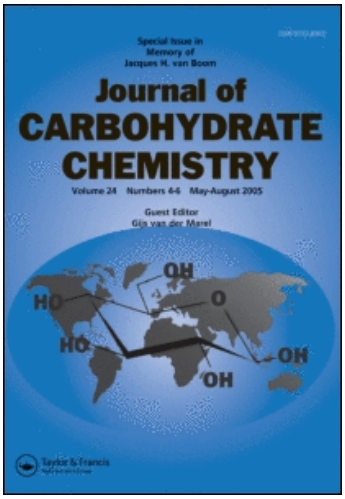


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# SYNTHESIS, CRYSTAL STRUCTURE, AND SOME REACTIONS OF 2,3,4-TRI-O-ACETYL-ß-D-GALACTOPYRANURONO-6,1-LACTONE[1]

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## ABSTRACT

**D**-*Gluco*-, **D**-*galacto*-, and **D**-*manno*-configurated 6,1-lactones of uronic acid were synthesized. A new synthetic approach based on the photobromination, hydrolysis, and oxidation of the corresponding 1,6-anhydro sugars is reported. Conformational studies of 2,3,4-tri-*O*-acetyl-ß-**D**-galactopyranurono-6,1-lactone in crystalline form and in solution were undertaken. First results of selective deacetylation catalyzed by wheat germ lipase are reported.

## INTRODUCTION

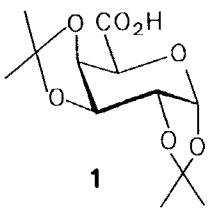
In order to understand the physiological role of dietary fibre in man[2] and the defense mechanisms of plants which can be induced by cell-wall material,[3] oligosaccharides, especially pectin fragments, should be available for biological studies. The isolation procedure of such fragments of defined structure from natural sources is often cumbersome and thus, several research groups are investigating effective approaches for the synthesis of oligosaccharides composed mainly of **D**-galacturonic acid.

Two fundamental routes of synthesis of **D**-galacturonic acid oligomers are known. The method of T. Ogawa et al.[4-7] is based on the formation of **D**-galactose oligomers with the desired *O*-glycosidic bonds while the hydroxymethyl group is

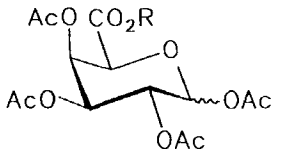
temporarily protected. In a separate operation oxidation of hydroxymethyl to carboxyl leads to the **D**-galacturonic acid oligomers.

The second principal route for oligomer preparation starts with intact **D**-galacturonic acid precursors suitable for use in glycosidation reactions. Accepting the challenge of the second route, we began a program aimed at the synthesis of **D**-galacturonic acid derivatives which can be used either as glycosyl donors or as glycosyl acceptors.

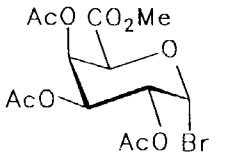
**SCHEME 1**



**1**



**2** : R= Me
**3** : R= Bn
**4** : R= p-$NO_2$-Bn



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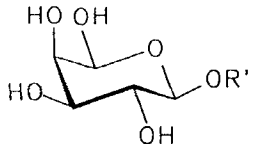
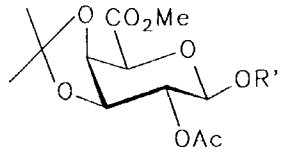


**6** : R'= All
**7** : R'= Bn



**8** : R'= All
**9** : R'= Bn

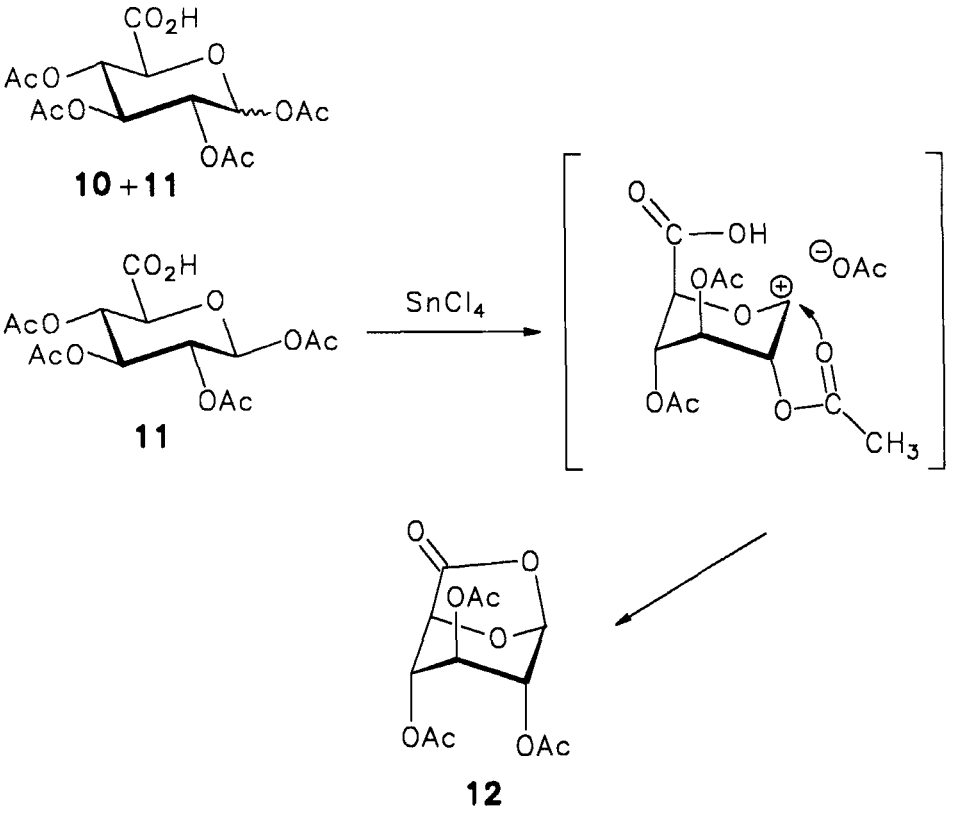
Several approaches based on **D**-galactose precursors have been previously reported. We reinvestigated the oxidation of 1,2;3,4-di-*O*-isopropylidene-α-**D**-galactopyranose to the corresponding **D**-galacturonic acid derivative **1**. However, only when conditions of acetolysis were used for the de-*O*-isopropylidenation step, alkyl (1,2,3,4-tetra-*O*-acetyl-α/ß-**D**-galactopyranose) uronates (**2**-**4**) could be obtained in an acceptable yield.[8] The latter compounds are simple starting-materials for glycosyl donors (e.g. **5**). By using allyl and benzyl **D**-galactopyranosides (**6** and **7**) as precursors we were able to prepare "standardized intermediates" (**8** and **9**) for glycoside synthesis with galacturonic acid derivatives.[9]

The present article describes the synthesis of acetylated **D**-galacturonic acid 6,1-lactone using the corresponding 1,6-anhydro-ß-**D**-galactopyranose as a precursor and the possibility of extending this reaction to *gluco*- and *manno*- configurated 1,6-anhydropyranoses.

## RESULTS AND DISCUSSION

Repeating units in pectin fragments are often α-(1 → 4) linked. For activation of the axial hydroxyl group in the 4-position the natural $^4C_1$ conformation of *galacto*-configurated glycosyl acceptors is converted into the $^1C_4$ conformation fixed as a 1,6-anhydro-structure leading to a more reactive equatorial hydroxyl group in the 4-position.[10] Therefore, we turned our attention to uronic acid 6,1-lactones, where previously only the acetylated **D**-glucopyranurono-6,1-lactone **12** was described. The approach by E. M. Fry was based on the conversion of tetra-*O*-acetyl-ß-**D**-glucuronic acid **11** in the presence of stannic chloride into the corresponding 6,1-lactone.[11]

**SCHEME 2**

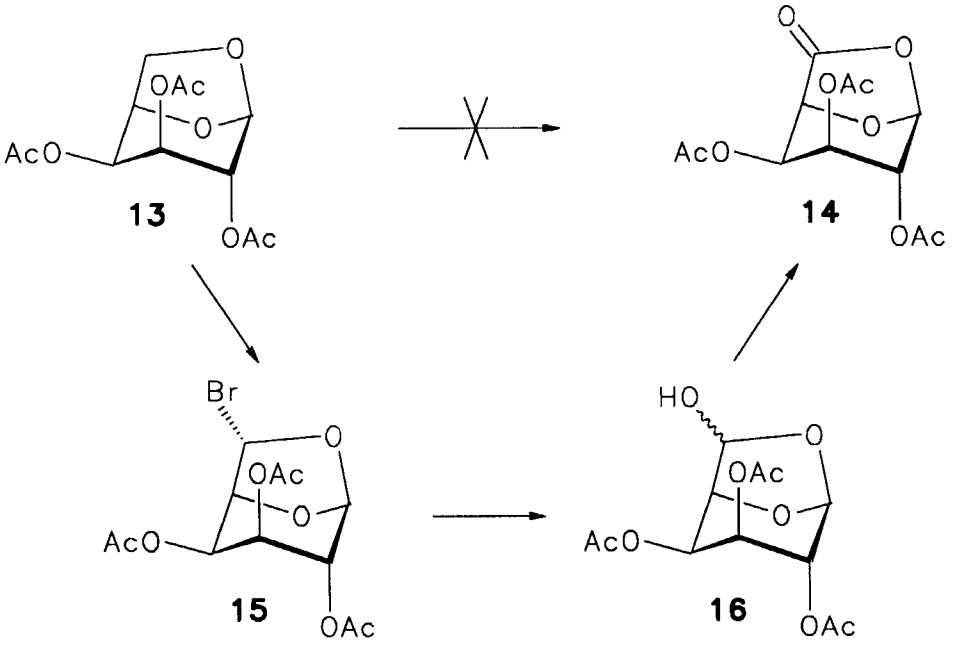



Our initial experiments focused on the ruthenium (VIII) oxide oxidation of the readily available 2,3,4-tri-*O*-acetyl-1,6-anhydro-ß-**D**-galactopyranose **13**[12] to the lactone **14**. This reagent has been applied successfully to oxidation of methylene

groups adjacent to ether oxygens in other fused ring systems.[13-15] Unfortunately, in our case no reaction occurred, although several modified reaction conditions were tested.

The key step in an alternative route to **14** was the introduction of bromine into the 6-position of compound **13** by the Ferrier-Ohrui reaction[16-19] which led to the derivative **15**. Consequently, the carbon-6-atom of compound **15** was anticipated to have comparable properties to a bromine-substituted carbon atom at the anomeric centre. As expected, in $^{13}C$ NMR spectra the carbon-6-signal at $\delta$ 64.3 ppm from **13** was shifted downfield at $\delta$ 82.6 ppm for compound **15**. These data are consistent with our experimental results wherein **15** was readily hydrolyzed to **16** on treatment with moist silver salts.

**SCHEME 3**

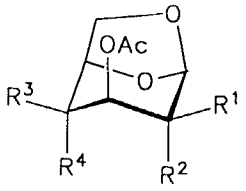


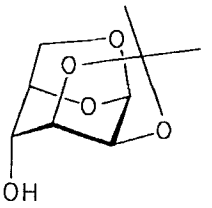

Subsequent oxidation of hemiacetal **16** with pyridinium dichromate (PDC) gave the desired 6,1-lactone **14** in 71% yield based on 1,6-anhydro derivative **13**. The same procedure was applied to the synthesis of glycuronic acid 6,1-lactones **12** and **22** derived from corresponding acetylated 1,6-anhydropyranoses of **D**-glucose (**17**) and **D**-mannose (**18**) in 59% and 52% yield, respectively. In order to obtain pure acetylated 1,6-anhydro-ß-**D**-mannopyranose **18** the crude product was deacetylated,

and then treated with acetone to afford 1,6-anhydro-2,3-*O*-isopropylidene-ß-**D**-mannopyranose **19**, purified by crystallization. De-*O*-isopropylidenation followed by acetylation led to the clean starting material for the preparation of **22**.[20]

This method of synthesis of 6,1-lactones compares favorably to the earlier reported synthesis of **12**[11] which has several troublesome steps, especially the separation by fractional crystallization of the ß-anomer of tetra-*O*-acetyl-glucuronic acid from the α-anomer which gave only moderate yields of the desired product.

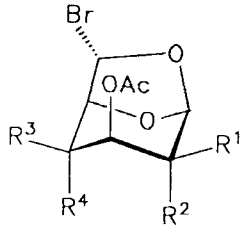
**SCHEME 4**

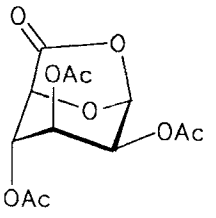


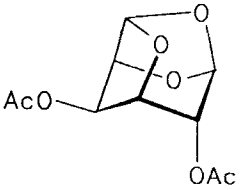




**17** : $R^1$, $R^3$= H; $R^2$, $R^4$= OAc
**18** : $R^2$, $R^3$= H; $R^1$, $R^4$= OAc

**19**




**20** : $R^1$, $R^3$= H; $R^2$, $R^4$= OAc
**21** : $R^2$, $R^3$= H; $R^1$, $R^4$= OAc

**22**

**23**

The IR spectra, the $^1$H NMR data and $^{13}$C NMR data for the 6,1-lactone **14** are in full accord with the proposed structure. The IR spectrum contained a new peak at 1835 cm$^{-1}$ assignable to C=O band and the C-O stretching vibrations of a γ-lactone indicating that this compound is a 6,1-lactone.

A comparison with the $^1$H NMR spectra of the 1,6-anhydro sugar **13** shows that for **14** both signals of H-$6_{exo}$ and H-$6_{endo}$ disappeared. Coupling between pyranosyl proton resonances were assigned by using homonuclear correlated spectroscopy (COSY, 500 MHz) and all sets of resonances were determined by tracing out the

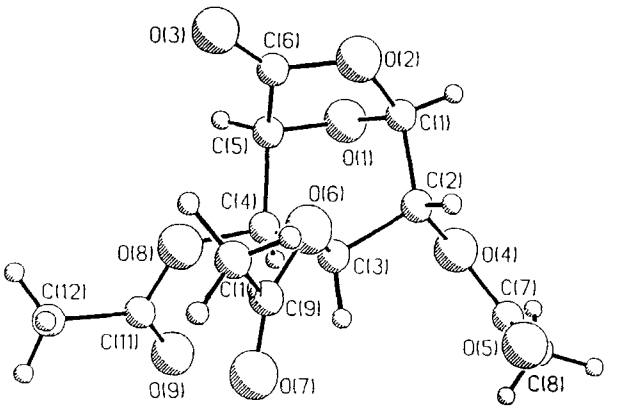



**Fig. 1**. Parallel projection of **14** oriented optimally.



coupling network. The anomeric proton is at the lowest magnetic field at δ 5.86 ppm exhibiting a strong correlation with the signal at δ 4.98 ppm (H-2) and a weak correlation with the signal at δ 5.43 ppm (H-3). The signal at δ 4.34 ppm (H-5) is linked with the signals at δ 5.36 ppm (strong; H-4) and at δ 5.43 ppm (weak; H-3). Further, a connection exists between signals at δ 5.43 ppm (H-3), at δ 4.98 ppm (H-2) and at δ 5.36 ppm (H-4).

X-ray studies were undertaken to establish the structure of **14** and to obtain information on the conformation of the pyranose ring. The crystals of **14** are trigonal, uncommon for a simple organic molecule. A good view (Fig. 1) is difficult to find, from every view point some atom lies in front of another. According to the X-ray studies, the $^1C_4$ conformation is present in the crystal state for **14**. From the literature is known that the 1,6-anhydro sugars have rigid skeletons in solution,[21,22] illustrated by nearly temperature independence of $^1$H-chemical shifts, coupling constants, and spectral line width of the spectra for **13** and **14**. In no case was a chair-boat transition ($^1C_4 \rightarrow B_{0,3}$) observed.

Nevertheless, conformational differences exist between crystal state and solution as suggested by poor agreement of calculated vicinal coupling constants for the pyranosyl protons of **14** ( particularly, $J_{2,3}$ and $J_{4,5}$) obtained by application of Karplus type equations and observed coupling constants from the $^1$H NMR spectra. Thus, a more distored $^1C_4$ chair conformation of the pyranose ring in solution is indicated. The values of $^3J_{H,H}$ were obtained from the following equation:

$$^3J = 5.95 - 1.35 \cos \phi + 5.45 \cos 2\phi - 0.45 \cos 3\phi.$$[21]

**TABLE 1**

Conformational Parameters for Hydrogen Atoms of Compound **14**

| | X-ray data | | | $^1$H NMR data | |
|---|---|---|---|---|---|
| | detected dihedral angle | calculated vic.-coupling constants | | detected vic.-coupling constants | calculated dihedral angle |
| | | Karplus | Altona | | Karplus |
| H-1/H-2 | -66.4° | 1.3 Hz | 1.1 Hz | 1.4 Hz | -65° |
| H-2/H-3 | 85.8° | 0.4 Hz | 0.4 Hz | 1.6 Hz | 117° |
| H-3/H-4 | 40.4° | 4.3 Hz | 5.0 Hz | 4.6 Hz | 43° |
| H-4/H-5 | -71.0° | 1.2 Hz | 0.3 Hz | 4.3 Hz | -45° |



This equation was corrected for the electronegativities, according to the relationship $^3J_{cor} = {}^3J \times (1 - 0.1 \Sigma \Delta E_i)$ utilizing Pauling's values for electronegativities $E_H = 2.1$, $E_C = 2.5$ and $E_O = 3.5$. A second set of calculated $^3J_{H,H}$ values was obtained by using the equation proposed by Altona.[23] Calculated vicinal-coupling constants $^3J_{H,H}$ (Hz) from values for dihedral angle $\phi_{H,H}$ from crystal-structure data for **14** were compared with the observed vicinal coupling constants from $^1$H NMR spectra. Additionally, the appropriate dihedral angle $\phi_{H,H}$ was determined from the $^1$H NMR data by application of the Karplus type equations,[24] $^3J_{H,H} = 9.26 \cos^2 \phi - 28$ (Table 1).

It seems noteworthy that the values $J_{4,5}$ in **D**-*galacto*, **D**-*gulo*, **D**-*talo* and **D**-*ido* configurated 1,6-anhydro sugars show nearly equal magnitudes (4.2 ± 0.3 Hz) attributed to the effects of oxygen substituent in equatorial orientation, revealing a chair deformation to a $E_0$ envelope form.[21] The same trend is apparent in the determined $J_{2,3} = 1.6$ Hz and the corresponding calculated dihedral angle $\phi_{2,3} \approx 117°$. In contrast, a decrease in the magnitude of $\phi_{3,4}$ expected from models, was not observed.

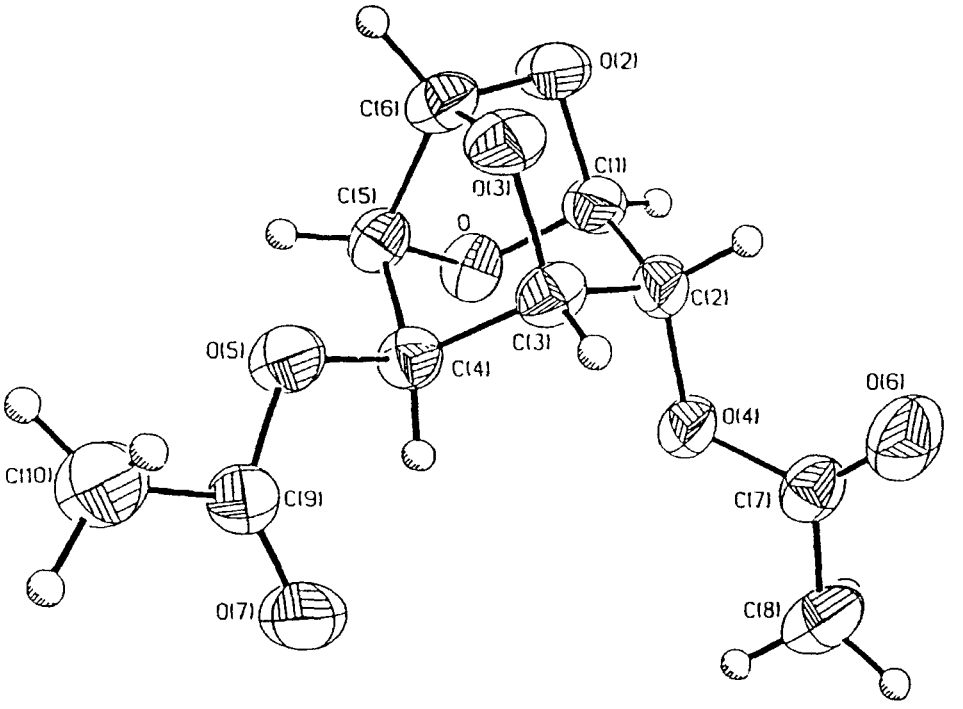



**FIG. 2.** An ORTEP diagram of **23**.

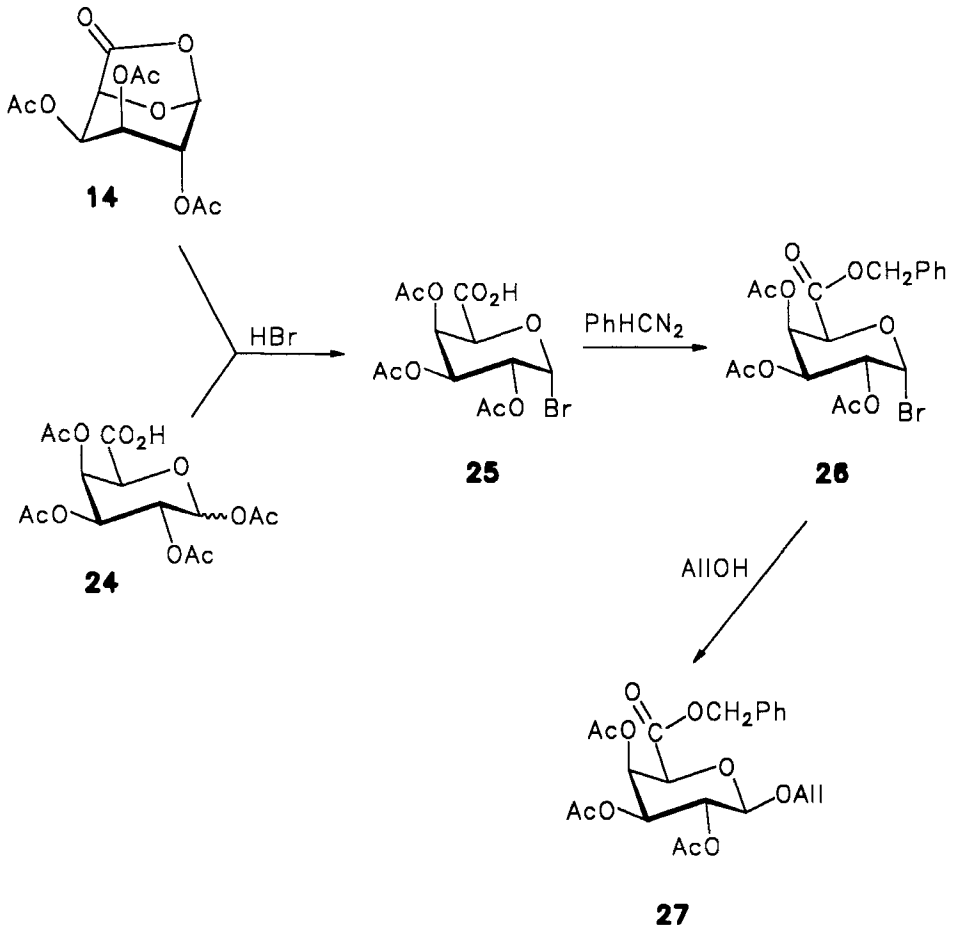


Consideration of the results of a computer modeling of **14** has demonstrated that the Karplus equation is to be used only with restriction in such conformationally locked ring-systems. Nevertheless, we can draw the conclusion that the pyranose ring of **14** is more flattened from the $^1C_4$ chair to the envelope form in solution than in crystal, following the common trend in 1,6-anhydro sugar series.[25] Hence, a $B_{0,3}$ boat form should be ruled out.

We also began a separate investigation to elongate the carbon skeleton of 1,6-anhydropyranoses by nucleophilic displacement of bromine in compound **15**. However, treatment of **15** with mercury(II)cyanide in nitromethane gave the crystalline tricyclic acetal **23**.

The $^1H$ NMR spectra of compound **23** showed only two acetyl methyl proton signals, while the $^{13}C$ NMR spectra showed two carbonyl carbon and two acetyl methyl carbon resonances. Additionally, a strong down-field shift of C-6 to $\delta$ 100.4 ppm compared to compound **13** suggested that C-1 and C-6 in **23** have more or less the same magnetic environments.

**SCHEME 5**




Finally, the structure of **23** has been verified by X-ray diffraction analysis with direct methods by use of a modified version of the NICOLET P3 program. A steric view of the molecular structure of **23** is shown in Fig. 2. The pyranose ring has the expected chair conformation $^1C_4$(D).

The reported new method for the preparation of **D**-*gluco*-, **D**-*galacto*-,and **D**-*manno* configurated 6,1-lactones of uronic acid based on the photobromination, hydrolysis, and oxidation of the corresponding 1,6-anhydro sugars has significantly improved the access to this class of substances. Unfortunately, our attempts to use **D**-galacturonic acid 6,1-lactone as a fertile synthetic intermediate in nucleophilic reactions failed completely.

Treatment of **14** with alcohols, amines or hydrazine resulted in either a complex mixture of products or with no reaction occurring. Only the conversion into the known (tri-*O*-acetyl-α-**D**-galactopyranosyl bromide) uronic acid **25** by the action of hydrogen bromide in acetic acid succeeded. The reaction proceeded in full analogy to that described by Fry,[11] however, an alternative approach to **25** illustrated in Scheme 5 has been shown[26] to be much shorter.

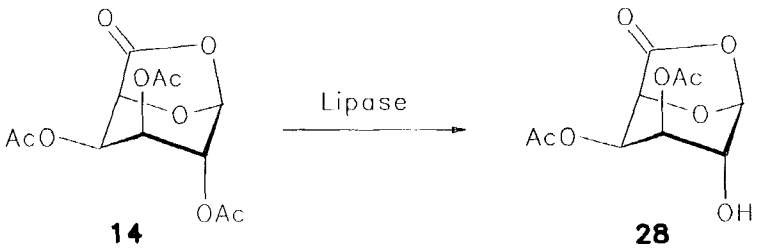
It may be noted that compound **25** offers the possibility for introduction of an acid-labile ester group into pyranosyl bromides of uronic acid (e.g. **26**). As expected, the reaction of **26** with allyl alcohol in the presence of $Hg(CN)_2$ / $HgBr_2$ afforded successfully the corresponding acetylated benzyl (allyl ß-**D**-galactopyranosid) uronate **27**.

In attempting to prepare a galacturonic acid glycosyl acceptor from **14** *O*-deacetylation of **14** was unavoidable. All attemps with chemical methods to achieve either a partial regioselective deacetylation or a complete deacetylation were unsuccessful and lead to an unidentified mixture of products. We propose a rapid lactone ring opening reaction under these conditions because in the IR spectra of the products the C=O band at 1835 $cm^{-1}$ disappeared.

Only enzyme-catalyzed hydrolysis[27-29] resulted in partially esterified derivatives. When compound **14** dissolved in *N,N*-dimethylformamide was treated with wheat germ lipase purchased from SIGMA Chemical Co. in $K_2HPO_4$ buffer (pH 7) formation of a new product was observed after 4 hours. The *O*-2 acetyl group of **14** was removed to give 3,4-di-*O*-acetyl-ß-**D**-galactopyranurono-6,1-lactone **28** in 19% yield.

Inspection of the $^1H$ NMR spectrum supported this conclusion, as only two methyl acetyl proton signals were observed. The assignment of the proton coupling network in **28** was accomplished from a two-dimensional COSY spectrum, which provided the key information that the chemical shift of H-2 of **28** was at significantly higher field compared with the signal of the starting material **14**.

**SCHEME 6**




**14** **28**

Thus, the general applicability of partial deprotected galacturono-6,1-lactones in glycosidation reactions has to be tested, and then we hope to find enzymes for selective *O*-deacetylation at C-4.

## EXPERIMENTAL

**General Procedures**. See ref. 9. NMR spectra were recorded with a Bruker spectrometer model WH-250 at 250 MHz for $^{1}H$, and 62.89 MHz for $^{13}C$, Bruker WH 270 at 270 MHz for $^{1}H$, and 67.89 MHz for $^{13}C$, Bruker WM-400 (400 MHz), and Bruker WM-500 (500 MHz) for $^{1}H$. X-ray diffraction measurements were performed on a Nicolet R3m/E diffractometer system, the diffraction data were collected with intensities from $\Theta$:2$\Theta$ scans by use of a profile-fitting procedure. A graphite monochromatic MoK$\alpha$ radiation (wavelength = 0.71073 Å) was used. A modified version of the Nicolet P3 data collection progam was applied and the SHELXTL 5.1 system was used for data reduction, structure solution, refinement, and preparation of graphics and tables [Sheldrick, G. M. (1985). SHELXTL. Release 5.1. Nicolet Analytical Instruments, Madison, Wisconsin, U.S.A.].

The crystals of compound **14** are trigonal, space group $P3_2$, with 3 molecules in a hexagonal cell of dimensions a= 8.4902 (0.033) and c= 17.1980 (0.100) Å. The crystals of compound **23** are monoclinic, space group $P2_1$, Z= 2, a= 10.065(2), b= 5.488(1), c= 10.214(2) Å, ß= 105.21(2)°, V= 544.4(2) Å$^3$. Additional diffractometer parameters are as follows: linear absorption coefficient, $\mu$= 1.20 cm$^{-3}$; F(000), 256; temperature of measurement, room temperature; final R-factors, R = 0.039, wR = 0.047; number of unique observed reflections, 1356; lattice parameters from 25 reflections in the range 25<2$\Theta$< 30°, no absorption correction; maximum (sin $\Theta/\lambda$ in intensity measurements, 0.65 Å$^{-1}$); range of h, k, l were 0 - 14, 0 - 8, -14 - 14; standard reflections, 3 standards after every 97 reflections; number of reflections measured, 1518 including standards; number of unique observed reflections, 1356 with I > 1.0 $\sigma$(I); value of $R_{int}$= $\Sigma$ | F -<F> | / $\Sigma$ F, from merging equivalent reflections, 0.013. All H atoms were found on the difference map (with some uncertainty in the methyl groups). H atoms inserted in theoretical positions and refined in the model, except methyl groups which were treated as rigid groups. The isotropic thermal parameter was taken as about 20% more than for the C atom to which the H atom was bonded. Parameters refined, 160; goodness of fit, 2.13; structure-factor weights, w= 1/[$\sigma^2$(F) + 0.00020 F$^2$] with $\sigma^2$(F) from counting statistics; maximum ratio of least-squares shift to e.s.d in final cycle, 0.16; no extinction correction; $f_0$, f', f" from International Tables for X-Ray Crystallography (1974) Vol. IV. Detailed X-ray data will be supplied on request to authors.

The following solvent systems (v/v) were used for chromatography: ($A_1$) 3:2, ($A_2$) 2:1, ($A_3$) 2:3 and ($A_4$) 4:1 PhMe-EtOAc.

**Photobromination of 1,6-Anhydro-2,3,4-tri-O-acetyl-D-hexopyranose.** A mixture of 1,6-anhydro-2,3,4-tri-*O*-acetyl-ß-**D**-hexopyranose (**13**, **17** or **18**, 2.02 g, 7 mmol) and bromine (0.8 mL, 16 mmol) in dry $CCl_4$ (100 mL) was refluxed over a 250 W heat lamp for 3-4 h (TLC, solvent $A_1$). The cooled solution was successively washed with 10% aq. $Na_2S_2O_3$ (2 x 50 mL), sat. aq. $NaHCO_3$ (2 x 50 mL), water (2 x 50 mL), and dried over $MgSO_4$. Evaporation of the solvent in vacuo below 30 °C gave a syrup which was dissolved twice in dry ether (50 mL) and reconcentrated to give the corresponding 6-bromo compound.

**(6S)-1,6-Anhydro-2,3,4-tri-O-acetyl-6-bromo-ß-D-galactopyranose (15).** (2.18 g, 85%), syrup, $[\alpha]_D^{21}$ -61.7° (*c* 0.98, chloroform); $^{13}C$ NMR ($CDCl_3$) δ 20.4, 20.6 (3 C, $\underline{C}H_3CO$), 64.4, 66.9, 69.3, 79.9 (C-2, C-3, C-4, C-5), 82.6 (C-6), 101.7 (C-1),168.8, 169.2, 169.5 (3C, $CH_3\underline{C}O$).

Anal. Calcd for $C_{12}$ $H_{15}$ $O_8$ Br: C, 39.26; H, 4.12. Found: C, 39.5 ; H, 4.2 .

**(6S)-1,6-Anhydro-2,3,4-tri-O-acetyl-6-bromo-ß-D-glucopyranose (20).** (2.49 g, 97%), syrup, $[\alpha]_D^{21}$ -69.1° (*c* 0.85, chloroform).

Anal. Calcd for $C_{12}$ $H_{15}$ $O_8$ Br: C, 39.26; H, 4.12. Found: C, 39.6 ; H, 4.3 .

**(6S)-1,6-Anhydro-2,3,4-tri-O-acetyl-6-bromo-ß-D-mannopyranose (21).** (2.42 g, 94%), mp 86 °C (from light petroleum); $[\alpha]_D^{21}$ -80.8° (*c* 1.0 , chloroform).

Anal. Calcd for $C_{12}$ $H_{15}$ $O_8$ Br: C, 39.26; H, 4.12. Found: C, 39.3 ; H, 4.1 .

**Tri-O-acetyl-ß-D-glycopyranurono-6,1-lactones.** To a suspension of 1,6-anhydro-2,3,4-tri-*O*-acetyl-6-bromo-ß-**D**-hexopyranose (**15**, **20** or **21**, 1.84 g, 5 mmol) and freshly prepared $Ag_2CO_3$ (1.25 g, 4.5 mmol) in dry acetone (10 mL) was added water (0.1 mL, 5.5 mmol). After vigorous stirring for 2 h at ambient temperature in the dark (TLC, solvent $A_1$), the mixture was filtered through Celite and concentrated. The residue was successively co-evaporated with toluene (2 x 50 mL) and ethanol (2 x 50 mL) and then dried in high vacuum for 3 h. A freshly prepared solution of pyridinium dichromate (1.13 g, 3 mmol) and acetic anhydride (1.4 mL) in dry dichloromethane (10 mL) was added to a solution of the obtained syrup, which was sufficiently pure for the next step, in dry dichloromethane (10 mL). After the reaction mixture was stirred for 3 h at ambient temperature (TLC, solvent $A_1$) it was passed through a layer of silica gel (2 x 3 cm) with ethyl acetate, and the eluent gave a syrupy residue, which was co-evaporated several times with toluene and then crystallized from ethanol.

**2,3,4-Tri-O-acetyl-ß-D-glucopyranurono-6,1-lactone (12).** (0.91 g, 60.1%), mp 122-123 °C, $[\alpha]_D^{20}$ -73.6° (*c* 1.0, chloroform); lit.[11] mp 123-124 °C, $[\alpha]_D^{19}$

-78.6° (*c* 0.9, chloroform); lit.[25] mp 127 °C, $[\alpha]_D^{20}$ -66.0° (*c* 2, chloroform); IR max 1825 (C=O str. 6,1-lactone) 1755, 1770 $cm^{-1}$ (C=O str. OAc); $^1H$ NMR ($CDCl_3$) δ 2.11, 2.18 (2 s, 9 H, $C\underline{H}_3CO$), 4.62 (t, 1 H, H-5), 4.79 (dd, 1 H, $J_{2,3}$= 2.4 Hz, $J_{2,4}$= 1.4 Hz, H-2), 4.83 (dd, 1 H, $J_{4,5}$= 1.6 Hz, H-4), 4.97 (m, 1 H, $J_{3,4}$= 2.6 Hz, H-3), 5.94 (t, 1 H, $J_{1,2}$ = $J_{1,3}$= 1.2 Hz, H-1); $^{13}C$ NMR ($CDCl_3$) δ 20.7 (3 C, $\underline{C}H_3CO$), 65.9 (C-2), 66.1 (C-4), 69.0 (C-3), 71.2 (C-5), 100.5 (C-1), 167.9 (C-6), 168.7, 169.4 (3 C, $CH_3\underline{C}O$).

Anal. Calcd for $C_{12}$ $H_{14}$ $O_9$: C, 47.69; H, 4.67. Found: C, 47.8 ; H, 4.8.

**2,3,4-Tri-O-acetyl-ß-D-galactopyranurono-6,1-lactone (14).** (1.27 g, 83.5%), mp 99-100 °C, $[\alpha]_D^{20}$ -11.5° (*c* 1.0, chloroform); IR max 1835 (C=O str. 6,1-lactone) 1750, 1770 $cm^{-1}$ (C=O str. OAc); $^1H$ NMR ($CDCl_3$) δ 2.08, 2.12, 2.18 (3 s, 9 H, $C\underline{H}_3CO$), 4.34 (dd, 1 H, H-5), 4.97 (t, 1 H, $J_{2,3}$= 1.6 Hz, $J_{2,4}$= 1.6 Hz, H-2), 5.35 (t, 1 H, $J_{4,5}$= 1.6 Hz, H-4), 5.42 (m, 1 H, $J_{3,4}$= 4.6 Hz, $J_{3,5}$= 1.6 Hz, H-3), 5.86 (t, 1 H, $J_{1,2}$= $J_{1,3}$= 1,5 Hz, H-1); $^{13}C$ NMR ($CDCl_3$) δ 20.5, 20.7 (3 C, $\underline{C}H_3CO$), 64.7 (C-2), 67.5 (C-3), 68.5 (C-4), 68.9 (C-5), 100.2 (C-1), 169.0 (C-6), 169.3 (3 C, $CH_3\underline{C}O$).

Anal. Calcd for $C_{12}$ $H_{14}$ $O_9$: C, 47.69; H, 4.67. Found: C, 47.7 ; H, 4.9 .

**2,3,4-Tri-O-acetyl-ß-D-mannopyranurono-6,1-lactone (22).** (0.84 g, 55.3%), syrup, $[\alpha]_D^{20}$ +7.9° (*c* 0.8, chloroform); IR max 1835 (C=O str. 6,1-lactone) 1720, 1730 $cm^{-1}$ (C=O str. OAc); $^1H$ NMR ($CDCl_3$) δ 2.10, 2.12, 2.19 (3 s, 9 H, $C\underline{H}_3CO$), 4.59 (t, 1 H, H-5), 4.94 (t, 1 H, $J_{4,5}$= 1.6 Hz, H-4), 5.26 (dd, 1 H, $J_{2,3}$= 5.2 Hz, H-2), 5.47 (m, 1 H, $J_{3,4}$= $J_{3,5}$= 1.6 Hz, H-3), 5.87 (t, 1 H, $J_{1,2}$= $J_{1,3}$= 1.6 Hz, H-1); $^{13}C$ NMR ($CDCl_3$) δ 20.4, 20.6, 20.7 (3 C, $\underline{C}H_3CO$), 64.5 (C-2), 67.3 (C-3), 67.6 (C-4), 71.3 (C-5), 101.6 (C-1), 168.1 (C-6), 169.1, 169.2, 169.4 (3 C, $CH_3\underline{C}O$).

Anal. Calcd for $C_{12}$ $H_{14}$ $O_9$: C, 47.69; H, 4.67. Found: C, 47.8 ; H, 5.0 .

**2,4-Di-O-acetyl-1,6;3,6-dianhydro-D-galacto-hexo-di-aldopyranose (23).** To a stirred solution of 2,3,4-tri-*O*-acetyl-1,6-anhydro-6-bromo-ß-D-galactopyranose (2.50 g, 8.7 mmol) in dry nitromethane (30 mL) was added dry $Hg(CN)_2$ (1.75 g, 8.7 mmol). A not quite clear solution resulted, and the reaction was complete in 2 h at room temperature (TLC, solvent $A_1$). The suspension was fitered through a bed of Celite, and the filtrate was concentrated to a small volume, diluted with dichloromethane (50 mL), washed with aq. 10 % KI (3 x 20 mL) then water (3 x 20 mL), dried over $MgSO_4$, and concentrated. Crystallization of the residue from ethanol gave the product (1.10 g, 65%), mp 93 °C, $[\alpha]_D^{21}$ -27.9° (*c* 1.0,chloroform); $^1H$ NMR ($CDCl_3$) δ 2.08, 2.12 (2 s, 6 H, $C\underline{H}_3CO$), 4.60 (m, 1 H, $J_{3,4}$= 0.2

Hz, $J_{3,5}$= 0.7 Hz, H-3), 4.70 (ddd, 1 H, $J_{5,6}$= 2.5 Hz, H-5), 4.93 (dd, 1 H, $J_{2,3}$= 4.3 Hz, H-2), 5.34 (d, 1 H, $J_{4,5}$= 0.2 Hz, H-4), 5.50 (t, 1 H, $J_{1,2}$= 1.4 Hz, H-1), 5.90 (d, 1 H, H-6); $^{13}$C NMR ($CDCl_3$) δ 20.7, 20.8 (2C, $\underline{C}H_3CO$), 67.2 (C-2), 73.6 (C-4), 79.2 (C-3), 79.5 (C-5), 100.3 (C-1), 100.4 (C-6), 169.6, 169.9 (2 C, $CH_3\underline{C}O$).

Anal. Calcd for $C_{10}H_{12}O_7$: C, 49.18; H, 4.95. Found: C, 49.3 ; H, 5.2 .

**Benzyl (2,3,4-Tri-O-acetyl-α-D-galactopyranosyl bromide) uronate (26).** Lactone **14** (1.06 g, 3.5 mmol) was dissolved in 55% hydrogen bromide in glacial acid (25 mL) at O °C. After 50 h at room temperature (TLC, solvent $A_1$), chloroform (50 mL) was added, the organic phase was washed with water (3 x 20 mL), dried over $MgSO_4$, and concentrated. This material was used without further purification in subsequent experiments. A stirred solution of the residue in dry dichloromethane (20 mL) was treated dropwise with a solution of phenyl diazomethane[30] (708 mg, 6.0 mmol) in dichloromethane (8 mL). Stirring was continued for 1 h (TLC, solvent $A_1$) at ambient temperature. The excess of diazo compound was discharged by the addition of glacial acid, and the mixture was concentrated in vacuo to yield an oil. Chromatography of the residue on silica gel (solvent $A_4$) gave, after concentration of the appropriate fractions and drying of the residue under high vacuum, the title compound (0.70 g, 42% from **14**) as a foam, $[\alpha]_D^{22}$ +179.2° (*c* 0.55, chloroform); $^1$H NMR ($CDCl_3$) δ 1.82, 2.00, 2.10 (3 s, 9 H, $C\underline{H}_3CO$), 4.88 (d, 1 H, H-5), 5.06 (dd, 1 H, $J_{2,3}$= 10.6 Hz, H-2), 5.18 (dd, 2 H, J= 11.8 Hz, $C\underline{H}_2Ph$), 5.44 (dd, 1 H, $J_{3,4}$= 3.3 Hz, H-3), 5.82 (dd, 1H, $J_{4,5}$= 1.6 Hz, H-4), 6.76 (d, 1 H, $J_{1,2}$= 3.8 Hz, H-1), 7.37 (m, 5 H, $CH_2\underline{Ph}$); $^{13}$C NMR ($CDCl_3$) δ 20.1, 20.5, 20.7 (3 C, $\underline{C}H_3CO$), 67.2, 67.6, 67.9, 72.4 (C-2, C-3, C-4, C-5), 67.9 ($\underline{C}H_2Ph$), 87.2 (C-1), 128.2, 128.7, 128.8, 129.0, 129.2 ($CH_2\underline{Ph}$), 165.1 (C-6), 169.4, 169.7, 169.9 (3 C, $CH_3\underline{C}O$).

**Benzyl (Allyl 2,3,4-tri-O-acetyl-ß-D-galactopyranosid) uronate (27).** A suspension of benzyl (2,3,4-tri-*O*-acetyl-α-**D**-galactopyranosyl bromide) uronate (**26**) (0.47 g, 1 mmol), Drierite (1 g), $Hg(CN)_2$ (130 mg, 0.5 mmol), and $HgBr_2$ ( 18 mg, 0.05 mmol) in dry allyl alcohol (5 mL) was stirred for 24 h at ambient temperature (TLC, solvent $A_4$). The mixture was concentrated in vacuo, diluted with chloroform (20 mL), and filtered. The filtrate was washed with aq. 10% KBr (3 x 5 mL) and water (2 x 5 mL), dried ($MgSO_4$), and concentrated. The crude material was purified by column chromatography (EtOAc gradient 9% - 17% in toluene) to yield **27** (0.33 g, 74%), $[\alpha]_D^{28}$ -9.6° (*c* 1.7, chloroform); $^1$H NMR ($CDCl_3$) δ 1.85, 1.95, 2.05 (3 s, 9 H, $C\underline{H}_3CO$), 4.09, 4.39 (2 m, 2 H, $OCH_2CH{=}CH_2$), 4.31 (d, 1 H, H-5), 4.53 (d, 1H, $J_{1,2}$= 7.8 Hz, H-1), 5.16 (dd, 2 H, J= 11.8 Hz, $C\underline{H}_2Ph$) 5.18, 5.29, 5.84 (3

m, 3 H, $OCH_2CH=CH_2$), 5.24 (dd, 1 H, $J_{2,3}$= 10.4 Hz, H-2), 5.05 (dd, 1 H, $J_{3,4}$= 3.2 Hz, H-3), 5,69 (dd, 1 H, $J_{4,5}$= 1.3 Hz, H-4), 7.34 (m, $CH_2Ph$); $^{13}C$ NMR ($CDCl_3$) δ 20.4, 20.6, 20.8 (3 C, $CH_3CO$), 67.6 ($CH_2Ph$), 68.3 (C-4), 68.5 (C-2), 69.7 ($CH_2$-$CH=CH_2$), 70.6 (C-3), 72.3 (C-5), 99.8 (C-1), 117.4, 133.7 ($CH_2CH=CH_2$), 128.7, 128.8, 129.2, 134.9 (CH2Ph), 166.5 (C-6), 169.3, 169.9, 170.0 (3 C, $CH_3CO$).

**3,4-Di-O-acetyl-ß-D-galactopyranurono-6,1-lactone (28).** 2,3,4-Tri-*O*-acetyl-ß-**D**-galactopyranurono-6,1-lactone (**14**) (0.61 g, 2 mmol) in *N*,*N*-dimethylformamide (5 mL) and wheat germ lipase (0.10 g, No. L-3001 Sigma Chemical Co.) were added to $K_2HPO_4$ buffer (50 mL, pH 7), then the mixture was stirred at 25 °C. The reaction was monitored on TLC (solvent $A_2$). After 4 h, the reaction mixture was extracted with ethyl acetate, dried, and concentrated to give a syrupy residue, which was subjected to column chromatography on silica gel (solvent $A_3$) to afford **28** (0.10 g, 19%) as a syrup, $[\alpha]_D^{20}$ -5.2° (*c* 1.0, chloroform); $^1H$ NMR ($CDCl_3$) δ 2.08, 2.12 (2 s, 6 H, $CH_3CO$), 4.03 (t, 1 H, H-2), 4.35 (t, 1 H, H-5), 5.42 (d, 2 H, $J_{4,5}$= 2.8 Hz, H-3, H-4), 5.83 (d, 1 H, $J_{1,2}$= 0.7 Hz, H-1); $^{13}C$ NMR ($CDCl_3$) δ 20.5 (2 C, $CH_3CO$), 64.8, 68.1, 69.1, 69.9 (C-2, C-3, C-4, C-5), 102.4 (C-1), 168.9 (C-6), 169.3, 169.4 (2 C, $CH_3CO$).

Anal. Calcd for $C_{10}H_{12}O_8$: C, 46.16; H, 4.65. Found: C, 46.2 ; H, 4.9.



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