Synthesis of Galactofuranosides by Regioselective Ring Opening of a 1,4-Anhydrogalactopyranose Derivative: A Possible Chemical Model for an Unprecedented Enzymatic Reaction

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The known 1,4-anhydro-2,3,6-tri-*O*-benzyl-β-D-galactopyranose (**1**) has been regioselectively ring-opened using a variety of nucleophiles under acidic conditions to exclusively afford furanosides.

Introduction

D-Galactofuranose is known to be a typical monosaccharide component of various polysaccharides and glycoconjugates isolated from infectious bacteria, protozoa, and fungi.[1] These include *Mycobacterium tuberculosis*,[2] the causative agent of tuberculosis, *Trypanosoma cruzii*,[3] the causative agent of Chagas' disease, and various *Penicillium*, *Aspergillus*, and *Neurospora*.[4] Fortunately, galactofuranose residues have not hitherto been identified in mammals. Uridine 5′-(α-D-galactofuranosyl pyrophosphate) (UDP-Gal*f*) has been isolated by Trejo et al.[5] from *Penicillium charlesii* and has been shown to act as donor in various Gal*f* transferases.[6] UDP-Gal*f* is formed[7,8] through an unprecedented enzymatic reaction catalyzed by UDP-galactopyranose mutase (EC 5.4.99.9), an enzyme that has recently been expressed, purified,[9,10] and crystallized.[11] The structure of this Leloir-type intermediate has been unambiguously confirmed by NMR spectroscopy.[7] Since galactofuranose is not found in humans, its biosynthesis would seem to be an appealing target for inhibition and drug development.

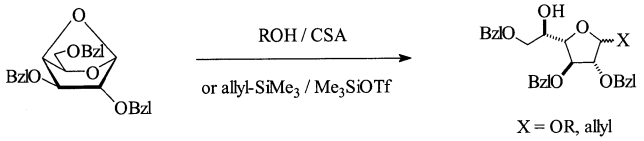
Although various potential inhibitors of galactofuranose metabolism have already been synthesized,[12−14] a rational design requires an understanding of the mode of action of the mutase.

Recently, it has been demonstrated by isotope exchange that the anomeric C−O bond is cleaved during UDP-Gal*p*/UDP-Gal*f* interconversion.[15] A hypothetical mechanism proposed for this process involves initial direct nucleophilic attack of the axial 4′-hydroxy group of UDP-Gal*p* at C-1′ with displacement of UDP and generation of a bicyclo acetal. In the second step, selective cleavage of the bond between O-5 and C-1 (and not of that between O-4 and C-1) must take place to give a furanose. Such selectivity has indeed recently been observed in the case of a 2-deoxy derivative using a chemical model.[16]

In order to study the regioselectivity of the O−C cleavage, we have treated the known benzylated bicyclic compound **1**[17] with various nucleophiles and our results are presented herein.

Results and Discussion

Reaction of anhydro compound **1** with alcohols, such as the monosaccharide derivative **2**[18] or 3-hydroxypropionitrile, in the presence of camphorsulfonic acid (CSA) resulted in regioselective ring opening of the bicyclic system to give galactofuranosides (Scheme 1). Reaction with the sugar alcohol **2** and subsequent acetylation gave the β-disaccharide **3** as the sole product. The β configuration of the anomeric linkage in **3** was confirmed by the 1′-H/2′-H coupling constant, $J_{1',2'} < 1$ Hz, a value typically observed for 1,2-*trans* protons in furanosides. The obtained disaccharide was found to be 1→6-linked as a result of attack of the more reactive 6-OH of **2**. No 1→4-linked disaccharide was detected. Moreover, when the bicyclic compound **1** was treated with various less reactive sugar-derived secondary alcohols, only unchanged starting material **1** was recovered from the reaction mixtures. This selectivity probably implies an S_N1 process, the furanosyl oxycarbenium species being glycosylated in a 1,2-*trans* fashion.



Scheme 1. Regioselective ring opening of the benzylated bicyclic compound **1** by various nucleophiles under acidic conditions

A 1:4 mixture of α- and β-galactofuranosides **4a** ($J_{1,2}$ = 4.3 Hz) and **4b** ($J_{1,2} < 1$ Hz) was obtained upon reaction with 3-hydroxypropionitrile. These products are potentially useful for the coupling of galactofuranose units with

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immunogenic proteins or their attachment on immobilized matrices.

Total regioselectivity was observed when compound **1** was treated with allyltrimethylsilane in acetonitrile in the presence of trimethylsilyl triflate. Only the *C*-galactofuranosides **5a** and **5b** were obtained, the stereochemistry being assigned on the basis of the NMR spectra. In the ^{1}H NMR spectrum, the anomeric proton of **5a** appears as a double triplet, being coupled to both allylic protons (J = 7.1 Hz) and to 2-H (J = 3.2 Hz). On the other hand, the signal due to the anomeric proton of **5b** is overlapped with that of 3-H, but from the 2-H signal it is possible to determine a $J_{1,2}$ of 2.4 Hz. These data are consistent with the assignment of α configuration for **5a**, and therefore *C*-galactofuranoside **5b** must be the β anomer. In this reaction, the α/β ratio is close to 1, the α-*C*-galactofuranoside **5a** being slightly favoured over the β anomer. Therefore, the attack of the intermediate oxycarbenium species proceeds without stereoselection.

It is interesting to note that the reaction of **1** with alcohols requires the presence of a protic acid such as CSA. When **1** was treated with alcohols in the presence of Lewis acids (trimethylsilyl triflate, $BF_3{\cdot}Et_2O$, $SnCl_4$), no nucleophilic attack occurred. Instead, a rearrangement of the bicyclic compound was observed. In order to clarify this point, compound **1** was allowed to react with trimethylsilyl triflate in the absence of a nucleophile and a new product was isolated from the reaction mixture in 52% yield. NMR spectroscopy allowed identification of this new compound as the *C*-galactofuranoside **6**. This compound stems from attack of the aromatic ring of the 2-*O*-benzyl group at the anomeric carbon atom. Such intramolecular *C*-arylation has previously been observed[19] in methyl 2-*O*-benzyl-D-xylofuranosides, but to the best of our knowledge this is the first example of internal participation of a 2-*O*-benzyl group in the ring opening of a 1,4-anhydro derivative.

Conclusion

In this work, we have demonstrated that the acid-catalysed ring opening of **1** invariably leads to D-galactofuranosides. This is in complete agreement with an earlier related study.[16] Activation at O-5 followed by cleavage of the C-1−O-5 bond is expected to be classically favoured by an antiperiplanar arrangement of the appropriate lone pair at O-4 with this broken bond, whereas neither orbital at O-5 is suitably oriented to allow the cleavage of the C-1−O-4 bond.

This work (i) constitutes a non-classical approach to the synthesis of galactofuranosides from galactopyranosides and (ii) may tentatively be viewed as the chemical counterpart of the unique enzymatic UDP-Gal*p*/UDP-Gal*f* interconversion, the mechanism of which is as yet unknown. We are currently trying to bridge this gap between chemistry and biochemistry in an effort to explain the so far intriguing benefit resulting from the introduction of an oxido-reductive step in the overall biosynthetic process. Such oxidation, probably involving the C-2 position,[20] may favour an S_N2 reaction at the anomeric center, thereby explaining the α selectivity observed in the mutase reaction.

Experimental Section

General Remarks: All reactions were performed in septum-sealed flasks under argon or nitrogen. Distilled anhydrous solvents were used. All reagents were used as purchased. − Silica gel (200−400 mesh) was used for column chromatography. − ^{1}H and ^{13}C NMR spectra were recorded with Bruker AC 200 or AM 400 spectrometers with samples in $CDCl_3$ solution. ^{1}H NMR assignments are based on COSY experiments. − Elemental analyses were performed at the UMYMFOR (CONICET) or the Centre Regional de Microanalyse (Jussieu, Paris). − Optical rotations were measured with a Perkin−Elmer polarimeter 241.

1,4-Anhydro Compound 1: Compound **1** was prepared according to a literature procedure.[17] − $[\alpha]_D^{20}$ = +57 (c = 1.02, $CHCl_3$); ref.[17] $[\alpha]_D^{23}$ = +57 (c = 1.0, $CHCl_3$); ref.[21] $[\alpha]_D^{25}$ = +57.6 (c = 1.0, $CHCl_3$). − The oil obtained could be crystallized from cyclohexane/ethyl acetate; m.p. 36 °C.

Disaccharide 3: A solution of **1** (35.2 mg, 0.08 mmol), alcohol **2**[18] (130.0 mg, 0.42 mmol), and CSA (80.0 mg, 0.34 mmol) in dry dichloromethane (5.0 mL) was stirred at room temperature for 4 d. The mixture was then diluted with further dichloromethane, the solution was washed with satd. aq. $NaHCO_3$ and water, and dried ($MgSO_4$). After evaporation of the solvent, the residue was purified by flash chromatography on silica gel (cyclohexane/ethyl acetate, 4:1) to give a disaccharide, which was directly acetylated (pyridine/acetic anhydride, 2:1; overnight). After evaporation of the volatiles, the residue was chromatographed on silica gel (cyclohexane/ethyl acetate, 9:1 → 5:1) to afford **3** (34.0 mg, 59% yield) as a colourless oil. − $[\alpha]_D^{20}$ = +10 (c = 0.67, $CHCl_3$). − ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.92 and 2.02 (2 s, 6 H, Ac), 3.39 (s, 3 H, OMe), 3.40−3.56 (m, 2 H, 6b-H), 3.64 (d, J = 6.0 Hz, 2 H, 6′a-H, 6′b-H), 3.69−3.80 (m, 2 H, 5-H, 6a-H), 3.85 (dd, J = 3.1 Hz, J = 7.0 Hz, 1 H, 3′-H), 3.95 (dd, J = 9.1 Hz, J = 10.1 Hz, 1 H, 3-H), 4.04 (dd, J = 1.2 Hz, J = 3.1 Hz, 1 H, 2′-H), 4.24 (dd, J = 3.6 Hz, J = 7.0 Hz, 1 H, 4′-H), 4.43−4.86 (m, 8 H, CH_2Ph), 4.79 (d, J = 3.5 Hz, 1 H, 1-H), 5.04 (dd, J = 9.1 Hz, J = 10.1 Hz, 1 H, 4-H), 5.06 (br. s, 1 H, 1′-H), 5.34 (dt, J = 3.6 Hz, J = 6.0 Hz, 1 H, 5′-H), 7.24−7.37 (m, 20 H, arom. H). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 20.7, 20.9 (Ac), 55.3 (OMe), 63.2 (C-6), 66.2 (C-2), 68.7 (C-6′), 69.3, 70.5, 70.8, 72.0, 72.3, 73.1, 74.8, 78.0, 79.6, 83.2 (C-3, C-4, C-5, C-3′, C-4′, C-5′, *C*H_2Ph), 87.8 (C-2′), 98.4 (C-1), 106.8 (C-1′), 127.5−128.3 and 137.5−138.0 (arom. C), 169.5 (C=O). − $C_{45}H_{51}N_3O_{12}\cdot H_2O$ (843.9): calcd. C 64.05, H 6.33; found C 63.95, H 6.29.

Glycosides 4a and 4b: A solution of **1** (131.7 mg, 0.30 mmol), 3-hydroxypropionitrile (125 μL, 1.82 mmol), and CSA (212.0 mg, 0.91 mmol) in dry dichloromethane (10.0 mL) was stirred under argon at room temperature for 3 d. The mixture was then diluted with further dichloromethane and the solution was washed with satd. aq. $NaHCO_3$ and water. After concentration, flash chromatography of the residue on silica gel (cyclohexane/ethyl acetate, 3:1 → 3:2) sequentially furnished the glycosides **4a** and **4b** (141.3 mg, 92% yield).

α-Glycoside 4a: Minor isomer, 27.7 mg. − $[\alpha]_D^{20}$ = +21 (c = 0.9, $CHCl_3$). − ^{1}H NMR (250 MHz, $CDCl_3$): δ = 2.51−2.67 (m, 3 H, OH, CH_2CN), 3.44−3.50 (m, 2 H, 6a-H, 6b-H), 3.55−3.91 (m, 3

H, 5-H, CH_2CH$_2$CN), 3.98 (dd, J = 5.1 Hz, J = 6.5 Hz, 1 H, 4-H), 4.10 (dd, J = 4.3 Hz, J = 7.2 Hz, 1 H, 2-H), 4.30 (dd, J = 6.5 Hz, J = 7.2 Hz, 1 H, 3-H), 4.50−4.80 (m, 6 H, CH_2Ph), 4.90 (d, J = 4.3 Hz, 1 H, 1-H), 7.20−7.45 (m, 15 H, arom. H). − ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 18.7 (*C*H$_2$CN), 62.9 (O*C*H$_2$CH$_2$CN), 70.6, 70.9, 72.3, 72.6, 73.2 (C-5, C-6, *C*H$_2$Ph), 80.7, 81.0, 84.0 (C-2, C-3, C-4), 100.7 (C-1), 117.5 (CN), 127.5−128.3 and 137.2−137.7 (arom. C).

β-Glycoside 4b: Major isomer, 113.6 mg. − $[\alpha]_D^{20}$ = −50 (c = 1.1, $CHCl_3$). − ^{1}H NMR (250 MHz, $CDCl_3$): δ = 2.60 (m, 2 H, CH_2CN), 3.52−3.71 (m, 3 H, 6a-H, 6b-H, CH_bCH$_2$CN), 3.76−3.98 (m, 2 H, 5-H, CH_aCH$_2$CN), 4.03−4.20 (m, 3 H, 2-H, 3-H, 4-H), 4.44−4.61 (m, 6 H, CH_2Ph), 5.16 (s, 1 H, 1-H), 7.20−7.45 (m, 15 H, arom. H). − ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 19.0 (*C*H$_2$CN), 62.3 (O*C*H$_2$CH$_2$CN), 69.9 (C-6), 71.4, 72.2, 72.3, 73.4 (C-5, CH_2Ph), 81.9, 83.1, 87.6 (C-2, C-3, C-4), 106.6 (C-1), 117.7 (CN), 127.7−128.5 and 137.2−137.9 (arom. C). − FAB MS; m/z: 526 [M$^+$ + Na]. − $C_{30}H_{33}NO_6$ (503.6): calcd. C 71.55, H 6.61, N 2.78; found C 71.42, H 6.74, N 2.71.

***C*-Glycosides 5a and 5b:** A solution of **1** (17.1 mg, 0.04 mmol) in dry acetonitrile (1.0 mL) was cooled to −40 °C under argon. Allyltrimethylsilane (40 μL, 0.20 mmol) and trimethylsilyl triflate (10 μL, 0.04 mmol) were added, and the mixture was allowed to warm slowly to room temperature. Satd. aq. $NaHCO_3$ was then added, and the resulting mixture was extracted with dichloromethane. The combined organic extracts were washed with satd. aq. NaCl, dried ($MgSO_4$), and concentrated to a syrup. Flash chromatography on silica gel (cyclohexane/ethyl acetate, 9:1 → 3:1) sequentially furnished the *C*-glycosides **5a** and **5b** (15.9 mg, 85% yield).

α-*C*-Glycoside 5a: Major isomer, 9.0 mg. − $[\alpha]_D^{20}$ = −22 (c = 0.86, $CHCl_3$). − ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.46−2.59 (m, 2 H, CH_2CH=CH$_2$), 2.88 (d, J = 6.1 Hz, 1 H, OH), 3.54−3.61 (m, 2 H, 6a-H, 6b-H), 3.83 (d, J = 3.2 Hz, 1 H, 2-H), 3.92 (m, 1 H, 5-H), 4.07 (dt, J = 3.2 Hz, J = 7.1 Hz, 1 H, 1-H), 4.11 (dd, J = 2.6 Hz, J = 3.3 Hz, 1 H, 4-H), 4.13 (d, J = 2.6 Hz, 1 H, 3-H), 4.41−4.63 (m, 6 H, CH_2Ph), 5.13 (m, 2 H, =CH$_2$), 5.83 (m, 1 H, CH=), 7.20−7.45 (m, 15 H, arom. H). − ^{13}C NMR (100 MHz, $CDCl_3$): δ = 29.7 (*C*H$_2$CH=CH$_2$), 70.7 (C-5), 71.4, 71.6, 71.8, 73.3 (C-6, *C*H$_2$Ph), 80.9, 81.9, 83.2, 84.1 (C-1, C-2, C-3, C-4), 117.1 (=CH$_2$), 127.6−128.5 (arom. C), 134.6 (CH=), 137.3−138.2 (arom. C).

β-*C*-Glycoside 5b: Minor isomer, 6.9 mg. − $[\alpha]_D^{20}$ = −28 (c = 0.6, $CHCl_3$). − ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.42−2.46 (m, 2 H, CH_2CH=CH$_2$), 2.72 (d, J = 5.0 Hz, 1 H, OH), 3.56−3.64 (m, 2 H, 6a-H, 6b-H), 3.93 (dd, J = 2.4 Hz, J = 2.7 Hz, 1 H, 2-H), 3.95 (m, 1 H, 5-H), 4.16 (dd, J = 4.0 Hz, J = 4.2 Hz, 1 H, 4-H), 4.17−4.21 (m, 2 H, 1-H, 3-H), 4.53−4.62 (m, 6 H, CH_2Ph), 5.12 (m, 2 H, =CH$_2$), 5.83 (m, 1 H, CH=), 7.20−7.45 (m, 15 H, arom. H). − ^{13}C NMR (100 MHz, $CDCl_3$): δ = 29.7 (*C*H$_2$CH=CH$_2$), 70.4 (C-5), 71.5, 71.6, 72.0, 73.4 (C-6, CH_2Ph), 82.7, 83.0, 84.7, 86.0 (C-1, C-2, C-3, C-4), 117.6 (=CH$_2$), 127.6−128.5 (arom. C), 134.1 (CH=), 137.5−138.0 (arom. C). − CI MS: m/z = 492 [M + NH_4^+], 475 [M + H$^+$]. − $C_{30}H_{34}O_5$ (474.6): calcd. C 75.92, H 7.22; found C 75.83, H 7.82.

***C*-Glycoside 6:** A solution of **1** (100.0 mg, 0.23 mmol) in dry dichloromethane (15.0 mL) was cooled to −20 °C under argon. Trimethylsilyl triflate (83 μL, 0.46 mmol) was added, and the mixture was allowed to warm slowly to 0 °C. After 3 h at this temperature, diisopropylethylamine (0.3 mL) was added and the mixture was concentrated to a syrup. Flash chromatography on silica gel (cyclohexane/ethyl acetate, 3:1) gave the *C*-glycoside **6** (52.3 mg, 52% yield). − $[\alpha]_D^{20}$ = −19 (c = 1.0, $CHCl_3$). − ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.55−3.63 (m, 3 H, 6a-H, 6b-H, OH), 3.97 (dt, J = 4.2 Hz, J = 6.0 Hz, 1 H, 5-H), 4.17 (t, J = 4.2 Hz, 1 H, 4-H), 4.20 (d, J = 4.2 Hz, 1 H, 3-H), 4.22 (d, J = 3.0 Hz, 1 H, 2-H), 4.57−4.73 (m, 5 H, CHPh), 4.78 (d, J = 3.0 Hz, 1 H, 1-H), 4.84 (d, J = 15.0 Hz, 1 H, CHPh), 7.05−7.50 (m, 14 H, arom. H). − ^{13}C NMR (50 MHz, $CDCl_3$): δ = 67.2, 71.5, 72.5, 73.0, 73.5 (C-4, C-5, C-6, CH_2Ph), 81.1, 83.9, 86.0 (C-1, C-2, C-3), 127.0−128.5 (arom. C), 137.8−138.2 (arom. C). − HRMS (CI): $C_{27}H_{29}O_5$ (433.5): calcd. m/z 433.2015; found 433.2012.

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