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Note

Synthesis of 4-*O*-glycosylated 1,5-anhydro-D-fructose and of 1,5-anhydro-D-tagatose from a common intermediate 2,3-*O*-isopropylidene-D-fructose

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ABSTRACT

Four novel disaccharides of glycosylated 1,5-anhydro-D-ketoses have been prepared: 1,5-anhydro-4-*O*-β-D-glucopyranosyl-D-fructose, 1,5-anhydro-4-*O*-β-D-galactopyranosyl-D-fructose, 1,5-anhydro-4-*O*-β-D-glucopyranosyl-D-tagatose, and 1,5-anhydro-4-*O*-β-D-galactopyranosyl-D-tagatose. The common intermediate, 1,5-anhydro-2,3-*O*-isopropylidene-β-D-fructopyranose, was prepared from D-fructose and was converted into the D-tagatose derivative by oxidation followed by stereoselective reduction to the 4-epimer. The anhydroketoses thus prepared were glycosylated and deprotected to give the disaccharides.

The naturally occurring monosaccharide 1,5-anhydro-D-fructose (AF, Fig. 1) has attracted much attention due to its interesting biological and chemical properties.[1] For the vast amounts of investigations resulting in patents on its properties and potential use in both food and non-food applications, as summarized in our patent,[2] 1,5-anhydro-D-fructose has been prepared mainly by enzymatic degradation of starch using an α-glucan lyase.[1,3]

One of the more interesting properties of AF might be its antioxidant activity,[4] and consequently its ability to prevent enzymatic browning and pigment discoloration, which is of interest in the food industry.[5] Compared to the commonly used antioxidant, ascorbic acid, AF prevents the oxidation of linoleic acid even more effectively.[6] In contrast to ascorbic acid, AF has great stability in aqueous solutions since it is not oxidized by molecular oxygen.[7] Due to its non-toxic character[8] the biological properties of AF in pharmaceutical and healthcare areas are an ongoing research topic.[2,9]

The use of carbohydrates as chiral building blocks for synthesizing more complex asymmetric structures in enantiomerically pure form[10] has also been considered for AF, and previous examples have been summarized.[1] Recent examples are the conversion of AF into deoxymannojirimycin (DMJ), an important mannosidase inhibitor,[11] and into ascopyrone P, a compound with antioxidant and antibacterial activities.[12] For these purposes a synthetic alternative to the enzymatic procedure mentioned above for the preparation of AF has been published recently.[2] This is based on the simplest reaction, namely a successful basic deacetylation (Zemplén) of tetra-*O*-acetyl-2-hydroxy-D-glucal (1,5-anhydro-2,3,4,6-tetra-*O*-acetyl-D-*arabino*-hex-1-enitol), the acetylated enol ether of AF,[2] a reaction which previously had been met with many drawbacks.[1] Lichtenthaler and his group also have now prepared AF[13] in a very similar fashion successfully.[2] The paper[13] contains descriptions of the preparation of (−)-bissetone and (−)-palythazine, which have already appeared as notes during the 1980s, as previously reviewed.[1]

In addition to its chemical synthesis, the enzymatic synthesis of AF has been improved significantly.[14]

AF has been detected exclusively as a monomeric compound in natural sources,[1] but recently novel oligosaccharides having AF at

Figure 1. Structure of 1,5-anhydro-D-fructose.

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the reducing end have been synthesized by enzymatic methods. Thus, glucosyl 1→3 1,5-anhydrofructose (1,5-anhydro-3-*O*-α-D-glucopyranosyl-D-fructose) has been prepared enzymatically from AF and β-cyclodextrin employing cyclodextrin glucanotransferase and glucoamylase.[15] Likewise, a series of oligosaccharides have been prepared by glycosylation of the 6-position of AF with dextran oligomers using dextransucrase for enzymatic transglycosylation.[16]

Following our successful deacetylation of the fully acetylated 2-hydroxy-D-glucal to 1,5-anhydro-D-fructose (AF),[2] it was obvious to investigate similar deacetylation reactions of other 2-acyloxy-glycals. In the light of the interest in disaccharide analogues of AF, investigation of deacetylation of hepta-*O*-acetyl-2-hydroxy-D-lactal (1,5-anhydro-4-*O*-(β-D-galactopyranosyl)-D-*arabino*-hex-1-enitol) was undertaken. The reaction did not meet with much success since several compounds were formed, among which D-galactose was isolated. This might indicate a β-elimination of this sugar. Thus, this approach was not investigated further.

Introducing glycosyl moieties onto AF at specific positions by chemical synthesis requires partly protected AF derivatives. For this purpose our recently reported method for preparation AF from D-fructose is suitable.[17] The method involves five steps giving nicely crystalline intermediates and yielding AF in yields of around 35% using a tosylated intermediate. By using a mesylated intermediate, AF can be isolated in a 65% yield based on D-fructose.[17] This chemical route has the advantage of giving access to the 1,5-anhydro-2,3-*O*-isopropylidene-D-fructose (**1**), the last intermediate before the final deprotection to AF. Compound **1** has one free OH-group, which is the OH-4 in AF. Thus, unlike the direct access to AF by the deacetylation method discussed above, this method is unique for derivatization of AF at the 4-position, including conversion to the C-4 epimeric 1,5-anhydro-D-tagatose derivative.

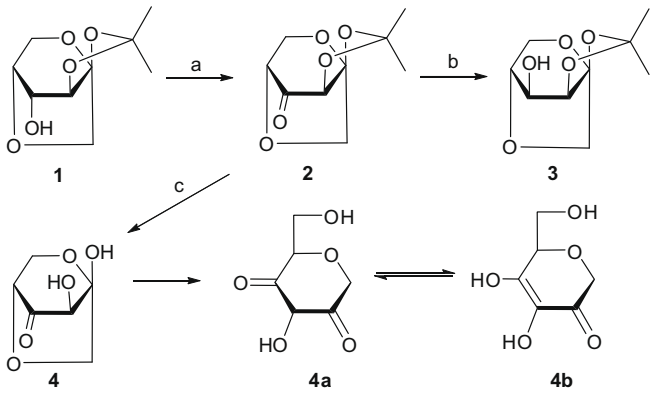
In this paper we describe the use of 1,5-anhydro-2,3-*O*-isopropylidene-β-D-fructopyranose (**1**) for glycosylation to give 4-*O*-glycosylated AF disaccharides. Furthermore, **1** was successfully converted to the 4-epimer by oxidation and stereoselective reduction to give the 1,5-anhydro-2,3-*O*-isopropylidene-β-D-tagatopyranose (**3**). Similar glycosylations and deprotection gave 4-*O*-glycosylated 1,5-anhydro-D-tagatose derivatives. Thus, four new 4-*O*-glycosylated 1,5-anhydro-ketoses have been prepared.

The antioxidant properties of the previously prepared oligosaccharides[15,16] have been claimed to be similar to those of AF, while glucosylated AF derivatives have also been claimed as anti-inflammatory drugs and health foods[18] as well as an immunosuppressant and anti-allergy agents.[19]

Thus, the 2,3-*O*-isopropylidene derivative of AF **1**[17] was oxidized with Dess–Martin periodinane in dichloromethane to give the 4-keto derivative **2** (Scheme 1). Experiments using Swern type oxidation methods (DMSO/oxalyl chloride, DMSO/Ac_2O) afforded significantly lower yields due to the formation of the methylthiomethyl ether side product. Acidic cleavage of the isopropylidene function of compound **2** gave the deprotected derivative **4**. Interpretation of the NMR data suggested that during the reaction and/or work-up subsequent opening of the cyclic hemiketal function occurred to give the diketo derivative **4a**, which is in equilibrium with the more stable conjugated derivative **4b** (Scheme 1). This confirmed further the structure of **2**.

Sodium borohydride reduction of the keto function in **2** resulted in formation of the epimeric 1,5-anhydro-D-tagatose derivative **3** in high stereoselectivity and yield. Only traces of the fructo epimer **1** could be detected by TLC of the crude material.

Glucosylation and galactosylation were performed using the 1,5-anhydrofructose **1** and 1,5-anhydrotagatose **3** derivatives as glycosyl acceptors (Scheme 2). The acid stability of the isopropylidene groups on the acceptor turned out to be high enough to resist acid-catalyzed glycosylation conditions.



Scheme 1. Reagents and conditions: (a) Dess–Martin periodinane, CH_2Cl_2, ambient temperature, 3 h (80%); (b) $NaBH_4$, MeOH, ambient temperature, 30 min (89%); (c) 80% AcOH, 65 °C, 30 min (95%).

After some preliminary experiments the use of imidate leaving groups on the glycosyl donors was selected since a glycosylation reaction with thioalkyl leaving groups afforded the formation of corresponding orthoesters using NIS/AgOTf activation. Efforts to rearrange the orthoesters formed in situ with more acid resulted in low yields due to decomposition. Using trichloroacetimidate leaving groups, there was no sign of orthoester formation.

Four glycosyl 1,5-anhydro-ketoses were prepared using tetra-*O*-acetyl-α-D-glucopyranosyl trichloroimidate (**5**)[20] and tetra-*O*-acetyl-α-D-galactopyranosyl trichloroimidate (**6**)[21] as donors and the two protected 1,5-anhydro-ketoses **1** and **3** as acceptors. The glycosylations were performed in dichloromethane in the presence of molecular sieves. The donors were activated with $BF_3{\cdot}Et_2O$, and the reaction temperature was varied from −50 to −20 °C to give the fully protected β-linked disaccharides **7**, **8**, **9**, and **10** in high anomeric purity. Deacetylation using sodium methoxide in methanol gave the disaccharides **11**, **12**, **13**, and **14**. Acidic hydrolysis to remove the isopropylidene groups gave the free disaccharides **15**, **16**, **17**, and **18**, respectively. The disaccharides were then dissolved in water and lyophilized to afford solid materials. Structures of the products were proven by 1D and 2D NMR spectroscopy, MS, and elemental analyses. Elemental analyses data suggest that the fructose-containing disaccharides in our case were isolated as their monohydrates.

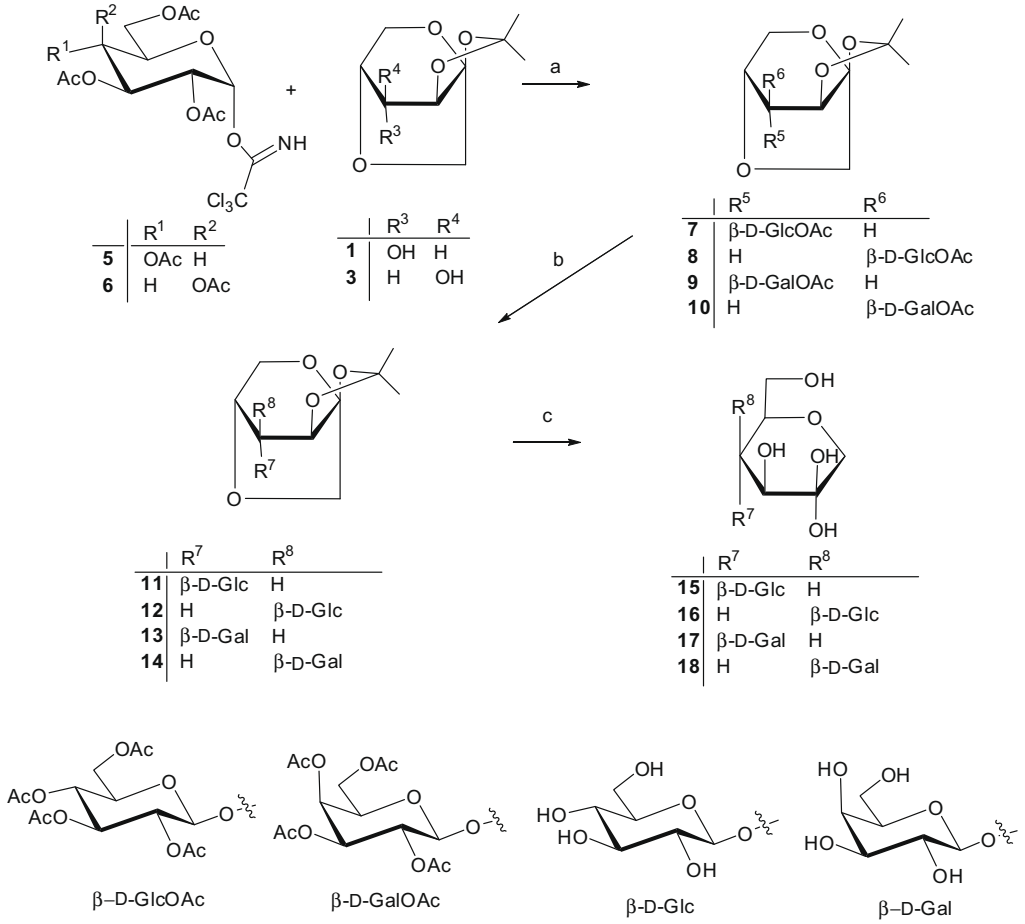
It is known that 1,5-anhydro-D-fructose exists as the hydrated form in aqueous solution, but the hydration process is slow.[1] Recording an NMR spectrum immediately after dissolving AF in D_2O gives a complicated spectrum showing monomeric as well as dimeric structures,[1] while after 5 h only the hydrated form is present. 1,5-Anhydro-D-tagatose also forms a hydrate in water slowly, while this monosaccharide shows no tendency to form dimeric structures.[22] The NMR spectra presented in Section 1 were measured after complete hydration.

In conclusion, four novel disaccharides were prepared by glycosylating 1,5-anhydro-ketoses. The disaccharides thus prepared probably have antioxidant properties similar to those of AF;[15,16] furthermore, they will be suitable for conjugation to proteins via their keto function as indicated in the literature.[15]

1. Experimental

1.1. General methods

Commercially available starting materials were used without further purification. Solvents were dried according to standard methods. Compound **1** was prepared according to literature procedures.[17] NMR spectra were recorded on a Bruker AMX-400 (100.62 MHz for ^{13}C) or DRX-500 (125.83 MHz for ^{13}C) spectrometer



Scheme 2. Reagents and conditions: (a) $BF_3{\cdot}EtO_2$, CH_2Cl_2, 4 Å, −50 to −20 °C, 1 h; (b) NaOMe, MeOH, ambient temperature, 30 min; (c) 80% AcOH, 65 °C, 30 min.

using DMSO-d_6 as the solvent. All chemical shifts are quoted in ppm downfield using solvent peaks as references (DMSO-d_6: ^{1}H: 2.50 ppm, ^{13}C: 39.4 ppm). Kieselgel 60 (E. Merck, Darmstadt, Germany) was used for column chromatography. MALDI-TOFMS measurements were carried out on a Bruker Biflex III mass spectrometer. 2,5-Dihydroxybenzoic acid was used as matrix, and 100–200 laser shots were applied for each spectrum. Optical rotations were measured on a Perkin–Elmer 241 polarimeter, and the concentrations are given in units of g 100 mL^{-1}. $[\alpha]_D^{25}$ values are given in units of 10^{-1} deg cm^2 g^{-1}.

1.2. 1,5-Anhydro-2,3-*O*-isopropylidene-D-*threo*-hex-4-ulopyran-2-ose (2)

Dess–Martin periodinane (2.62 g, 6.18 mmol) was added to a solution of 1,5-anhydro-2,3-*O*-isopropylidene-β-D-fructopyranose (**1**,[17] 1.00 g, 4.95 mmol) in dry CH_2Cl_2 (40 mL), and the reaction mixture was stirred for 3 h at ambient temperature. The reaction was monitored by TLC (1:1 hexane–EtOAc), and after completion the mixture was diluted with EtOAc (200 mL) and was washed with aq $NaHCO_3$ (3 × 50 mL). The organic phase was dried (Na_2SO_4), filtered, and concentrated to a syrup, which was purified by column chromatography (6:4 hexane–acetone) to give the title compound **2** (800 mg, 80%) as a colorless solid: mp 83–87 °C; $[\alpha]_D^{25}$ +37.8 (*c* 0.19, MeOH); ^{1}H NMR (DMSO-d_6): δ 4.83 (s, 1H, H-3), 4.55 and 4.13 (2d, each 1H, J_{gem} 9.14 Hz, H-1, H-1′), 4.39 and 4.16 (2m, each 1H, H-6, H-6′), 4.15 (m, 1H, H-5), 1.63 and 1.48 (2s, each 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ 204.3 (C-4), 117.4 ($C(CH_3)_2$), 103.0 (C-2), 86.8 (C-3), 76.4 (C-5), 70.8 (C-1), 68.4 (C-6), 28.3 and 26.3 (2 × –CH_3). Anal. Calcd for $C_9H_{12}O_5$: C, 54.00; H, 6.04. Found: C, 54.11; H, 6.12.

1.3. 1,5-Anhydro-2,3-*O*-isopropylidene-β-D-tagatopyranose (3)

To a solution of **2** (200 mg, 1.00 mmol) in MeOH (10 mL) $NaBH_4$ (75.6 mg, 2.00 mmol) was added at ambient temperature. The mixture was stirred for 30 min, and was then diluted with EtOAc (200 mL), and the organic phase was washed with water (3 × 50 mL). The combined washings were extracted with EtOAc (3 × 50 mL), and the combined organic phases were dried (Na_2SO_4), filtered, and concentrated to a residue. Column chromatography (6:4 hexane–EtOAc) gave 1,5-anhydro-2,3-*O*-isopropylidene-β-D-tagatopyranose (**3**) (180 mg, 89%) as a colorless syrup: $[\alpha]_D^{25}$ +44.2 (*c* 0.16, acetone); ^{1}H NMR (DMSO-d_6): δ 5.14 (d, 1H, $J_{4,OH}$ 5.34 Hz, 4-OH), 4.24 (m, 1H, H-4), 4.20 and 3.97 (2dd, each 1H, J_{gem} 9.92 Hz, $J_{5,6}$ 1.78 and ∼1 Hz, H-6, H-6′), 4.13 and 3.72 (2d, each 1H, J_{gem} 8.40 Hz, H-1, H-1′), 4.02 (d, 1H, $J_{3,4}$ 8.14 Hz, H-3), 3.80 (m, 1H, H-5), 1.49 and 1.37 (2s, each 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ 114.8 (–$C(CH_3)_2$), 98.9 (C-2), 77.8 (C-3), 69.6 (C-5), 69.6 (C-1), 65.0 (C-4), 64.0 (C-6), 28.1 and 26.0 (2 × –CH_3). Anal. Calcd for $C_9H_{14}O_5$: C, 53.46; H, 6.98. Found: C, 53.07; H, 7.02.

For comparison, spectra were also measured in MeOH-d_4, and they showed **3** to be different from **2**.[17]

^{1}H NMR (MeOH-d_4): δ 4.38 (m, 1H, H-4), 4.36 and 3.87 (2 × d, each 1H, J_{gem} 10.21 Hz, H-6), 4.25 and 3.78 (2 × dd, each 1H, J_{gem} 8.85 Hz, H-1), 3.87 (m, 1H, H-3), 3.79 (m, 1H, H-5), 1.58 and 1.42 (2s, each 3H, CH_3); ^{13}C NMR (MeOH-d_4): δ 116.9 (–$C(CH_3)_2$),

100.4 (C-2), 78.9 (C-3), 71.3 and 70.9 (C-1 and C-5), 66.5 (C-4), 65.3 (C-6), 28.0 and 26.0 (2 × –CH_3).

1.4. (*R*)-4,5-dihydroxy-6-(hydroxymethyl)-2*H*-pyran-3(6*H*)-one (4b)

A mixture of **2** (1.00 g, 4.95 mmol) and 80% aq AcOH (10 mL) was stirred for 30 min at 65 °C, and was then concentrated and co-concentrated with toluene. The residue was then dissolved in water (10 mL) and lyophilized to afford **4b** (850 mg, 95%) as a colorless glass: ^{1}H NMR (D_2O): δ 4.35 (m, 3H, H-1 and H-5), 3.92 and 3.78 (2dd, each 1H, J_{gem} 12.61 Hz, $J_{5,6}$ 2.52 and 5.36 Hz, H-6, H-6′); ^{13}C NMR (D_2O): δ 178.1 (C-4), 173.9 (C-2), 130.0 (C-3), 79.0 (C-5), 66.6 (C-1), 61.0 (C-6).

1.5. General procedures

1.5.1. Glycosylation

A solution of glycosyl acceptor (300 mg, 1.48 mmol) and glycosyl donor (875 mg, 1.77 mmol) in dry CH_2Cl_2 (5 mL), containing 4 Å molecular sieves (500 mg) was cooled to −50 °C. Then, a solution of $BF_3{\cdot}Et_2O$ (100 μL) in CH_2Cl_2 (1 mL) was added, and the reaction mixture was stirred for 1 h at −20 °C. After addition of pyridine (200 μL) and EtOAc (100 mL), the mixture was filtered, and the filtrate was washed with aq $NaHCO_3$ (3 × 50 mL). The organic phase was dried (Na_2SO_4), filtered, and concentrated to a residue that was purified by column chromatography (95:5 CH_2Cl_2–acetone) to give the disaccharide.

1.5.2. Deacetylation

To a solution of fully protected disaccharide (400 mg, 0.75 mmol) in dry MeOH (5 mL) was added NaOMe (50 mg), and the mixture was stirred for 30 min at ambient temperature. The solution was neutralized with Amberlite IR 120 (H^+) resin, filtered, and concentrated. Column chromatography (8:2 CH_2Cl_2–MeOH) of the residue gave the products.

1.5.3. Acid hydrolysis

A mixture of starting compound (100 mg, 0.27 mmol) and 80% aq AcOH (10 mL) was stirred for 30 min at 65 °C, and was then concentrated and co-concentrated with toluene. Finally, the residue was dissolved in water (3 mL) and lyophilized to afford the products.

1.6. 1,5-Anhydro-2,3-*O*-isopropylidene-4-*O*-β-(2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl)-D-fructopyranose (7)

Coupling of donor **5** with acceptor **1** according to the general procedure resulted in **7** (68%) as a syrup: $[\alpha]_D^{25}$ −16.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (DMSO-d_6): δ 5.24 (dd, 1H, $J_{2',3'}$ 9.66 Hz, $J_{3',4'}$ 9.66 Hz, H-3′), 4.99 (d, 1H, $J_{1',2'}$ 8.14 Hz, H-1′), 4.95 (dd, 1H, $J_{4',5'}$ 9.66 Hz, H-4′), 4.79 (dd, 1H, H-2′), 4.21 and 4.03 (2dd, each 1H, J_{gem} 12.30 Hz, $J_{5',6'}$ 4.41 Hz, and 2.21 Hz, H-6′), 4.19 (m, 1H, H-4), 4.13 and 3.87 (2d, each 1H, J_{gem} 8.51 Hz, H-1), 4.08 and 3.97 (2m, each 1H, H-6), 4.07 (d, 1H, $J_{3,4}$ 3.78 Hz, H-3), 3.97 (m, 1H, H-5), 3.93 (m, 1H, H-5′), 2.03, 1.98, 1.98 and 1.94 (4s, each 3H, 4 × OAc), 1.46 and 1.34 (2s, each 3H, 2 × –CH_3); ^{13}C NMR (DMSO-d_6): δ 97.8 (C-1′), 83.9 (C-3), 80.2 (C-4), 71.7 (C-3′), 70.5 (C-5′), 70.4 (C-2′), 70.2 (C-5), 69.3 (C-1), 67.7 (C-4′), 65.8 (C-6), 61.3 (C-6′), 27.4 and 25.2 (2 × –CH_3), 20.22 (4 × OAc); MALDI-TOFMS: Calcd for $C_{23}H_{32}O_{14}$: 532. Found: 555 $[M+Na]^+$, 571 $[M+K]^+$.

1.7. 1,5-Anhydro-2,3-*O*-isopropylidene-4-*O*-β-(2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl)-D-tagatopyranose (8)

Coupling of donor **5** with acceptor **3** according to the general procedure resulted in **8** (75%) as a syrup: $[\alpha]_D^{25}$ +7.3 (*c* 0.8, $CHCl_3$); ^{1}H NMR (DMSO-d_6): δ 5.24 (dd, 1H, $J_{2',3'}$ 9.78 Hz, $J_{3',4'}$ 9.78 Hz, H-3′), 4.92 (dd, 1H, $J_{4',5'}$ 9.78 Hz, H-4′), 4.86 (d, 1H, $J_{1',2'}$ 7.88 Hz, H-1′), 4.77 (dd, 1H, H-2′), 4.53 (d, 1H, $J_{3,4}$ 6.31 Hz, H-3), 4.47 (dd, 1H, $J_{4,5}$ 1.57 Hz, H-4), 4.18 and 4.04 (2dd, each 1H, J_{gem} 12.30 Hz, H-6′), 4.09 (m, 2H, H-1), 4.05 (m, 1H, H-5), 3.99 (m, 1H, H-5′), 3.92 and 3.64 (2dd, each 1H, J_{gem} 11.35 Hz, $J_{5,6}$ 3.47 Hz and 8.20 Hz, H-6), 2.02, 2.00, 1.98 and 1.94 (4s, each 3H, 4 × OAc), 1.29 (s, 6H, 2 × –CH_3); ^{13}C NMR (DMSO-d_6): δ 99.8 (C-1′), 75.5 (C-4), 75.3 (C-3), 74.6 (C-5), 72.3 (C-1), 71.7 (C-3′), 70.4 (C-2′), 70.1 (C-5′), 68.7 (C-6), 68.0 (C-4′), 61.2 (C-6′), 26.4 and 25.4 (2 × –CH_3), 20.2 (4 × OAc); MALDI-TOFMS: Calcd for $C_{23}H_{32}O_{14}$: 532. Found: 555 $[M+Na]^+$, 571 $[M+K]^+$.

1.8. 1,5-Anhydro-2,3-*O*-isopropylidene-4-*O*-β-(2,3,4,6-tetra-*O*-acetyl-D-galactopyranosyl)-D-fructopyranose (9)

Coupling of donor **6** with acceptor **1** according to the general procedure resulted in **9** (65%) as a syrup: $[\alpha]_D^{25}$ −23.1 (*c* 0.6, $CHCl_3$); ^{1}H NMR (DMSO-d_6): δ 5.27 (dd, 1H, $J_{3',4'}$ 3.57 Hz, $J_{4',5'}$ <1 Hz, H-4′), 5.14 (dd, 1H, $J_{2',3'}$ 10.18 Hz, H-3′), 4.96 (dd, 1H, $J_{1',2'}$ 7.89 Hz, H-2′), 4.90 (d, 1H, H-1′), 4.19 (m, 1H, H-4), 4.16 (m, 1H, H-5′), 4.13 and 3.87 (2d, each 1H, J_{gem} 8.83 Hz, H-1), 4.09 and 3.99 (2m, each 1H, H-6), 4.06 (d, 1H, H-3), 4.05 (m, 2H, H-6′), 3.95 (m, 1H, H-5), 2.12, 2.01, 1.99 and 1.92 (4s, each 3H, 4 × OAc), 1.46 and 1.35 (2s, each 3H, 2 × –CH_3); ^{13}C NMR (DMSO-d_6): δ 98.4 (C-1′), 84.0 (C-3), 80.3 (C-4), 70.1 (C-5), 69.7 (C-5′), 70.0 (C-3′), 69.6 (C-1), 68.5 (C-2′), 66.8 (C-4′), 65.8 (C-6), 60.7 (C-6′), 27.9 and 24.9 (2 × –CH_3), 20.3, 20.3, 20.2 and 20.2 (4 × OAc); MALDI-TOFMS: Calcd for $C_{23}H_{32}O_{14}$: 532. Found: 555 $[M+Na]^+$, 571 $[M+K]^+$.

1.9. 1,5-Anhydro-2,3-*O*-isopropylidene-4-*O*-β-(2,3,4,6-tetra-*O*-acetyl-D-galactopyranosyl)-D-tagatopyranose (10)

Coupling of donor **6** with acceptor **3** according to the general procedure resulted in **10** (73%) as a syrup: $[\alpha]_D^{25}$ −10.4 (*c* 0.9, $CHCl_3$); ^{1}H NMR (DMSO-d_6): δ 5.26 (dd, 1H, $J_{3',4'}$ 3.47 Hz, $J_{4',5'}$ <1 Hz, H-4′), 5.14 (dd, 1H, $J_{2',3'}$ 10.40 Hz, H-3′), 4.94 (dd, 1H, $J_{1',2'}$ 7.88 Hz, H-2′), 4.78 (d, 1H, H-1′), 4.53 (d, 1H, $J_{3,4}$ 6.30 Hz, H-3), 4.47 (dd, 1H, $J_{4,5}$ 1.26 Hz, H-4), 4.19 (m, 1H, H-5′), 4.08 (m, 2H, H-1), 4.05 (m, 2H, H-6′), 4.04 (m, 1H, H-5), 3.92 and 3.64 (2dd, each 1H, J_{gem} 11.35 Hz, $J_{5,6}$ 3.78 Hz and 7.88 Hz, H-6), 2.13, 2.02, 2.00 and 1.90 (4s, each 3H, 4 × OAc), 1.29 (s, 6H, 2 × –CH_3); ^{13}C NMR (DMSO-d_6): δ 100.2 (C-1′), 75.6 (C-4), 75.4 (C-3), 74.8 (C-5), 72.2 (C-1), 70.0 (C-3′), 69.8 (C-5′), 68.7 (C-6), 68.4 (C-2′), 67.0 (C-4′), 61.0 (C-6′), 22.0 (2 × –CH_3), 20.6, 20.6, 20.4 and 20.2 (4 × OAc); MALDI-TOFMS: Calcd for $C_{23}H_{32}O_{14}$: 532. Found: 555 $[M+Na]^+$, 571 $[M+K]^+$.

1.10. 1,5-Anhydro-2,3-*O*-isopropylidene-4-*O*-β-D-glucopyranosyl-D-fructopyranose (11)

Zemplén deacylation of compound **7** according to the general procedure resulted in **11** (80%) as a syrup: $[\alpha]_D^{25}$ −18.4 (*c* 1.2, pyridine); ^{1}H NMR (DMSO-d_6): δ 5.08 (d, 1H, $J_{2',OH}$ 5.09 Hz, 2′OH), 4.99 (d, 1H, $J_{3',OH}$ 4.83 Hz, 3′OH), 4.94 (d, 1H, $J_{4',OH}$ 5.34 Hz, 4′OH), 4.34 (d, 1H, $J_{1',2'}$ 7.88 Hz, H-1′), 4.29 (dd, 1H, $J_{6',OH}$ 4.83 Hz and 7.63 Hz, 6′OH), 4.20 (m, 1H, H-4), 4.18 (d, 1H, H-3), 4.14 and 3.86 (2d, each 1H, J_{gem} 8.51 Hz, H-1), 4.07 and 3.94 (2m, each 1H, H-6), 4.06 (m, 1H, H-5), 3.66 and 3.44 (2m, each 1H, H-6′), 3.13 (m, 2H, H-3′ and H-5′), 3.06 (m, 1H, H-4′), 3.02 (m, 1H, H-2′), 1.45 and 1.34 (2s, each 3H, 2 × –CH_3); ^{13}C NMR (DMSO-d_6): δ 101.6 (C-1′), 84.8 (C-3), 79.8 (C-4), 76.5 (C-3′ and C-5′), 73.2 (C-2′), 70.0 (C-5), 69.9 (C-4′), 69.4 (C-1), 66.1 (C-6), 61.0 (C-6′), 27.5 and 25.2 (2 × –CH_3). Anal. Calcd for $C_{15}H_{24}O_{10}$: C, 49.45; H, 6.64. Found: C, 48.99; H, 6.47.

1.11. 1,5-Anhydro-2,3-*O*-isopropylidene-4-*O*-β-D-glucopyranosyl-D-tagatopyranose (12)

Zemplén deacylation of compound **8** according to the general procedure resulted in **12** (85%) as a syrup: $[\alpha]_D^{25}$ +5.8 (*c* 1, pyridine); ^{1}H NMR (DMSO-d_6): δ 5.00 (d, 1H, $J_{2',OH}$ 5.04 Hz, 2′OH), 4.95 (d, 1H, $J_{3',OH}$ 4.72 Hz, 3′OH), 4.89 (d, 1H, $J_{4',OH}$ 5.36 Hz, 4′OH), 4.55 (dd, 1H, $J_{3,4}$ 6.30 Hz, $J_{4,5}$ 1.58 Hz, H-4), 4.53 (d, 1H, H-3), 4.50 (dd, 1H, $J_{6',OH}$ 5.67 Hz and 5.99 Hz, 6′OH), 4.20 (d, 1H, $J_{1',2'}$ 7.57 Hz, H-1′), 4.11 (m, 1H, H-5), 4.09 (m, 2H, H-1), 3.93 and 3.63 (2dd, each 1H, J_{gem} 11.35 Hz, $J_{5,6}$ 4.10 Hz and 7.57 Hz, H-6), 3.68 and 3.42 (2m, each 1H, H-6′), 3.12 (m, 2H, H-3′ and H-5′), 3.02 (m, 1H, H-4′), 2.96 (m, 1H, H-2′), 1.29 (s, 6H, 2 × –CH_3); ^{13}C NMR (DMSO-d_6): δ 102.9 (C-1′), 76.5 (C-3′ and C-5′), 75.7 (C-5), 75.7 (C-3 and C-4), 73.1 (C-2′), 72.3 (C-1), 69.9 (C-4′), 67.9 (C-6), 60.7 (C-6′), 26.2 (2 × –CH_3). Anal. Calcd for $C_{15}H_{24}O_{10}$: C, 49.45; H, 6.64. Found: C, 49.87; H, 6.55.

1.12. 1,5-Anhydro-2,3-*O*-isopropylidene-4-*O*-β-D-galactopyranosyl-D-fructopyranose (13)

Zemplén deacylation of compound **9** according to the general procedure resulted in **13** (85%) as a syrup: $[\alpha]_D^{25}$ −28.4 (*c* 0.5, pyridine); ^{1}H NMR (DMSO-d_6): δ 4.90 (d, 1H, $J_{2',OH}$ 4.73 Hz, 2′OH), 4.73 (d, 1H, $J_{3',OH}$ 5.67 Hz, 3′OH), 4.38 (m, 2H, 4′OH and 6′OH), 4.28 (d, 1H, $J_{1',2'}$ 7.56 Hz, H-1′), 4.18 (m, 2H, H-4 and H-3), 4.15 and 3.86 (2d, each 1H, H-1), 4.08 and 3.98 (2m, each 1H, H-6), 4.05 (m, 1H, H-5), 3.64 (m, 1H, H-4′), 3.51 (m, 2H, H-6′), 3.36 (m, 1H, H-5′), 3.34 (m, 1H, H-2′), 3.28 (m, 1H, H-3′), 1.46 and 1.29 (2s, each 3H, 2 × –CH_3); ^{13}C NMR (DMSO-d_6): δ 103.5 (C-1′), 85.9 (C-3), 80.9 (C-4), 76.2 (C-5′), 74.7 (C-3′), 71.5 (C-2′), 71.2 (C-5), 70.7 (C-1), 69.2 (C-4′), 67.5 (C-6), 61.4 (C-6′), 28.8 and 26.1 (2 × –CH_3). Anal. Calcd for $C_{15}H_{24}O_{10}$: C, 49.45; H; 6.64. Found: C, 49.11; H, 6.61.

1.13. 1,5-Anhydro-2,3-*O*-isopropylidene-4-*O*-β-D-galactopyranosyl-D-tagatopyranose (14)

Zemplén deacylation of compound **10** according to the general procedure resulted in **14** (76%) as a syrup: $[\alpha]_D^{25}$ −16.4 (*c* 0.6, pyridine); ^{1}H NMR (DMSO-d_6): δ 4.83 (d, 1H, $J_{2',OH}$ 4.73 Hz, 2′OH), 4.70 (d, 1H, $J_{3',OH}$ 5.36 Hz, 3′OH), 4.55 (m, 3H, 4′OH, H-3 and H-4), 4.33 (dd, 1H, $J_{6',OH}$ 4.41 Hz and 10.09 Hz, 6′OH), 4.15 (d, 1H, $J_{1',2'}$ 7.25 Hz, H-1′), 4.09 (m, 3H, H-1 and H-5), 3.90 and 3.46 (2m, each 1H, H-6), 3.61 (m, 1H, H-4′), 3.50 (m, 2H, H-6′), 3.32 (m, 1H, H-5′), 3.28 (m, 1H, H-2′), 3.25 (m, 2H, H-3′), 1.31 and 1.30 (2s, each 3H, 2 × –CH_3); ^{13}C NMR (DMSO-d_6): δ 103.2 (C-1′), 75.4 (C-3 and C-4), 74.8 (C-5′), 74.6 (C-5), 73.1 (C-3′), 72.1 (C-1), 69.9 (C-2′), 67.9 (C-4′), 67.5 (C-6), 60.2 (C-6′), 26.3 and 25.5 (2 × –CH_3). Anal. Calcd for $C_{15}H_{24}O_{10}$: C, 49.45; H; 6.64. Found: C, 49.21; H, 6.71.

1.14. 1,5-Anhydro-4-*O*-β-D-glucopyranosyl-D-fructose (15)

Acid hydrolysis of compound **11** according to the general procedure resulted in **15** (95%) as a glass: $[\alpha]_D^{25}$ −21.0 (*c* 0.5, water); ^{1}H NMR (D_2O): δ 4.44 (d, 1H, $J_{1',2'}$ 7.88 Hz, H-1′), 3.88 and 3.69 (2m, each 2H, H-6′ and H-6), 3.68 and 3.40 (2m, each 1H, H-1), 3.62 (m, 1H, H-3), 3.59 (m, 1H, H-4), 3.47 (m, 1H, H-5), 3.45 (m, 2H, H-3′ and H-5′), 3.35 (m, 1H, H-4′) 3.25 (m, 1H, H-2′); ^{13}C NMR (D_2O): δ 102.9 (C-1′), 92.9 (C-2), 79.7 (C-5), 79.3 (C-4), 76.0 (C-3′ and C-5′), 75.8 (C-3), 73.7 (C-2′), 72.0 (C-1), 70.1 (C-4′), 61.0 (C-6 and C-6′). Anal. Calcd for $C_{12}H_{22}O_{11}\cdot H_2O$: C, 40.00; H, 6.71. Found: C, 40.09; H, 6.65.

1.15. 1,5-Anhydro-4-*O*-β-D-glucopyranosyl-D-tagatose (16)

Acid hydrolysis of compound **12** according to the general procedure resulted in **16** (96%) as a syrup: $[\alpha]_D^{25}$ +3.6 (*c* 0.6, water); ^{1}H NMR (D_2O): δ 4.44 (d, 1H, $J_{1',2'}$ 7.86 Hz, H-1′), 3.89 (m, 1H, H-4), 3.85 and 3.65 (2m, each 2H, H-6 and H-6′), 3.76 (m, 1H, H-3), 3.73 and 3.38 (2m, each 1H, H-1), 3.69 (m, 1H, H-5), 3.41 (m, 2H, H-3′ and H-5′), 3.32 (m, 1H, H-4′), 3.23 (m, 1H, H-2′); ^{13}C NMR (D_2O): δ 102.9 (C-1′), 92.5 (C-2), 78.8 (C-3), 76.1 (C-3′ and C-5′), 73.6 (C-2′), 72.9 (C-1), 72.0 (C-5), 71.2 (C-4′), 70.0 (C-4), 61.2 (C-6 and C-6′). Anal. Calcd for $C_{12}H_{22}O_{11}$: C, 42.11; H, 6.48. Found: C, 41.73; H, 6.42.

1.16. 1,5-Anhydro-4-*O*-β-D-galactopyranosyl-D-fructose (17)

Acid hydrolysis of compound **13** according to the general procedure resulted in **17** (95%) as a glass: $[\alpha]_D^{25}$ −21.2 (*c* 0.8, water); ^{1}H NMR (D_2O): δ 4.28 (d, 1H, $J_{1',2'}$ 7.88 Hz, H-1′), 3.79 and 3.60 (2dd, each 1H, J_{gem} 12.30 Hz, H-6′), 3.76 (m, 1H, H-5′), 3.60 (m, 2H, H-6), 3.59 (m, 1H, H-5), 3.57 and 3.29 (2d, each 1H, J_{gem} 11.98 Hz, H-1), 3.54 (m, 1H, H-4′), 3.50 (m, 1H, H-3′) 3.49 (m, 1H, H-3), 3.38 (m, 1H, H-2′), 3.37 (m, 1H, H-4); ^{13}C NMR (D_2O): δ 103.4 (C-1′), 93.0 (C-2), 79.7 (C-4), 79.2 (C-3), 75.9 (C-4′ and C-5), 72.9 (C-3′), 71.9 (C-1), 71.1 (C-2′), 69.2 (C-5′), 61.5 (C-6), 60.9 (C-6′). Anal. Calcd for $C_{12}H_{22}O_{11}\cdot H_2O$: C, 40.00; H, 6.71. Found: C, 39.94; H, 6.72.

1.17. 1,5-Anhydro-4-*O*-β-D-galactopyranosyl-D-tagatose (18)

Acid hydrolysis of compound **14** according to the general procedure resulted in **18** (96%) as a syrup. $[\alpha]_D^{25}$ −6.4 (*c* 0.5, water); ^{1}H NMR (D_2O): δ 4.38 (d, 1H, $J_{1',2'}$ 7.89 Hz, H-1′), 3.91 (m, 1H, H-4), 3.86 (m, 1H, H-4′), 3.77 (m, 1H, H-3), 3.74 and 3.39 (2d, each 1H, J_{gem} 11.98 Hz, H-1), 3.70 (m, 4H, H-6 and H-6′), 3.69 (m, 1H, H-5), 3.63 (m, 1H, H-5′), 3.58 (m, 1H, H-3′), 3.47 (m, 1H, H-2′); ^{13}C NMR (D_2O): δ 103.6 (C-1′), 92.4 (C-2), 78.9 (C-3), 75.6 (C-5′), 73.2 (C-3′), 73.0 (C-1), 72.0 (C-5), 71.3 (C-2′), 70.0 (C-4), 69.1 (C-4′), 61.5 (C-6 and C-6′). Anal. Calcd for $C_{12}H_{22}O_{11}$: C, 42.11; H, 6.48. Found: C, 41.70; H, 6.40.

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