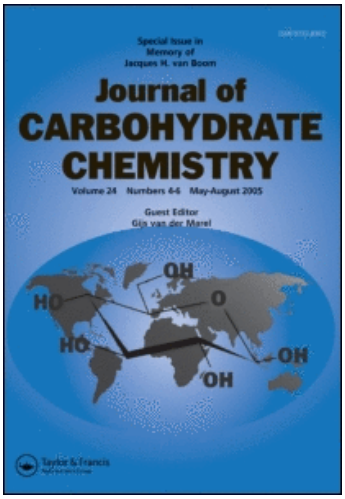


Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

ENZYMATIC *O*-GALACTOSYLATION OF SELECTED FREE AS WELL AS PARTIALLY PROTECTED KETOHEXOSES

Brigitte Eder[a]; Inge Lundt[b]; Arnold E. Stütz[a]; Tanja M. Wrodnigg[a]
[a] Glycogroup, Institut für Organische Chemie, Technische Universität Graz, Graz, Austria [b] Department of Chemistry, Laboratory of Organic Chemistry, Technical University of Denmark, Lyngby, Denmark

Online publication date: 30 November 2001

To cite this Article Eder, Brigitte , Lundt, Inge , Stütz, Arnold E. and Wrodnigg, Tanja M.(2001) 'ENZYMATIC *O*-GALACTOSYLATION OF SELECTED FREE AS WELL AS PARTIALLY PROTECTED KETOHEXOSES', Journal of Carbohydrate Chemistry, 20: 7, 647 — 657
To link to this Article: DOI: 10.1081/CAR-100108279
URL: http://dx.doi.org/10.1081/CAR-100108279

J. CARBOHYDRATE CHEMISTRY, 20(7&8), 647–657 (2001)

ENZYMATIC *O*-GALACTOSYLATION OF SELECTED FREE AS WELL AS PARTIALLY PROTECTED KETOHEXOSES

Brigitte Eder,[1] Inge Lundt,[2] Arnold E. Stütz,[1] and Tanja M. Wrodnigg*

[1]Glycogroup, Institut für Organische Chemie, Technische Universität Graz, Stremayrgasse 16, A-8010 Graz, Austria
[2]Technical University of Denmark, Department of Chemistry, Laboratory of Organic Chemistry, Building 201, DK-2800 Lyngby, Denmark

Dedicated to Prof. Dr. Joachim Thiem on the happy occasion of his 60th birthday.

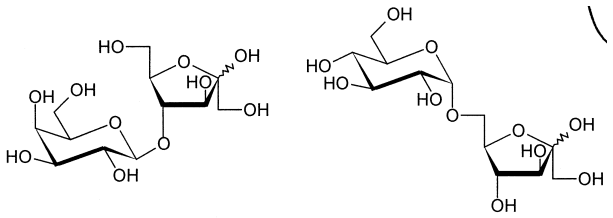
ABSTRACT

A range of partially protected ketoses was *O*-galactosylated with β-galactosidase from *Aspergillus oryzae* employing (4-nitro)phenyl β-D-galactopyranoside as the donor. This enzyme also accepted free D-tagatose as a substrate.

INTRODUCTION

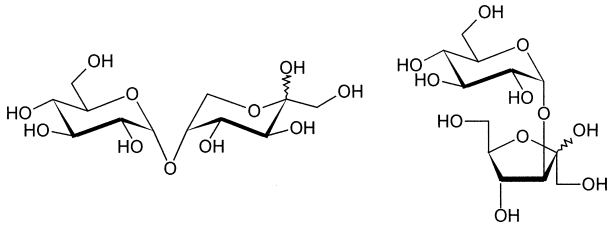
Despite all efforts ketose chemistry, including synthetic applications, is by far not as advanced as the chemistry of aldoses. Even in the area of the most common ketohexoses including D-fructose, L-sorbose or D-tagatose, synthetically useful transformations are relatively scarce as compared to those for the corresponding aldohexose counterparts, for example, D-glucose or D-galactose. In particular, little is known about the synthesis and synthetic applications of open-chain ketoses[1,2] as well as the chemistry of di- and oligosaccharides containing D-fructose or other ketoses at the reducing end.[1]

Nevertheless, several disaccharides with D-fructose at the reducing end are the subject of commercial interest and exhibit a wide range of useful properties.



1 **2**

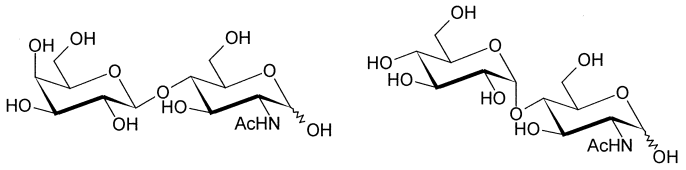
For example, lactulose (4-*O*-β-D-galactopyranosyl-D-fructose **1**) is employed as a mild agent for gastrointestinal protection and ulcer prevention.[3] Palatinose or isomaltulose (6-*O*-α-D-glucopyranosyl-D-fructofuranose **2**) is a biocatalytically obtained bulk product as a precursor to low calorie sweeteners.[1]



3 **4**

Other representative fructo disaccharides are leucrose (5-*O*-α-D-glucopyranosyl-D-fructopyranose **3**),[4] as well as turanose (3-*O*-α-D-glucopyranosyl-D-fructose **4**), the latter being a constituent of melezitose, a highly crystalline non-reducing trisaccharide found in certain types of honey.[5]

In context with our interest in preparatively useful applications of the Heyns rearrangement, we recently reported a very short and efficient synthesis of *N*-acetyl-D-lactosamine (**5**) from lactulose (**1**).[7]

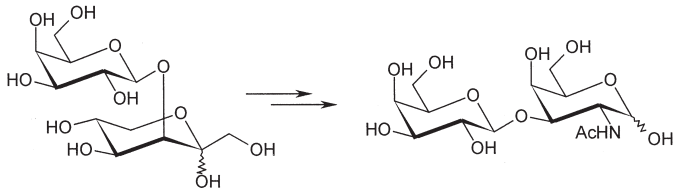


5 **6**

A range of other *O*-glycosylated D-glucosamine derivatives such as *N*-acetyl-D-maltosamine (**6**) could also be obtained from the corresponding *O*-aldopyranosylketoses by this approach, thereby demonstrating its fairly general applicability to disaccharides with D-fructose at the reducing end.

In order to extend our knowledge on this preparatively interesting reaction sequence additional examples of *O*-glycosylated ketoses were deemed to be interest-

ing targets, in particular D-fructose as well as D-tagatose derivatives with β-D-galactopyranosyl substituents.



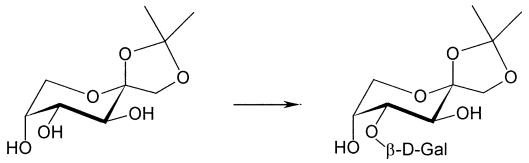
7 8

For example, 3-*O*-β-D-galactopyranosyl-D-tagatose (**7**), if made easily available, could be considered a valuable starting material for the preparation of *N*-acetyl-3-*O*-β-D-galactopyranosyl-D-galactosamine (**8**), a constituent of the tumor marker Thomsen-Friedenreich (TF-) antigen.[8]

For short and efficient approaches to the desired disaccharides, reasonably well-established β-galactosidase mediated glycosylation appeared to be an interesting method of choice.[9] Despite the fact that enzymatic *O*-glycosylation was one of the early synthetic applications of sugar-related biocatalysis and has remained one of the most frequently employed, ketoses have rarely been used as substrates.[10] However, in one example, 1-*O*-α-D-glucopyranosyl-D-tagatose was prepared by *O*-glucosylation of free tagatose employing the *Protaminobacter rubrum* enzyme with 4-nitrophenyl α-D-glucopyranoside as the donor.[11] Wild-type *Kluyveromyces lactis* β-galactosidase was found to transfer galactosyl units from lactose. Products identified included fructose derivatives β-galactosylated at positions 1 or 6.[12] A further example is the synthesis of a 1→6 connected β-D-galactosyl-D-fructose with immobilised *E. coli* β-galactosidase as well as with the *A. oryzae* enzyme.[13] *O*-Galactosylations at other positions were also found to be occurring under the conditions employed in this study. The same workers exploited a *Saccharomyces* α-glucosidase to obtain *O*-α-D-glucosylated fructoses.[14] Interestingly, fructose was also found to be an acceptor for the endo-β-*N*-acetylglucosaminidase mediated transfer of a $Man_9GlcNAc$ decasaccharide.[15]

RESULTS AND DISCUSSION

In order to have positions 1 and 2 of a suitable ketose available for subsequent reactions such as the Heyns rearrangement, 1,2-*O*-protected ketoses were our substrates of choice. For initial feasibility experiments, 1,2-*O*-isopropylidene-β-D-fructopyranose (**9**),[16] obtained from controlled hydrolysis of 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose[17], as well as 2,3-*O*-isopropylidene-α-L-sorbofuranose (**11**), obtained from partial hydrolysis of 2,3:4,6-di-*O*-isopropylidene-α-L-sorbofuranose,[18,19] were chosen due to their easy availability.

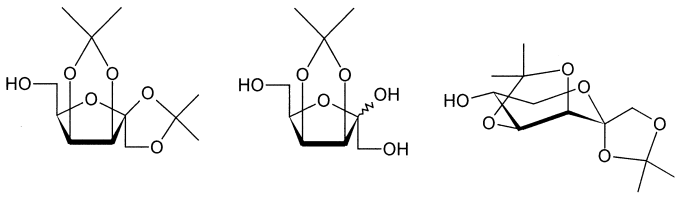


9 **10**

11 **12**

Upon reaction with 4-nitrophenyl β-D-galactopyranoside in the presence of β-galactosidase from *Aspergillus oryzae*, galactosyl transfer occurred to O-4 of the partially protected fructopyranose **9** to afford 1,2-*O*-isopropylidene protected lactulose derivative **10** in 63% yield based on the donor. By the same protocol, sorbofuranose derivative **12** was obtained from compound **11** in 50% yield.

These encouraging results prompted our attempts to prepare 1,2-*O*-protected tagatose as a precursor to (eventually *O*-galactosylated) D-galactosamine derivatives.

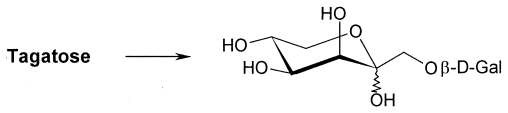


13 **14** **15**

Despite intense efforts, we were not able to obtain the desired compound from controlled hydrolysis of 1,2:3,4-di-*O*-isopropylidene-α-D-tagatofuranose (**13**),[20] the main product of acid catalysed reactions of D-tagatose with acetone. In all cases, the 3,4-*O*-isopropylidene group proved to be equally stable as the 1,2-*O*-protecting group leading to mixtures of products. 1,2:3,4-Di-*O*-isopropylidene-D-tagatopyranose (**15**) was only obtained in preparatively insignificant amounts as a side product from the *O*-isopropylidenation reaction.

Subsequent attempts of *O*-galactosylation of free D-tagatose were met by, albeit limited, success as per-*O*-acetylation of the product mixture containing the disaccharide **16** gave, after chromatography, a 10% yield of the 1-*O*-galactosylated octa-*O*-acetyl derivative **16a** (see Experimental).

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

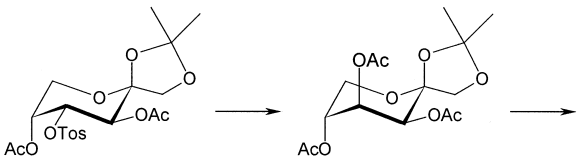


16

However, although this is an interesting finding, this product is unsuitable for the Heyns rearrangement.

As an alternative to a controlled hydrolysis procedure, we envisaged a simple synthesis of 1,2-*O*-protected D-tagatose from the corresponding D-fructose or L-sorbose derivatives.

Following work reported by Buchanan and co-workers,[21] 3,5-di-*O*-acetyl-1,2-*O*-isopropylidene-4-*O*-tosyl-β-D-fructopyranose (**17**) was prepared from compound **9** in good yield.



17 **18**

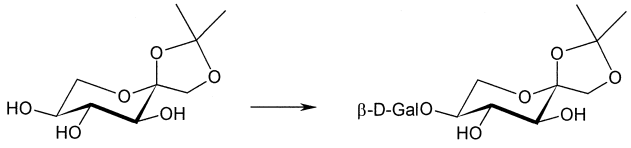
19 **20**

Treatment of **17** with sodium acetate in DMF at 140 °C furnished the corresponding 3,4,5-tri-*O*-acetyl-D-tagatose derivative **18** in 60% yield. This was subsequently deprotected employing Zemplen conditions to give 1,2-*O*-isopropylidene-β-D-tagatopyranose (**19**).

This simple approach represents a new access to 1,2-*O*-isopropylidene-D-tagatopyranose.

This substrate was accepted by the β-galactosidase furnishing a low yield of 8–10% of the corresponding 5-*O*-β-D-galactopyranosylketose **20**.

In order to gain insight into the reactivities of the more common 1,2-*O*-isopropylidene protected ketohexoses, the respective L-sorbopyranose (**21**)[22] was also examined as a substrate.



21 **22**

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Gratifyingly, this compound was accepted by the enzyme to give the 5-*O*-galactosylated ketodisaccharide **22** in 15% yield.

In conclusion, it was found that a range of partially protected ketohexopyranoses as well as an example of a free ketose and a selected 2,3-*O*-protected furanoid system were accepted by the β-galactosidase from *Aspergillus oryzae*. Isolated yields of *O*-galactosyl ketoses ranged from "preparatively unacceptable" to "fair" but unfortunately the desired 1→3 connections could not be achieved with the substrates reported here and the enzyme employed in this study. At this point, it is interesting to note that analogous experiments employing two different batches of β-glucosidase from almonds and 4-nitrophenyl β-D-glucopyranoside as the donor did not lead to any detectable disaccharide formation with the ketoses probed in this study.

EXPERIMENTAL

General Methods. TLC was performed on precoated aluminum plates (Merck 5554) employing 5% vanillin/sulfuric acid as well as ceric ammonium molybdate as staining agents. For column chromatography, silica gel 60, 230–400 mesh (Merck 9385), was used. ^{1}H NMR spectra were recorded on a Varian INOVA 500 operating at 499.925 MHz. ^{13}C NMR spectra were recorded at 75.47 or 50.29 MHz. Residual non-deuterated solvent was used as internal standard for determination of chemical shifts. Signals of protecting groups (*O*-isopropylidene, *O*-acetyl) were found in the expected regions and are not listed explicitly. MS was conducted on a HP 1100 series MSD, Hewlett Packard with methanol as the solvent. Samples were dissolved in acetonitrile or acetonitrile/water mixtures. The scan mode for positive ions (mass range 100–1000 D) was employed varying the fragmentation voltage from 50 to 250 V with best molecular peaks observed at 150 V.

3,4,5-Tri-*O*-acetyl-1,2-*O*-isopropylidene-β-D-tagatopyranose (18). To a 6% solution of 3,5-di-*O*-acetyl-1,2-*O*-isopropylidene-4-*O*-tosyl-D-fructopyranose (**17**,[21] 6.20 g, 13.5 mmol) in DMF, sodium acetate (43.2 g, 40 equiv) was added and the mixture was stirred at 140 °C for 10–14 d (TLC: cyclohexane/ethyl acetate 1:1, v/v). Solids were removed by filtration, the solvent was evaporated under reduced pressure and the remaining oily residue was treated with pyridine (80 mL) and acetic anhydride (40 mL). Methanol was added after 3 h and the reaction mixture was concentrated under reduced pressure. After addition of dichloromethane to the residue, the organic layer was consecutively washed with 5% aqueous HCl and satd aqueous sodium bicarbonate and dried (Na_2SO_4). After filtration, the solvent was removed and the residue was subjected to chromatography to yield compound **18** (3.90 g, 83%): $[\alpha]_D^{20}$ −105.2° (*c* 1.5, dichloromethane); ^{13}C NMR ($CDCl_3$) δ 103.1 (C-2), 72.7 (C-1), 69.6, 67.1, 66.1 (C-3, C-4, C-5), 60.5 (C-6). ^{1}H NMR δ 5.27 (dd, 1 H, $J_{4,5}$ 3.5 Hz, H-4), 5.15 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-3), 4.85 (m, 1 H, H-5), 4.28 (dd, 1 H, $J_{5,6a}$ 1.3 Hz, $J_{6a,6b}$ 13.2 Hz, H-6a), 3.96 (d, 1H, $J_{1a,1b}$ 9 Hz, H-1a), 3.88 (d, 1 H, *J* 9 Hz, H-1b) 3.69 (d, 1 H, H-6b).

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Anal. Calcd for $C_{12}H_{22}O_9$ (346.37): C, 52.02; H, 6.41. Found: C, 51.89; H, 6.45.

1,2-*O*-Isopropylidene-β-D-tagatopyranose (19). To a 5% methanolic solution of compound **18** (3.87 g, 10.9 mmol), methanolic sodium methoxide (1M, 0.3 mL) was added, and the mixture was kept at ambient temperature for 1 h. Ion exchange resin (IR 120, pre-washed with dry methanol) was added. After filtration, the solution was concentrated under reduced pressure. The crude product was purified by chromatography (cyclohexane/ethyl acetate 1:10, v/v) to furnish compound **19** (1.32 g, 55%): $[\alpha]_D^{20}$ −83.0° (*c* 2.3, MeOH); ^{13}C NMR (CD_3OD) δ 105.7 (C-2), 72.2, 71.3, 70.2, 64.4 (C-1, C-3, C-4, C-5), 61.1 (C-6). ^{1}H NMR δ 4,15 (d, 1 H, $J_{6a,6b}$ 12,5 Hz, H-6a), 4,10 (d, 1 H, $J_{1a,1b}$ 8.2 Hz, H-1a), 3.89 (d, 1 H, H-1b), 3.87 (m, 1 H, H-4), 3.77 (d, 1 H, $J_{3,4}$ 3.5 Hz, H-3), 3.72 (m, 1 H, H-5), 3.48 (d, 1 H, H-6b).

Anal. Calcd for $C_9H_{16}O_6$ (220.25): C, 49.08; H, 7.34. Found: C, 49.01; H, 7.40.

General Procedure for the β-D-Galactosidase Catalysed Glycosylation Reaction. To an 8% solution of the respective acceptor (2.5 equiv) in phosphate buffer (50 mM; K_2HPO_4/KH_2PO_4), β-D-galactosidase (EC 3.2.1.23, *Aspergillus oryzae*; Sigma; Specific activity: 5.1 units/mg enzyme; 0.125 units/mg of donor) was added at pH 5. To this mixture, 4-nitrophenyl β-D-galactopyranoside (1 equiv) was added over a period of 2–4 h. After complete reaction of the donor, the solution was co-distilled with toluene several times and concentrated under reduced pressure. The remaining material was chromatographed in the respective solvent system given below.

4-*O*-(β-D-Galactopyranosyl)-1,2-*O*-isopropylidene-β-D-fructopyranose (10) and 3,5,2′,3′,4′,6′-Hexa-*O*-acetyl-4-*O*-(β-D-galactopyranosyl)-1,2-*O*-isopropylidene-β-D-fructopyranose (10a). Following the General Procedure, from the reaction of 1,2-*O*-isopropylidene-β-D-fructopyranose (**9**, 5.0 g, 22.7 mmol), disaccharide **10** (307 mg, 63% by conversion of **9**) was isolated ($CHCl_3$/MeOH 10:1 to 0:1, v/v). 4.72 g of starting monosaccaride **9** were recovered. For **10**: $[\alpha]_D^{20}$ −52.6° (*c* 3.1, DMSO); ^{13}C NMR (DMSO-d_6) δ 106.1 (C-2), 100.9 (C-1′), 78.0 (C-4), 75.5 (C-5′), 73.1 (C-3′), 71.0 (C-2′), 70.6 (C-4′), 68.2 (C-5), 66.1 (C-3), 65.6 (C-1), 63.5 (C-6), 60.4 (C-6′). ^{1}H NMR δ 5.20 (d, 1 H, *J* 2.7 Hz), 4.81 (d, 1 H, *J* 4.7 Hz), 4.70−4.61 (m, 2 H), 4.53−4.46 (m, 2 H), 4.30 (d, 1 H, *J* 6.8 Hz), 4.05 (d, 1 H, *J* 8.3 Hz), 3.82−3.34 (m, 6 H). Due to the poor resolution of the ^{1}H NMR spectra, a sample of **10** was conventionally per-*O*-acetylated (**10a**): $[\alpha]_D^{20}$ −70.1° (*c* 2.1, acetone); ^{13}C NMR ($CDCl_3$) δ 104.7 (C-2), 100.3 (C-1′), 75.2 (C-4), 72.0 (C-1), 71.2 (C-3′), 70.7 (C-5′), 69.0 (C-2′), 68.9 (C-5), 67.6 (C-3), 67.0 (C-4′), 62.5 (C-6), 61.2 (C-6′). ^{1}H NMR δ 5.35 (d, 1 H, $J_{3',4'}$ 3.0 Hz, H-4′), 5.29 (d, 1 H, $J_{3,4}$ 10.2 Hz, H-3), 5.19 (m, 1 H, H-5), 5.07 (dd, 1 H, $J_{1',2'}$ 7.4 Hz, $J_{2',3'}$ 10.4 Hz, H-2′), 4.95 (dd, 1 H, H-3′), 4.50 (d, 1 H, H-1′), 4.17 (dd, 1 H, $J_{5',6'}$ 6.5 Hz, $J_{6'a,6'b}$ 11.1 Hz, H-6′a), 4.11 (m, 1 H, H-4), 4.07 (dd, 1 H, $J_{5'6'b}$ 5.6

Hz, H-6′b), 3.95 (d, 1 H, $J_{6a,6b}$ 13.1 Hz, H-6a), 3.97−3.88 (m, 2 H, H-1a, H-5′), 3.92 (d, 1 H, $J_{1a,1b}$ 11.6 Hz, H-1b), 3.74 (dd, 1 H, $J_{5,6}$ 1.9 Hz, H-6b). MS: *m/z* 657.3 ($M+Na^+$).

Anal. Calcd for $C_{27}H_{38}O_{17}$ (634.67): C, 51.10; H, 6.05. Found: C, 51.02; H, 6.12.

6-*O*-(β-D-Galactopyranosyl-2,3-*O*-isopropylidene-α-L-sorbofuranose (12) and 1,4,2′,3′,4′,6′-hexa-*O*-acetyl-6-*O*-(β-D-galactopyranosyl-2,3-*O*-isopropylidene-α-L-sorbofuranose (12a). 2,3-*O*-Isopropylidene-α-L-sorbofuranose (**11**, 5.0 g, 22.7 mmol) was subjected to the General Procedure, furnishing disaccharide **12** (640 mg, 50%, calculated by conversion of **11**). 4.27 g of sorbofuranose **11** were recovered. Due to difficulties with purification, compound **12** was characterised as the hexa-*O*-acetyl derivative **12a**: $[\alpha]_D^{20}$ −1.0° (*c* 4.3, dichloromethane); ^{13}C NMR ($CDCl_3$) δ 112.3 (C-2), 101.2 (C-1′), 83.4 (C-3), 77.8 (C-5), 75.9 (C-4), 70.9 (C-3′), 70.8 (C-5′), 68.5 (C-2′), 67.0 (C-4′), 66.0 (C-6), 63.1 (C-1), 61.2 (C-6′). ^1H NMR δ 5.37 (d, 1 H, $J_{3',4'}$ 3.2 Hz, H-4′), 5.22 (d, 1 H, $J_{4,5}$ 2.4 Hz, H-4), 5.17 (dd, 1 H, $J_{1',2'}$ 8.3 Hz, $J_{2',3'}$ 10.3 Hz, H-2′), 4.99 (dd, 1 H, H-3′), 4.56 (m, 1 H, H-5), 4.44 (d, 1 H, H-1′), 4.39 (s, 1 H, H-3), 4.32 (d, 1 H, $J_{1a,1b}$ 12.2 Hz, H-1a), 4.18 (d, 1 H, H-1b), 4.15−4.10 (m, 2 H, H-6′a, H-6′b), 4.04 (dd, 1 H, $J_{6a,6b}$ 10.2 Hz, H-6a), 3.88 (dd, $J_{5'6'a}$ 6.8 Hz, $J_{5'6'b}$ 6.8 Hz, H-5′), 3.62 (dd, 1 H, H-6b). MS: *m/z* 657.3 ($M+Na^+$).

Anal. Calcd for $C_{27}H_{38}O_{17}$ (634.67): C, 51.10; H, 6.05. Found: C, 51.15; H, 6.11.

2,3,4,5,2′,3′,4′,6′-Octa-*O*-acetyl-1-*O*-(β-D-Galactopyranosyl)-D-tagatopyranose (16a). Applying the General Procedure to free D-tagatose (2.5 g, 13.9 mmol) led to a mixture of compounds containing disaccharide **16**. Purification by per-*O*-acetylation and chromatography (cyclohexane/ethyl acetate 2:1, v/v) gave the octa-*O*-acetyl derivative **16a** (750 mg, 8.6%): main anomer: $[\alpha]_D^{20}$ −2.3° (*c* 1.6, dichloromethane); ^{13}C NMR ($CDCl_3$) δ 101.6 (C-1′), 95.8 (C-2), 71.8 (C-1), 71.2 (C-5′), 70.8 (C-3′), 70.3 (C-3), 69.4 (C-4), 68.8 (C-2′), 67.0 (C-4′), 66.6 (C-5), 61.5 (C-6′), 60.4 (C-6).). ^1H NMR δ 5.36 (dd, 1 H, $J_{3,4}$ 3.4 Hz, $J_{4,5}$ 10.3 Hz, H-4), 5.31 (m, 1 H, H-4′), 5.27 (d, 1 H, H-3), 5.17−5.09 (m, 2 H, H-5, H-2′), 4.94 (dd, 1 H, $J_{2',3'}$ 10.3 Hz, $J_{3',4'}$ 3.4 Hz, H-3′), 4.40 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.10−4.03 (m, 2 H, H-6′a, H-6′b), 3.89−3.79 (m, 2 H, H-6a, H-5′), 3.75 (d, 1 H, $J_{1a,1b}$ 10.7 Hz, H-1a), 3.70 (d, 1 H, $J_{6a,6b}$ 10.7 Hz, H-6b), 3.50 (d, 1 H, H-1b). MS: *m/z* 701.2 ($M+Na^+$).

Anal. Calcd for $C_{28}H_{38}O_{19}$ (678.67): C, 49.55; H, 5.65. Found: C, 49.46; H, 5.70.

5-*O*-(β-D-Galactopyranosyl)-1,2-*O*-isopropylidene-β-D-tagatopyranose (20) and 3,4,2′,3′,4′,6′-Hexa-*O*-acetyl-5-*O*-(β-D-galactopyranosyl)-1,2-*O*-isopropylidene-β-D-tagatopyranose (20a). Following the General Procedure, compound **19** (400 mg, 1.82 mmol) was *O*-galactosylated to yield, after chro-

matography ($CHCl_3$/MeOH 10:1 to 7:1, v/v), disaccharide **20** (13 mg, 7.7% by conversion). 303 mg of tagatose derivative **19** were recovered. For **20**: $[\alpha]_D^{20}-49.5°$ (*c* 0.5, MeOH); ^{13}C NMR (CD_3OD) δ 105.7 (C-2), 103.5 (C-1′), 78.1, 75.7, 73.6, 72.2, 71.3, 70.0, 69.1, 64.5, 61.4, 59.0 (C-1, C-3, C-4, C-5, C-6, C-2′, C-3′, C-4′, C-5′, C-6′). ^1H NMR δ 4.35 (d, 1 H), 4.18−4.07 (m, 3 H), 3.90−3.27 (m, 10 H).

Due to the poor resolution of the ^1H NMR spectra of the hexahydroxydisaccharide, compound **20** was per-*O*-acetylated to **20a**: $[\alpha]_D^{20}$ −50.7° (*c* 1.6, dichloromethane); ^{13}C NMR ($CDCl_3$) δ 103.4 (C-2), 100.2 (C-1′), 74.9 (C-5), 72.7 (C-1), 71.1 (C-5′), 71.0 (C-3′), 68.9 (C-2′), 68.2 (C-4), 67.1 (C-4′), 65.6 (C-3), 61.5 (C-6′), 59.8 (C-6). ^1H NMR δ 5.44 (m, 1 H, H-4), 5.38 (d, 1 H, H-4′), 5.24 (dd, 1 H, $J_{2',3'}$ 10.3 Hz, $J_{1',2'}$ 7.9 Hz, H-2′), 5.16 (d, 1 H, $J_{3,4}$ 3.7 Hz, H-3), 5.02 (dd, 1 H, $J_{3',4'}$ 3.4 Hz, H-3′), 4.60 (d, 1 H, H-1′), 4.21−4.15 (m, 2 H, H-6a, H-6′a), 4.12 (dd, 1 H, $J_{5',6'b}$ 6.8 Hz, $J_{6'a,6'b}$ 11.2 Hz, H-6′b), 3.91 (d, 1 H, $J_{1a,1b}$ 9.1 Hz, H-1a), 3.91 (m, 1 H, H-5′), 3.87 (d, 1 H, H-1b), 3.81 (m, 1 H, H-5), 3.67 (d, 1 H, $J_{6a,6b}$ 12.0 Hz, H-6b).

Anal. Calcd for $C_{27}H_{38}O_{17}$ (634.67): C, 51.10; H, 6.05. Found: C, 50.99; H, 6.11.

3,4,2′,3′,4′,6′-Hexa-*O*-acetyl-5-*O*-(β-D-galactopyranosyl)-1,2-*O*-isopropylidene-α-L-sorbopyranose (22a). 1,2-*O*-Isopropylidene-α-L-sorbopyranose (**21**, 200 mg, 0.91 mmol) was reacted according to the General Procedure to give, after chromatography ($CHCl_3$/MeOH 10:1 to 7:1, v/v), disaccharide **22** (25 mg, 17.6% by recovery of **21**) as a colourless syrup.

118 mg of starting material **21** were recovered.

To allow for better resolution of the NMR spectra, compound **22** was per-*O*-acetylated (**22a**): $[\alpha]_D^{20}-35.7°$ (*c* 1.4, dichloromethane); ^{13}C NMR ($CDCl_3$) δ 103.8 (C-1′), 101.9 (C-2), 75.9 (C-5), 73.2 (C-4), 71.8, 71.1, 71.0 (C-1, C-3′, C-5′), 69.6 (C-3), 68.7 (C-2′), 67.2 (C-4′), 62.3 (C-6), 61.5 (C-6′). ^1H NMR δ 5.39−5.36 (m, 2 H, H-4, H-4′), 5.15 (dd, 1 H, $J_{1',2'}$ 7.8 Hz, $J_{2',3'}$ 10.3 Hz, H-2′), 4.96 (dd, 1 H, $J_{3',4'}$ 3.4 Hz, H-3′), 4.94 (d, 1 H, $J_{3,4}$ 10.3 Hz, H-3), 4.55 (d, 1 H, H-1′), 4.17 (dd, 1 H, $J_{5',6'a}$ 6.3 Hz, $J_{6'a,6'b}$ 11.2 Hz, H-6′a), 4.11 (dd, 1 H, $J_{5',6'b}$ 6.3 Hz, H-6′b), 3.93 (d, 1 H, $J_{1a,1b}$ 9.3 Hz, H-1a), 3.93−3.82 (m, 4 H, H-5, H-6a, H-6b, H-5′), 3.82 (d, 1 H, H-1b). MS: *m/z* 657.3 ($M+Na^+$).

Anal. Calcd for $C_{27}H_{38}O_{17}$ (634.67): C, 51.10; H, 6.05. Found: C, 50.98; H, 6.01.

ACKNOWLEDGMENT

MS measurements by Dr. M. Murkovic are appreciated. Studies were kindly supported by the Austrian *Fonds zur Förderung der wissenschaftlichen Forschung (FWF)*, Project P-13593 CHE. T. M. W. thanks the FWF for a Hertha Firnberg Fellowship (T-18 CHE).

REFERENCES

1. Lichtenthaler, F. W. Towards Improving the Utility of Ketoses as Organic Raw Materials. Carbohydr. Res. **1998**, *313*, 69–89 and references cited there.
2. Brigl, P.; Schinle, R. Über Benzoyl- und Benzalderivate der Fructose. Ber. Dtsch. Chem. Ges., **1933**, *66*, 325–330; Schlubach, H. H.; Vorwerk, J. Untersuchungen über L-Sorbose. Ber. Dtsch. Chem. Ges., **1933**, *66*, 1251–1253; Cramer, F. B.; Pacsu, E. Studies in the Ketone Sugar Series. VIII. The Structure of L-Sorbose Pentaacetate. J. Am. Chem. Soc. **1937**, *59*, 1467–1469; Zinner, H.; Schneider, U. Mercaptale und Tritylverbindungen der L-Sorbose. Chem. Ber. **1963**, *96*, 2159–2164; Lichtenthaler, F. W.; Klotz, J.; Flath, F.-J. Acylation and Carbamoylation of D-Fructose: Acyclic, Furanoid and Pyranoid Derivatives and Their Conformational Features. Liebigs Ann., **1995**, 2069–2080 and references cited there.
3. Conn, H. O. Lactulose, a Drug in Search for a Modus Operandi. Gastroenterology **1978**, *74*, 135–138.
4. Schwengers, D. Leucrose, a Ketodisacchride of Industrial Design, In *Carbohydrates as Organic Raw Materials*; Lichtenthaler, F. W., Ed; VCH Publ.: Weinheim, New York, 1991; 183–195.
5. Siddiqui, I. R. The Sugars of Honey. Adv. Carbohydr. Chem. Biochem. **1970**, *25*, 285–309; Hudson, C. S. Melezitose and Turanose. Adv. Carbohydr. Chem. **1946**, *2*, 1–36.
6. Heyns, K.; Koch, W. Über die Bildung eines Aminozuckers aus D-Fructose und Ammoniak. Z. Naturforsch. **1952**, 7, 486–488; Wrodnigg, T. M.; Eder, B. The Amadori and Heyns Rearrangements: Landmarks in the History of Carbohydrate Chemistry or Unrecognized Synthetic Opportunities. Topics Curr. Chem. (Glycoscience) **2001**, *215*, 115–152.
7. Wrodnigg, T. M.; Stütz, A. E. An Exceptionally Simple Chemical Synthesis of *N*-Acetyl-D-lactosamine from Lactulose. Angew. Chem., **1999**, *111*, 854–856; Int. Ed. Engl. **1999**, *38*, 827–828.
8. For a recent review see: Hanisch, F.-G.; Baldus, S. E. The Thomsen-Friedenreich (TF) Antigen: A Critical Review on the Structural, Biosynthetic and Histochemical Aspects of a Pancarcinoma-Associated Antigen. Histol. Histopathol. **1997**, *12*, 263–281. For recent synthetic approaches see: Gambert, U.; Thiem, J. Chemoenzymatic Preparation of the Thomsen-Friedenreich Antigen Determinant. Carbohydr. Res. **1997**, *299*, 85–89; Gambert, U.; Thiem, J. Multi-Enzyme System for the Synthesis of the Sialylated Thomsen-Friedenreich Antigen Determinant. Eur. J. Org. Chem. **1999**, *1*, 107–110.
9. Kren, V.; Thiem, J. Glycosylation Employing Bio-Systems: from Enzymes to Whole Cells. Chem. Soc. Rev. **1997**, *26*, 463–473 and references cited there; Fernandez-Mayoralas, A. Synthesis and Modification of Carbohydrates Using Glycosidases and Lipases. Topics Curr. Chem. (Glycoscience) **1997**, *186*, 1–20 and references cited there.
10. Vocadlo, D. J.; Withers, S. G. Glycosidase-Catalysed Oligosaccharide Synthesis. In *Carbohydrates in Chemistry and Biology*, Ernst B., Hart, G. W., Sinaÿ, P., Eds; Wiley-VCH: Weinheim, 2000; Part 1, Vol. 2, 723–844.
11. Kawamoto M.; Fujii, S.; Suzuki, K. Glucosyltagatose and Its Manufacture. Japanese Patent JP 85-275070 19851209; Chem. Abstr. **1989**, *110*:133719.
12. Kieweg, R.; Kaehling H.; Haltrich, D.; Weber, A.; Nidetzky, B.; Kulbe, K. D. Synthesis of Positional Isomers of Lactulose by Transgylcosylation. Abstract of Papers,

214th ACS National Meeting,, Las Vegas, NV, Sept. 7–11 (1997), American Chemical Society: Washington DC, 1997; Carb-050.

13. Ajisaka, K.; Fujimoto, H.; Nishida, H. Enzymic Synthesis of Disaccharides by the Use of the Reversed Hydrolysis Activity of β-D-Galactosidases. Carbohydr. Res. **1988**, *180*, 35–42.
14. Fujimoto, H.; Ajisaka, K. Syntheses of α-D-Glucosyl-D-fructoses by Use of a Reversed Hydrolysis Activity of α-Glucosidase. Biotechnol. Lett.**1988**, *10*, 107–112.
15. Fan, J. Q.; Takegawa, K.; Iwahara, S.; Kondo, A.; Kato, I.; Abeygunawardana, C.; Lee, Y. C. Enhanced Transglycosylation Activity of *Arthrobacter protophormiae* endo-β-*N*-Acetylglucosaminidase in Media Containing Organic Solvents. J. Biol. Chem. **1995**, *270*, 17723–17729.
16. Irvine, J.-C.; Garrett, C. S.; Acetone Derivatives of D-Fructose. J. Chem. Soc. **1910**, *97*, 1277–1284; Barry, C. P.; Honeyman, J. Fructose and Its Derivatives. Adv. Carbohydr. Chem. **1952**, *7*, 53–98 and references cited there.
17. Fischer, E. Ueber die Verbindungen der Zucker mit Alkoholen und Ketonen. Ber. **1895**, *28*, 1145–1167; Brady, Jr., R. F. Cyclic Acetals of Ketoses. Carbohydr. Res. **1970**, *15*, 35–40; Kang, J.; Lim, G. J.; Yoon, S. K.; Kim, M. Y. Asymmetric Cyclopropanation Using New Chiral Auxiliaries Derived from D-Fructose. J. Org. Chem. **1995**, *60*, 564–577 and references cited there;.
18. Reichstein, T.; Grüssner, A. Eine Ergiebige Synthese der L-Ascorbinsäure (C-Vitamin). Helv. Chim. Acta **1934**, *17*, 311–328.
19. Szarek, M. A.; Wu, X.; Szarek, W. A. Synthesis and Evaluation of 1,5,6-Trideoxy-6,6-difluoro-1,5-imino-D-glucitol as a Glucosidase Inhibitor. Carbohydr. Res. **1997**, *299*, 165–170.
20. Reichstein, T.; Bosshard W. D-Tagatose, Diaceton-D-tagatose und Tagaturonsäure. Helv. Chim. Acta **1934**, *17*, 755–761; Cubero, I. I.; Lopez-Espinosa M. T. P. Synthetic Application of Partially Protected Fructopyranoses, J. Carbohydr. Chem. **1986**, 5, 299–311 and references cited there; Eyrisch, O.; Sinerius, G.; Fessner, W.-D. Facile Enzymic De Novo Synthesis and NMR Spectroscopic Characterization of D-Tagatose 1,6-biphosphate, Carbohydr. Res. **1993**, *238*, 287–306 and references cited there.
21. Ataie, M.; Buchanan, J. G.; Edgar, A. R.; Kinsman, R. G.; Lyssikatou, M.; Mahon, M. F.; Welsh, P. M. 3,4-Anhydro-1,2-*O*-isopropylidene-β-D-tagatose and 4,5-Anhydro-1,2-*O*-isopropylidene-β-D-fructopyranose. Carbohydr. Res. **2000**, 323, 36–43.
22. Gizaw, Y.; BeMiller, J. N. Application of a Phase Transfer Reaction to the Synthesis of L-Fructose. Carbohydr. Res. **1995**, *266*, 81–85; Tokuyama, K.; Honda, E.; Hoki, N. Sorboses. II. Reaction Mechanism of Acetonization of 1,2-*O*-isopropylidene-α-L-sorbopyranose. J. Org. Chem. **1964**, *29*, 133–136 and references cited there.

Received April 4, 2001
Accepted July 12, 2001

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Request Permission or Order Reprints Instantly!