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Lipase-mediated 'Meso-Tricks' to Transform Latently Symmetrical D-Aldoses into L-Aldoses *via* Alditols

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(Dedicated to the glorious memory of Sir Derek H. R. Barton, great man and great scientist)

Abstract: L-Fucose and L-xylose were synthesized from dulcitol and xylitol, respectively, using CCL- or PPL-mediated kinetic resolution as the key step. In the first case dissymmetrization was achieved directly by acylating a meso derivative, while in the second it was preceded by the conversion of the meso polyol into a racemic intermediate.
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The realm of carbohydrates is inhabited mainlly by D-aldoses. However, recent decades witnessed rapidly growing interest in biological properties of L-aldoses, e.g., L-fucose (L-**1**) and L-xylose (L-**2**). The former is important for the cell surface recognition in mammalians, and can be used as a marker or a remedy for several immune defence system pathologies,[1] while the "unnatural' L-**2** is increasingly used as a starting material for the synthesis of anticancer, antiviral and antidiabetes drugs, or else as an ingredient of the corresponding pharmaceutical formulations.[2] Here we propose two conceptually related protocols for the syntesis of L-**1** and L-**2** from dulcitol (**3**) and xylitiol (**4**), respectively, where lipase-mediated optical kinetic resolution is the pivotal step. Since **3** and **4** are manufactured from D-galactose and D-xylose, this path is equivalent to chemo-enzymatic conversion of these sugars into L-**1** and L-**2.**

Lipase-mediated disymmetrization of acyclic meso alditols was not always successful.[3] By contrast, the hydrolysis of 1-*O*-Acetyl-2,4:3,5-di-*O*-methylene-DL-xylitol (*rac*-**5**) using porcine pancreatic lipase (PPL) is highly enantioselective,[4] which prompted us to employ cyclic alditol derivatives at the lipase-mediated kinetic resolution stage. Two variants of the protocol were envisaged: direct kinetic resolution of a meso substrate (*I*) and the conversion of a meso polyol into racemic monohydric alcohol (and its carboxylate} with subsequent kinetic resolution (*II*) and optional recycling of unconverted part of the meso precursor.

I. Direct optical resolution: Synthesis of L-fucose. Previous chemical syntheses of L-**1** started from D-galactose,[5] L- or D-arabinose,[6] D-glucose,[7] and D-mannose.[8] Overall yields in these multistep schemes were within 1-15%. Impressive enzymatic synthesis of L-**1** requires expensive precursors and serious involvement in enzymology.[9]

As the overture of our synthesis of L-**1** (Scheme 1, **A**) dulcitol **3** was converted into a mixture of two isomeric diacetonides (ref. 10), the major one being symmetric (**6**) and the other racemic(*rac*-**7**). The required diol **6** was separated from *rac*-**7** both by fractional crystallyzation and by obtaining the corresponding mixture of diacetates (**6a** + *rac*-**7a**), isolating the poorly soluble **6a** by recrystallization, and saponifying it back to **6.**

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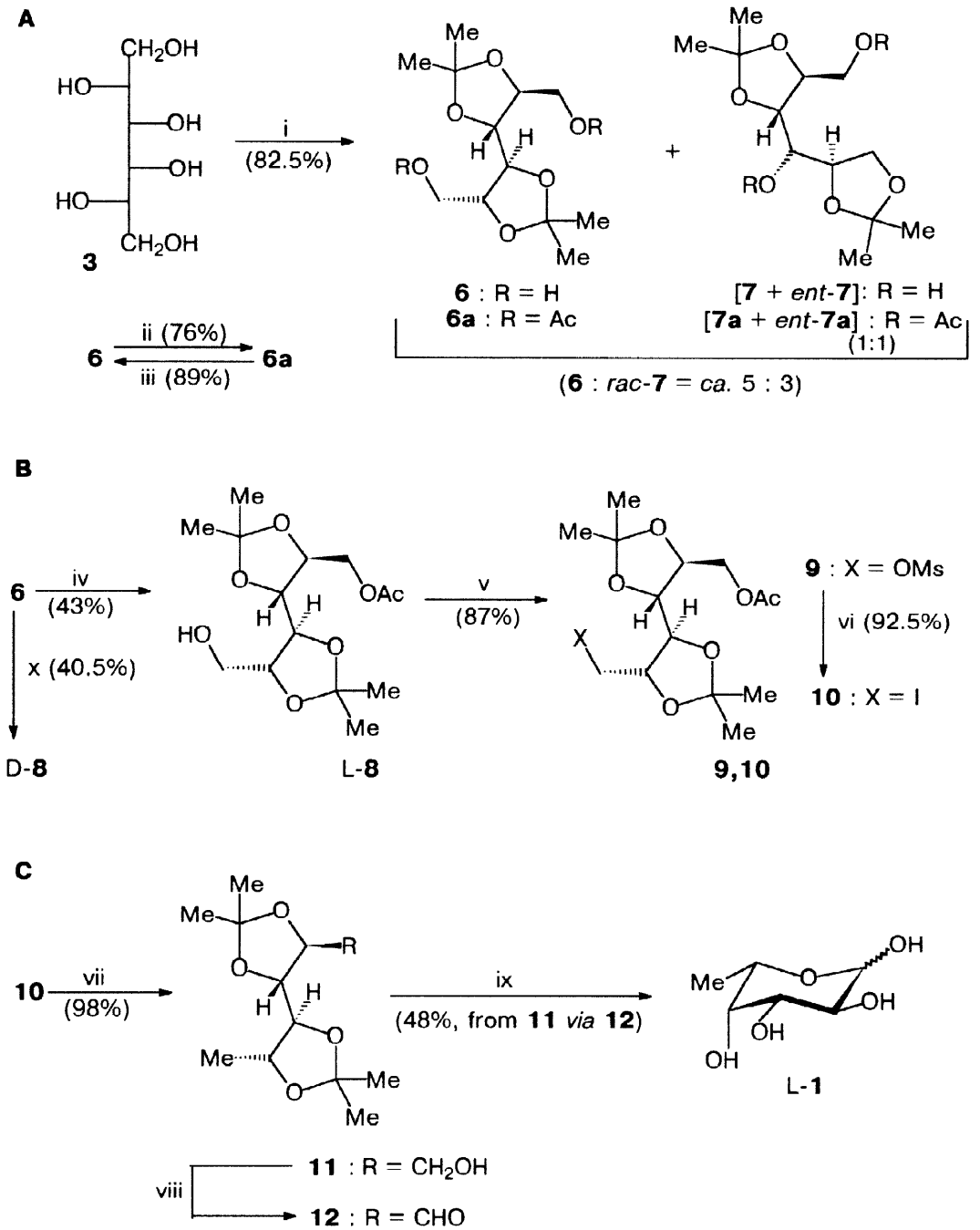


Scheme 1. *Reagents and Conditions*: i, Me_2CO—conc.H_2SO_4(cat.)/$CuSO_4$, RT; ii, Ac_2O/Py; iii, KOH/MeOH, RT; iv, H_2C=CHOAc—CCL / Et_2O, RT; v, MsCl / Py—$CHCl_3$, RT; vi, NaI / Me_2CO, 60°C; vii, H_2—Ni (cat.)—K_2CO_3/MeOH, RT, 1 atm; viii, DCC—H_3PO_4 (cat.)—DMSO, RT; ix, AcOH—H_2O (6:4), 100°C; x, H_2C=CHOAc—PPL/Et_2O, RT.

Originally, we tried to dissymmetrize diacetate **6a** by PPL-catalysed hydrolysis. However, even at high enzyme-to-substrate ratio (PPL:**6a** = 2:1, w/w) and long exposures (3-4 days) no conversion of **6a** was observed. By contrast, acylation of diol **6** with vinyl acetate in the presence of PPL proceeded rather smoothly: at ~47% conversion it gave a chiral monoacetate ($[\alpha]_D^{22}$ +12.7°, in $CHCl_3$) in 40.5% isolated yield *vs.* ≤2.5% yield of **6a**. The configuration of this monoacetate (D-**8**, ee ~100%) was opposite to what was required.

Then controlled acylatyion of diol **6** using vinyl acetate and the lipase from *Candida rugosa* (CCL) was undertaken (Schme 1, **B**). At optimal exposures (19-20 h) the yield of crystalline monoacetate (L-**8**) with $[\alpha]_D^{22}$ –9.05 ($CHCl_3$) amounted to 43%, the recovery of **6** and the yield of diacetate **6a** being 47 and 8%, respectively.[11] Longer exposures increased the yield of L-**8** up to 73%, but at the expense of ee ($[\alpha]_D^{22}$ –6.4° after 44 h). Mesylation of L-**8** and subsequent treatment of crystalline mesylate **9** with NaI led to the wax-like iodide **10** (m.p. 45-47°C) that was hydrogenolysed (with concomitant deacylation) over skeletal Ni in the presence of K_2CO_3 in MeOH to give 2,3:4,5-di-*O*-isopropylidene-L-fucitol (**11**).[5b,13] Finally (Scheme 1,**C**), alcohol **11** was oxidized into the corresponding oxo diketal (**12**), and the latter was immediately hydrolysed to give the target sugar L-**1** {m.p. 138-140°C (EtOH), $[\alpha]_D^{22}$ –74.7°(4 h) (H_2O)}. Lit.[5b,8]: m.p. 137-139°C (EtOH), $[\alpha]_D$ –75°(24 h) (*c* 0.95, H_2O). Taking into account the content of **6** in the starting mixture of isomeric diols, the yield of L-fucose from **3** was *ca.* 7% over nine steps. This compares favorably with earlier syntheses of L-**1** from sugars.[5-8] In spite of only fair ee of monoacetate L-**8** (~75% as follows from the comparison with $[\alpha]_D$ of L-**8** or D-**8** possessing 98% ee[12]), optical purity of the intermediate alcohol **11** and of target L-**1** is practically the same as reported earlier for compounds **11**[5b,13] and L-**1**[5-8] prepared from homochiral precursors. This must be the effect of recrystallizing intermediates **9** and **11** on the way from L-**8** to L-**1**.

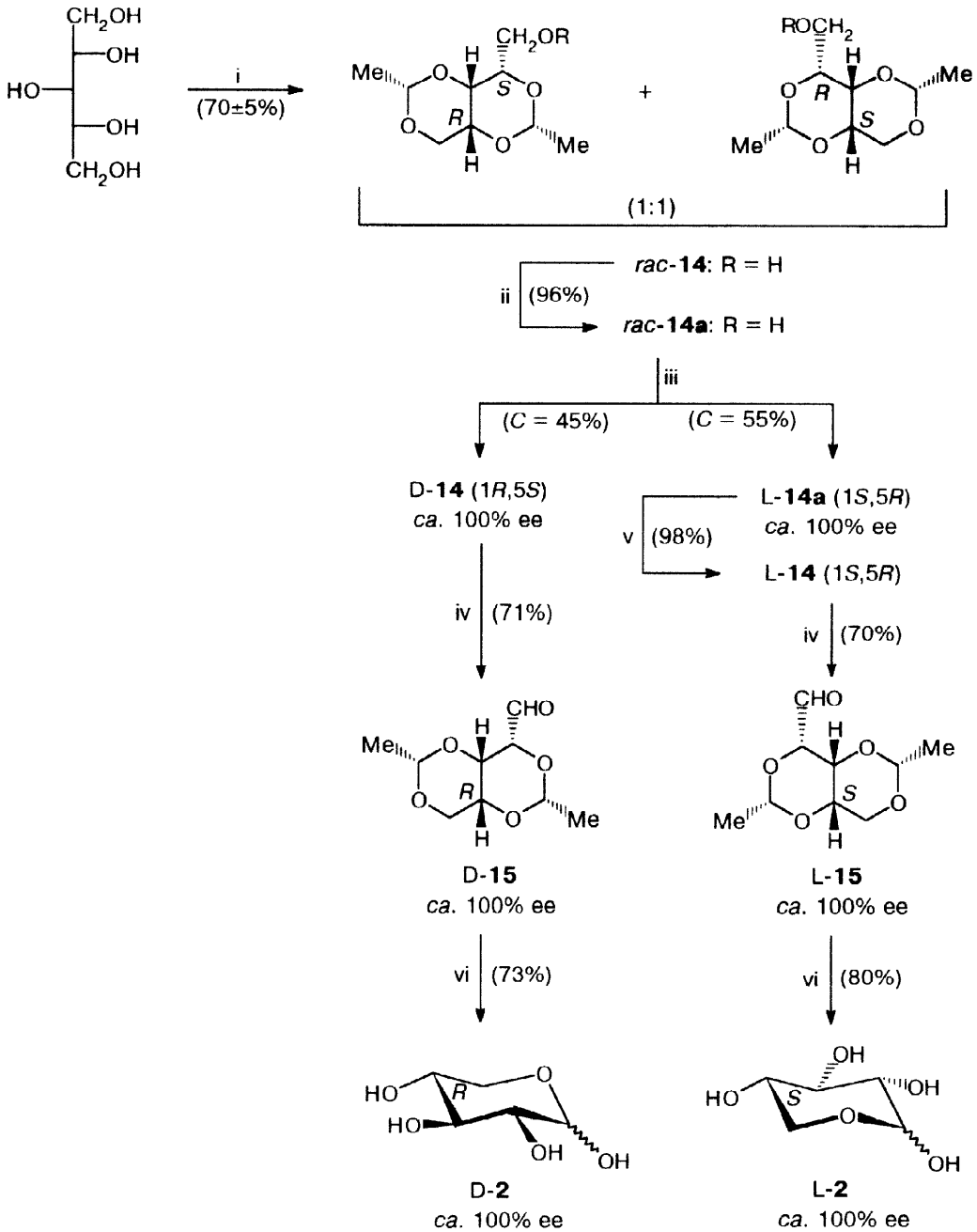
II. Chemical dissymmetrization—enzymatic resolution sequence: Synthesis of L-xylose. Previous syntheses of L-**2** were performed by oxidative scission of D-glucose[14] or D-glucitol,[15,16] by epimerization of L-arabinose,[17] and using aldol-type condensation of (*S*)-2,3-*O*-cyclohexylideneglyceral.[18] Two syntheses of L-**2**, employng more than one enzyme consecutively[19] or in tandem,[20] promise to be the best ones, provided that the cost-to-effect problem is solved..

Although PPL-catalysed hydrolysis of *rac*-**5** gave 2,4:3,5-di-*O*-methylene-L-xylitol with ~100% ee,[4] it was not a good path to L-xylose because of the difficulty of removing the protecting methylene groups. PPL-catalysed hydrolysis of the corresponding di-*O*-benzylidene derivative, *rac*-**13,** gave only racemic products.

So we tried to transform xylitol **4** into L-**2** by employing PPL-catalysed hydrolysis of 1-*O*-acetyl-2,4:3,5-di-*O*-ethylidene-DL-xylitol (*rac*-**14a**) as the key step (Scheme 2). In contrast with the case of *rac*-**5**, the conversion of *rac*-**14a** (*C* = 45%) gave alcohol D-**14** with $[\alpha]_D^{22}$ +3.6°C (H_2O).[21] Using Pfitzner—Moffatt oxidation, the latter was converted into aldehyde D-**15** {$[\alpha]_D^{22}$ +13.4°(H_2O)} that gave enantiopure D-xylose (D-**2**) upon hydrolysis. Alcohol D-**14** had ~100% ee, as follows from NMR spectra of (*S*)-MTPA ester, D-**14b**.

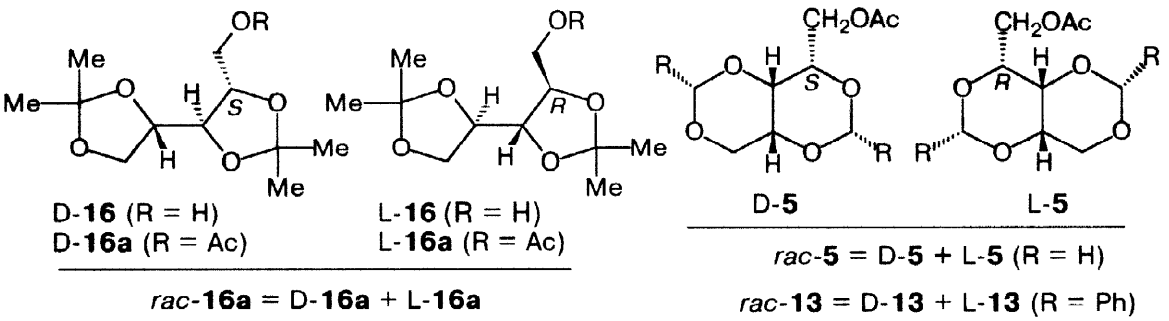
In order to obtain enantiopure alcohol L-**14**, the hydrolysis of *rac*-**14a** was extended to 55% conversion. Chromatographic separation of the products afforded acetate L-**14a** {m.p. 55-56°C, $[\alpha]_D^{22}$ +7.30° ($CHCl_3$)}that was saponified to give enantiopure L-**14** {$[\alpha]_D^{22}$–3.5°(H_2O)}.[21] ^{19}F and ^1H NMR spectra of its (*S*)-MTPA ester, L-**14b,** had no signals attributable to D-**14b** . Pfitzner—Moffat oxidation of L-**14** gave 2,4:3,5-di-*O*-ethylidene-L-xylose (L-**15**) with m.p. 162—164°C and $[\alpha]_D^{22}$ –13.4° (H_2O). Lit.[22]: m.p. 152-160°C, $[\alpha]_D^{20}$ –13.2° (H_2O). Acid-catalysed hydrolysis of diacetal L-**15** gave the target L-**2** with m.p. 145-146°C and $[\alpha]_D^{22}$ –18.4° (H_2O). Lit.[15a]: m.p.144°C, $[\alpha]_D^{20}$ –18.6°(H_2O).

Allowing for 55% conversion of *rac*-**14a** in the kinetic resolution step, material yield of L-**2** from **4** is ~16% over six steps of the synthesis. This compares well with earlier syntheses of L-**2** from other sugars.[5-8]



Scheme 2. *Reagents and Conditions.* i, MeCHO/conc. HCl, 50°C; ii, Ac_2O—DMAP/Py; iii, H_2O/PPL, (pH 7), RT; iv, DMSO—DCC—H_3PO_4 (cat.), RT; v, KOH/MeOH, RT; vi, H_2O —H_2SO_4 (cat.)/Me_2CO, 60°C.

Alternative PPL-mediated approach from **4** to L-**2,** involving the transformation of **4** into 1-*O*-acetyl-2,3:4,5-di-*O*-isopropylidene-DL-xylitol (*rac*-**16a**) *via* the corresponding alcohol (*rac*-**16**)[23] and enzymatic hydrolysis of *rac*-**16a** to 35-45% conversion, proved to be inefficient. In this case the ee of the resulting alcohol L-**16** {m.p. 34-35°C, $[\alpha]_D^{22}$ +3.25°(EtOH)} was only 26-28%. Lit.(for L-**16**)[24]: m.p. 33-35°C, $[\alpha]_D^{18}$ +12.5°(EtOH). When the unconverted fraction of the acetate (i.e., mainly D-**16a**) was again hydrolysed in the presence of PPL to 38% conversion, and residual D-**16a** was saponified, alcohol D-**16** thus obtained {m.p. 33-35°C, $[\alpha]_D^{22}$ –3.6° (EtOH)} had about 29% ee.



Concluding remarks. The protocols outlined in this paper are "one-enzyme sequences" that employ only most common, inexpensive, easy-to-handle lipases. They could be useful in preparing other L-aldoses from those of relatively common D-aldoses, that are latently symmetric and hence readily convertible into meso alditols.

A point of interest is the variation of PPL enantioselectivity with the size of remote substituents R in acetates *rac*-**5**,[25] *rac*-**13** and *rac*-**14a** (R = Me). The lack of enantioselectivity in the case of *rac*-**13** is not due to the non-catalytic hydrolysis, since the rate of the latter is quite negligible.[26] The opposite enantioselectivity of hydrolysis of *rac*-**5** and *rac*-**14a** is difficult to explain in terms of existing "static" active-site models of PPL.[27] Perhaps, it could be better interpreted by taking into account present-day knowledge of PPL[28] and possible competition for the active site between the staggered conformation of one enantiomer and the lowest-energy *gauche* conformation of another.[4]

EXPERIMENTAL

General. Porcine pancreatic lipase (PPL) was used as purchased from Serva (14 U/mg, Specimen A) or from Olainfarm, Latvia (47 U/mg, specimen B). The lipase from *Candida rugosa* (≡ *C.cylindracea*, CCL) with specific activity 2U/mg, N,N'-dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), vinyl acetate and (*S*)-(−)-Mosher's acid [(*S*)-MTPA] were purchased from Fluka AG. TLC: pre-coated Silufol plates (SiO_2). GLC: LKhM-8 MD instrument (FSU), stainless steel column (150×0.3 cm, FI detector; 5% SE-30 on Chromaton N-AW-DMCS; N_2 as the carrier, 1 atm, 60 mL/min) . Column chromatography: Silica gel 60. Optical rotations: JASCO-DIP 360 polarimeter. NMR spectra: Bruker AC-300 spectrometer (for ^1H nuclei) and Bruker AC-200 instrument (for ^{19}F or ^{13}C nuclei, respectively). Unless stated otherwise, NMR spectra were taken in $CDCl_3$ (δ in ppm relative to $SiMe_4$ for H and C, or to $FCCl_3$ for F); all coupling constants (*J*) are given in Hertz.

2,3:4,5-Di-*O*-isopropylidenedulcitol (6). Dulcitol (**3**) (5.0 g, 27.5 mmol), anhydrous $CuSO_4$ (8.4 g) and conc. H_2SO_4 (80 mcL) in dry acetone (100 mL) were mixed together and agitated for 65 h at RT. The reaction mass was filtered, the filtrate was neutralised by stirring with K_2CO_3 (5 g) for 1 h, separated from the solid phase, and cooled to deposit the first crop of crystals (972 mg, 27%) consisting of practically pure *rac*-**7**, m.p. 144-145°C. The mother liquor was concentrated to *ca.* ½ of its volume to give the second crop (774 mg, 21.5%) with m.p. 110—112°C, mainly meso diol **6**. The mother liquor was concentrated to 15 mL to afford on standing, the third crop of crystals (1.224 g, 34%) with m.p. 108-110°C; recrystallization quantitatively gave pure meso diol **6**, m.p. 111-112°C(acetone—hexane). Lit.[10a]: m.p. 110-112°C.

Intermediate fractions from several runs were combined (2.5 g), dissolved in Py (12.5 mL) and acetylated with Ac_2O (12.5 mL) at 20°C. After 2 h of stirring the solution was left at 4-5°C for 12 h, the deposit was collected and

recrystallized from abs. EtOH to give pure diacetate **6a**, m.p.135-136° (2.6 g, 76%). Lit.[10a]: m.p. 135-136°C. ^{1}H NMR: 1.32 (s, 6H); 1.37 (s, 6H); 2.18 (s, 3H); 3.51 (dd, 2H, $J_{3,4}$ =4.5, $J_{2,3} \equiv J_{4,5}$ = 1.6, 3-H and 4-H); 4.0-4.2 (m, 4H, 2-H, 5-H and 1-H_B and 6-H_B parts of ABC); 4.38 (dd, 2H, $J_{1,1} \equiv J_{6,6}$ =11.5, $J_{1,2} \equiv J_{5,6}$ = 1.8; 1-H_A and 6-H_A parts of ABC). The mother liquor was concentrated to give the second crop of crystals with m.p. 87-89°C (0.6 g, 17.5%). Recrystallization gave diacetate *rac*-**7a**, m.p. 89-90°C (EtOH—H_2O, 1:1). Lit.[10a]: m.p. 89-90°C.

Diacetate **6a** (2 g, 5.8 mmol) and KOH (647 mg, 11.6 mmol) in 25 mL of MeOH were stirred together at RT. The reaction was monitored by TLC (hexane—AcOEt, 1:1, R_f 0.75 for **6a** and R_f 0.25 for **6**). After 90 min, the mixture was neutralised to pH 7 (AcOH) and concentrated. The oily residue was washed with hot abs. acetone (5x5 mL), the extract was concentrated to 7 mL and diluted with hexane (14 mL). On standing overnight this solution deposited the crystals of pure diol **6** with m.p. 111-112°C, identical with the above specimen (1.35 g, 89%). When the aqueous work-up was attempted, the yield of **6** was only 62%.

(2*S*,5*R*)-1-*O*-Acetyl-2,3:4,5-di-*O*-isopropylidenedulcitol (L-8). A mixture of diol **6** (1.0 g, 3.8 mmol), vinyl acetate (1.1 mL, 11.4 mmol) and powdered CCL (1 g) in abs. Et_2O (30 mL) was stirred at RT for 19 h. By that time it contained diol **6** (R_f 0.25) and a monoacetate (R_f 0.55) in nearly equal amounts while the formation of diacetete **6a** (R_f 0.75) was insignificant. CCL was removed by filtration and washed with small portions of AcOEt. The filtrate and washings were combined, and concentrated to leave an oily residue that was chromatographed on a column of SiO_2 (28x2 cm). Fractions eluted with PhH—Et_2O (4:1, 100 mL) contained diacetate **6a** (100 mg, *ca.* 8%). Subsequent elution with PhH—Et_2O (2:1, 200 mL) gave monoacetate L-**8** (503 mg, 43%) as prisms with m.p. 71-72°C (from hexane—Et_2O) and $[\alpha]_D^{20}$ −9.05° (*c* 1.0, $CHCl_3$). For a specimen with 98% ee lit.[12]: $[\alpha]_D^{RT}$ −12° (*c* 2.2, $CHCl_3$). Anal. Calcd. for $C_{14}H_{24}O_7$: C, 55.26; H, 7.89. Found: C, 55.41; H, 8.03. ^{1}H NMR: 1.32 (s, 3H); 1.45 (br. s, 6H); 1.48 (s, 3H); 2.10 (s, 3H); 2.35 (br.s, 1H, OH); 3.7-3.9 (m, 4H, 3-H, 4-H and 6-H_2); 4.03-4.27 (m, 3H, 2-H, 5-H and 1-H_B); 4.40 (A-part of ABC, 1H, $J_{1,1}$ = 11, $J_{1,2}$ = 2; 1-H_A). ^{13}C NMR: 20.81 (**Me**C=O); 26.88 (**Me**$_2$C); 62.36 (C-1); 64.36 (C-6); 76.35, 76.99, 78.63, 78.79 (C-2, C-3, C-4, C-5); 109.84 (O**C**Me$_2$O); 110.49 (OC**Me**$_2$O); 170.74 (O-**C**=O).

Diacetate **6a** was saponified as above, and the resulting specimen of **6** was combined with crystalline fractions (m.p. 110-111°C) eluted from the column with pure Et_2O (470 mg, 47% recovery). This material was recrystallized from acetone—hexane and used again in the CCL-catalysed acylation step.

(2*S*,5*R*)-1-*O*-Acetyl-6-*O*-methylsulfonyl-2,3:4,5-di-*O*-isopropylidenedulcitol (9). Monoacetate L-**8** (200 mg, 0.65 mmol) was dissolved in dry $CHCl_3$ (4 mL) and treated with freshly distilled MsCl (82 mg, 0.71 mmol) in abs. Py (0.67 mL, 0.71 mmol). The mixture was stirred at RT for 48 h, left overnight, and washed with aqueous solutions of $CuSO_4$ (3x2 mL) and $NaHCO_3$ (2x2 mL) and with water(2 mL). The organic layer was dried (Na_2SO_4) and concentrated. The solid residue (240 mg) was recrystallized to give the acetoxy mesylate **9** (220 mg, 87%), m.p. 109-110°C (abs. EtOH) . Anal. Calcd. for $C_{15}H_{26}O_9S$: C, 47.12; H, 6.81. Found: C, 47.31; H, 6.93. ^{1}H NMR: 1.35 (s, 3H); 1.38 (s, 3H); 1.41 (br. s, 6H); 2.10 (s, 3H); 3.06 (s, 3H); 3.76 (pseudo-quintet, 2H, $J_{3,4}$ =5.7, $J_{2,3}$ (or $J_{4,5}$) =2, $J_{4,5}$ (or $J_{2,3}$) = 1.6; 3-H and 4-H); 4.05-4.36 [m, 4H; 2-H, 5-H, and 6-H_B overlapping with 1-H_B (B-part of ABC, $J_{1,1}$ = 11.4) at δ 4.10]; 4.40 (A-part of ABC, 1H, $J_{1,1}$ = 11.4, $J_{1,2}$ = 2.1; 1-H_A); 4.50 (A'-part of A'B'C', 1H, $J_{6,6}$ = 11.1, $J_{5,6}$ =1.9; 6-H_A). ^{13}C NMR: 20.81 (**Me**C=O); 26.78 (**Me**$_2$C); 26.86 (**Me**$_2$C); 37.62 (**Me**SO_2}; 64.20 (C-1); 68.82 (C-6); 76.38, 77.38, 78.52, 78.76 (C-2, C-3, C-4, C-5); 110.64 (Me$_2$**C**); 110.82 (Me$_2$**C**); 170.72 (O-**C**=O).

(2*S*,5*R*)-1-*O*-Acetyl-6-deoxy-6-iodo-2,3:4,5-di-*O*-isopropylidenedulcitol (10). A mixture of mesylate **9** (250 mg, 0.65 mmol) and dry NaI (~20-fold excess) in abs. acetone (12 mL) was refluxed for 48 h under argon. The solid phase was removed by filtration and washed with benzene (2x10 mL), the filtrate and washings were combined and concentrated; the oily residue was mixed with water (5 mL). The emulsion was extracted with PhH (5x5 mL), the combined organic layer

was washed with saturated aqueous solution of $Na_2S_2O_3$ (2x5 mL) and water (5 mL), dried (Na_2SO_4), and concentrated. The remaining gum (270 mg), consisting of a single product (TLC), was dissolved in hexane. The solution was filtered to remove the traces of inorganic material, and the volatile products were stripped off uner vacuum to leave a pale-yellow wax with m.p. 45-47°C (250 mg, 92.5%). Due to high solubility in many solvents, this substance could not be recrystallized. ^{1}H NMR: 1.23 (s, 3H); 1.27 (s, 3H); 1.34 (s, 3H), 1.40 (s, 3H); 1.71 (s, 3H); 3.17 (B-part of ABC, 1H, $J_{6,6}$ = 12, $J_{5,6}$ = 4.3; 6-H_B); 3.32 (A-part of ABC, 1H, $J_{6,6}$ = 12, $J_{5,6}$ = 4.3; 6-H_A); 3.62-3.80 (m, 3H, 3-H, 4-H, 5-H); 4.15-4.35 (m, 2H, 2-H and 1-H_B); 4.49 (A'-part of A'B'C', $J_{1,1}$ =12, $J_{1,2}$ = 2; 1-H_A). ^{13}C NMR(in C_6D_6): 7.33 (C-6); 20.45 (**Me**C=O); 27.10 (**Me**$_2$C); 27.33 (**Me**$_2$C); 27.90 (**Me**$_2$C); 64.45 (C-1); 78.90, 79.12, 79.90, 82,30 (C-2, C-3, C-4, C-5); 110.90 (Me$_2$**C**); 111.15 (Me$_2$**C**); 170.43 (O-**C**=O).

(2*S*,5*R*)-6-Deoxy-2,3:4,5-di-*O*-isopropylidenedulcitol (11). To a solution of iodide **10** (200 mg, 0.48 mmol) in 8 mL of abs. MeOH freshly prepared skeletal Ni (120 mg) and powdered K_2CO_3 (70 mg) were added. The reaction vessel was flushed with H_2, and a slow stream of H_2 (1 atm, 20°C) was continuously passed through the magnetically agitated reaction mixture for 3 h. The solid phase was removed by filtration, the filtrate was neutralized with AcOH to pH 7 and concentrated to leave chromatographically pure oil that solidified on standing. Recrystallization from hexane afforded pure alcohol **11** (119 mg, ≥98%), m.p. 57-58°C, $[\alpha]_D^{22}$ +11.6° (*c* 1.0, EtOH). Lit.: m.p. 54-59°C (petroleum ether)[5b], $[\alpha]_D^{23}$ +11.7° (*c* 0.9, EtOH)[13]. ^{1}H NMR (C_6D_6): 1.30 (d, 3H, $J_{5,6}$ = 5.2; 6-H_3); 1.41 (br.s, 6H, overlapping signals of two **Me**-C groups); 1.48 (s, 3H); 2.45 (br. s, 1H; OH); 3.52 (dd, 1H, $J_{3,4}$ = 5.2, $J_{4,5}$ = 5.5; 4-H); 3.45-4.15 (m, 5H, 1-H_2, 2-H, 3-H, 5-H). ^{13}C NMR (in C_6D_6): 18.98 (C-6); 27.21 (**Me**$_2$C); 27.43 (**Me**$_2$C);27.58 (**Me**$_2$C); 63.63 (C-1); 77.85, 80.01, 82.35, 83.76 (C-2, C-3, C-4, C-5); 109.58 (Me$_2$**C**); 110.02 (Me$_2$**C**).

L-Fucose (L-1). A solution of DCC (300 mg, 1.45 mmol) in 0.5 mL of benzene and that of anhydrous H_3PO_4 (24 mg, *ca.* 0.24 mmol) in abs. DMSO (0.24 mL) were added to a solution of **11** (119 mg, 0.48 mmol) in abs. DMSO (1.9 mL). The reaction mixture was stirred (RT, 4 h) and left overnight. Precipitated N,N'-dicyclohexylurea was removed by filtration, the filtrate was concentrated at 55-60°C (bath)/1 Torr to leave crude oxo diketal **12** (200 mg) that was immediately hydrolysed with 4.8 mL of 60% aqueous AcOH (100°C, Ar, 2 h,). The hydrolysate was concentrated at 50°C (bath)/10 Torr (with periodical addition and evaporation of 2-3 mL of distilled water); the remainder was dissolved in AcOEt—MeOH (3:1, 10 mL, v/v) and purified by column chromatography on SiO_2 pre-washed with the same mixed solvent. Early fractions contained unidentified side products and traces of dicyclohexylurea and DMSO (TLC, GLC), and the main fraction afforded pure L-**1** (as slowly solidifying syrup; R_f 0.53, AcOEt—MeOH, 3:1). Recrystallization from abs. EtOH gave prisms (38 mg, 48%) with m.p. 138-140°C and $[\alpha]_D^{22}$ −110° (in 15 min) → −75°(in 24 h) (*c* 0.95, H_2O). Lit.[5b,8]: m.p. 137-139°C (from EtOH), $[\alpha]_D^{RT}$ −73° ÷ −77° (at equilibrium, *c* 0.8-1.0, H_2O). ^{1}H NMR:(in D_2O): 1.27 (d, 3H, $J_{5,6}$ = 6.3; 6-H_3); 3.43 (dd, 1H, $J_{2,3}$ = 10, $J_{1,2}$ = 7.5; 2-H); 3.67 (dd, 1H, $J_{2,3}$ = 10, $J_{3,4}$ = 3.6; 3-H); 3.73-3.85 (m, 2H, 4-H and 5-H); 4.32 (d, 1H, $J_{1,2}$ = 7.5; 1-H). These data are almost identical with those reported earlier.[5b,8]

Attempted PPL-catalysed hydrolysis of diacetate 6a. Three experiments were performed at RT using vigorously stirred suspensions of **6a** (100 mg) in 0.1 M phosphate buffer (pH 7, 10 mL). Other experimental parameters are given below (enumeration order: PPL,specimen; PPL to **6a** ratio, w/w; exposure; substrate recovery, mg): A, 1:1, 99, 98; B, 1:1, 72, 100; B, 2:1, 115, 98. No spots of monoacetate **8** could be detected by TLC.

(2*R*,5*S*)-1-*O*-Acetyl-2,3:4,5-di-*O*-isopropylidenedulcitol (D-8). A solution of diol **6** (100 mg, 0.38 mmol) in abs. Et_2O (4 mL), vinyl acetate (0.11 mL, 1.14 mmol) and PPL (specimen A, 100 mg) were mixed and agitated at RT for 21 h and left overnight. Only diol **6** (R_f 0.25, R_t 4.75 min) and its monoacetate (R_f 0.55, R_t 9.0 min) were detected by TLC (AcOEt—MeOH, 3:1) and GLC (130°C) in the reaction mass. Work-up: the same as for L-**8**. Elution with PhH—Et_2O (3:1) gave 3 mg (≤2.5%) of diacetate **6a**, while the late fractions, eluted with pure Et_2O, contained 53 mg of diol **6** with

m.p. 111-112°C (47% conversion). Intermediate fractions, eluted with PhH—Et_2O (2:1), afforded monoacetate D-**8** (38 mg, 40.5%), m.p. 67-70°C, $[\alpha]_D^{22}$ +12.8° (*c* 1.0, $CHCl_3$). ^1H NMR spectrum of this compound practically coincided with that of its antipode L-**8**. Lit.[12]: $[\alpha]_D^{RT}$ +12.5° (*c* 1.2, $CHCl_3$).

2,4:3,5-Di-*O*-ethylidene-DL-xylitol (*rac*-14). By analogy with a known procedure,[25] a mixture of MeCHO (5.6 mL, 99 mmol), xylitol **4** (5g, 32.7 mmol) and conc. HCl (2.5 mL) in water (5 mL) was stirred at 50°C for 3 h, neutralized to pH 7 ($NaHCO_3$), and extracted with $CHCl_3$ (5x25 mL). The extract was dried (Na_2SO_4) and concentrated to give a rapidly solidifying syrup. Recrystallization from petroleum ether—AcOEt (5:1, v/v) afforded *rac*-**14** (5 g, 75%), m.p. 139-140°C. ^1H NMR: 1.35 (d, 3H, J = 5.2; **MeCHO$_2$**); 1.45 (d, 3H, J = 5.1; **MeCHO$_2$**); 2.25 (s, 1H, OH); 3.49 (dd, 1H, $J_{4,5B}$ and/or $J_{3,4}$ = 1.5, $J_{4,5A}$ = 1.2; 4-H); 3.65 (m, 1H, 3-H); 3.72-3.90 (m, superimposed on the B-part of ABC with J_{AB} = 12.5; 4H, 1-H_2, 2-H and 5-H_B); 4.12 (A-part of ABC, J_{AB} = 12.5, $J_{4,5A}$ = 1.2; 5-H_A); 4.70 (q, 1H, J = 5.2; Me**CHO$_2$**); 4.83 (q, 1H, J = 5.1; Me**CHO$_2$**).

1-*O*-Acetyl-2,4:3,5-di-*O*-ethylidene-D,L-xylitol (*rac*-14a). A mixture of *rac*-**14** (1.5 g, 7.35 mmol), Ac_2O (1.38 mL, 14.7 mmol), DMAP (30 mg) and Py (0.6 mL, 7.35 mmol) was agitated at 20±2°C for 16 h, diluted with ice-cold water (10 mL), neutralised with solid K_2CO_3, extracted with $CHCl_3$ (4x15 mL). The extract was washed with aqueous solutions of $CuSO_4$, HCl (1 N) and $NaHCO_3$, then with water, dried (Na_2SO_4), and concentrated. The oily residue was dissolved in petroleum ether and cooled with liquid N_2 (vapors). The crystalline precipitate (1.74g, 96%) melted at 54-55°C and was free of GLC-detectable contaminants (a single peak, R_t 7.1 min). ^1H NMR: 1.30 (d, 3H, J = 5.1; **MeCHO$_2$**); 1.40 (d, 3H, J = 5.2; **MeCHO$_2$**); 2.05 (s, 3H, **MeC=O**); 3.50 (m, 1H, 4-H); 3.63 (m, 1H; 3-H); 3.80-4.25 (m, 5H; 2-H, 5-H_2, 1-H_2); 4.70 (q, 1H, J = 5.1; Me**CHO$_2$**); 4.78 (q, 1H, J = 5.2; Me **CHO$_2$**). ^{13}C NMR: 20.65 (**MeC=O**); 21.11 (**MeCHO$_2$**); 63.02 (C-1); 69.86, 69.93, 70.11, 75.83 (C-2, C-3, C-4, C-5);98.62 (Me**CHO$_2$**); 98.81 (Me**CHO$_2$**); 170.83 (O-C=O).

PPL-Catalysed hydrolysis of *rac*-14a. Standard procedure. Finely ground acetate *rac*-**14a** (2.0 g) was dispersed in 17 mL of 1 N phosphate buffer (pH 7) by vigorous magnetic stirring, at 20°C. Then PPL (specimen B) was added in one portion, and the stirring was continued under argon. The reaction was monitored (TLC, GLC) and arrested either at 45% conversion (in 19 h) or at 55% conversion (in 25 h). The reaction mass was concentrated at 45-50°C (bath)/10 Torr, the resulting slurry was diluted with $CHCl_3$ (20 mL), and hydrophilic substances were re-extracted with distilled water (4x5 mL). The organic layer was dried (Na_2SO_4) and concentrated; the oily residue was chromatographed on a column (30x2 cm). Elution with hexane—AcOEt (2:1) gave unconverted acetate (~100% recovery). Elution with pure AcOEt gave the dextrorotatory alcohol, the recovery of which from chloroformic extract was 85-87 % of theoretically possible; additional amount of the alcohol was extracted with $CHCl_3$ (3x5mL) from aqueous phase.

(1*R*,3*S*,5*S*,6*R*,8*S*)-3,8-Dimethyl-2,4,7,9-tetraoxabicyclo[4.4.2]dec-5-ylmethanol (D-14). The reaction was arrested at 45±2% conversion and worked-up as described above. Fractions, eluted with AcOEt, gave crystalline diol D-**14** (1.427 g, 86%), m.p. 135-138°C, $[\alpha]_D^{22}$ +3.5° (*c* 1.0, H_2O). Recrystallization gave a specimen with m.p. 139-140°C (AcOEt) and $[\alpha]_D^{22}$ +3.6° (*c* 1.02, H_2O). ^1H NMR: spectrum: the same as for *rac*-**14**.

(1*S*,3*R*,5*R*,6*S*,8*R*)-3,8-Dimethyl-2,4,7,9-tetraoxabicyclo[4.4.2]dec-5-ylmethanol (L-14). The reaction was stopped at 55% conversion and worked-up as above. Fractions, eluted from the column with hexane—AcOEt (2:1), were concentrated to give 902 mg (~100%) of acetate L-**14a** as chromatographically pure (TLC, GLC) colourless oil with $[\alpha]_D^{22}$ +7.30° (*c* 1.0, $CHCl_3$) and R_t (130°C) 4.4 min. ^1H NMR spectrum: the same as for *rac*-**14a**.

This material (492 mg, 2 mmol) was stirred with KOH (112 mg, 2 mmol) in 5 mL of MeOH (RT, 3 h); by that time only alcohol L-**14** was present (TLC, GLC). The mixture was neutralised and concentrated at 40°C (bath) to leave an oil that was diluted with water (6 mL) and extracted with $CHCl_3$ (5x10 mL). The extract was dried (Na_2SO_4) and evaporated to

give a solid residue (400 mg, 98%) with m.p. 135-138°C and $[\alpha]_D^{22}$ -3.5° (*c* 1.0, H_2O). Recrystallization (AcOEt) or sublimation (145°C/7 Torr) gave two identical specimens of L-**14** with m.pp. 139-140°C and $[\alpha]_D^{22}$ –3.5° (*c* 1.03, H_2O). Lit.[21]: m.p. 164-165°C, $[\alpha]_D^{RT}$ –3.5° (H_2O). ^1H NMR: the same as *rac*-**14**.

(*S*)-MTPA esters of L-14 (L-14b) and D-14 (D-14b) were obtained conventionally[29] as viscous gums. **L-14b.** ^{19}F NMR : –71.87 (s, the only signal). ^1H NMR : 1.25 (d, 3H, *J* = 5.2; **MeCHO$_2$**); 1.29 (d, 3H, *J* = 5.1; **MeCHO$_2$**); 3.45 (dd, 1H; 4-H); 3.55 (s, 3H; OMe), superimposed on 3.50-3.70 (m, 1H; 3-H); 3.76-4.15 (m, 2H; 2-H and 5-H_B); 4.30-4.60 (m, 3H, 5-H_A, 1-H_2); 4.70 (q, 1H, *J* = 5.1; MeC**HO$_2$**); 4.83 (q, 1H, *J* = 5.2; MeC**HO$_2$**); 7.35-7.60 (m, 5H; Ph). **D-14b.** ^{19}F NMR : –71.82 (s, the only signal). ^1H NMR: 1.23 (d, 3H, *J* = 5.1; **MeCHO$_2$**); 1.27 (d, 3H, *J* = 5.2; **MeCHO$_2$**); 3,46(br. s, 1H, 4-H);3.55 (s, 3H, OMe) 3.61 (m, 1H; 3-H); 3.70 (m, 1H; 2-H); 3.90 (m, 1H, 5-H_B); 4.10 (m, 1H; 5-H_A); 4.42 and 4.46 (B-part and A-part of ABC, 2H, 1-H_2); 4.72 (q, 1H, *J* = 5.1; MeC**HO$_2$**); 4.82 (q, 1H, *J* = 5.2; MeC**HO$_2$**); 7.35-7.6 (m, 5H; Ph).

2,4:3,5-Di-*O*-ethylidene L-xylose (L-15). A solution of DCC (1.514 g, 7.35 mmol) in abs. benzene (1 mL) was mixed with that of anhydrous H_3PO_4 (119 mg, 1.22 mmol) in abs. DMSO (0.4 mL). Then L-**14** (500 mg, 2.45 mmol) in DMSO (3.5 mL) was added, and the mixture was stirred at RT for 16 h. and left overnight. The bulk of N,N'-dicyclohexylurea was removed by filtration, the filtrate was concentrated at 80°C (bath)/1 Torr, and the oily residue was extracted with hot hexane (5x5 mL). The extract was left for 24 h to deposit additional crop of dicyclohexylurea. The mother liquor was filtered, refluxed (1 h) and left overnight to give crystalline aldehyde L-**15** [360 mg, m.p.145-155°C; R_t 3.0 min (130°C)] which still contained DMSO and other impurities (GLC). Sublimation at 190°C (bath)/1 Torr afforded pure L-**15** {m.p. 162-164°C, $[\alpha]_D^{20}$ –13.2° (*c* 1.0, H_2O)}. Yield: 340 mg (70%). Lit.[22]: m.p. 161-165°C, $[\alpha]_D^{RT}$ –13.2° (H_2O). ^1H NMR: 1.22 (d, 3H, *J* = 5.2; **MeCHO$_2$**); 1.39 (d, 3H, *J* = 5.1; **MeCHO$_2$**); 2.75 (dd, 1H, $J_{4,5B}$ = 1.9, $J_{4,5A}$ = 1.6; 4-H); 3.33 (B-part of ABC, 1H, J_{AB} = 12.6, $J_{4,5B}$ = 1.9; 5-H_B); 3.45-3.49 (m, 2H, 2-H and 3-H); 3.85 (A-part of ABC, 1H, J_{AB} = 12.6, $J_{4,5A}$ = 1.6; 5-H_A); 4.30 (q, 1H, *J* = 5.1; MeC**HO$_2$**); 4.37 (q, 1H, *J* = 5.2; MeC**HO$_2$**); 9.50 (s, 1H, 1-H). ^{13}C NMR : 20.49 (two **MeCHO$_2$**); 76.35, 76.98, 77.62 (C-2, C-3, C-4); 97.94 (MeC**HO$_2$**); 98.53 (MeC**HO$_2$**); 199.89 (C-1). Aqueous work-up[5b] was tedious and gave lower yields.

2,4:3,5-Di-*O*-ethylidene-D-xylose (D-15) was obtained from D-**14** in the same manner. Yield: 71%. M.p. 164°C (sublimation), $[\alpha]_D^{22}$ +13.4° (*c* 1, H_2O). ^1H NMR spectrum of D-**15** was identical with that of L-**15**.

L-Xylose (L-2). Diacetal L-**15** (202 mg, 1 mmol) was dissolved in acetone—water (3 mL, 2:1) and treated with conc. H_2SO_4 (40 mcL). The reaction mixture was refluxed for 7 h, cooled to RT, and stirred for 3 h with 1 g of AN-2 FH ion exchanger (OH form) to adjust pH to 7.0. The solution was filtered from the resin, the resin was washed with hot EtOH (3x3 mL), the filtrate and washings were combined and concentrated to leave a viscous oil. The oil was dissolved in water (4 mL), the solution was refluxed with activated charcoal (2x1 g) and filtered. The filtrate was evaporated to dryness, the amorphous residue was dissolved in hot EtOH. This solution was filtered, and concentrated to a syrup that was kept for a week in a vacuum dessicator over P_2O_5. The solidified residue was recrystallized from abs. EtOH to give pure L-**2** (120 mg, 80%) with m.p. 145-146°C (prisms) and $[\alpha]_D^{22}$ –76.3° and –18.6° (in 15 min and 2 h after dissolution, respectively) (*c* 1.0, H_2O). Lit.[15a]: m.p. 144°C, $[\alpha]_D^{20}$ –79.3° (initial) → –18.6 (at equilibrium) (H_2O).

D-Xylose (D-2) was obtained from diacetal D-**15** in exactly the same manner. Yield: 109 mg (73%). M.p. 144-145°C (from abs. EtOH), $[\alpha]_D^{22}$ +73.9° (15 min) → +18.8° (2 h) (*c* 1.0, H_2O).

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