gel 60 Å (35–70 μm, Amicon) and TLC on pre-coated plates (E. Merck Silica Gel 60 F_{254}), detection was afforded with 5% H_2SO_4 in EtOH with heating. Solvents were used as purchased. The pD of the buffers was measured with a pH calibrated electrode and the value was corrected by adding 0.4 units to the obtained pH value [13]. NMR spectra were recorded at 400 MHz (^{1}H) and 100 MHz (^{13}C) with a Bruker DRX 400 instrument except for NOE-difference spectra, which were obtained at 600 MHz with a Bruker DRX 600 instrument. Chemical shifts are given in ppm using acetone (δ_H 2.225, δ_C 31.07) as an internal reference for samples measured in D_2O solutions. Assignments of signals were done from standard H,H-COSY and HSQC experiments. ^{1}H NMR chemical shifts of overlapping signals were obtained from the center of the cross peaks in the 2D spectra. High resolution mass spectrometry (HRMS) was recorded in FAB positive mode on a JEOL JMS-SX/SX-102A instrument using glycerol as a matrix. Synthesis, work up and purification of the lactones was performed essentially as described for compounds **1** and **2**. Differences in reaction temperature or solvents used in chromatography are indicated when appropriate.

*Methyl 3-*O*-[(*R*)-1-carboxyethyl]-α-*D*-glucopyranosid-1′,2-lactone* (**1**) *and methyl 3-*O*-[(*R*)-1-carboxyethyl]-α-*D*-glucopyranosid-1′,4-lactone* (**2**).—Acetic acid (45 mL) was added to lyophilized methyl 3-*O*-[(*R*)-1-carboxyethyl]-α-D-glucopyranoside (45 mg) in a 100 mL flask. The flask was sealed and the reaction stirred for 9 h at 60 °C when TLC (EtOAc) showed that the starting material had disappeared and two spots (R_f 0.35 and 0.25) were visible as the only products. The reaction mixture was transferred to a 500 mL round bottomed flask, cooled to −60 °C, and freeze-dried with the flask kept at 0 °C with crushed ice. Separation on a column of silica gel, eluted with EtOAc, gave **1** (30 mg, 72%); $[\alpha]_D$ +98° (*c* 1.4, acetone); HRMS: Calcd for $C_{10}H_{16}O_7$ + H: 249.0974. Found: 249.0979 $[M+H]^+$, and **2** (7 mg, 17%); $[\alpha]_D$ +218° (*c* 0.35, acetone); HRMS: Calcd for $C_{10}H_{16}O_7$ + H: 249.0974. Found: 249.0976 $[M+H]^+$.

*Methyl 3-*O*-[(*S*)-1-carboxyethyl]-α-*D*-glucopyranosid-1′,2-lactone* (**3**) *and methyl 3-*O*-[(*S*)-1-carboxyethyl]-α-*D*-glucopyranosid-1′,4-lactone* (**4**).—Acetic acid (50 mL) was added to lyophilized methyl 3-*O*-[(*S*)-1-carboxyethyl]-α-D-glucopyranoside (49 mg) in a 100 mL flask. The reaction was performed as described above. After 8 h TLC (EtOAc) showed only one spot (R_f 0.28). The solvent was removed and the products were separated from traces of starting material on a silica gel column (EtOAc) yielding a 1:12 mixture of **3** and **4** (30 mg, 66%), as determined from ^{1}H NMR spectroscopy; HRMS: Calcd for $C_{10}H_{16}O_7$ + H: 249.0974. Found: 249.0971 $[M+H]^+$.

*Methyl 3-*O*-[(*R*)-1-carboxyethyl]-α-*D*-galactopyranosid-1′,2-lactone* (**5**) *and methyl 3-*O*-[(*R*)-1-carboxyethyl]-α-*D*-galactopyranosid-1′,4-lactone* (**6**).—Acetic acid (25 mL) was added to lyophilized methyl 3-*O*-[(*R*)-1-carboxyethyl]-α-D-galactopyranoside (24 mg) in a 100 mL flask. The reaction was performed as described above. After 5 h TLC (5:1 EtOAc–MeCN) showed only one spot (R_f 0.42). The solvent was removed and the products were separated from traces of starting material on a silica gel column (5:1 EtOAc–MeCN). Compounds **5** and **6** were isolated as a 4:5 mixture as determined from ^{1}H NMR spectroscopy, (19 mg, 92%); HRMS: Calcd for $C_{10}H_{16}O_7$ + H: 249.0974. Found: 249.0981 $[M+H]^+$.

*Methyl 3-*O*-[(*S*)-1-carboxyethyl]-α-*D*-galactopyranosid-1′,2-lactone* (**7**) *and methyl 3-*O*-[(*S*)-1-carboxyethyl]-α-*D*-galactopyranosid-1′,4-lactone* (**8**).—Acetic acid (40 mL) was added to lyophilized methyl 3-*O*-[(*S*)-1-carboxyethyl]-α-D-galactopyranoside (40 mg) in a 100 mL round bottomed flask. The reaction was performed as described above for 17 h when TLC (5:1 EtOAc–MeCN) showed one product spot (R_f 0.42) and one spot of starting material at the baseline. The temperature was raised to 80 °C and the reaction proceeded for 6 h until TLC showed only one product spot (R_f 0.42). The solvent was removed and the products were purified on a silica gel column

(5:1 EtOAc–MeCN) to yield **7** (13 mg, 37%); $[\alpha]_D$ +54° (*c* 0.51, acetone); HRMS: Calcd for $C_{10}H_{16}O_7$ + H: 249.0974. Found: 249.0964 $[M+H]^+$. The rest of the material (11 mg, 32%) was isolated as a 1:0.9 mixture of **7** and **8** as determined from ^{1}H NMR spectroscopy.

*Methyl 3-O-[(*R*)-1-carboxyethyl]-α-*D*-manno-pyranosid-1′,2-lactone* (**9**) *and methyl 3-O-[(*R*)-1-carboxyethyl]-α-D-mannopyranosid-1′,4-lactone* (**10**).—Acetic acid (70 mL) was added to lyophilized methyl 3-*O*-[(*R*)-1-carboxyethyl]-α-D-mannopyranoside (73 mg) in a 100 mL round bottomed flask. The reaction was performed as described above at 80 °C for 6 h when TLC (5:1 EtOAc–MeCN) indicated complete reaction (one spot; R_f 0.43). The solvent was removed and the crude product was purified on a silica gel column (5:1 EtOAc–MeCN) to yield **10** (25 mg, 37%); $[\alpha]_D$ +203° (*c* 1.25, acetone); HRMS: Calcd for $C_{10}H_{16}O_7$ + H: 249.0974. Found: 249.0967 $[M+H]^+$; and a 1:2 mixture of **9** and **10** as determined from a ^{1}H NMR spectrum (15 mg, 22%).

*Methyl3-O-[(*S*)-1-carboxyethyl]-α-*D*-manno-pyranosid-1′,2-lactone* (**11**) *and methyl 3-O-[(*S*)-1-carboxyethyl]-α-D-mannopyranosid-1′, 4-lactone* (**12**).—Acetic acid (60 mL) was added to lyophilized methyl 3-*O*-[(*S*)-1-carboxyethyl]-α-D-mannopyranoside (64 mg) in a 100 mL flask. The reaction was performed as described above. After 5 h TLC (5:1 EtOAc–MeCN) indicated complete reaction (one spot R_f 0.45) and the solvent was removed. Purification on a silica gel column (5:1 EtOAc–MeCN) yielded a 1:3 mixture (as determined from a ^{1}H NMR spectrum) of **11** and **12** (49 mg, 77%). Analytical samples (< 2 mg) of separated **11** and **12** were isolated. HRMS: Calcd for compound **11** $C_{10}H_{16}O_7$ + H: 249.0974. Found: 249.0983 $[M+H]^+$; Calcd for compound **12** $C_{10}H_{16}O_7$ + H: 249.0974. Found: 249.0960 $[M+H]^+$.

Formation of lactones.—The formation of lactones at 60 °C was monitored with ^{1}H NMR spectroscopy (~5 mM samples) by performing the reactions in the NMR tube using acetic acid-d_4 as solvent. Spectra were recorded at appropriate time intervals, depending on the formation rate. The relative amounts of starting material and lactones were calculated by integration of separated signals, mainly the signals of the OCH_3 group. The sum of the signals for the lactones and opened form was set to 100% and the relative proportions were then calculated. In cases with overlapping OCH_3 signals other separated signals were used for integration.

Hydrolysis of lactones.—The rate of hydrolysis of lactones (~5 mM samples) was studied by ^{1}H NMR spectroscopy in D_2O solutions at 22 °C (0.1 M phosphate buffer pD 2.4, 0.1 M acetate buffer pD 4.6 and 0.1 M phosphate buffer pD 7.4, respectively). Spectra were recorded at appropriate time intervals, depending on the rate of hydrolysis. The amounts of lactones and product were calculated from integrated signals in the same way as described for the lactone formations above. The hydrolysis rates were estimated from plots of lactone concentration versus time by adapting linear functions to the obtained graphs.

Separated 2- and 4-lactone-(*S*)-Gal*p* were hydrolyzed by 1 M aqueous NaOH. The lactone was added to the basic solution at 80 °C and kept at that temperature for 1 h. The samples were then neutralized (Dowex H^+) and cation exchanged (Dowex Na^+) before analysis by ^{1}H NMR spectroscopy.

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