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Note

Enzymatic procedures in the preparation of regioprotected D-fructose derivatives☆

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Abstract—A combination of different lipases from *Pseudomonas cepacia, Candida antarctica* B, *Candida rugosa* and *Mucor miehei*, aided the regioesterification of the free fructose allowing the synthesis of 1,6-di-*O*-acetyl-D-fructofuranose, 1,4,6-tri-*O*-acetyl-D-fructofuranose, 1,6-di-*O*-acetyl-4-*O*-benzoyl-D-fructofuranose and 1,6-di-*O*-benzoyl-D-fructofuranose. Using *C. antarctica* B and *C. rugosa* lipases the alcoholysis of fructose peracetate (α, β-form) has furnished 1,2,3,4-tetra-*O*-acetyl-α-D-fructofuranose and 2,3,4,6-tetra-*O*-acetyl-β-D-fructofuranose. 1,4,6-Tri-*O*-acetyl-D-fructofuranose was successfully employed to produce a rare ketohexose, namely D-psicose.

Keywords: Lipase; Fructose; Regioprotection; Biocatalysis

Biocatalysis of hydrolases in organic solvents is today a well-known area in organic synthesis for the preparation of regioprotected polyfunctionalised molecules.[1] Sugars have received particular attention in this context because of their structural features, stereochemistry and biological properties. Using lipases (EC 3.1.1.3) different selective modifications have been achieved on free sugars,[2] aminosugars,[3] sugar component of natural compounds[4] and the sugar moiety of nucleosides.[5] Although there are many examples of the use of hydrolases in the regioprotection of compounds containing fructose as a structural moiety,[6] there are few studies on biocatalysed protection–deprotection of pure fructose. These studies report the differentiation between the hydroxyl groups of fructose by esterification in the presence of porcine pancreatic lipase, as well as protease subtilisin, but only mixtures of 1-*O*-acyl-D-fructose and 6-*O*-acyl-D-fructose were obtained.[7] Furthermore to prepare surfactants and food additives, *Pseudomonas* sp. lipase and *Candida antarctica* lipase have been exploited for the synthesis of fatty acid fructose mono and diesters. In all the cases only the primary hydroxyl functions were recognised, as in previous studies.[8]

The aim of the present studies was to further develop a route towards the synthesis of differently regioprotected sugar derivatives, to establish the potential of hydrolases operating in organic solvent. We report the results obtained using four different lipases in esterification and alcoholysis processes of D-fructose **1** and peracetate **6**. One of the regioprotected derivatives obtained has been used as a starting material to obtain the rare fructose isomer D-psicose.

At the beginning of our investigation, different lipases were tested for the esterification of D-fructose in tetrahydrofuran (THF) using vinyl acetate as an acyl donor. In the conditions tested, lipase from *C. antarctica* B (CAL-B) was able to catalyse in 2 h the esterification of **1** at primary hydroxyl groups giving 1,6-di-*O*-acetyl-D-fructofuranose (**2**) as the sole product in quantitative yields. No further esterification was observed on prolonging the reaction time, neither was any further reaction observed after addition of a fresh amount of catalyst. Most

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likely the stereochemistry hindered the recognition of CAL-B of hydroxyl groups at C-3 and C-4 such as at the anomeric position. Esterification experiments in the presence of lipase from *Pseudomomas cepacia* (PSL), *Mucor miehei* (MML) and *Candida rugosa* (CRL) were unsuccessful, obtaining no or poor reactivity.

Regiodiscrimination of secondary hydroxyl groups has been attempted by exploiting **2** as starting material; this compound has the advantage of being constrained in furanosic forms, so consequently lowering the number of possible isomers present and giving a well-behaved solubility in apolar solvents.

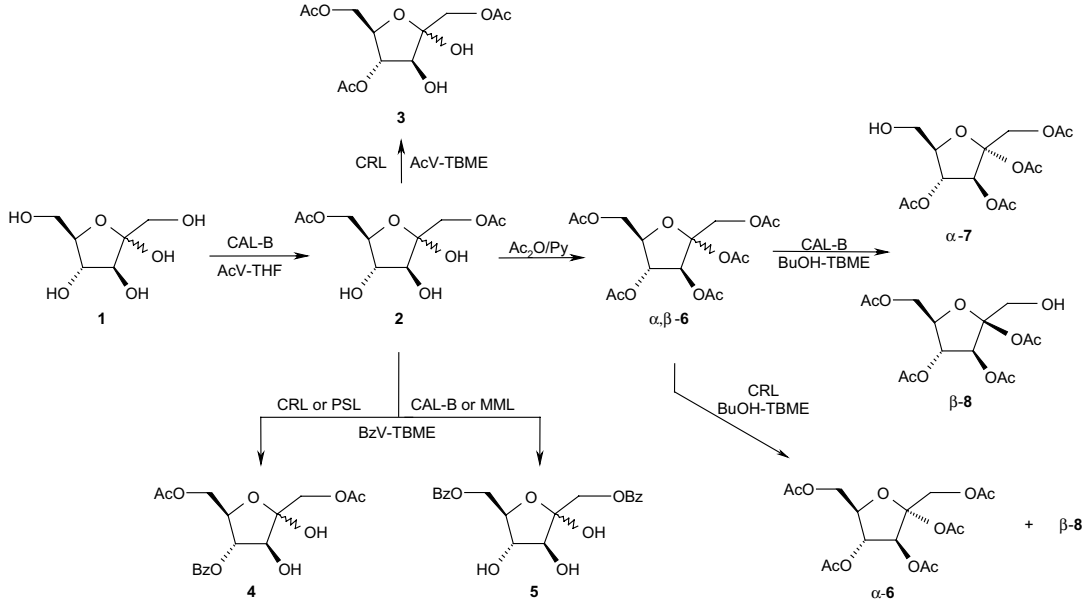
The four lipases were screened for the esterification of **2** in *tert*-butylmethylether (TBME), and in presence of vinyl acetate as acyl donor. Under these conditions, CRL gave excellent recognition of the hydroxyl group at C-4, affording 1,4,6-tri-*O*-acetyl-D-fructofuranose (**3**) as the sole product. An analogous catalytic action was evidenced for PSL, but associated with a very low conversion. MML and CAL left the substrate **2** untransformed. The real action, a transesterification at primary hydroxyl groups exclusively, of these last two lipases was revealed when esterification using vinyl benzoate as acyl donor was tried: catalysis in the presence of CRL or PSL gave 1,6-di-*O*-acetyl-4-*O*-benzoyl-D-fructofuranose **4** as expected; conversely ester **5** was the product obtained under the catalysis of MML and CAL.

For derivatives bearing unprotected primary hydroxyl functions, the alcoholysis of D-fructose peracetate **6** was examined. The preparation of this pentacetate in α and β furanose form, was obtained by subjecting **2**, prepared by the use of CAL-B as previously described, to treatment with acetic anhydride and pyridine. ^{1}H NMR analysis of the obtained product **6** indicated a α/β ratio of 1:1. The anomeric mixture of compound **6** was submitted to alcoholysis in the presence of CAL-B, with butanol as the nucleophilic agent, and TBME as solvent. The reaction proved highly regiospecific and stereoselective, giving differential CAL-B recognition of α/β anomers and affording 1,2,3,4-tetra-*O*-acetyl-α-D-fructofuranose (**7**) and 2,3,4,6-tetra-*O*-acetyl-β-D-fructofuranose (**8**) (Scheme 1).

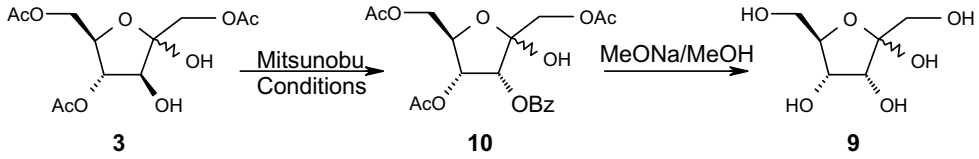
A different outcome was observed on alcoholysis employing CRL as catalyst, with recognition of the ester group at C-1β exclusively, to give 2,3,4,6-tetra-*O*-acetyl-β-D-fructofuranose (**8**): the α-anomer remained as an unreacted substrate.

These data proved the synthetic potential of ester **3** in the preparation of D-psicose, **9**. Triester (**3**) was subjected to inversion of configuration at C-3 in Mitsunobu conditions, furnishing the corresponding 1,4,6-tri-*O*-acetyl-3-*O*-benzoyl-D-psicose (**10**) that gave the desired free D-psicose (**9**) in 80% overall yield (Scheme 2).

In conclusion, we have developed a set of reactions catalysed by different lipases that allow access to different fructose derivatives. Lipase from *C. antarctica* B has high recognition for primary hydroxyl groups in the esterification. Conversely, in the reverse alcoholysis process of peracetate D-fructose, the configuration at the anomeric centre strongly influenced the regioselectivity of this biocatalyst. Lipase from *C. rugosa* showed preference for esterification at C-4, whilst able to affect the



Scheme 1.



3 10 9

Scheme 2.

ester function at C-1 only in the alcoholysis of peracetate ins β-anomeric form. The obtained fructose derivatives are suitable starting materials for further transformation and synthesis of valuable rare ketohexose sugars.

1. Experimental

1.1. General methods

^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO solution at 400.13 MHz, on a Bruker AMX-400 instrument using TMS as internal reference. The coupling constants (J) are reported in hertz and the chemical shifts (δ) in parts per million downfield from TMS. The different products obtained by chemical or enzymatic routes were characterised by 2D ^{1}H NMR experiments. Optical rotations were measured on a DIP 135 JASCO instrument. Mass spectra were recorded on a ESI-MS spectrometer Waters-Micromass ZQ2000 dissolving compounds in a 0.001 M methanolic solution of LiCl: in positive mode adducts ions with Li^+ were observed, in negative mode adducts ions with Cl^- were observed. GC analysis was performed using a Shimatsu 17A gas chromatograph equipped with a Zebron ZB-5 column; temp progr.: 100–300 °C 10 °C/min, inj. 180 °C, det. 220 °C. Elemental analyses were carried out on a Perkin–Elmer 240 elemental analyser. Lipase from *P. cepacia* was obtained from Amano international Enzyme. *C. rugosa* lipase was from Sigma (St. Louis, MO). Novozym 435 (immobilised lipase from *C. antarctica* B) and Lipozyme IM (immobilised lipase from *Mucor miehei*) were a kind gift of Novo Nordisk. Flash chromatography was performed on 60 mm mesh silica gel (Merck, Darmstadt, Germany); analytical TLC (silica gel 60-F254 precoated plates) and preparative TLC (Kieselgel F254, 0.5 and 1.0 mm thickness) materials were furnished by Merck and compounds were visualised by spraying with molybdophosphoric acid.

1.2. 1,6-Di-*O*-acetyl-D-fructofuranose (2)

Lipase from *C. antarctica* B (40 mg) was added to a solution of D-fructose (**1**) (40 mg) in tetrahydrofuran (4 mL) containing vinyl acetate (3 equiv) as acyl donor. The suspension was shaken (300 rpm) at 45 °C, aliquots were drawn at regular time intervals and monitored by TLC (Et_2O–MeOH, 99:1). After 2 h the reaction was quenched, filtering off the catalyst and the filtrate evaporated to dryness in vacuum to give compound **2** (54 mg, 95%) as a slightly yellow syrup. No further purification was necessary. Anomeric ratio 1:2; $R_f = 0.4$ (Et_2O–MeOH, 99:1); ^{1}H NMR (400 MHz, $CDCl_3$) major anomer: 4.32 (d, 1H, $J_{3,4}$ 2.1 Hz, H-3), 4.29 (br d, 1H, $J_{4,5}$ 3.3 Hz, H-4), 4.23 (d, 1H, $J_{1a,1b}$ 7.2 Hz, H-1$_a$), 4.12 (d, 1H, H-1$_b$), 4.03 (d, 2H, $J_{6,5}$ 7.2 Hz, H-6), 3.98 (dt, 1H, H-5), 2.13 (s, 3H, $COOCH_3$), 2.11 (s, 3H, $COOCH_3$); minor anomer: 4.29 (br d, 1H, H-4) 4.21–4.13 (m, 3H, H-3, H-1$_a$ and H-1$_b$), 4.07 (br d, 1H, H-6), 3.98 (dt, 1H, H-5), 2.15 (s, 3H, $COOCH_3$), 2.10 (s, 3H, $COOCH_3$). ESI-MS negative mode CV 5 eV (MeOH+ LiCl 0.001 M) *m/z*: 299.2 ($M + Cl^-_{35}$, 100%), 301.2 ($M + Cl^-_{37}$, 32.6%). Anal. Calcd for $C_{10}H_{16}O_8$: C, 45.46; H, 6.10. Found: C, 45.66; H, 6.06.

1.3. General procedure for the synthesis of 1,4,6-tri-*O*-acyl-D-fructofuranose (3–4)

Lipase of choice (from *C. rugosa* or *P. cepacia*, 40 mg) was added to a solution of **2** (40 mg) in *tert*-butylmethylether (4 mL) containing appropriate vinyl ester (3 equiv). The suspension was shaken (300 rpm) at 45 °C, aliquots were drawn at regular time intervals and monitored by TLC (100% Et_2O). After 7 h the reaction was quenched, filtering off the catalyst and the filtrate evaporated to dryness in vacuum. The residue was purified by flash chromatography on silica gel (petroleum ether/diethyl ether) or by PTLC (100% Et_2O).

1.3.1. 1,4,6-Tri-*O*-acetyl-D-fructofuranose (3). Yield 41 mg (90%) obtained as a slight yellow syrup from compound **2** and vinyl acetate, as described in the general procedure: anomeric ratio 1:4; $R_f = 0.52$ (Et_2O); ^{1}H NMR (400 MHz, $CDCl_3$) major anomer: 5.01 (t, 1H, $J_{4,5}$ and $J_{4,3}$ 5.2 Hz, H-4), 4.31 (d, 1H, H-3), 4.17 (d, 1H, $J_{1a,1b}$ 10.8 Hz, H-1$_a$), 4.11 (d, 2 H, $J_{6,5}$ 7.1 Hz, H-6), 4.16–4.09 (m, 2H, H-5 and H-1$_b$), 2.14 (s, 3H, $COOCH_3$), 2.12 (s, 3H, $COOCH_3$), 2.10 (s, 3H, $COOCH_3$); minor anomer: 4.77 (br dd, 1H, H-4), 4.30–4.20 (m, 2H, H-3 and H-1$_a$), 4.11 (br d, 2H, H-6), 4.16–4.09 (m, 1H, H-5), 4.01 (m, 1 H, H-1$_b$), 2.14 (s, 3H, $COOCH_3$), 2.12 (s, 3H, $COOCH_3$), 2.10 (s, 3H,

$COOCH_3$). ESI-MS negative mode CV 5 eV (MeOH+LiCl 0.001 M) *m/z*: 341.4 ($M + Cl^-_{35}$, 100%), 343.4 ($M + Cl^-_{37}$, 32.6%). Anal. Calcd for $C_{12}H_{18}O_9$: C, 47.06; H, 5.92. Found: C, 47.25; H, 5.87.

1.3.2. 1,6-Di-*O*-acetyl-4-*O*-benzoyl-D-fructofuranose (4). Yield 50 mg (90%) obtained as a slightly yellow syrup from compound **2** and vinyl benzoate, as described in the general procedure: anomeric ratio 1:4; $R_f = 0.60$ (Et_2O); ^1H NMR (400 MHz, $CDCl_3$) major anomer: 8.04 (d, 2H, $J_{2',3'}$ 7.2 Hz, H-2′), 7.60 (d, 1H, $J_{4',3'}$ 7.5 Hz, H-4′), 7.47 (t, 2H, H-3′), 5.24 (t, 1H, $J_{4,5}$ and $J_{4,3}$ 5.4 Hz, H-4), 4.44–4.41 (m, 1H, H-5), 4.40 (d, 1H, H-3), 4.33 (d, 2H, $J_{6,5}$ 5.6 Hz, H-6), 4.25 (d, 1H, $J_{1a,1b}$ 11.8 Hz, H-1_a), 4.18 (d, 1H, H-1_b), 2.12 (s, 3H, $COOCH_3$), 2.10 (s, 3H, $COOCH_3$); minor anomer: 8.04 (d, 2H, $J_{2',3'}$ 7.2 Hz, H-2′), 7.60 (d, 1H, $J_{4',3'}$ 7.5 Hz, H-4′), 7.47 (t, 2H, H-3′), 5.02 (br dd, 1H, H-4), 4.47-4.46 (m, 2H, H-3 and H-5), 4.33 (br d, 2H, H-6), 4.30 (br dd, 2H, H-1), 2.11 (s, 3H, $COOCH_3$), 2.10 (s, 3H, $COOCH_3$). ESI-MS negative mode CV 5 eV (MeOH+LiCl 0.001 M) *m/z*: 403.3 ($M + Cl^-_{35}$, 100%), 405.3 ($M + Cl^-_{37}$, 32.6%). Anal. Calcd for $C_{17}H_{20}O_9$: C, 55.43; H, 5.47. Found: C, 55.15; H, 5.49.

1.4. 1,6-Di-*O*-benzoyl-D-fructofuranose (5)

Lipase of choice (from *C. antarctica* B or *M. miehei*, 40 mg) was added to a solution of **2** (40 mg) in *tert*-butylmethylether (4 mL) containing vinyl benzoate (3 equiv). The suspension was shaken (300 rpm) at 45 °C, aliquots were drawn at regular time intervals and monitored by TLC (100% Et_2O). After 7 h the reaction was quenched, filtering off the catalyst and the filtrate evaporated to dryness in vacuum. The residue was purified by flash chromatography on silica gel (petroleum ether/diethyl ether) or by PTLC (100% Et_2O) to afford compound **5** (46 mg, 80%) as a slightly yellow syrup. $R_f = 0.36$ (Et_2O); ^1H NMR (400 MHz, $CDCl_3$) major anomer: 8.04 (br s, 4H, H-2′), 7.55 (br t, 2H, H-4′), 7.42 (br t, 4H, H-3′), 4.51 (br d, 1H, $J_{3,4}$ 2.3 Hz, H-3), 4.41 (br d, 1 H, H-4), 4.33–4.27 (m, 2H, H-1), 4.20–4.14 (m, 3H, H-5 and H-6). ESI-MS negative mode CV 5 eV (MeOH+LiCl 0.001 M) *m/z*: 423.3 ($M + Cl^-_{35}$, 100%), 425.2 ($M + Cl^-_{37}$, 32.6%). Anal. Calcd for $C_{20}H_{20}O_8$: C, 61.85; H, 5.19. Found: C, 62.11; H, 5.21.

1.5. D-Fructose pentacetate (6)

1,6-Di-*O*-acetyl-D-fructofuranose **2** (500 mg) was dissolved in 10 mL of pyridine and 10 mL of Ac_2O. The solution was stirred at room temperature for 6 h and then evaporated to dryness in vacuo to give compound **6** (724 mg, 98%) in α/β 1:1 mixture as a slightly yellow syrup. $R_f = 0.84$ (100% Et_2O). Chemical synthesis of pure α- and β-**6** using as starting material compounds **7** and **8** (see below) was confirmed by NMR signals. ^1H NMR (400 MHz, $CDCl_3$) α anomer: 5.85 (d, 1H, $J_{3,4}$ 4.1 Hz, H-3), 5.14 (dd, 1H, $J_{4,5}$ 6.3 Hz, H-4), 4.62 (d, 1H, $J_{1a,1b}$ 12.0 Hz, H-1_a), 4.51 (ddd, 1H, $J_{5,6a}$ 3.6, $J_{5,6b}$ 5.7, H-5), 4.40 (dd, 1H, $J_{6a,6b}$ 12.2 Hz, H-6_a), 4.35 (d, 1H, H-1_b), 4.17 (dd, 1H, H-6_b), 2.16 (s, 3H, $COOCH_3$), 2.11 (s, 3H, $COOCH_3$), 2.10 (s, 6H, $2 \times COOCH_3$), 2.07 (s, 3H, $COOCH_3$); β anomer: 5.69 (dd, 1H, $J_{4,3}$ 2.2, $J_{4,5}$ 9.0 Hz, H-4), 5.50 (d, 1H, H-3), 5.22 (ddd, 1H, $J_{5,6a}$ 2.7, $J_{5,6b}$ 4.9 Hz, H-5), 4.92 (d, 1H, $J_{1a,1b}$ 17.3 Hz, H-1_a), 4.68 (d, 1H, H-1_b), 4.29 (dd, 1H, $J_{6a,6b}$ 12.6 Hz, H-6_a), 4.12 (dd, 1H, H-6_b), 2.20 (s, 3H, $COOCH_3$), 2.11 (s, 3H, $COOCH_3$), 2.10 (s, 6H, $2 \times COOCH_3$), 2.08 (s, 3H, $COOCH_3$). ESI-MS positive mode CV 5 eV (MeOH+LiCl 0.001 M) *m/z*: 391.4 ($M+H^+$, 8%), 331.4 ($M-CH_3COO^-$, 100%), 271.4 ($M-CH_3COO^- - CH_3COOH$, 3%), 211.4 ($M-CH_3COO^- - 2 \times CH_3COOH$, 3%). Anal. Calcd for $C_{16}H_{22}O_{11}$: C, 49.23; H, 5.68. Found: C, 49.55; H, 5.71.

1.6. General procedure for the alcoholysis of D-fructose pentacetate (6)

Lipase of choice (from *C. antarctica* B or *C. rugosa*, 40 mg) was added to a solution of **6** (40 mg) in *tert*-butylmethylether (4 mL) containing butanol (3 equiv) as nucleophilic agent. The suspension was shaken (300 rpm) at 45 °C, aliquots were drawn at regular time intervals and monitored by TLC (100% Et_2O). After 7 h the reaction was quenched, filtering off the catalyst and the filtrate evaporated to dryness in vacuum. The residue was purified by flash chromatography on silica gel (petroleum ether/diethyl ether) or by PTLC (100% Et_2O).

1.6.1. 1,2,3,4-Tetra-*O*-acetyl-α-D-fructofuranose (7). Yield 14 mg (41%) obtained as a slight yellow syrup from compound **6** and butanol in presence of *C. antarctica* B lipase, as described in the general procedure: $[\alpha]_D^{22}$ +22.86 (*c* 0.385, $CHCl_3$) R_f 0.59 (100% Et_2O); ^1H NMR (400 MHz, $CDCl_3$): 5.90 (d, 1H, $J_{3,4}$ 4.6 Hz, H-3), 5.30 (dd, 1H, $J_{4,5}$ 6.6 Hz, H-4), 4.68 (d, 1H, $J_{1a,1b}$ 12.0 Hz, H-1_a), 4.39 (m, 1H, H-5), 4.28 (d, 1H, H-1_b), 3.89 (dd, 1H, $J_{6a,5}$ 2.8, $J_{6a,6b}$ 12.7 Hz, H-6_a), 3.71 (dd, 1H, $J_{6b,5}$ 2.8, H-6_b), 2.17 (s, 3H, $COOCH_3$), 2.10 (s, 6H, $2 \times COOCH_3$), 2.07 (s, 3H, $COOCH_3$). ESI-MS positive mode CV 5 eV (MeOH+LiCl 0.001 M) *m/z*: 355.4 ($M+Li^+$, 100%). Anal. Calcd for $C_{14}H_{20}O_{10}$: C, 48.28; H, 5.79. Found: C, 48.55; H, 5.75.

1.6.2. 2,3,4,6-Tetra-*O*-acetyl-β-D-fructofuranose (8). Yield 12 mg (35%) when using *C. antarctica* B lipase and 14 mg (39%) in presence of *C. rugosa* lipase, obtained as a slightly yellow syrup from compound **6** and butanol, as described in the general procedure: $[\alpha]_D^{22}$ −220.37 (*c* 0.362, $CHCl_3$); R_f 0.72 (100% Et_2O);

^{1}H NMR (400 MHz, $CDCl_3$): 5.62 (dd, 1H, $J_{4,3}$ 2.1, $J_{4,5}$ 9.0 Hz, H-4), 5.49 (d, 1H, H-3), 5.25 (m, 1H, H-5), 4.37 (d, 2H, $J_{1a,1b}$ 5.4 Hz, H-1), 4.29 (dd, 1H, $J_{6a,5}$ 2.5, $J_{6a,6b}$ 12.2 Hz, H-6_a), 4.14 (dd, 1H, $J_{6b,5}$ 4.5, H-6_b), 2.16 (s, 9H, $3 \times COOCH_3$), 2.07 (s, 3 H, $COOCH_3$). ESI-MS positive mode CV 5 eV (MeOH+LsiCl 0.001 M) *m/z*: 355.4 ($M+Li^+$, 100%). Anal. Calcd for $C_{14}H_{20}O_{10}$: C, 48.28; H, 5.79. Found: C, 48.49; H, 5.82.

1.7. Preparation of D-psicose (9)

1.7.1. 1,4,6-Tri-*O*-acetyl-3-*O*-benzoyl-D-psicose (10). To a solution of 1,4,6-tri-O-acetyl-D-fructofuranose **3** (130 mg, 0.42 mmol), Ph_3P (240 g, 0.9 mmol) and benzoic acid (111.0 mg, 0.9 mmol) in toluene (4.5 mL) diethyl azodicarboxylate (0.150 mL, 0.9 mmol) was added dropwise. After being stirred at room temperature for 4 h, the mixture was chromatographed (EtOAc–hexane) to afford compound **10** (148 mg, 85%). Anomeric ratio 1:7.5; R_f 0.35 (Et_2O–MeOH = 98:2); ^{1}H NMR (400 MHz, $CDCl_3$) major anomer: 8.04 (d, 2H, $J_{2',3'}$ 7.4 Hz, H-2′), 7.60 (d, 1H, $J_{4',3'}$ 7.4 Hz, H-4′), 7.47 (t, 2H, H-3′), 5.08 (dd, 1H, $J_{4,3}$ 2.58, $J_{4,5}$ 4.57 Hz, H-4), 4.81 (d, 1H, $J_{1a,1b}$ 12.2 Hz, H-1_a), 4.73 (d, 1H, H-1_b), 4.69 (d, 1H, H-3), 4.47 (m, 1H, H-5), 4.42 (dd, 1H, $J_{6a,5}$ 3.7, $J_{6a,b}$ 15.6 Hz, H-6_a), 4.14 (dd, 1H, $J_{6b,\ 5}$ 5.5, H-6_b), 2.12 (s, 3H, $COOCH_3$), 2.09 (s, 3H, $COOCH_3$), 2.02 (s, 3H, $COOCH_3$). ESI-MS positive mode CV 5 eV (MeOH+LiCl 0.001 M) *m/z*: 417.3 ($M+Li^+$, 100%). Anal. Calcd for $C_{19}H_{22}O_{10}$: C, 55.61; H, 5.40. Found: C, 55.83; H, 5.38.

1.7.2. Hydrolysis of 1,4,6-tri-*O*-acetyl-3-*O*-benzoyl-D-psicose (10). To a solution of compound **10** (110 mg) in MeOH (3 mL) 25 wt% sodium methoxide (in MeOH, three drops) was added. After stirring at room temperature for 2 h, Amberlite IRC 50 was added to the mixture till neutralisation, filtered and evaporated under reduced pressure to obtain pure D-psicose **9** (45.8 mg, 95%). ^{1}H NMR data results indistinguishable by data obtained with a commercial sample of D-psicose and from data reported in literature.[9] To obtain another proof of its nature, compound **9** (10 mg) was dissolved in 1 mL of a silylating mixture (HMDS–TMCS–pyridine = 3:1:9) and compared by gas chromatography to samples of commercially available D-fructose and D-psicose analogously silylated; retention times: D-fructose persilylated 15.17, D-psicose persilylated 15.40 min.

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