Synthesis of Carbohydrates via Tandem Use of the Osmium-Catalyzed Asymmetric Dihydroxylation and Enzyme-Catalyzed Aldol Addition Reactions

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Abstract: A new strategy is described for the asymmetric synthesis of carbohydrate derivatives via the tandem use of the osmium-catalyzed asymmetric dihydroxylation (AD) and aldolase-catalyzed aldol addition reactions. Both D- and L-forms of fructose, 6-deoxy-*galacto*-2-heptulose, and 6-phenyl-*galacto*-2-hexulose were synthesized to illustrate this methodology.

Introduction

The synthesis of carbohydrates and their analogues is currently of considerable interest in organic chemistry, as these compounds are important components of many important natural products such as complex oligosaccharides,[1] glycoconjugates,[2] and antibiotics.[3] Most existing syntheses are based on chemical manipulation of naturally occurring sugars, where most, if not all, of the stereocenters in the product are derived directly from the starting material.[4] Such an approach is obviously limited to starting materials from the carbohydrate chiral pool and often necessitates elaborate selective protection/deprotection and activation/deactivation procedures to obtain the desired products. An alternative strategy previously described utilizes the Sharpless asymmetric epoxidation to synthesize a number of D- and L-sugars from achiral progenitors.[5a,b] Another elegant approach based on asymmetrization of *meso*-cyclic triols with lipase also leads to interesting sugars.[5c] A new strategy is described here which allows the quick and facile synthesis of carbohydrates from simple achiral precursors with complete stereocontrol. This powerful new approach is based on the tandem use of the osmium-catalyzed asymmetric dihydroxylation (AD) and enzyme-catalyzed aldol addition reactions.

Results and Discussion

The osmium-catalyzed AD of olefins is now routinely used to synthesize chiral vicinal diols of known relative and absolute configuration.[6] With α,β-unsaturated aldehydes, protected as the acetals, the AD followed by deprotection produces chiral dihydroxyaldehydes,[7] which are generally good acceptor substrates for aldolases.[8] Aldolases are enzymes which catalyze stereospecific aldol reactions between specific donor substrates and a wide variety of acceptor aldehydes.[9] Over 20 aldolases are known, most of which generate aldol products containing either one or two new hydroxymethylene stereocenters with defined stereochemistry.[10] Tandem use of the AD and aldolase-catalyzed reactions on alkenals allows direct access to numerous carbohydrate derivatives containing up to four new hydroxymethylene stereocenters. For example, eight stereoisomers of the corresponding 6-substituted hexulose can be synthesized when a particular (*E*)-alkenal is subjected to all permutations of the AD followed by an aldolase-catalyzed aldol addition reaction with dihydroxyacetone phosphate (DHAP) (Scheme 1). To illustrate this new methodology, the D- and L-forms of the hexuloses, fructose (**7a** and **6a**), 6-deoxy-*galacto*-2-heptulose (**6b** and **7b**), and 6-phenyl-*galacto*-2-hexulose (**6c** and **7c**), were synthesized from the appropriate α,β-unsaturated aldehydes: acrolein, (*E*)-crotonaldehyde, and (*E*)-cinnamaldehyde, respectively.

Acetals **1a–c** were easily prepared from the corresponding α,β-unsaturated aldehydes and 1,2-benzenedimethanol as previously described.[11] The use of the 1,2-benzenedimethanol acetal protecting group imparts several advantages to the overall methodology. It is relatively sterically demanding and can result in higher ee's in the AD, especially when R is hydrogen.[12] The vicinal diols produced are generally crystalline, which allows potential enhancement of ee via recrystallization, as well as facilitating purification. Also, the acetal deprotection can efficiently be carried out by $Pd^{II}O$-catalyzed hydrogenolysis, as well as acid-catalyzed hydrolysis. However, 1,2-benzenedimethanol is relatively expensive, and in some cases it may be convenient to use simpler acetals for this methodology. The essentially neutral pH of the latter deprotection conditions are important for compounds that are prone to acid-catalyzed racemization and polymerization.

The AD of **1a–c** utilizing AD-mix-β and AD-mix-α produced the 3-(1,2-dihydroxyalkyl)-1,5-dihydro-3*H*-2,4-benzodioxepine derivatives **2a–c** and **3a–c**, respectively, in almost quantitative

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(5) (a) Ko, S. Y.; Lee, A. W. M.; Masamune, S.; Reed, L. A.; Sharpless, K. B.; Walker, F. J. *Tetrahedron* **1990**, *46*, 245 and references therein. (b) Ko, S. Y.; Lee, A. W. M.; Masamune, S.; Reed, L. A.; Sharpless, K. B.; Walker, F. J. *Science* **1983**, *220*, 949. (c) Johnson, C. R.; Golebiowski, A.; Steensma, D. H. *J. Am. Chem. Soc.* **1992**, *114*, 9414.

(6) Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K.-S.; Kwong, H.-L.; Morikawa, K.; Wang, Z.-M.; Xu, D.; Zhang, X.-L. *J. Org. Chem.* **1992**, *57*, 2768 and references therein. Wang, L.; Sharpless, K. B. *J. Am. Chem. Soc.* **1992**, *114*, 7568.

(7) The synthesis of **2a** via the AD has been reported, see: Oi, R.; Sharpless, K. B. *Tetrahedron Lett.* **1992**, *33*, 2095.

(8) Polyhydroxyaldehydes are generally good substrates for adolases, see examples in reference 9.

(9) (a) Whitesides, G. M.; Wong, C.-H. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 617. (b) Toone, E. J.; Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. *Tetrahedron* **1989**, *45*, 5365. (c) Look, G. C.; Fotsch, C. H.; Wong, C.-H. *Acc. Chem. Res.* **1993**, *26*, 182 and references cited therein.

(10) With chiral substrates, the majority of adolases catalyze the aldol reaction with high enzyme-controlled stereoselectivity. Exceptions include *N*-acetylneuraminic acid aldolase and D-tagatose diphosphate aldolase, where the stereoselectivity is substrate controlled.

(11) Machinga, N.; Kibayashi, C. *Tetrahedron Lett.* **1989**, *30*, 4165.

(12) Unpublished results (K.B.S.).

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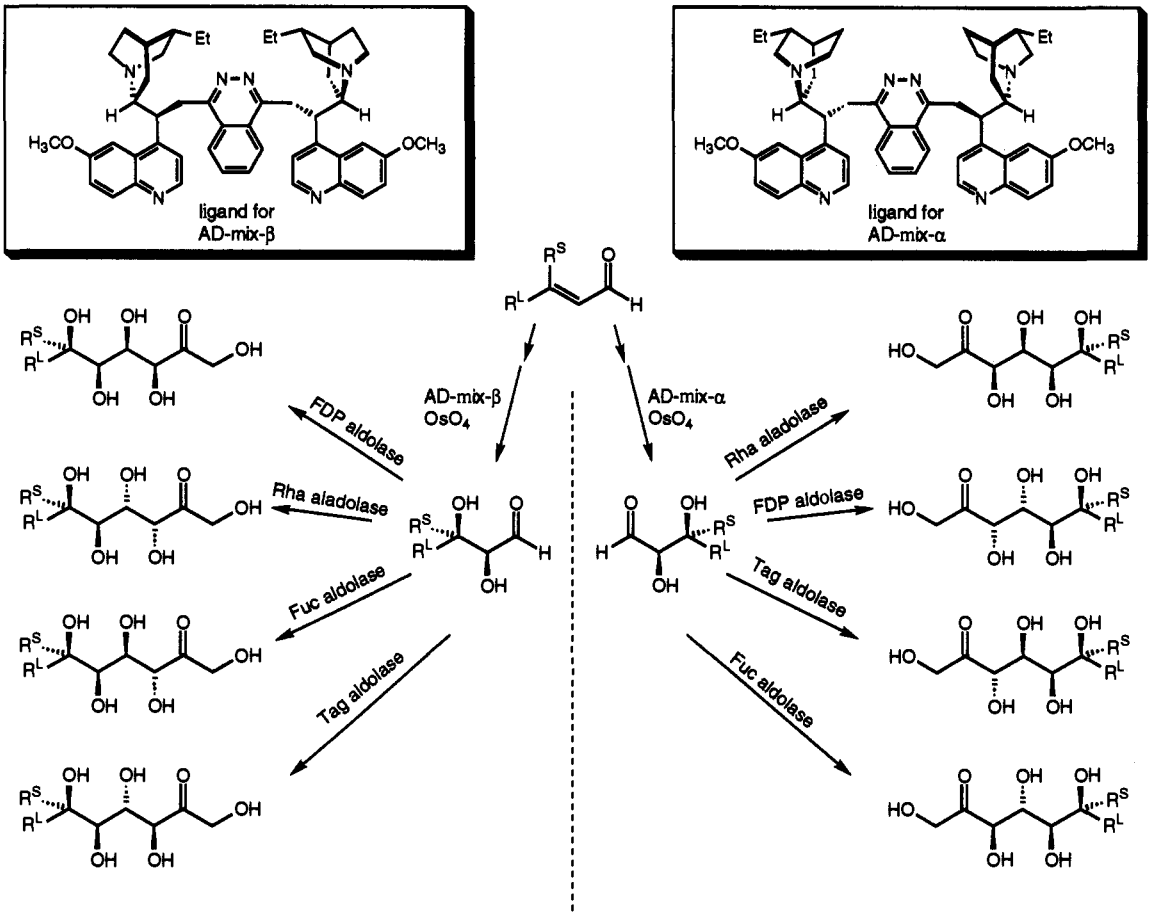
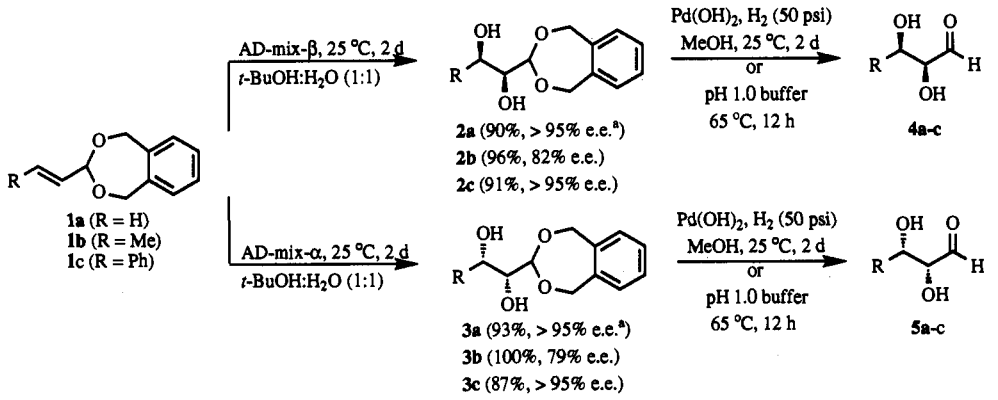
Scheme 1



Scheme 2[a]

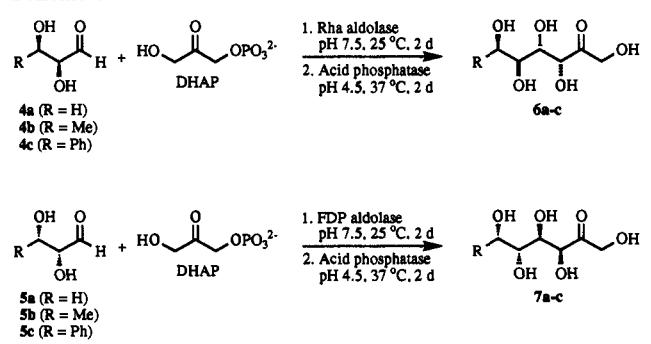


[a] After recrystallization from benzene.

yield and high ee (Scheme 2).[13] Subsequent removal of the acetal from **2a–c** and **3a–c** gave the aldehydes **4a–c** and **5a–c**. The deprotection of **2b,c** and **3b,c** was easily achieved by acid-catalyzed hydrolysis, although $Pd^{II}O$-catalyzed hydrogenolysis was used for **2a** and **3a** due to the acid sensitivity of the products L- and D-glyceraldehyde (**4a** and **5a**).[11,14]

The final step of each synthesis was the aldolase-catalyzed asymmetric aldol reaction of aldehydes **4a–c** and **5a–c** with DHAP

Scheme 3



to give the carbohydrate products **6a–c** and **7a–c** (Scheme 3). *In vivo*, L-rhamnulose 1-phosphate (Rha) aldolase catalyzes the

(13) The ee of each dihydroxylated product was determined by ^1H NMR analysis of the corresponding *bis*-MTPA ester. Compounds **2b** and **3b** also contained a small amount of diastereomeric product (3%) resulting from contaminating *cis* α,β-unsaturated aldehyde. The aldol reactions of **2b** and **3b** were subsequently performed with 0.88 and 0.87 equiv of DHAP, respectively. This avoided reaction of the minor enantiomer and diastereomer, since the products resulting from the major enantiomer of **2b** and **3b** are the thermodynamically most stable.

(14) The deprotection of **3** by hydrogenolysis sometimes required extra $Pd^{II}O$ catalyst (up to 1.0 equiv) for complete reaction.

reversible condensation of DHAP with L-lactaldehyde to give Rha.[15] Similarly, D-fructose diphosphate (FDP) aldolase catalyzes the aldol addition between DHAP and D-glyceraldehyde 3-phosphate to give FDP.[15] Both aldolases are specific for DHAP as the nucleophilic donor but have wide substrate specificity for the electrophilic acceptor aldehyde.[9,16-19] Rha aldolase generates 3*R*/4*S* stereochemistry in the product, while FDP aldolases gives the 3*S*/4*R* product.

The Rha aldolase-catalyzed reaction of aldehydes **4a–c**, derived using AD-mix-β, gave D-sugar derivatives **6a–c**.[20,21] Similarly, the FDP aldolase-catalyzed reaction of aldehydes **5a–c**, obtained using AD-mix-α, gave the enantiomeric L-sugar derivatives **7a–c**. These complementary synthetic sequences illustrate the flexibility of the methodology and also provide an example of enantiocomplementary tandem asymmetric catalysis. The aldehyde substrates used in the enzymatic aldol reactions need not be enantiomerically pure, if the major enantiomer is the preferred substrate for the enzyme. For example, reaction of DHAP (1 eq) with L-glyceraldehyde (3 equiv, 55% ee) catalyzed by Rha aldolase gave enantiomerically pure L-fructose in 75% yield.

In summary, the tandem use of the AD and aldolases has successfully been used for the facile asymmetric synthesis of L and D-forms of three hexuloses. By suitable choice of alkenal, AD-mix, and aldolase, numerous other carbohydrate derivatives and their steroisomers are potentially accessible via this approach. Though FDP and Tag aldolases prefer aldehydes with the *R*-configuration at the α-position, and Fuc and Rha aldolases prefer the *S*-configuration, they also accept the corresponding enantiomeric aldehydes as substrates. Many other pyruvate and acetaldehyde aldolases also behave similarly.[9] Use of aldehydes prepared from *cis*-olefins will give another class of sugars. Furthermore, this new strategy of asymmetric catalysis may also be extended to the synthesis of derivatized AD products, such as azidoaldehydes, for use in the aldol reaction, that will allow access to heterosubstituted carbohydrate derivatives. Similarly, using α-hydroxy aldehydes, potentially available from the AD of enol ethers, will lead to yet another class of carbohydrates.[22] The flexibility and reliability of this approach may also make it amenable to the construction of diverse combinatorial carbohydrate libraries. These and other studies on the development of this methodology are currently in progress.

Experimental Section

Melting points are uncorrected. Optical rotations were measured with a Perkin Elmer 241 polarimeter. Infrared spectra were recorded with a Perkin Elmer 1600 Series FTIR spectrometer. High-field NMR spectra were recorded on a 300- or 500-MHz instrument. Unless otherwise stated, $CDCl_3$ was used as solvent for NMR experiments, with chemical shifts reported in δ ppm relative to $CHCl_3$ as an internal reference (7.25 ppm for ^1H and 77.3 ppm for ^{13}C). When D_2O and CD_3OD were used as NMR solvents, MeOH (3.35 ppm for ^1H and 49.6 ppm for ^{13}C) was the internal reference. When abbreviated DEPT sequence experiments were carried out during ^{13}C NMR experiments, the carbon multiplicities are listed as (C) quaternary, (CH_2) methylene, and (CH/CH_3) methine/methyl. The purity of all products was assessed as >95% via ^1H and ^{13}C NMR analyses. Thin-layer chromatography was performed on silica gel 60 F254 plates. Flash chromatography was performed on silica gel 60 (230–400 mesh ASTM).

3-Vinyl-1,5-dihydro-3*H*-2,4-benzodioxepine (1a). Compound **1a** was prepared from acrolein as previously described.[7]

3-(1-Propenyl)-1,5-dihydro-3*H*-2,4-benzodioxepine (1b). Triethyl orthoformate (41.1 mL, 376 mmol, 10.0 equv) was added to a solution of 1,2-benzenedimethanol (5.20 g, 37.6 mmol, 1.00 equiv) and *p*-toluenesulfonic acid (0.358 g, 1.88 mmol, 0.05 equiv) in anhydrous toluene (50 mL) at 25 °C under N_2. The reaction was stirred at 25 °C for 21 h. Et_2O (100 mL) was added and the resultant organic fraction washed with saturated aqueous $NaHCO_3$ (100 mL) and H_2O (100 mL) and then dried ($MgSO_4$). Removal of the volatiles *in vacuo* gave crude 3-methoxy-1,5-dihydro-3*H*-2,4-benzodioxepine (6.22 g, 92%) as a colorless oil.[11]

(*E*)-Crotonaldehyde (4.29 mL, 51.8 mmol, 1.50 equiv) was added to a solution of crude 3-methoxy-1,5-dihydro-3*H*-2,4-benzodioxepine (6.22 g, 34.6 mmol, 1.00 equiv) and *p*-toluenesulfonic acid (0.329 g, 1.73 mmol, 0.05 equiv) in 50 mL of anhydrous toluene at 25 °C under N_2. The reaction was stirred at 25 °C for 23 h. Et_2O (200 mL) was added and the resultant organic fraction washed with saturated aqueous $NaHCO_3$ (200 mL) and then dried ($MgSO_4$). Removal of the volatiles *in vacuo* and purification by flash chromatography (5–10% EtOAc in hexanes) gave the product **1b** (2.35 g, 36%) as a colorless oil: R_f 0.2 (5% EtOAc in hexanes); ^1H NMR δ 7.21–7.14 (m, 4H), 5.98 (dqd, J = 15.5, 6.5 and 1.0 Hz, 1H), 5.64 (ddq, J = 15.5, 4.5, and 1.5 Hz, 1H), 5.33 (br d, J = 4.5 Hz, 1H), 4.93 (d, J = 15.0 Hz, 2H), 4.87 (d, J = 15.0 Hz, 2H), 1.76 (ddd, J = 6.5, 1.5, and 1.0 Hz, 3H); ^{1} ^3C NMR δ 138.8 (C), 130.1 (CH/CH_3), 127.8 (CH/CH_3), 127.1 (CH/CH_3), 127.0 (CH/CH_3), 104.7 (CH/CH_3), 70.0 (CH_2), 17.6 (CH/CH_3); IR (neat) 1720 (md), 1685 (st with shoulders), 1495 (wk) cm^{-1}; MS (LSIMS$^+$) m/z (relative intensity) 213 (37, M + Na^+), 121 (100); HRMS calcd for $C_{12}H_{14}O_2$ + Na 213.0892, found 213.0892. Note there were also some (*Z*)-isomer of **1b** present (~6% from ^1H NMR) derived from contaminating (*Z*)-crotonaldehyde.

3-(3-Phenyl-1-propenyl)-1,5-dihydro-3*H*-2,4-benzodioxepine (1c). (*E*)-Cinnamaldehyde (3.15 mL, 25.0 mmol, 1.00 equiv) was added to a solution of crude 3-methoxy-1,5-dihydro-3*H*-2,4-benzodioxepine (4.50 g, 25.0 mmol, 1.00 equiv) (for preparation, see synthesis of **1b**) and *p*-toluenesulfonic acid (0.238 g, 1.25 mmol, 0.05 equiv) in anhydrous DME (100 mL) at 25 °C under N_2. The reaction was stirred at 25 °C for 2 h. Et_2O (250 mL) was added and the resultant organic fraction washed with saturated aqueous $NaHCO_3$ (50 mL and then dried ($MgSO_4$). Removal of the volatiles *in vacuo* and purification by flash chromatography (5% EtOAc in hexanes) gave the product **1c** (3.50 g, 56%) as colorless crystals (recrystallized from hexanes): mp 102–103 °C; R_f 0.4 (5% EtOAc in hexanes); ^1H NMR δ 7.42–7.15 (m, 9H), 6.85 (br d, J = 16.0 Hz, 1H), 6.27 (dd, J = 16.0 and 4.0 Hz, 1H), 5.55 (dd, J = 4.0 and 1.0 Hz, 1H), 4.99 (d, J = 14.0 Hz, 2H), 4.92 (d, J = 14.0 Hz, 2H); ^{13}C NMR δ 1 38.8 (C), 136.1 (C), 133.2 (CH/CH_3), 128.6 (CH/CH_3), 128.2 (CH/CH_3), 127.3 (CH/CH_3), 127.1 (CH/CH_3), 126.9 (CH/CH_3), 125.6 (CH/CH_3), 104.2 (CH/CH_3), 70.0 (CH_2); IR ($CHBr_3$) 1655 (wk), 1600 (wk), 1575 (wk), 1490 (md) cm^{-1}; MS (LSIMS$^+$) m/z (relative intensity) 253 (100, M + H^+); HRMS calcd for $C_{17}H_{16}O_2$ + H 253.1229, found 253.1235.

3-(1*S*,2-Dihydroxyethyl)-1,5-dihydro-3*H*-2,4-benzodioxepine (2a). Compound **2a** was prepared in >95% ee from **1a** using AD-mix-β as previously described.[7]

3-(1*S*,2*R*-Dihydroxypropyl)-1,5-dihydro-3*H*-2,4-benzodioxepine (2b). Compound **1b** (0.475 g, 2.50 mmol, 1.00 equiv) was added to a well-stirred mixture of AD-mix-β (3.50 g) and methanesulfonamide (0.238 g, 2.50 mmol, 1.00 equiv) in a 1:1 mixture of t-BuOH/H_2O (25 mL) at 25 °C. The reaction was stirred for 2 days, CH_2Cl_2 (100 mL) was then added, and the organic layer was collected after shaking. The aqueous phase was extracted with a further 2 × 50 mL of CH_2Cl_2, and the combined organic fractions were dried ($MgSO_4$). Removal of the volatiles *in vacuo* and purification by flash chromatography (40–80% EtOAc in hexanes) gave the product **2b** (0.538 g, 96%) as a viscous oil: R_f 0.5 (80% EtOAc in hexanes); $[\alpha]^{25}D$ –7.4° (*c* 5.2, $CHCl_3$); 82% ee (from ^1H NMR of *bis*-Mosher ester); ^1H NMR δ 7.26–7.19 (m, 4H), 5.00 (d, J = 5.0 Hz, 1H), 4.97 (d, J = 14.0 Hz, 2H), 4.90 (d, J = 14.0 Hz, 1H), 4.89 (d, J

(15) FDP aldolase from rabbit muscle is commercially available. Rha aldolase is not, but it is an inducible enzyme of *E. coli* and has recently been cloned and overexpressed in *E. coli*.[18] For this study, it was overproduced by growth of *E. coli* K40 in M9 minimal medium with L-rhamnose as the sole carbon source.[20] The enzyme was released just prior to use by treatment of the whole cells with lysozyme.

(16) Takagi, Y. *Methods Enzymol.* **1966**, *9*, 542. Chiu, T. H.; Feingold, D. S. *Biochem. Biophys. Res. Commun.* **1965**, *19*, 511.

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(18) Fessner, W.-D.; Sinerius, G.; Schneider, A.; Dreyer, M.; Schulz, G. E.; Badia, J.; Aguilar, J. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 555.

(19) Liu, K. K.-C.; Kajimoto, T.; Chen, L.; Zhong, Z.; Ichikawa, Y.; Wong, C.-H.: *J. Org. Chem.* **1991**, *56*, 6280 and references therein.

(20) The aldolase-catalyzed aldol additions followed by acid phosphatase-catalyzed phosphate hydrolysis generally proceeded in modest to good yield. The resulting carbohydrates were isolated and fully characterized. In solution, these compounds exist in several different forms.

(21) By convention, tandem use of AD-mix-β/Rha aldolase and AD-mix-α/FDP aldolase produces D- and L-sugars, respectively. The one exception involves the smallest substrate acrolein, whereby the D/L assignment of the product fructose is made at C-5 rather than C-6. Thus, AD-mix-β/Rha aldolase gives L-fructose, and AD-mix-α/FDP aldolase gives D-fructose.

(22) Hashiyama, T.; Morikawa, K.; Sharpless, K. B. *J. Org. Chem.* **1992**, *57*, 5067.

= 14.0 Hz, 1H), 4.07 (m, 1H), 3.43 (m, 1H), 2.67 (d, J = 6.0 Hz, 1H), 2.48 (d, J = 5.5 Hz, 1H), 1.24 (d, J = 6.5 Hz, 3H); ^{13}C NMR δ 139.0 (C), 128.1 (CH/CH_3), 128.0 (CH/CH_3), 109.2 (CH/CH_3), 74.6 (CH_2), 73.8 (CH/CH_3), 73.5 (CH/CH_3), 66.2 (CH_2), 19.7 (CH/CH_3); IR ($CHBr_3$) 3445 (br st) cm^{-1}; MS (LSIMS$^+$) m/z (relative intensity) 247 (100, M + Na$^+$); HRMS calcd for $C_{12}H_{16}O_4$ + Na 247.0946, found 247.0951. Note there was also a *cis* isomer of **2b** present (~6% from ^{1}H NMR) derived from contaminating *cis*-**1b**.

3-(3-Phenyl-1*S*,2*R*-dihydroxypropyl)-1,5-dihydro-3*H*-2,4-benzodioxepine (2c). Using an experimental procedure analogous to that described for **2b**, **1c** gave **2c** in 91% yield after flash chromatography (20–40% EtOAc in hexanes) as colorless crystals (recrystallized from benzene): mp 125.5–126.5 °C; R_f 0.5 (60% EtOAc in hexanes); $[\alpha]^{25}_D$ +25.4° (c 3.0, $CHCl_3$); >95% ee (from ^{1}H NMR of *bis*-Mosher ester); ^{1}H NMR δ 7.43–7.23 (m, 9H), 5.01–4.91 (m, 6H), 3.81 (m, 1H); ^{13}C NMR δ 141.0 (C), 138.9 (C), 128.3 (CH/CH_3), 127.9 (CH/CH_3), 127.8 (CH/CH_3), 127.6 (CH/CH_3), 126.4 (CH/CH_3) 108.2 (CH/CH_3), 75.6 (CH/CH_3), 73.6 (CH_2), 73.5 (CH_2), 72.3 (CH/CH_3); IR ($CHBr_3$) 3550 (st), 3455 (br st), 1495 (wk) cm^{-1}; MS (LSIMS+) m/z (relative intensity) 309 (100, M + Na$^+$); HRMS calcd for $C_{17}H_{18}O_4$ + Na 309.1103, found 309.1105.

3-(1*R*,2-Dihydroxyethyl)-1,5-dihydro-3*H*-2,4-benzodioxepine (3a). Using AD-mix-α and an experimental procedure analogous to that described for **2a**, **1a** gave **3a** in >95% ee: all other data were equivalent to that given for enantiomer **2a**.

3-(1*R*,2*S*-Dihydroxypropyl)-1,5-dihydro-3*H*-2,4-benzodioxepine (3b). Using AD-mix-α and an experimental procedure analogous to that described for **2b**, **1b** gave **3b** in 100% yield after flash chromatography (40–80% EtOAc in hexanes) as a viscous oil: $[\alpha]^{25}_D$ +7.30° (c 3.2, $CHCl_3$); 79% ee (from ^{1}H NMR of *bis*-Mosher ester): all other data were equivalent to that given for enantiomer **2b**.

3-(3-Phenyl-1*R*,2*S*-dihydroxypropyl)-1,5-dihydro-3*H*-2,4-benzodioxepine (3c). Using AD-mix-α and an experimental procedure analogous to that described for **2b**, **1c** gave **3c** in 87% yield after flash chromatography (20–50% EtOAc in hexanes) as colorless crystals (recrystallized from benzene): mp 126–127 °C; $[\alpha]^{25}_D$ –26.1° (c 3.0, $CHCl_3$); >95% ee (from ^{1}H NMR of *bis*-Mosher ester): all other data were equivalent to that given for enantiomer **2c**.

L-Fructose (6a). A solution of **2a** (0.315 g, 1.50 mmol, 1.00 equiv) in MeOH (15 mL) containing $Pd^{II}O$ catalyst (45.9 mg, 0.375 mmol, 0.250 equiv) was placed under 50 psi of H_2 in a Parr hydrogenation apparatus and shaken at 25 °C for 2 days. The catalyst was then removed by filtration, and the volatiles were removed *in vacuo* to give L-glyceraldehyde (**4a**) as an oil.

K12 *Escherichia coli* containing excess Rha aldolase were obtained by growth of the cells in M9 minimal medium containing L-rhamnose as the sole carbon source. The cells were collected by centrifugation and stored at –70 °C. Just prior to use, 0.6 g (wet cells) were thawed, suspended in 10 mL of 0.1 M Tris buffer (pH 7.5), and lysed by incubation with 3 mg of lysozyme at 37 °C for 1 h.

To a solution of **4a** in 7.0 mL of 0.1 M Tris buffer (pH 7.5) was added 41.7 mL of a 0.036 mM solution of DHAP (1.50 mmol, 1.00 equiv), and the pH was adjusted to 7.5 with 1 M NaOH. The lysed cell suspension containing Rha aldolase was then added and the reaction stirred at 25 °C for 3 days under N_2. The pH was adjusted to 4.0–5.0 using 6 M HCl, and acid phosphatase (600 units) added. After the mixture was shaken at 37 °C in an incubator for 2 days, the pH was adjusted to 7.0 and H_2O removed by lyophilization to yield a yellow solid. MeOH was added, and the resulting yellow solution containing solid was filtered through Celite. Removal of volatiles *in vacuo* from the filtrate and purification by flash chromatography (10–30% MeOH in $CHCl_3$) gave the product **6a**: data were identical with those of commercially available fructose. $[\alpha]^{20}_D$ +133° to +92° (C2, H_2O).

6-Deoxy-D-*galacto*-2-heptulose (6b). Using an experimental procedure analogous to that described for **6a**, with acetal deprotection to give **4b** achieved via acid-catalyzed hydrolysis (see preparation of **7b**), and 0.88 equiv of DHAP, **2b** gave **6b** as a viscous oil in 60% after two rounds of flash chromatography (10–30% MeOH in $CHCl_3$): R_f 0.2 (30% MeOH in $CHCl_3$); ^{1}H NMR (CD_3OD) [major α-pyranose form and minor furanose form detected in a ratio of ~4:1] α-pyranose δ 4.16 (qd, J = 6.5 and 1.0 Hz, H-6), 3.82 (dd, J = 9.5 and 3.0 Hz, H-4), 3.78 (d, J = 9.5 Hz, H-3), 3.72 (dd, J = 3.0 and 1.0 Hz, H-5), 3.66 (d, J = 11.0 Hz, H-1), 3.56 (d, J = 11.0 Hz, H-1'), 1.23 (d, J = 6.5 Hz, 3H-7); furanose δ 4.11 (dd, J = 7.0 and 7.5 Hz, H-4), 4.06 (d, J = 7.5 Hz, H-3), 3.84 (m, H-6), 3.59 (s, H-1 and H-1'), 3.56 (m, H-5), 1.23 (d, J = 6.5 Hz, 3H-7); ^{13}C NMR (CD_3OD) [major α-pyranose form and minor furanose form detected in a ratio of ~4:1] δ 102.5 (C), 98.1 (C), 86.4 (CH/CH_3), 78.8 (CH/CH_3), 77.2 (CH/CH_3), 73.5 (CH/CH_3), 72.5 (CH/CH_3), 71.2 (CH/CH_3), 70.0 (CH/CH_3), 67.9 (CH/CH_3), 66.5 (CH_2), 65.1 (CH_2), 19.2 (CH/CH_3), 16.5 (CH/CH_3); MS (LSIMS$^+$) m/z (relative intensity) 149 (100); $[\alpha]^{22}_D$ +16.9° (c 1.5, MeOH).

6-Phenyl-D-*galacto*-2-hexulose (6c). Using an experimental procedure analogous to that described for **6a**, with acetal deprotection to give **4c** achieved via acid-catalyzed hydrolysis rather than hydrogenolysis (see preparation of **7b**), **2c** gave **6c** as a viscous oil in 18% yield after two rounds of flash chromatography (10–30% MeOH in $CHCl_3$): R_f 0.5 (30% MeOH in $CHCl_3$); ^{1}H NMR (CD_3OD) [mixture of forms—73% α-pyranose form, 27% of both anomeric furanose forms in a 4.4:1 ratio] α-pyranose δ 7.47–7.23 (m, 5H), 5.13 (s, H-6), 4.01 (d, J = 3.0 Hz, H-5), 3.98 (dd, J = 10.0 and 3.0 Hz, H-4), 3.88 (d, J = 10.0 Hz, H-3), 3.77 (d, J = 11.5 Hz, H-1), 3.61 (d, J = 11.5 Hz, H-1'); major furanose δ 7.47–7.23 (m, 5H), 4.71 (d, J = 4.0 Hz, H-6), 4.22 (dd, J = 7.0 and 6.5 Hz, H-4), 4.03 (d, J = 7.0 Hz, H-3), 3.90 (dd, J = 6.5 and 4.5 Hz, H-5), 3.50 (d, J = 11.5 Hz, H-1), 3.47 (d, J = 11.5 Hz, H-1'); minor furanose δ 7.47–7.23 (m, 5H), 4.77 (d, J = 3.5 Hz, H-6), 4.12 (dd, J = 5.0 and 6.5 Hz, H-4), 4.08 (dd, J = 6.5 and 3.5 Hz, H-5), ~3.98 (m, H-3), 3.70 (d, J = 11.5 Hz, H-1), 3.57 (d, J = 11.5 Hz, H-1'); ^{13}C NMR (D_2O) [major α-pyranose form and minor furanose form detected in a ratio ~4:1] δ 139.8 (C), 138.7 (C), 129.5 (CH/CH_3), 129.3 (CH/CH_3), 129.1 (CH/CH_3), 128.5 (CH/CH_3), 127.8 (CH/CH_3), 127.9 (CH/CH_3), 127.1 (CH/CH_3), 102.3 (C), 98.6 (C), 84.4 (CH/CH_3), 76.7 (CH/CH_3), 76.4 (CH/CH_3), 76.0 (CH/CH_3), 73.5 (CH/CH_3), 73.1 (CH/CH_3), 71.3 (CH/CH_3), 68.1 (CH/CH_3), 64.8 (CH_2), 63.1 (CH_2); MS (LSIMS$^+$) m/z (relative intensity) 279 (100, M + Na$^+$); HRMS calcd for $C_{12}H_{16}O_6$ + Na 279.0845, found 279.0839, $[\alpha]^{20}_D$ –4.9° (c 3.2, MeOH).

D-Fructose (7a). Using an experimental procedure analogous to that described for **6a** with commercially available FDP aldolase in place of the Rha aldolase obtained from K12 *E. coli*, **3a** was converted into **7a** (via **5a**): $[\alpha]^{20}_D$ –133° to –92° (c 2, H_2O).

6-Deoxy-L-*galacto*-2-hexulose (7b). Compound **3b** (94.0 mg, 0.420 mmol, 1.00 equiv) was added to 4.20 mL of 0.05 M pH 1.0 KCl–HCl buffer and stirred at 65–75 °C for 10 h. The reaction containing **5b** was then adjusted to pH 3.0–7.0 using 1 M NaOH, prior to adding 5.24 mL of 0.071 M DHAP (0.365 mmol, 0.869 equiv). After readjusting the pH to 6.5–7.0 using 1 M NaOH, FDP aldolase from rabbit muscle (188 units) was added, and the solution was stirred slowly at 25 °C for 25 h under N_2. The pH was then adjusted to 4.0–5.0 using 6 M HCl. Acid phosphatase (168 units) was added, and the reaction mixture was shaken at 37 °C in an incubator for 45 h. The pH was then readjusted to 7.0 and H_2O removed by lyophilization to yield a yellow solid. MeOH was added, and the resulting yellow solution containing solid was filtered through Celite. Removal of volatiles *in vacuo* from the filtrate and purification by two rounds of flash chromatography (10–30% MeOH in $CHCl_3$) gave the product **7a** (46.2 mg, 65%) as an oil: all other data were equivalent to that given for enantiomer **6b**; $[\alpha]^{22}_D$ –17.0° (c 1.7, MeOH).

6-Phenyl-L-*galacto*-2-hexulose (7c). Using an experimental procedure analogous to that described for **7b**, **3c** was converted into **7c** (via **5c**) in 18% yield after two rounds of flash chromatography (10–30% MeOH in $CHCl_3$) as a viscous oil: all other data were equivalent to that given for enantiomer **6c**; $[\alpha]^{20}_D$ +5.0° (c 3.0, MeOH).

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