Carbohydrate Research, 190 (1989) 65–76
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

SYNTHESIS OF GALACTOFURANOSE DISACCHARIDES OF BIOLOGICAL SIGNIFICANCE

CARLA MARINO, OSCAR VARELA, AND ROSA M. DE LEDERKREMER
Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón II, 1428 Buenos Aires (Argentina)

(Received August 11th, 1988; accepted for publication December 18th, 1988)

ABSTRACT

Methyl β-D-galactofuranoside (**3**) was readily obtained by tin(IV) chloride-catalyzed glycosylation of penta-*O*-benzoyl-α,β-D-galactofuranose (**1**), followed by debenzoylation with sodium methoxide. Glycosylation of **1** with 2,3,5-tri-*O*-benzoyl-D-galactono-1,4-lactone (**4**) or with the 6-*O*-trityl-lactone derivative **5** gave the benzoylated β-D-galactofuranosyl-(1→6)-D-galactono-1,4-lactone **6** in excellent yield. The structure of disaccharide **6** was confirmed by borohydride reduction to the glycosyl-alditol **7**. A byproduct of the condensation reaction of **1** with **4** or **5** was identified as the benzoylated (1→1)-β,β′-D-galactofuranosyl disaccharide **8**. Compound **8** was readily prepared (88% yield) by controlled addition of water to **1**, in the presence of stannic chloride. *O*-Debenzoylation of **8** afforded crystalline β′-D-galactofuranosyl-(1→1)-β-D-galactofuranoside (**9**). The glycosyl-lactone **6** constitutes a key intermediate for the synthesis of a disaccharide derivative having both units in the furanoid form. Thus, diisoamylborane reduction of the lactone function of **6** led to the disaccharide derivative **10**, from which the methyl glycoside **12** was prepared. *O*-Debenzoylation of **12** gave the corresponding methyl β-D-galactofuranosyl-(1→6)-β-D-galactofuranoside (**13**). The free disaccharide β-D-Gal*f*-(1→6)-D-Gal*p* (**14**) and its acetylated derivative (**15**) were also synthesized from **10**.

INTRODUCTION

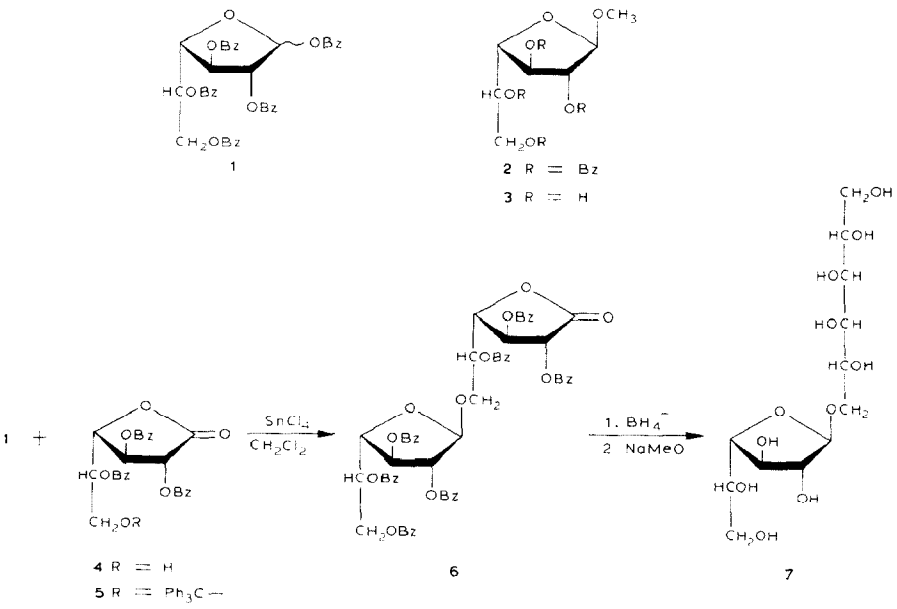
We have recently described[1] a novel use of partially protected aldono-1,4-lactones as glycosylating agents of monosaccharides. The resulting glycosyl-lactone can be converted into a substituted disaccharide having the reducing end in a furanose form. With the aim of extending the scope of this method, we have now synthesized disaccharides composed of two galactofuranose units. In our laboratory, we have shown that D-galactofuranose is a constituent of the glycoconjugates obtained from the parasite *Trypanosoma cruzi*[2], and from the fungus *Ascobolus furfuraceous*[3]. The partial hydrolysis of immunologically specific polysaccharides from *Mycoplasma mycoides*[4] or from *Mycobacterium tuberculosis*[5] gave

0008-6215/89/$ 03.50

β-D-Gal*f*-(1→6)-D-Gal*p*, indicating that at least the terminal non-reducing galactose was in the furanose form. McNeil *et al.* have recently demonstrated that the highly immunogenic arabinogalactans of *Mycobacterium leprae* and *Mycobacterium tuberculosis* contain, exclusively, arabinofuranosyl and galactofuranosyl residues[6]. It was also established that some of the galactofuranose units were (1→6)-linked. In this paper we report the synthesis of β-D-Gal*f*-(1→6)-D-Gal*f* derivatives.

RESULTS AND DISCUSSION

Benzoylation of D-galactose at high temperature[7] gave a crystalline anomeric mixture of 1,2,3,5,6-penta-*O*-benzoyl-α,β-D-galactofuranoses (**1**) whose ^{1}H-n.m.r. spectrum indicated an approximately 1:1 ratio for the α:β anomers. It has been shown that peracylated derivatives of sugars may be converted[1,8] into the corresponding 1,2-*trans* glycosides on treatment with tin(IV) chloride followed by reaction with an alcohol. Therefore, the anomeric mixture **1** was allowed to react with methanol in the presence of stannic chloride, to afford the crystalline methyl 2,3,5,6-tetra-*O*-benzoyl-β-D-galactofuranoside (**2**) in 85% yield. The β-anomeric configuration for the methyl glycoside **2** was established[9] from the small $J_{1,2}$ value (<1.0 Hz). The coupling constants for the other protons of the furanoid ring ($J_{2,3}$ 1.1, $J_{3,4}$ 5.4 Hz) suggested a preferential E_0(D) conformation for **2**, as proposed[10] for β-galactofuranosides. The ^{13}C-n.m.r. spectrum of **2** showed the signal of C-1 at 106.7 p.p.m., and two low-field signals (82.0 and 81.1 p.p.m.) corresponding to C-2 and C-4, characteristic of β anomers of galactofuranose derivatives[1,7,11]. The high selectivity in favor of the β anomer in reactions of peracylated sugars with stannic chloride has been attributed[12] to the formation of acyloxonium ions by removal of the C-1 substituent and anchimeric participation of the C-2 acyloxy group.



O-Debenzoylation of **2** gave methyl β-D-galactofuranoside (**3**) in 91% yield. The values of the chemical shifts of the ^{13}C-n.m.r. spectrum of **3** were identical to those already reported[13]. The synthetic route described herein is a more efficient and high-yielding method for the preparation of methyl β-D-galactofuranoside than has been previously reported[14,15].

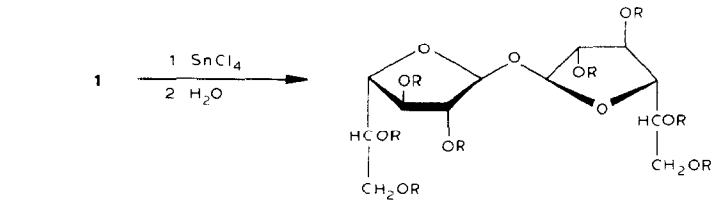
In view of the success obtained for the stereoselective formation of a simple 1,2-*trans* glycoside by this method, we decided to explore this reaction further, and studied the glycosylation of **1** with conveniently protected derivatives of D-galactono-1,4-lactone, employing $SnCl_4$ as catalyst. The resulting glycosyl-aldono-1,4-lactone would then be the precursor of a disaccharide derivative formed by two galactofuranose residues.

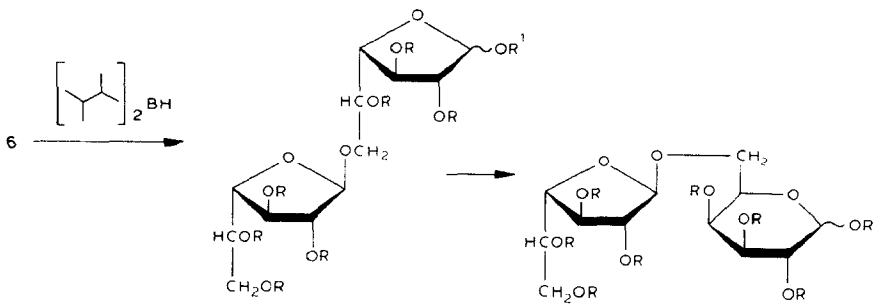
We first employed 2,3,5-tri-*O*-benzoyl-D-galactono-1,4-lactone (**4**), having a free 6-hydroxyl group, for the glycosylation of **1**. Compound **4** may be obtained by boron trifluoride-catalyzed detritylation of the readily available[1] 2,3,5-tri-*O*-benzoyl-6-*O*-trityl-D-galactono-1,4-lactone (**5**). Reaction of **1** with **4** in the presence of $SnCl_4$ afforded the glycosyl-lactone **6**, in 91% yield. A 2D-COSY ^{1}H-n.m.r. experiment[16,17] allowed the complete assignment of the spectrum of **6**. The signals for the protons of the lactone moiety were readily identified by their connectivities starting from H-2, which appeared as a doublet at 5.93 p.p.m. Signals for the protons of the galactofuranosyl residue were assigned by comparison with the spectrum of glycoside **2**. These assignments were also confirmed by the connectivities of the protons starting from the lower-field signal (6.08 p.p.m.), corresponding to H-5′. The broad singlet for H-1′ ($J_{1',2'}$ <1.0 Hz) would dictate[9] the β configuration for the galactofuranoside ring. The lowest-field signal in the ^{13}C-n.m.r. spectrum of **6** was the lactone carbonyl (C-1) at 168.5 p.p.m. The seven carbonyl groups of the benzoates were found between 165.8 and 164.6 p.p.m. The anomeric carbon (C-1′) appeared at 105.5 p.p.m., and the signals at 82.1 and 81.8 p.p.m. corresponded to C-2′ and C-4′.

We have previously observed[1] that $SnCl_4$ was an effective catalyst for condensing peracylated derivatives of sugars with hydroxyl groups protected as trityl ethers, and therefore condensation of **1** with **5** was performed. Although the reaction was slower than with compound **4**, and a larger amount of $SnCl_4$ was required, the condensation afforded compound **6** in 85% yield. The formation of the glycosidic bond in reactions of **1** with **4** or **5** stereoselectively gave the isomer having the β configuration. Confirmation of the structure of disaccharide **6** was achieved by borohydride reduction, followed by deacylation with sodium methoxide, which gave the glycosyl-alditol **7**. The ^{13}C-n.m.r. spectrum of **7** showed at lower fields the C-1′, C-4′, and C-2′ signals for the furanose moiety at 108.6, 83.6, and 81.8 p.p.m., respectively. The signals for the carbon atoms of the acyclic moiety appeared shifted upfield (71.7–69.6 p.p.m.), being distinguishable from those of the hydroxymethyl carbons (C-1 and C-6′) at 64.2 and 63.6 p.p.m. The C-6 atom, involved in the glycosidic linkage, was shifted downfield. Trimethylsilylation of a sample of **7**, gave the trimethylsilyl (Me_3Si) derivative, which showed a mass

spectrum identical to that of the Me_3Si-alditol derived from the galactan of *Mycobacterium tuberculosis*[5].

During the condensation of **1** with the partially protected lactone derivatives **4** or **5**, a by-product was detected, whose proportions increased when the reaction were not conducted under strict anhydrous conditions, indicating possible hydrolysis of the anomeric benzoate. However, the expected formation of 2,3,4,6-tetra-*O*-benzoyl-D-galactofuranose was ruled out according to the ^{1}H-n.m.r. spectrum, which showed an unique signal (a broad singlet) for H-1, at 5.77 p.p.m. The other coupling constants for the ring-protons indicated the presence of a furanoid system, having the β configuration. A (1→1)-linked non-reducing disaccharide structure **8** was proposed for this side-product. This finding was supported by the ^{13}C-n.m.r. spectrum, which resembled that of **2**, although the C-1 signal of the latter was deshielded by 5.8 p.p.m. with respect to the C-1 signal in **8**. Similar chemical shifts have been reported in the literature for related compounds[18]. The structure of **8** was further confirmed by debenzoylation to give the crystalline disaccharide **9**. Compound **9** showed only six signals, in the ^{13}C-n.m.r. spectrum, as expected for such a symmetrical structure, and a single anomeric signal was detected. Furthermore, compound **9** did not mutarotate, and on acidic hydrolysis (monitored by ^{1}H-n.m.r. spectroscopy) a gradual decrease of the anomeric signal at 5.20 p.p.m. was observed, together with the appearance of the doublets at δ 5.22 ($J_{1,2}$ 3.0 Hz) and 4.56 ($J_{1,2}$ 7.6 Hz) corresponding to the α and β anomers of galactopyranose[19].





A good yield (88%) of crystalline **8** was obtained by reaction of **1** with $SnCl_4$ followed by the controlled addition of water. Compound **8** could be produced by condensation of the benzooxonium ion intermediate[8,12] with 2,3,4,6-tetra-*O*-benzoyl-β-D-galactofuranose. The latter product is obtained by the nucleophilic attack of water on C-1 of the benzooxonium ion. We have also previously observed the formation of a nonreducing disaccharide by the self-condensation of 2,5,6,7-tetra-*O*-benzoyl-3-deoxy-β-D-*gluco*-heptofuranose[18], catalysed by Lewis acids. Dyong *et al.*[20] have described similar dimerization on treatment of acetylated reducing aldofuranoses with $BF_3 \cdot OEt_2$.

On the other hand, diisoamylborane reduction[21,22] of the glycosyl-lactone **6** afforded the disaccharide derivative **10**, in 86% yield, with a large preponderance of the β anomer, as indicated by the ^1H- and ^{13}C-n.m.r. spectra. Thus, the C-1 signal appeared at 100.7 p.p.m., comparable to the shift found for C-1 of β-D-galactofuranose[13].

Debenzoylation of **10** with NaOMe afforded the disaccharide **14** contaminated with galactose, as partial hydrolysis of the glycosidic bond occurred during the isolation. Purification of this latter was achieved by h.p.l.c. and afforded the pure disaccharide **14**, which had the same optical rotation as the one isolated from the galactan of *Mycoplasma mycoides*[4], from the arabinogalactan of *Mycobacterium tuberculosis*[5], and obtained by synthesis through condensation[23] of 3,5,6-tri-*O*-acetyl-1,2-*O*-(1-methoxyethylidene)-α-D-galactofuranose with benzyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranoside. In order to avoid the tedious h.p.l.c. purification of compound **14**, the product obtained by *O*-debenzoylation of **10** with NaOMe, was acetylated immediately after neutralization of the base (CO_2) and the crude, acetylated derivative **15** obtained was then purified by column chromatography (58% yield, from **10**). A 2D-COSY ^1H-n.m.r. experiment allowed the complete assignment for the spectrum of disaccharide **15**. The doublet located at lower field (5.69 p.p.m.) corresponded to H-1, and the large value for the coupling constant ($J_{1,2}$ 8.3 Hz) suggested the β configuration for the anomeric center. No signal for the α-anomeric proton was detected. The low value of δ for H-6a, H-6b (3.69 and 3.58 p.p.m.) confirmed that the HO-6 group of the galactopyranose was involved in the glycosidic linkage. The chemical shift for C-1 (92.1 p.p.m.) in the ^{13}C-n.m.r. spectrum of **15** agreed with the value reported for C-1 of penta-*O*-acetyl-β-D-galactopyranose[13]. The other signal in the anomeric region was that of C-1′ (105.5 p.p.m.), which was attributed to the β-D-galactofuranosyl residue. The electron-impact mass spectrum of **15** was in good agreement with that given for the acetate of the natural disaccharide[5]. Acetylation of disaccharide **10** gave the 1-acetate **11**, mainly the β anomer, as shown by its ^1H-n.m.r. spectrum. The anomeric proton gave two signals: a broad singlet at 6.48 p.p.m. ($J_{1,2}$ <1.0 Hz) and a doublet at 6.60 p.p.m. ($J_{1,2}$ 4.2 Hz), in 5:1 ratio, which corresponded respectively to the β and α anomers. The anomeric region of the ^{13}C-n.m.r. spectrum of **11** showed two intense signals at 105.5 (C-1′) and 99.4 p.p.m. (C-1 β anomer), and a signal of lower intensity at 93.5 p.p.m. was attributed to C-1 of the α anomer. Similar shifts have been reported for the anomers of per-*O*-benzoylated galactofuranose[7].

Attempts to preparing glycosides from compounds **10** or **11** employing $SnCl_4$ as catalyst were unsuccessful, as hydrolysis of the glycosidic linkage occurred.

Methylation of the free hydroxyl group of **10** with diazomethane catalysed by traces of $BF_3 \cdot OEt_2$ led to the corresponding disaccharide methyl glycoside **12**, isolated as a chromatographically homogeneous solid. The ^1H and ^{13}C-n.m.r. spectra of **12** showed that this product was actually an ~2.5:1 mixture of β and α anomers, respectively. The β anomer showed C-1 and C-1′ signals at lower field (106.8, 105.8 p.p.m.), values similar to those observed for the anomeric carbon atom of benzoylated methyl β-D-galactofuranoside (**2**). The α anomer showed C-1′ at 105.2 p.p.m. and C-1 shifted upfield (100.9 p.p.m.), comparable with the reported values for α-galactofuranosides[13]. Furthermore, the signal for the methyl group of each anomer appeared clearly differentiated, at 55.5 and 54.9 p.p.m., corresponding to the α and β anomers, respectively. The anomeric protons of the major anomer were readily identified in the ^1H-n.m.r. spectrum of **12**. Both protons (H-1 and H-1′) had a $J_{1,2}$ value of <1.0 Hz, consistent with the β configuration[9] at C-1 and C-1′. The anomeric mixture could not be separated by column chromatography, and therefore *O*-debenzoylation was performed. As observed previously for the debenzoylation of **10**, partial hydrolysis of the glycosidic linkage of the methyl disaccharide glycoside **12** also occurred during the neutralization of NaOMe with Dowex 50 W resin. Removal of the benzoyl groups using 5:2:1 MeOH–H_2O–Et_3N was more effective, and no hydrolysis of **12** occurred. Examination of the reaction product by t.l.c. showed two spots because of the presence of both, α and β anomers. Flash chromatography allowed the purification of the main component, the methyl β-glycoside of the disaccharide **13**, formed by two galactofuranose units. The ^{13}C-n.m.r. spectrum of **13** showed in the C-1,1′ region two close signals (109.7 and 109.4), which confirmed the presence of two furanose rings having the β-anomeric configuration. The electron-impact mass spectrum of the per-*O*-Me_3Si derivative supported the structure of **13**. It showed intense peaks at *m/z* 319, 217, and 205, typical of hexofuranosides[24].

The present work confirms the utility of partially protected aldonolactones as glycosylating agents for monosaccharides. The formation of the 1,2-*trans*-glycosidic linkage occurred stereoselectively with very high yield of the β anomer. Reduction of the lactone function afforded a disaccharide derivative having both units in the furanose form. The methyl glycoside of Gal*f*-β-D-(1→6)-D-Gal*f* (**13**) constitutes a synthetic analog of the terminal unit found[6] in the highly immunogenic arabinogalactans from *Myobacterium tuberculosis* and *Mycobacterium leprae*.

EXPERIMENTAL

General methods. — Melting points were determined with a Thomas–Hoover apparatus and are uncorrected. Optical rotations were recorded with a Perkin–Elmer 141 polarimeter. ^1H- and ^{13}C-n.m.r. spectra were recorded with a Varian XL-100 spectrometer operating in the Fourier-transform mode, at 100.1 and 25.2

MHz, respectively. For solutions in $CDCl_3$, tetramethylsilane was used as internal standard. 2D-COSY-^{1}H-n.m.r. experiments were performed with a Bruker 500-MHz spectrometer. G.l.c.–m.s. was performed with a Varian Aerograph 1400 gas chromatograph coupled to a Varian–MAT CH 7A spectrometer at 70 eV, using a glass column (180 × 0.2 cm) packed with 3% OV-17 and helium as carrier, at a flow rate of 28 mL/min; T_i 250°; T_c 100–290°, with a temperature gradient of 20°/min. H.p.l.c. was performed with a Micromeritics liquid chromatograph equipped with a refractive-index detector and a Micromeritics 730 injector, using a Lichrosorb NH_2 (10 μm) column (25 × 0.4 cm internal diameter) and 4:1 MeCN–H_2O at a flow rate of 3 mL/min. Column chromatography was performed on Silica Gel 60 (Merck). T.l.c. was carried out on precoated aluminum plates (0.2 mm) of Silica Gel 60F-254 (Merck), with (*a*) 3:1 hexane–EtOAc, (*b*) 9:1 PhMe–EtOAc, and (*c*) 7:1:2 PrOH–EtOH–H_2O. Detection was effected by exposure to u.v. light and by spraying the plates with 5% (v/v) H_2SO_4 in EtOH followed by heating. Descending paper chromatography was performed on Whatman No. 1 with 6:4:3 1-butanol–pyridine–water, detection being effected with $AgNO_3$–NaOH[25].

*1,2,3,5,6-Penta-*O*-benzoyl-*D*-galactofuranose* (**1**). — Compound **1** was prepared as described in the literature[7].

*Methyl 2,3,5,6-tetra-*O*-benzoyl-β-*D*-galactofuranoside* (**2**). — To a solution of compound **1** (1.40 g, 2 mmol) in dry CH_2Cl_2 (20 mL) cooled to 0°, $SnCl_4$ (0.25 mL, 2.1 mmol) was added. The solution was stirred for 10 min followed by the addition of MeOH (0.2 mL, 2.6 mmol). T.l.c. examination (solvent *a*) of the mixture after 15 h of stirring at room temperature, showed a main spot (R_F 0.40) faster-moving than the starting material (R_F 0.32). The mixture was diluted with CH_2Cl_2 (40 mL) and extracted with saturated aq. $NaHCO_3$. The organic layer was dried ($MgSO_4$) and evaporated. Purification of the residue, by column chromatography (3:1 hexane–EtOAc) afforded a syrup (1.26 g, 85%), which slowly crystallized from EtOH. Compound **2** had m.p. 90–92°, $[\alpha]_D^{20}$ −3° (*c* 1, $CHCl_3$); ^{1}H-n.m.r.: δ 8.15–7.20 (20 H, aromatic), 6.06 (H-5), 5.62 ($J_{3,4}$ 5.4 Hz, H-3), 5.46 ($J_{2,3}$ 1.1 Hz, H-2), 5.20 ($J_{1,2}$ <1.0 Hz, H-1), 4.77 (H-6,6′), 4.65 ($J_{4,5}$ 3.5 Hz, H-4), and 3.47 (CH_3O); ^{13}C-n.m.r.: δ 165.8, 165.4 (× 2), 165.2 (Ph*C*O), 133.3–128.2 (C-aromatic), 106.7 (C-1), 82.0 (C-4), 81.1 (C-2), 77.6 (C-3), 70.2 (C-5), 63.4 (C-6), and 54.9 (*C*H_3O).

Anal. Calc. for $C_{35}H_{30}O_{10}$: C, 68.85; H, 4.95. Found: C, 69.16; H, 5.18.

*Methyl β-*D*-galactofuranoside* (**3**). — To a suspension of compound **2** (0.52 g, 0.85 mml) in MeOH (40 mL), a 0.5M solution of NaOMe in MeOH (10 mL) was added. The mixture was stirred for 2 h at 0°, made neutral with Dowex 50 W (H^+), filtered, and the filtrate evaporated. Methyl benzoate was eliminated by distillation with water. The residue (0.15 g, 91%) showed a single spot by t.l.c. (R_F 0.36, 4:1 EtOAc–MeOH). Compound **3** had $[\alpha]_D^{20}$ −120° (*c* 1, MeOH), in agreement with data from the literature[15]; ^{13}C-n.m.r. (1:1 D_2O–H_2O): δ 109.0 (C-1), 83.8 (C-4), 81.6 (C-2), 77.5 (C-3), 71.8 (C-5), 63.6 (C-6), and 55.8 (*C*H_3O).

*2,3,5-Tri-*O*-benzoyl-6-*O*-(2,3,5,6-tetra-*O*-benzoyl-β-*D*-galactofuranosyl)-*D*-galactono-1,4-lactone* (**6**). — *(a) Starting from 2,3,5-tri-*O*-benzoyl-*D*-galactono-1,4-*

lactone[1] (**4**). To a solution of penta-*O*-benzoyl-D-galactofuranose (**1**; 1.01 g, 1.45 mmol) in dry CH_2Cl_2 (15 mL), $SnCl_4$ (0.18 mL, 1.5 mmol) was added, and the solution was stirred for 10 min at 0°. Compound **4** (0.71 g, 1.45 mmol) was then slowly added, and the mixture was stirred for 5 h at room temperature, when examination by t.l.c. (solvent *b*) showed a main spot (R_F 0.44), with intermediate mobility with respect to the starting materials **1** (R_F 0.52) and **4** (R_F 0.15). The solution was poured into aq. $NaHCO_3$, and extracted with CH_2Cl_2 (40 mL, three times). The organic extract was washed with water (40 mL, twice), dried ($MgSO_4$), filtered and evaporated. The syrup obtained was then dissolved in hot EtOH which yielded an amorphous solid upon cooling. Compound **6** was purified by dissolution and precipitation from EtOH; yield 1.41 g (91%); $[\alpha]_D$ −7° (*c* 2, chloroform); 2D-COSY-^{1}H-n.m.r. ($CDCl_3$): δ 8.15–7.15 (35 H, aromatic), 6.08 (H-5′), 5.93 ($J_{2,3}$ 6.0 Hz, H-2), 5.85 ($J_{3,4}$ 5.6 Hz, H-3), 5.84 (H-5), 5.61 ($J_{3',4'}$ 4.8 Hz, H-3′), 5.46 ($J_{2',3'}$ 1.0 Hz, H-2′), 5.41 ($J_{1',2'}$ <1.0 Hz, H-1′), 5.10 ($J_{4,5}$ 2.5 Hz, H-4), 4.72–4.80 (H-4′, H-6′a,6′b), 4.12 ($J_{5,6a}$ 7.7 Hz, H-6a), and 4.02 ($J_{5,6b}$ 6.0, $J_{6a,6b}$ 10.3 Hz, H-6b); ^{13}C-n.m.r.: δ 168.5 (C-1), 165.5, 165.3, 165.2, 165.1, 164.9, 164.6 (Ph*C*O), 133.4–127.8 (C-aromatic), 105.5 (C-1′), 82.1, 81.8 (C-2′,4′), 78.7, 77.5 (C-3′,4), 73.8, 72.3, 70.3, 70.1 (C-2,3,5,5′) and 63.8, and 63.4 (C-6,6′).

Anal. Calc. for $C_{61}H_{48}O_{18}$: C, 68.54; H, 4.53. Found: C, 68.72; H, 4.76.

(b) Starting from 2,3,5-tri-O-benzoyl-6-O-trityl-D-galactono-1,4-lactone[1] (**5**). Compound **5** (2.0 g, 2.73 mmol) was slowly added to a solution of **1** (1.91 g, 2.73 mmol) and $SnCl_4$ (0.33 mL, 2.75 mmol) in CH_2Cl_2 (25 mL), as before. T.l.c. examination showed that some of the startig material remained, even after 20 h of reaction time, therefore an additional amount of $SnCl_4$ (0.33 mL) was added. After stirring for an additional 20 h, the mixture was processed as described earlier. Compound **6** (2.48 g, 85%) had the same physical constants and spectral properties as the product from (*a*).

In the mother liquors of preparations (*a*) and (*b*), a by-product was detected on t.l.c. with a higher R_F (0.50, solvent *b*). This latter was isolated and characterized as octa-*O*-benzoyl-β-D-galactofuranosyl-(1→1)-β-D-galactofuranoside (**8**, see later).

β-D-Galactofuranosyl-(1→6)-D-galactitol (**7**). — To a suspension of compound **6** (0.50 g, 0.46 mmol) in MeOH (15 mL) was added $NaBH_4$ (0.35 g, 9.21 mmol) in 0.05 g portions every 0.5 h. After stirring for 20 h, the mixture showed by t.l.c. several spots (partially benzoylated products), but no starting material was detected. Debenzoylation was completed by addition of 0.5M NaOMe in MeOH (8 mL) and stirring for 3 h. The solution was deionized with Dowex 50 W (H^+) resin, filtered, and the filtrate evaporated. Methyl benzoate was eliminated by repeated evaporations with water. The syrup (0.12 g, 76%) showed a main spot by t.l.c., R_F 0.67 (solvent *c*), and by paper chromatography R_{Gal} 0.82. Compound **7** had $[\alpha]_D^{20}$ −41° (*c* 0.6, H_2O); ^{13}C-n.m.r. (1:1 D_2O–H_2O): δ 108.6 (C-1′), 83.6 (C-4′), 81.8 (C-2′), 77.6 (C-3′), 71.7, 71.1, 70.8, 70.5, 70.2, 69.6 (C-5′,2,3,4,5,6) and 64.2, and 63.6 (C-1,6′).

Compound **7** (5 mg) dissolved in pyridine (0.5 mL) was silylated with Tri-Sil (Pierce) (0.5 mL). G.l.c.–m.s. (only values of m/z >300 and >5% of the base peak are given): 525(61), 451(67), 422(5), 361(63), 345(67), 332(66), 319(100), and 307(65).

2,3,5,6-Tetra-O-benzoyl-(2,3,5,6-tetra-O-benzoyl-β-D-galactofuranosyl)-(1→1)-β-D-galactofuranoside (**8**). — A solution of compound **1** (1.0 g, 1.43 mmol) in dry CH_2Cl_2 (15 mL), was allowed to react at 0° with $SnCl_4$ (0.17 mL, 1.43 mmol) for 10 min. Water (0.025 mL, 1.43 mmol) was added, and the mixture was stirred for 3 h at room temperature. The solution was poured into saturated aq. $NaHCO_3$ and extracted with CH_2Cl_2. The extract was dried ($MgSO_4$), filtered, and evaporated, to afford compound **8** (0.74 g, 88%), which crystallized from EtOH. Compound **8** was recrystallized from EtOH and had m.p. 79–82°, $[\alpha]_D^{20}$ −18° (*c* 1, $CHCl_3$); ^{1}H-n.m.r.: δ 8.15–7.20 (H-aromatic), 6.10 (H-5), 5.77 ($J_{1,2}$ <1.0 Hz, H-1), 5.73 ($J_{3,4}$ 4.9 Hz, H-3), 5.56 ($J_{2,3}$ 1.0 Hz, H-2), 4.92 (H-4), and 4.78 (H-6,6′); ^{13}C-n.m.r.: δ 165.8, 165.4, 165.3, 165.2 (Ph*C*O), 133.2–128.1 (C-aromatic), 100.9 (C-1), 82.3, 82.0 (C-2,4), 77.3 (C-3), 70.2 (C-5), and 63.6 (C-6).

Anal. Calc. for $C_{68}H_{54}O_{19}$: C, 69.50; H, 4.63. Found: C, 69.53; H, 4.75.

β-D-Galactofuranosyl-(1→1)-β-D-galactofuranoside (**9**). — To a suspension of **8** (0.49 g, 0.41 mmol) in anhydrous MeOH (30 mL) at 0°, 0.5M NaOMe in MeOH (7.8 mL) was added. A solid was formed after stirring overnight at room temperature. It was filtered (0.08 g) and the mother liquor was made neutral with Dowex 50 W, filtered and the filtrate evaporated. The residue obtained (0.03 g) was crystallized from MeOH. Both solids had the same R_F value (0.64, solvent *c*) and m.p. They were pooled (0.11 g, 78%) and recrystallized from MeOH, to afford pure compound **9**; it had m.p. 206–208°, $[\alpha]_D^{20}$ −150° (*c* 1, H_2O); ^{1}H-n.m.r. (D_2O): δ 5.20 (b.s, H-1,1′); ^{13}C-n.m.r. (1:1 D_2O–H_2O): δ 104.8 (C-1), 84.6 (C-4), 82.4 (C-2), 78.1 (C-3), 72.1 (C-5), and 64.0 (C-6).

Anal. Calc. for $C_{12}H_{22}O_{11}$: C, 42.11; H, 6.48. Found: C, 41.79; H, 6.46.

Hydrolysis of **9** (0.02 g) was performed with 2M deuterium chloride in D_2O (0.5 mL) at room temperature, and monitored by ^{1}H-n.m.r. spectroscopy with 1,4-dioxane as the internal reference (3.70 p.p.m. downfield from Me_4Si). After 24 h the anomeric region showed: δ 5.22 (H-1, α-Gal*p*), 5.20 (H-1,1′, compound **9**), and 4.56 (H-1, β-Gal*p*).

2,3,5-Tri-O-benzoyl-6-O-(2,3,5,6-tetra-O-benzoyl-β-D-galactofuranosyl)-D-galactofuranose (**10**). — To a solution of freshly prepared bis(3-methyl-2-butyl)-borane[21,22] (6.75 mmol) in tetrahydrofuran (5 mL), under a nitrogen atmosphere, compound **6** (1.80 g, 1.68 mmol) in tetrahydrofuran (5 mL) was added. The solution was stirred for 16 h at room temperature, under a static nitrogen atmosphere, and then processed as already described[22]. Boric acid was eliminated by co-evaporation with MeOH, and the resulting syrup showed on t.l.c. (solvent *b*) a main spot (R_F 0.26), which was slower-moving than the starting material (**6**). The syrup was dissolved in hot EtOH and precipitation occurred upon cooling, affording a chromatographically homogeneous, amorphous solid (1.55 g, 86%);

$[\alpha]_D^{20}$ +3° (*c* 1, $CHCl_3$); ^{1}H-n.m.r.: δ 8.18–7.15 (35 H, aromatic), 6.14–5.94 (H-5,5′), 5.77 ($J_{1,2}$ <1.0 Hz, H-1,3), 5.60 ($J_{3',4'}$ 5.0 Hz, H-3′), 5.55, 5.46 ($J_{2,3} \simeq J_{2',3'} \simeq$1.0 Hz, H-2,2′), 5.36 ($J_{1',2'}$ <1.0 Hz, H-1′), 5.08–4.65 (H-4,4′,6′a,6′b), 4.24 (H-6a), and 3.97 (H-6b); ^{13}C-n.m.r.: δ 166.0, 165.6 (× 2), 165.4 (× 3), 165.0 (Ph*C*O), 133.7–128.2 (C-aromatic) 104.7 (C-1′), 100.7 (C-1), 83.2, 81.8, 81.6, 80.6 (C-2,2′,4,4′), 77.4, 77.1 (C-3,3′), 70.6, 70.3 (C-5,5′), and 64.1, 63.3 (C-6,6′).

Anal. Calc. for $C_{61}H_{50}O_{18}$: C, 68.40; H, 4.70. Found: C, 68.12; H, 4.97.

1-O-Acetyl-2,3,5-tri-O-benzoyl-6-O-(2,3,5,6-tetra-O-benzoyl-β-D-galactofuranosyl)-D-galactofuranose (**11**). — Acetic anhydride (0.5 mL) was added dropwise to a solution of compound **10** (0.15 g, 0.13 mmol) in dry pyridine (0.5 mL), previously cooled at −5°. The mixture was kept for 20 h at −5°, and then MeOH (1 mL) was slowly added. After 0.5 h of stirring at room temperature, the solution was evaporated with PhMe to eliminate the pyridine. The residue showed a single spot by t.l.c. (R_F 0.42, solvent *b*). Compound **11** was obtained as an amorphous solid (0.14 g, 87%) by dissolution in hot EtOH, followed by cooling. The n.m.r. spectra indicated a 5:1 ratio for the β:α anomers. It had $[\alpha]_D^{20}$ −13° (*c* 0.7, $CHCl_3$); ^{1}H-n.m.r. (β anomer): δ 8.15–7.15 (35 H, aromatic), 6.48 ($J_{1,2}$ <1.0 Hz, H-1), 6.18–5.82 (H-5,5′), 5.70–5.55 (H-2,3,3′), 5.42 ($J_{2',3'}$ 1.0 Hz, H-2′), 5.39 ($J_{1',2'}$ <1.0 Hz, H-1′), 4.90–4.66 (H-4,4′,6′a,6′b), 4.33–3.91 (H-6a,6b), and 2.06 (CH_3CO). The α anomer showed a doublet at δ 6.60 ($J_{1,2}$ 4.2 Hz). ^{13}C-N.m.r. (β anomer): δ 168.9 (CH_3CO), 165.4–164.9 (Ph*C*O), 133.3–128.1 (C-aromatic), 105.5 (C-1′), 99.4 (C-1), 83.5, 81.7 (× 2), 81.4 (C-2,2′,4,4′), 77.5, 77.1 (C-3,3′), 70.6, 70.2 (C-5,5′), 64.9, 63.8 (C-6,6′), and 20.9 (CH_3CO). The α-anomeric carbon appeared at 93.5 p.p.m.

Anal. Calc. for $C_{63}H_{51}O_{19}$: C, 68.05; H, 4.62. Found: C, 68.15; H, 4.73.

Methyl 2,3,5-tri-O-benzoyl-6-O-(2,3,5,6-tetra-O-benzoyl-β-D-galactofuranosyl)-D-galactofuranoside (**12**). — Compound **10** (0.50 g, 0.47 mmol) was methylated[26] with diazomethane–$BF_3 \cdot OEt_2$ in CH_2Cl_2. The reaction was monitored by t.l.c. (solvent *b*) until no starting material could be detected (120 mL of the diazomethane solution had been consumed). The mixture was filtered and evaporated. The syrup was purified by column chromatography using 24:1 PhMe–EtOAc as eluent. Fractions containing the product of R_F 0.46 (solvent *b*) were combined and evaporated; yield 0.39 g (77%). A solid was obtained by cooling a hot solution of the syrup in EtOH. The ^{13}C-n.m.r. spectrum of the product indicated a 2.5:1 β:α ratio of anomers. It had $[\alpha]_D^{20}$ −10° (*c* 1, $CHCl_3$); ^{1}H-n.m.r. (β anomer): δ 8.15–7.15 (35 H, aromatic), 6.09 (H-5′), 5.85 (H-5), 5.62 (H-3,3′), 5.43 (H-1′,2), 5.15 (H-1), 4.83–4.70 (H-4,4′,6′a,6′b), 4.31–3.94 (H-6a,6b), and 3.41 (CH_3O); ^{13}C-n.m.r. (β anomer): δ 165.8, 165.4 (× 4), 165.2, 164.9 (Ph*C*O), 133.2–128.2 (C-aromatic), 106.8, 105.8 (C-1,1′), 82.2, 81.8 (× 2), 80.9 (C-2,2′,4,4′), 77.4 (C-3,3′), 70.9, 70.1 (C-5,5′), 65.4, 63.4 (C-6,6′), and 54.9 (CH_3O). The α anomer showed, *inter alia*, signals at 105.2 (C-1′), 100.9 (C-1), 74.5, 72.1, and 55.5 (CH_3O).

Anal. Calc. for $C_{62}H_{52}O_{18}$: C, 68.63; H, 4.85. Found: C, 68.85, H, 4.94.

Methyl 6-O-(β-D-galactofuranosyl)-β-D-galactofuranoside (**13**). — A suspension of compound **12** (0.27 g, 0.25 mmol) in 5:2:1 MeOH–H_2O–Et_3N (15 mL) was heated at 60° with stirring. After 10 h complete dissolution was observed. Paper chromatography showed a single spot (R_{Gal} 0.72). The solvent was evaporated, the product obtained was dissolved in water (15 mL), and was extracted with ether (3 × 10 mL). The aqueous solution was freeze dried. The resulting product (76 mg, 86%) which showed two spots by t.l.c. (solvent *c*): R_F 0.73 (main, β anomer) and 0.66 (α anomer) was purified by flash chromatography on a column of silica gel with 2:1 EtOAc–MeOH. The β anomer (**13**, 42 mg) was isolated pure; $[\alpha]_D^{20}$ −90° (*c* 0.5, H_2O); ^{13}C-n.m.r. (1:1 D_2O–H_2O): δ 109.7, 109.4 (C-1,1'), 84.6 (C-4,4'), 82.7, 82.4 (C-2,2'), 78.5, 78.4 (C-3,3'), 72.5 (C-5), 71.3, 70.7 (C-5',6), 64.4 (C-6'), and 56.6 (CH_3O). The α anomer had: δ 103.6 (C-1), 83.0 (C-4), 78.0 (C-2), 76.1 (C-3), 73.2, 70.1 (C-5,6), and 56.8 (CH_3O). G.l.c.–m.s. (Me_3Si derivative, only values of *m/z* >200 and >3% of the base peak are given): 511(3), 451(3), 361(12), 332(62), 319(97), 271(73), 247(9), 243(9), 241(6), 231(61), 217(100), and 205(70).

Anal. Calc. for $C_{13}H_{24}O_{11}$: C, 43.82; H, 6.79. Found: C, 44.17; H, 6.86.

β-D-Galactofuranosyl-(1→6)-D-galactopyranose (**14**). — A solution of 0.5M NaOMe in MeOH (6 mL) was added to a suspension of **10** (0.35 g, 0.32 mmol) in dry MeOH (20 mL) previously cooled at 0°. After 2 h the mixture was processed as described for the preparation of compound **3**. T.l.c. of the deionized product (0.09 g, 81%) showed a main spot of R_F 0.52 (solvent *c*) and a spot with the same mobility as galactose (R_F 0.54). Purification by h.p.l.c., gave pure compound **14**, which had R_{Gal} 0.77; $R_{Lactose}$ 1.30; $[\alpha]_D^{20}$ −21° (*c* 0.4, water), in good agreement with data reported in the literature[4,5].

1,2,3,4-Tetra-O-acetyl-6-O-(2,3,5,6-tetra-O-acetyl-β-D-galactofuranosyl)-D-galactopyranose (**15**). — Compound **10** (0.43 g, 0.39 mmol) suspended in dry MeOH (30 mL) was debenzoylated with 0.5M NaOMe in MeOH (7 mL). After 2 h of stirring at room temperature, the mixture was made neutral by addition of solid carbon dioxide. The solvent was evaporated, and the residue was dried overnight *in vacuo* over P_2O_5 and then was acetylated by addition of pyridine (3 mL) and Ac_2O (3 mL). After 5 h, the mixture was poured into water, and extracted with CH_2Cl_2 (3 × 30 mL). The extract was washed with 1% aq. HCl, saturated aq. $NaHCO_3$, and water. The solution was dried ($MgSO_4$), filtered, and evaporated. The residue was chromatographed on a column of silica gel, with 2:1 hexane–EtOAc. Evaporation of the fraction having the product of R_F 0.48 (solvent *b*), afforded 0.16 g (58%) of compound **15**; $[\alpha]_D$ −13° (*c* 1, $CHCl_3$): 2D-COSY ^{1}H-n.m.r. ($CDCl_3$): δ 5.69 ($J_{1,2}$ 8.3 Hz, H-1), 5.47 ($J_{4,5}$ 2.9 Hz, H-4), 5.36 (H-5'), 5.33 ($J_{2,3}$ 10.4 Hz, H-2), 5.06 ($J_{3,4}$ 3.3 Hz, H-3), 5.01 (H-1' and H-2'), 4.99 ($J_{3',4'}$ 5.8 Hz, H-3'), 4.33 ($J_{5',6'a}$ 3.7 Hz, H-6'a), 4.18 ($J_{5',6'b}$ 7.1, $J_{6'a,6'b}$ 12.0 Hz, H-6'b), 4.12 ($J_{5,6a}$ 7.7, $J_{5,6b}$ 5.8 Hz, H-5), 3.69 ($J_{6a,6b}$ 9.6 Hz, H-6a), 3.58 (H-6b), and 2.18, 2.11 (× 2), 2.10, 2.09, 2.06, 2.04, 1.98 (CH_3CO); ^{13}C-n.m.r.: δ 170.3, 170.0, 169.9, 169.7, 169.3, 168.7 (CH_3CO), 105.5 (C-1'), 92.1 (C-1), 81.2, 80.7 (C-2',4'), 76.2 (C-3'), 72.3, 71.0, 69.6, 67.7, 66.7 (C-5',2,3,4,5), and 64.1, 62.7 (C-6,6'); m.s. (only val-

ues m/z >160 and >5% of the base peak are given): 533(8), 331(81), 317(6), 245(8), 240(6), 229(5), 210(12), and 169(100).

Anal. Calc. for $C_{28}H_{38}O_{19}$: C, 49.56; H, 5.64. Found: C, 49.30; H, 5.43.

ACKNOWLEDGMENTS

We are indebted to CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), the University of Buenos Aires, and UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases for financial support and to UMYMFOR (CONICET-FCEN, Buenos Aires) for the microanalyses.

REFERENCES

1 C. DU MORTIER, O. VARELA, AND R. M. DE LEDERKREMER, *Carbohydr. Res.*, 189 (1989) 79–86.
2 R. M. DE LEDERKREMER, O. L. CASAL, M. J. M. ALVES, AND W. COLLI, *FEBS Lett.*, 116 (1980) 25–29.
3 J. F. GROISMAN, AND R. M. DE LEDERKREMER, *Eur. J. Biochem.*, 165 (1987) 327–332.
4 P. PLACKETT AND S. H. BUTTERY, *Biochem. J.*, 90 (1964) 201–205.
5 E. VILKAS, C. AMAR, J. MARKOVITS, J. F. G. VLIEGENTHART, AND J. P. KAMERLING, *Biochim. Biophys. Acta*, 297 (1973) 423–435.
6 M. MCNEIL, S. J. WALLNER, S. W. HUNTER, AND P. J. BRENNAN, *Carbohydr. Res.*, 166 (1987) 299–308.
7 N. B. D'ACCORSO, I. M. E. THIEL, AND M. SCHÜLLER, *Carbohydr. Res.*, 124 (1983) 177–184.
8 S. HANESSIAN AND J. BANOUB, *Methods Carbohydr. Chem.*, 8 (1980) 243–245.
9 D. R. BUNDLE AND R. U. LEMIEUX, *Methods Carbohydr. Chem.*, 7 (1976) 79–86.
10 S. J. ANGYAL, *Carbohydr. Res.*, 77 (1979) 35–50.
11 O. VARELA, C. MARINO, AND R. M. DE LEDERKREMER, *Carbohydr. Res.*, 155 (1986) 247–251.
12 S. HANESSIAN AND J. BANOUB, *Carbohydr. Res.*, 59 (1977) 261–267.
13 K. BOCK AND C. PEDERSEN, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 27–66.
14 E. PACSU, *Methods Carbohydr. Chem.*, 2 (1963) 354–367.
15 I. AUGESTAD AND E. BERNER, *Acta Chem. Scand.*, 8 (1954) 251–256.
16 A. BAX AND R. FREEMAN, *J. Magn. Reson.*, 45 (1981) 177–181.
17 M. A. BERNSTEIN, L. D. HALL, AND S. SUKUMAR, *Carbohydr. Res.*, 103 (1982) c1–c6.
18 L. O. JERONIC, A. FERNANDEZ CIRELLI, AND R. M. DE LEDERKREMER, *Carbohydr. Res.*, 167 (1987) 175–186.
19 S. J. ANGYAL AND V. A. PICKLES, *Aust. J. Chem.*, 25 (1972) 1695–1710.
20 I. DYONG, L. BAUMEISTER, AND H. BENDLIN, *Chem. Ber.*, 112 (1979) 161–174.
21 L. M. LERNER, *Methods Carbohydr. Chem.*, 6 (1972) 131–134.
22 O. VARELA, A. FERNANDEZ CIRELLI, AND R. M. DE LEDERKREMER, *Carbohydr. Res.*, 70 (1979) 27–35.
23 J. C. JACQUINET AND P. SINAŸ, *Carbohydr. Res.*, 34 (1974) 343–349.
24 D. C. DEJONGH, T. RADFORD, J. D. HRIBAR, S. HANESSIAN, M. BIEBER, G. DAWSON, AND C. C. SWEELEY, *J. Am. Chem. Soc.*, 91 (1969) 1728–1740.
25 W. TREVELYAN, D. PROCTER, AND J. HARRISON, *Nature (London)*, 166 (1950) 444–445.
26 I. O. MASTRONARDI, S. M. FLEMATTI, J. O. DEFERRARI, AND E. G. GROS, *Carbohydr. Res.*, 3 (1966) 177–183.