### Abstract

A stereoselective synthesis of carbohydrates employs two key stereoselective steps, viz. an osmium-catalyzed asymmetric dihydroxylation (AD) of an alkene to form an aldehyde intermediate and an aldolase-catalyzed aldol addition reaction wherein the aldehyde intermediate is elongated by the addition of a ketone. Moreover, ketones having four new stereocenters can be synthesized without the use of chiral starting materials. The stereoselectivity of the asymmetric dihydroxylation (AD) reaction is determined by the cis or trans (Z or E) stereoisomeric configuration of the alkene and by the choice of AD-mix, i.e., an AD-mix-α or AD-mix-β. The stereoselectivity of the aldolase-catalyzed addition reaction is determined by the choice of aldolase and by the stereochemistry of the aldehyde intermediate. Four aldolases with broad substrate specificity are sufficient to produce all possible enantiomeric combinations of the two hydroxymethylene stereocenters formed during the aldol addition reaction. Accordingly, a synthetic scheme combining an osmium-catalyzed asymmetric dihydroxylation (AD) reaction and an aldolase-catalyzed aldol addition reaction enables direct access to a wide range of carbohydrate derivatives containing up to four new hydroxymethylene stereocenters.
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Catalytic Chemo-Enzymatic Asymmetric Synthesis of Carbohydrates

Specification

Field of Invention:
The invention relates to a catalytic chemo-enzymatic asymmetric synthesis of carbohydrates employing two key stereoselective steps. More particularly, the invention relates to a synthetic method employing a first key step for producing an aldol intermediate by stereoselective osmium-catalyzed asymmetric dihydroxylation of an alkene and a second key step for producing a carbohydrate by stereoselective aldolase-catalyzed addition to the aldol intermediate by a nucleophilic donor substrate of aldolase.

Background:
Many natural products have bioactivities that depend upon a carbohydrate component. Carbohydrates can have significant pharmaceutical and industrial value due to their contribution to such bioactivity. However, bioactive carbohydrates can also be relatively scarce and/or difficult to isolate in quantity from natural sources. Accordingly, there is a continuing interest in the synthesis of bioactive carbohydrates and their analogs. Carbohydrate analogs are of particular interest if such analogs have enhanced or altered utility over the corresponding natural product.

Techniques for the organic synthesis of carbohydrates have evolved rapidly. Conventional techniques for the organic synthesis of complex oligosaccharides are reviewed by N. Sharon in Complex
Carbohydrates (Addison-Wesley: Reading, MA, 1975). Progress with respect to the organic synthesis of glycoconjugates is reviewed in a series edited by Horowitz et al., i.e., The Glycoconjugates Vol. I-IV (Academic: New York, 1977-1982). Techniques for the organic synthesis of carbohydrate antibiotics are reviewed by Umezawa, i.e., Advances in Carbohydrate Chemistry and Biochemistry 1974, 30, 111. Moreover, the organic synthesis of carbohydrates by conventional methodologies has been characterized by its difficulty.

The principle difficulty posed by the synthesis of carbohydrates lies with the large number of stereocenters characteristically found in these compounds. Conventional methods of organic synthesis often achieve the required stereochemistry of carbohydrates by chemical manipulation of naturally-occurring sugars, i.e., the stereocenters in the product carbohydrate are derived directly from the stereocenters of the starting material. This approach is reviewed by S. Hanessian, i.e., Total Synthesis of Natural Products: The "Chiron" Approach (Organic Chemistry Series; Vol. 3), Pergamon Press: Oxford, 1983. This approach is limited by the availability of starting materials found in the carbohydrate chiral pool. Also, this approach often necessitates elaborate selective protection/deprotection and activation/deactivation procedures to obtain the desired products.

Ko et al. disclose a novel stereoselective method for carbohydrate synthesis which does not require chiral starting materials, i.e., Science 1983, 220, 949-951 and Tetrahedron 1990, 46, 245-264 and references therein. Ko's strategy utilizes a reiterative two-carbon extension cycle, i.e., each
cycle adds two carbons to a growing carbohydrate. Each cycle employs four key chemical transformations, including an asymmetric epoxidation of an achiral progenitor.

C. R. Johnson et al. disclose an enzyme assisted stereoselective organic synthesis of carbohydrates based on asymmetrization of meso-cyclic triols with lipase, i.e., Journal of the American Chemical Society. 1992, 114, 9414.

Moreover, it has been the direction of carbohydrate synthesis to endeavor to formulate synthetic strategies which maintain or enhance the stereoselectivity of the process while simplifying and/or shortening the synthetic process, i.e., reducing the number of steps. The disclosures by Ko and Johnson represent substantial advances in this regard over conventional methodologies.

It is disclosed herein that three factors should be considered when formulating a strategy for a stereoselective carbohydrate synthesis, viz.:

1. A synthetic scheme is considered stereoselectively advantageous if its reactions have kinetics which favor the formation of one stereoisomer over another.

2. A synthetic scheme is also considered stereoselectively advantageous if it employs catalysts or reactants having regioselective effects which favor the formation of one stereoisomer over another.

3. A synthetic scheme is also considered stereoselectively advantageous if it employs a donor which preferentially attacks one acceptor face over another.

It is also disclosed herein that, starting with achiral precursors, it is possible to achieve
substantially complete "either/or" stereoselective control over the entire synthesis of carbohydrates employing the above three factors.

What is needed is a simple facile strategy incorporating the above three factors for synthesizing carbohydrates and carbohydrate analogs employing simple achiral precursors and a minimal number of steps while providing excellent stereoselectivity and yield.

Summary:
The invention is a simple facile method for stereoselectively synthesizing carbohydrates, e.g., ketose sugars, aldoses, α-amino-β-hydroxyacids, and analogs thereof. The invention employs two key stereoselective steps. The first key stereoselective step employs an osmium-catalyzed asymmetric dihydroxylation (AD) of an alkene to produce an aldol intermediate. Preferred alkenes include α,β-alkenal and diolefins. However, other alkenals and polyolefins may also be employed. The second key stereoselective step employs an aldolase-catalyzed addition reaction for extending the aldol intermediate by the addition of a nucleophilic donor. The nucleophilic donor is selected to conform with the substrate specificity of the chosen aldolase. Preferred ketose sugars have at least six carbon atoms and three or four "new" stereocenters, i.e., stereocenters at positions C_{3,5} or C_{3,6} of such 2-ketohexoses are "new". Stereocenters are considered "new" if their chirality is not derived from the chirality of the starting material. The method achieves substantially complete stereoselectivity with respect to new stereocenters. The stereoselectivity of positions C_{5,6} of 2-ketohexoses is achieved during
the first key stereoselective step by the choice of catalyst and by the choice of the \( \alpha,\beta \)-alkenal stereoisomer. Stereoselectivity with respect to positions \( C_{3,4} \) of 2-keto hexose is achieved during the second key stereoselective step by the choice of aldolase. Accordingly, the invention discloses that substantially complete stereoselectivity with respect to the synthesis of all four stereocenters of 2-keto hexoses, i.e., positions \( C_{3,5} \), can be achieved by an appropriate choice of starting material stereoisomer and by two choices of catalysts.

The method of the invention achieves a rapid synthesis of ketose sugars utilizing a minimal number of steps while providing excellent stereospecificity, stereoselectivity, and yield. Stereoselectivity is achieved by the use of stereoselective catalysts. The method of the invention does not depend upon the chirality of the starting materials.

The preferred catalysts for the first key stereoselective step of the synthesis are regioselective organic catalysts which promote an asymmetric dihydroxylation (AD) reaction. The preferred regioselective catalysts are the commercially available AD-mix-\( \alpha \) or AD-mix-\( \beta \) disclosed by K.B. Sharpless in *Journal of Organic Chemistry* 1992, 57, 2768-2771, incorporated herein by reference, and in *The Journal of Organic Chemistry* 1993, 58, 3785-3786, also incorporated herein by reference. AD-mix-\( \alpha \) and AD-mix-\( \beta \) are available from Aldrich.

A preferred starting material for the osmium-catalyzed asymmetric dihydroxylation (AD) reaction is an \( \alpha,\beta \)-alkenal. The \( \alpha,\beta \)-alkenal may have multiple substituents on either side of the olefinic bond. If the \( \alpha,\beta \)-alkenal includes two non-hydrogen substituents, then it may form *cis* or *trans*
stereoisomers. If the α,β-alkenal includes more than two substituents, its stereoisomers may be categorized as zusammen (Z) or entgegen (E) according to the Cahn-Ingold-Prelog system. The stereoselectivity of the first key stereoselective step is determined by the combined choice of catalyst, i.e., the AD-mix-α or AD-mix-β, and by the choice of substrate stereoisomer, i.e., the zusammen (Z) or entgegen (E) stereoisomers of α,β-alkenal. The AD reaction requires only one protection/deprotection step, i.e., the conversion between an aldehyde and an acetal. The aldehyde is protected as an acetal prior to the asymmetric dihydroxylation (AD) reaction and is deprotected after the asymmetric dihydroxylation (AD), i.e., the acetal is re-converted to the aldehyde after the asymmetric dihydroxylation (AD) reaction so as to form the corresponding aldol.

A second preferred starting material for the osmium-catalyzed asymmetric dihydroxylation (AD) reaction is a polyolefin. A selective asymmetric dihydroxylation of a number of dienes using the Sharpless AD mix is disclosed by Xu et al., Journal of the American Chemical Society 1992, 114, 7570-7571. Dihydroxylated polyolefins can converted to the corresponding aldose by ozonization followed by treatment with NaI according to the method disclosed P.S. Bailey et al., Organic Synthesis 1961, 41, 41, incorporated herein by reference, or by periodation.

There are a number of other starting materials which can also be employed in an osmium-catalyzed asymmetric dihydroxylation (AD) reaction to stereospecifically produce an aldose. For example, an alkenol having a terminal alcohol can undergo an osmium-catalyzed asymmetric dihydroxylation. The newly added hydroxyls are selectively protected and
the terminal alcohol is then oxidized to form an aldehyde. The aldose is then produced by deprotection of the remaining alcohols. Other starting materials employable with the AD reaction to produce stereospecific aldoses would be known to those skilled in the art.

There are four preferred aldolases which may be employed to catalyze the second key stereoselective step, i.e., fructose 1,6-diphosphate (FDP) aldolase (EC 4.1.2.13), L-rhamnulose 1-phosphate (Rha 1-P) aldolase (EC 4.1.2.19), L-fuculose 1-phosphate (Fuc 1-P) aldolase (EC 4.1.2.17), and tagatose 1,6-diphosphate (Tag) aldolase. Each of the above aldolases employs dihydroxyacetone phosphate (DHAP) as a nucleophilic donor. Alternative aldolases employing DHAP include ketotetrose phosphate aldolase (EC 4.12.2) and phospho-5-keto-2-deoxygluconate aldolase (EC 4.1.2.29). DHAP aldolases catalyze the condensation of ketose sugars. The choice of aldolase determines the stereoselectivity of the aldol addition reaction. The four preferred aldolases, i.e., FCP, Tag, Fuc, and Rha aldolase, are sufficient to provide substantially all possible stereoselectivities to complete the production of essentially any 2-ketohehexose. Although DHAP is the preferred nucleophilic donor employable with this class of aldolase, alternative nucleophilic donors include a number of substrate analogs.

The second key stereoselective step may also be catalyzed by alternative aldolases employing nucleophilic donors other than DHAP for synthesizing sugars other than ketose. For example 2-ketoacids may be synthesized using aldolases which employ pyruvate or phosphoenolpyruvate as a nucleophilic donor.

Examples of aldolase which employ pyruvate include N-
acetylneuraminic aldolase (EC 4.1.3.3.), 3-deoxy-D-
manno-octulonosonate aldolase (EC 4.1.2.23), 2-keto-3-
deoxy-6-phosphogluconate aldolase (EC 4.1.2.14), 2-
keto-3-deoxy-6-phosphogalactonate aldolase (EC
4.1.2.21), 2-keto-3-deoxy-D-glucarate aldolase (EC
4.1.2.20), 2-keto-4-hydroxyglutarate aldolase (EC
4.1.2.31), 4-hydroxy-2-keto-4-methylglutarate aldolase
(EC 4.1.3.17), 2-keto-3-deoxy-D-xylonate aldolase (EC
4.1.2.28), 2-keto-3-deoxy-L-arabonate aldolase (EC
4.1.2.128), and tyrosinepheneol lyase (EC 4.1.99.2).
Examples of aldolase which employ phosphorolpyruvate
as a nucleophilic donor include N-acetylneuraminic
synthetase (EC 4.1.3.19.), 3-deoxy-D-arabino-2-
heptulosonic acid 7-phosphate synthetase (EC
4.1.2.15), and 3-deoxy-D-manno-octulonosonate synthetase
(EC 4.1.2.16).

The second key stereoselective step may also be
catalyzed by further alternative aldolases employing
acetaldehyde as a nucleophilic donors for synthesizing
aldose sugars and α-amino-β-hydroxyacids. For
example, 2-deoxyribose-5-phosphate aldolase (EC
4.1.2.4) can be employed in the second key
stereoselective step to synthesize 2-deoxyaldose
sugars. α-Amino-β-hydroxyacids can be synthesized by
this method using aldolases such as L-threonine
aldolase (EC4.1.2.5) which employ glycine or
hydroxyamine as nucleophilic donors.

The method of the invention provides excellent
yields and a product having high levels of
enantiomeric purity. The chirality of each of the C₅-6
stereocenters of product ketoses is determined as
follows:

1. The chirality of each of the two C₅-6
   stereocenters is determined by the cis/trans
   or E/Z stereoisomeric configuration of
substituents on the α,β-alkenal starting material and by the choice of catalyst, i.e., the preferred AD-mix-α or AD-mix-β. All four permutations of possible chirality combinations for the two C₅-6 stereocenters may be achieved by an appropriate selection of these variables.

2. The chirality of each of the two C₃-4 stereocenters is determined by the choice of aldolase and by the chirality of the α-carbon of the aldol. All four permutations of possible chirality combinations for the two C₃-4 stereocenters may be achieved by selecting an aldolase from the group consisting of FDP aldolase, RHA aldolase, Fuc aldolase, and Tag aldolase. However other aldolases may also be employed.

The method of the invention is quick, facile, and efficient, i.e., it employs only two key stereoselective steps. The method employs simple achiral starting materials and provides excellent yields with ketose sugars having a high level of enantiomeric purity. The two key stereoselective steps are synergistic because the product of the first key stereoselective step is employable as a reactant in the second stereoselective step. The combination of the two key stereoselective steps enables the creation of a product having four new stereocenters without employing chiral precursors.

Heterocarbohydrates may also be synthesized by the same method, except that appropriately derivatized starting material is employed.
Brief Description of the Drawings:

Figure 1 is a scheme illustrating the overall two step strategy for synthesizing 2-ketohexoses. $R_5$ represents a "small" R group; $R_1$ represents a "large" R group, as defined by the Sharpless selection rules. Only $E$-$\alpha,\beta$-alkenals are illustrated. Also illustrated in Fig. 1 are the preferred coordination structures of the osmium catalysts employed in the asymmetric dihydroxylation (AD) reactions, i.e., (DHQD)$_2$-PHAL (AD-mix-$\alpha$) and (DHQ)$_2$-PHAL (AD-mix-$\beta$).

Figure 2 is a scheme illustrating in greater detail the strategy of the first step illustrated in Fig. 1, i.e., the asymmetric dihydroxylation (AD) of $E$-$\alpha,\beta$-alkenals to form the corresponding aldol intermediate.

Figure 3 is a scheme illustrating in greater detail the strategy of the second step illustrated in Fig. 1, i.e., the aldolase-catalyzed aldol addition reaction for extending the aldol intermediate by the addition of DHAP.

Detailed Description:

It is disclosed herein that 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-methyl-galacto-hexulose (6b and 7b) and
6-phenyl-galacto-hexulose (6c and 7c), are synthesized
from the appropriate α,β-unsaturated aldehydes:
acrolein, E-crotonaldehyde and E-cinnamaldehyde,
respectively.

The osmium-catalyzed AD of olefins is
conventionally employed to synthesize chiral vicinal
diols of known relative and absolute configuration.
Improved catalysts (AD-mix-α and AD-mix-β) for
catalyzing the AD of olefins with enhanced yield and
stereochemical control is described by K.B. Sharpless
et al., viz., J. Am. Chem. Soc. 1992, 114, 7568 and J.
Sharpless also discloses that catalyzed AD of
α,β-unsaturated aldehydes may be achieved if the
aldehyde is first converted to an acetal (Tetrahedron
Lett. 1992, 33, 2095). Proper section of the acetal
may facilitate the catalyzed AD reaction and
subsequent recrystallizations. After completion of the
AD reaction, the acetal may be deprotected to provide
the corresponding dihydroxyaldehyde. For example,
Sharpless (Tetrahedron Lett. 1992, 33, 2095) reports
the synthesis of the dihydroxyaldehyde 2a via the AD
of the corresponding unsaturated acetal.

Dihydroxyaldehydes and polyhydroxyaldehydes are
preferred acceptor substrates for aldolases.
Aldolases are enzymes which catalyze stereospecific
aldol addition reactions between specific donor
substrates and a wide variety of acceptor aldehydes.
In an aldol addition reaction, the acceptor is
elongated by the formation of a carbon-carbon bond
with the acceptor aldehyde. Reviews of the chemistry
of aldolase catalyzed addition reactions are provided
1985, 24, 617), Toone et al., (Tetrahedron 1989, 45,
5365) and Look et al, (Acc. Chem. Res. 1993, 26, 182 and references cited therein). Over 20 aldolases are known, most of which generate aldol products containing either one or two new hydroxymethylene stereocenters with defined stereochemistry, i.e., the stereoselectivity of a majority of aldolase catalyzed addition reactions involving chiral substrates is highly enzyme-controlled and not substrate-controlled. Exceptions include N-acetylneuraminic acid aldolase and D-tagatose diphosphate aldolase, where the stereoselectivity is substrate controlled.

Acetals 1a-c can be prepared from the corresponding α,β-unsaturated aldehydes and 1,2-benzenedimethanol as described by Machinga and Kibayashi, Tetrahedron Lett. 1989, 30, 4165, incorporated herein by reference. The use of the 1,2-benzenedimethanol acetal protecting group imparts several advantages to the overall methodology. We have observed that it is relatively stericly demanding and can result in higher e.e.'s in the AD, especially when R is hydrogen. The vicinal diols produced are generally crystalline which allows potential enhancement of e.e. via recrystallization, as well as facilitating purification. Also, the acetal deprotection can be carried out efficiently by Pd(II)O-catalyzed hydrogenolysis, as well as acid-catalyzed hydrolysis. However, 1,2-benzenedi-methanol 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 produces the
3-(1,2-dihydroxyalkyl)-1,5-dihydro-3H-2,4-benzodioxepine derivatives 2a-c and 3a-c respectively in almost quantitative yield and high e.e. (Scheme 2). The e.e. of each dihydroxylated product can be determined by ¹H NMR analysis of the corresponding bis-MTPA ester. We found in one instance that compounds 2b and 3b also included a small amount of diastereomeric product (3%) resulting from contaminating cis α,β-unsaturated aldehyde. The aldol reactions of 2b and 3b can then be performed with 0.88 and 0.87 equivalents of DHAP respectively. This avoids reaction of the minor enantiomer and diastereomer, since the products resulting from the major enantiomer of 2b and 3b are the thermodynamically most stable. Subsequent removal of the acetal from 2a-c and 3a-c gives the aldehydes 4a-c and 5a-c. The deprotection of 2b,c and 3b,c can be achieved by acid-catalyzed hydrolysis. However, Pd(II)O-catalyzed hydrogenolysis is preferred for 2a and 3a because of the acid sensitivity of the products L- and D-glyceraldehyde (4a and 5a). (See: Machinga and Kibayashi, Tetrahedron Lett. 1989, 30, 4165.) The deprotection of 3 by hydrogenolysis sometimes required extra Pd(II)O catalyst (up to 1.0 equivalents) for complete reaction. The final step of each synthesis is the aldolase-catalyzed asymmetric aldol reaction of aldehydes 4a-c and 5a-c with DHAP to give the carbohydrate products 6a-c and 7a-c (Scheme 3). In vivo, L-rhamnulose 1-phosphate (Rha) aldolase catalyzes the reversible condensation of DHAP with L-lactaldehyde to give Rha. FDP aldolase from rabbit muscle is commercially available. Rha aldolase is not presently commercially available. However, it is an inducible enzyme of E. coli and has recently been cloned and overexpressed in E. coli. (See: Fessner et al., Angew. Chem. Int. Ed. Engl. 1991, 30, 555.) In
one instance, we overproduced Rha aldolase by growth of E. coli K40 in M9 minimal medium with L-rhamnose as the sole carbon source. The aldolase-catalyzed aldol additions followed by acid phosphatase-catalyzed phosphate hydrolysis generally proceeded in good yield. The resulting carbohydrates are isolated and fully characterized. In solution, these compounds exist in several different forms. The enzyme was released just prior to use by treatment of the whole cells with lysozyme. Similarly, D-fructose diphosphate (FDP)aldolase catalyzes the aldol addition between DHAP and D-glyceraldehyde 3-phosphate to give FDP. Both aldolases are specific for DHAP as the nucleophilic donor, but have wide substrate specificity for the electrophilic acceptor aldehyde. Substrate specificities for various aldolases are disclosed by numerous authors, including: Takagi, Method. Enzymol. 1966, 9, 542; Chiu, Biochem. Biophys. Res. Commun. 1965, 19, 511; Fessner, Tetrahedron Lett. 1992, 33, 5231 and Angew. Chem. Int. Ed. Engl. 1991, 30, 555; and by Liu, J. Org. Chem. 1991, 56, 6280 and references therein. Rha aldolase generates 3R/4S stereochemistry in the product, while FDP aldolase provides the 3S/4R product.

The Rha aldolase-catalyzed reaction of aldehydes 4a-c, derived using AD-mix-β, gave D-sugar derivatives 6a-c. The aldolase-catalyzed aldol additions followed by acid phosphatase-catalyzed phosphate hydrolysis generally proceeds in good yield. The resulting carbohydrates may be isolated and fully characterized. In solution, these compounds exist in several different forms. By convention, tandem use of AD-mix-β/Rha aldolase and AD-mix-α/FDP aldolase produce 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. Similarly, the FDP aldolase-catalyzed reaction of aldehydes 5a-c, obtained using AD-mix-α, gives 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 equivalent) with L-glyceraldehyde (3 equivalents, 55% ee) catalyzed by Rha aldolase gives enantiomerically pure L-fructose in 75% yield.

Use of aldehydes prepared from cis-olefins can 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 hetero-substituted carbohydrate derivatives.

The alkene may also be a polyolefin. Polyolefins can be stereoselectively converted an aldol intermediate by means of an osmium-catalyzed asymmetric dihydroxylation (AD) reaction. Selective asymmetric dihydroxylations of a number of dienes using the Sharpless AD mix are disclosed by Xu et al., Journal of the American Chemical Society 1992, 114, 7570-7571. Dihydroxylated polyolefins can be converted to the corresponding allose by ozonization followed by treatment with NaI according to the method disclosed P.S. Bailey et al., Organic Synthesis 1961,
41, 41, incorporated herein by reference, or by periodation.

Similarly, using α-hydroxy aldehydes, potentially available from the AD of enol ethers, will lead to yet another class of carbohydrates. (See: Hashiyama, T. et al., J. Org. Chem. 1992, 57, 5067.)

The flexibility and reliability of this approach may also make it amenable to the construction of diverse combinatorial carbohydrate libraries.

In summary, the tandem use of the AD and aldolases can be successfully been employed 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 stereoisomers are potentially accessible via this approach. Though FDP and Tag aldolases prefer aldehydes with R-configuration at 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. (See: Whitesides and Wong, Angew. Chem. Int. Ed. Engl. 1985, 24, 617; Toone, E.J. et al., Tetrahedron 1989, 45, 5365; and Look, G.C. et al. Acc. Chem. Res. 1993, 26, 182 and references cited therein.)

Examples:

Melting points are uncorrected. Optical rotations are measured with a Perkin Elmer 241 polarimeter. Infrared spectra are recorded with a Perkin Elmer 1600 Series FTIR spectrometer. High-field NMR spectra are recorded on a 300- or 500-MHz instrument. Unless otherwise stated, CDCl₃ was used as solvent for NMR experiments, with chemical shifts reported in δ ppm relative to CHCl₃ as an
internal reference (7.25 ppm for $^1$H and 77.3 ppm for $^{13}$C). When D$_2$O and CD$_3$OD are used as NMR solvents, MeOH (3.35 ppm for $^1$H and 49.6 ppm for $^{13}$C) was the internal reference. When abbreviated DEPT sequence experiments are 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 $^1$H 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).

**Example 1:**

3-Vinyl-1,5-dihydro-3H-2,4-benzodioxepine (1a).

Compound 1a was prepared from acrolein and physically characterized as previously described. The synthesis of 2a via the AD has been reported. (See: Oi and Sharpless, Tetrahedron Lett. 1992, 33, 2095.)

**Example 2:**

3-(1-Propenyl)-1,5-dihydro-3H-2,4-benzodioxepine (1b). Triethyl orthoformate (41.1 mL, 376 mmol, 10.0 equivalents) is added to a solution of 1,2-benzenedimethanol (5.20 g, 37.6 mmol, 1.00 equivalents) and p-toluenesulfonic acid (0.358 g, 1.88 mmol, 0.05 equivalents) in anhydrous toluene (50 mL) at 25°C under N$_2$. The reaction is stirred at 25°C for 21 h. Et$_2$O (100 mL) is added and the resultant organic fraction washed with saturated aq. NaHCO$_3$ (100 mL) and H$_2$O (100 mL), then dried (MgSO$_4$). Removal of the volatiles in vacuo gave crude 3-methoxy-1,5-dihydro-3H-2,4-benzodioxepine (6.22 g, 92%) as a colorless oil. (See: Machinga, N. et al., Tetrahedron Lett. 1989, 30, 4165.) E-Crotonaldehyde
(4.29 mL, 51.8 mmol, 1.50 equivalents) is added to a solution of crude 3-methoxy-1,5-dihydro-3H-2,4-benzodioxepine (6.22 g, 34.6 mmol, 1.00 equivalents) and p-toluenesulfonic acid (0.329 g, 1.73 mmol, 0.05 equivalents) in 50 mL of anhydrous toluene at 25°C under N₂. The reaction is stirred at 25°C for 23 h. Et₂O (200 mL) is added and the resultant organic fraction washed with saturated aq. NaHCO₃ (200 mL), then dried (MgSO₄). 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: Rₜ 0.2 (5% EtOAc in hexanes); ¹H NMR δ 7.21-7.14 (m, 4H), 5.98 (dqd, J = 15.5, 6.5 & 1.0 Hz, 1H), 5.64 (ddq, J = 15.5, 4.5 & 1.5 Hz, 1H), 5.33 (br dd, 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 & 1.0 Hz, 3H); ¹³C NMR δ 138.8 (C), 130.1 (CH/CH₃), 127.8 (CH/CH₃), 127.1 (CH/CH₃), 127.0 (CH/CH₃), 104.7 (CH/CH₃), 70.0 (CH₂), 17.6 (CH/CH₃); IR (neat) 1720 (md), 1685 (st with shoulders), 1495 (wk) cm⁻¹; MS (LSIMS⁺) m/z (rel. intensity) 213 (37, M+Na⁺), 121 (100); HRMS calcd for C₁₂H₁₄O₂⁺Na 213.0892, found 213.0892. Note there was also some Z-isomer of 1b present (⁰.⁶% from ¹H NMR) derived from contaminating Z-crotonaldehyde.

**Example 3:**

3-(3-Phenyl-1-propenyl)-1,5-dihydro-3H-2,4-benzodioxepine (1c). E-Cinnamaldehyde (3.15 mL, 25.0 mmol, 1.00 equivalents) is added to a solution of crude 3-methoxy-1,5-dihydro-3H-2,4-benzodioxepine (4.50 g, 25.0 mmol, 1.00 equivalents) (for preparation, see synthesis of 1b) and p-toluenesulfonic acid (0.238 g, 1.25 mmol, 0.05 equivalents) in anhydrous DME (100 mL) at 25°C under N₂. The reaction is stirred at 25°C for
2 h. Et₂O (250 mL) is added and the resultant organic fraction washed with saturated aq. NaHCO₃ (50 mL), then dried (MgSO₄). 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; R₆ 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 & 4.0 Hz, 1H), 5.55 (dd, J = 4.0 & 1.0 Hz, 1H), 4.99 (d, J = 14.0 Hz, 2H), 4.92 (d, J = 14.0 Hz, 2H); ¹³C NMR δ 138.8 (C), 136.1 (C), 133.2 (CH/CH₃), 128.6 (CH/CH₃), 128.2 (CH/CH₃), 127.3 (CH/CH₃), 127.1 (CH/CH₃), 126.9 (CH/CH₃), 125.6 (CH/CH₃), 104.2 (CH/CH₃), 70.0 (CH₂); IR (CHBr₃) 1655 (wk), 1600 (wk), 1575 (wk), 1490 (md) cm⁻¹; MS (LSIMS') m/z (rel. intensity) 253 (100, M⁺); HRMS calcd for C₁₇H₁₆O₂ + H 253.1229, found 253.1235.

Example 4:

3-(1S, 2-Dihydroxyethyl)-1, 5-dihydro-3H-2, 4-benzodioxepine (2a). Compound 2a is prepared in >95% e.e. from 1a using AD-mix-β as previously described. The synthesis of 2a via the AD has been reported by Oi and Sharpless, Tetrahedron Lett. 1992, 33, 2095.

Example 5:

3-(1S, 2R-Dihydroxypropyl)-1, 5-dihydro-3H-2, 4-benzodioxepine (2b). Compound 1b (0.475 g, 2.50 mmol, 1.00 equivalents) is added to a well-stirred mixture of AD-mix-β (3.50 g) and methanesulfonamide (0.238 g, 2.50 mmol, 1.00 equivalents) in a 1:1 mixture of t-BuOH:H₂O (25 mL) at 25°C, and the reaction stirred for 2 days. CH₂Cl₂ (100 mL) is then added and the organic layer collected after shaking. The aq. phase is extracted with a further 2 x 50 mL of CH₂Cl₂ and
the combined organic fractions dried (MgSO₄). 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: R₆ 0.5 (80% EtOAc in hexanes); [α]²⁵D -7.4° (c 5.2, CHCl₃); 82% e.e. (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 = 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); ¹³C NMR δ 139.0 (C), 128.1 (CH/CH₃), 128.0 (CH/CH₃), 109.2 (CH/CH₃), 74.6 (CH₂), 73.8 (CH/CH₃), 73.5 (CH/CH₃), 66.2 (CH₂), 19.7 (CH/CH₃); IR (CHBr₃) 3445 (br st) cm⁻¹; MS (LSIMS⁺) m/z (rel. intensity) 247 (100, M+Na⁺); HRMS calcd for C₁₇H₁₆O₄⁺Na²⁺ 247.0946, found 247.0951. Note there is also cis isomer of 2b present (≈6% from ¹H NMR) derived from contaminating cis 1b.

Example 6:
3-(3-phenyl-1S,2R-dihydroxypropyl)-1,5-dihydro-3H-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; R₆ 0.5 (60% EtOAc in hexanes); [α]²⁵D +25.4°C (c 3.0, CHCl₃); >95% e.e. (from ¹H NMR of bis-Mosher ester); ¹H NMR δ 7.43-7.23 (m, 9H), 5.01-4.91 (m, 6H), 3.81 (m, 1H); ¹³C NMR δ 141.0 (C), 138.9 (C), 128.3 (CH/CH₃), 127.9 (CH/CH₃), 127.8 (CH/CH₃), 127.6 (CH/CH₃), 126.4 (CH/CH₃), 108.2 (CH/CH₃), 75.6 (CH/CH₃), 73.6 (CH₂), 73.3 (CH₂), 72.3 (CH/CH₃); IR (CHBr₃) 3550 (st), 3455 (br st), 1495 (wk) cm⁻¹; MS (LSIMS⁺) m/z (rel. intensity) 309 (100, M+Na⁺); HRMS calcd for C₁₇H₁₆O₄⁺Na 309.1103, found
Example 7:
3-(1R,2-Dihydroxyethyl)-1,5-dihydro-3H-2,4-benzodioxepine (3a). Using AD-mix-α and an experimental procedure analogous to that described for 2a, 1a gave 3a in >95% e.e.: all other data are equivalent to that given for enantiomer 2a.

Example 8:
3-(1R,2S-Dihydroxypropyl)-1,5-dihydro-3H-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: [α]^{25}_{D} +7.30 (c 3.2, CHCl₃); 79% e.e (from ¹H NMR of bis-Mosher ester); all other data are equivalent to that given for enantiomer 2b.

Example 9:
3-(3-phenyl-1R,2S-dihydroxypropyl)-1,5-dihydro-3H-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; [α]^{25}_{D} -26.1 (c 3.0, CHCl₃); >95% e.e. (from ¹H NMR of bis-Mosher ester); all other data are equivalent to that given for enantiomer 2c.

Example 10:
L-Fructose (6a). A solution of 2a (0.315 g, 1.50 mmol, 1.00 equivalents) in MeOH (15 mL) containing Pd(II)O catalyst (4.59 x 10⁻² g, 0.375 mmol, 0.250 equivalents) is placed under 50 psi of H₂ in a Parr
hydogenation apparatus and shaken at 25°C for 2 days. The catalyst is then removed by filtration and volatiles removed in vacuo to give L-glyceraldehyde (4a) as an oil.

K12 E. coli containing excess Rha aldolase are obtained by growth of the cells in M9 minimal medium containing L-rhamnose as the sole carbon source. The cells are collected by centrifugation and stored at -70°C. Just prior to use, 0.6 g (wet cells) are 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 0.1 M Tris buffer (pH 7.5) is added 41.7 mL of a 0.036 mM solution of DHAP (1.50 mmol, 1.00 equivalents) and the pH adjusted to 7.5 with 1 M NaOH. The lysed cell suspension containing Rha aldolase is then added and the reaction stirred at 25°C for 3 days under N₂. The pH is adjusted to 4.0-5.0 using 6 M HCl, and acid phosphatase (600 U) added. After shaking at 37°C in an incubator for 2 days, the pH is adjusted to 7.0 and H₂O removed by lyophilization to yield a yellow solid. MeOH is added and the resulting yellow solution containing solid filtered through celite. Removal of volatiles in vacuo from the filtrate and purification by flash chromatography (10-30% MeOH in CHCl₃) gave the product 6a: data are identical with commercially available fructose.

Example 11:

6-Methyl-D-galacto-hexulose (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 equivalents of DHAP, 2b gave 6b as a viscous oil.
in 60% after two rounds of flash chromatography
(10-30% MeOH in CHCl₃); Rₚ 0.2 (30% MeOH in CHCl₃); ¹H
NMR (CD₃OD) [major α-pyranose form and minor furanose
form detected in a ratio of 4:1] α-pyranose δ 4.16
(qd, J = 6.5 & 1.0 Hz, H-6), 3.82 (dd, J = 9.5 & 3.0
Hz, H-4), 3.78 (d, J = 9.5 Hz, H-3), 3.72 (dd, J = 3.0
& 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, 3 H-7); major
furanose δ 4.11 (dd, J = 7.0 & 7.5 Hz, H-4), 4.06 (d,
J=7.5 Hz, H-3), 3.84 (m, H-6), 3.59 (s, H-1 & H-1'),
3.56 (m, H-5), 1.23 (d, J=6.5 Hz, 3 H-7); ¹³C NMR
(CD₃OD) [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/CH₃), 78.8 (CH/CH₃), 77.2 (CH/CH₃), 73.5
(CH/CH₃), 72.5 (CH/CH₃), 71.2 (CH/CH₃), 70.0 (CH/CH₃),
67.9 (CH/CH₃), 66.5 (CH₂), 65.1 (CH₂), 19.2 (CH/CH₃),
16.5 (CH/CH₃); MS (LSIMS⁺) m/z (rel. intensity) 149
(100).

Example 12:

6-Phenyl-D-galacto-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 CHCl₃); Rₚ 0.5 (30% MeOH in CHCl₃); ¹H
NMR (CD₃OD) [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 & 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 & 6.5 Hz, H-4), 4.03 (d, J = 7.0 Hz,
- 24 -

H-3), 3.90 (dd, J = 6.5 & 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 & 6.5 Hz, H-4), 4.08 (dd, J = 6.5 & 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'); 13C NMR (D₂O) [major α-pyranose form and minor furanose form detected-ratio ~4:1] δ 139.8 (C), 138.7 (C); 129.5 (CH/CH₃), 129.3 (CH/CH₃), 129.1 (CH/CH₃), 128.5 (CH/CH₃), 127.8 (CH/CH₃), 127.9 (CH/CH₃), 127.1 (CH/CH₃), 102.3 (C), 98.6 (C), 84.4 (CH/CH₃), 76.7 (CH/CH₃), 76.4 (CH/CH₃), 76.0 (CH/CH₃), 73.5 (CH/CH₃), 73.1 (CH/CH₃), 71.3 (CH/CH₃), 68.1 (CH/CH₃), 64.8 (CH₂), 63.1 (CH₂); MS (LSIMS⁺) m/z (rel. intensity) 279 (100, M+Na⁺); HRMS calcd for C₁₂H₁₆O₅+Na 279.0845, found 279.0839.

Example 13:

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 is converted into 7a (via 5a).

Example 14:

6-Methyl-L-galacto-hexulose (7b). Compound 3b (9.40 x 10⁻² g, 0.420 mmol, 1.00 equivalents) is 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 is 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 equivalents). After readjusting the pH to 6.5-7.0 using 1 M NaOH, FDP aldolase from rabbit muscle (188 U) is added, and the solution stirred slowly at 25°C for 25 h under N₂. The pH is then adjusted to 4.0-5.0
using 6 M HCl. Acid phosphatase (168 U) is added, and the reaction is shaken at 37°C in an incubator for 45 h. The pH is then readjusted to 7.0 and H₂O removed by lyophilization to yield a yellow solid. MeOH is added and the resulting yellow solution containing solid filtered through celite. Removal of volatiles in vacuo from the filtrate and purification by 2 rounds of flash chromatography (10-30% MeOH in CHCl₃) gave the product 7a (4.62 x 10⁻² g, 65%) as an oil: all other data are equivalent to that given for enantiomer 6b.

**Example 15:**

6-Phenyl-L-galacto-hexulose (7c). Using an experimental procedure analogous to that described for 7b, 3c is converted into 7c (via 5c) in 18% yield after 2 rounds of flash chromatography (10-30% MeOH in CHCl₃) as a viscous oil: all other data are equivalent to that given for enantiomer 6c.
What is claimed is:

1. A method for synthesizing a carbohydrate comprising the following steps:
   Step A: stereoselectively converting an alkene to an aldol intermediate by means of an osmium-catalyzed asymmetric dihydroxylation (AD) reaction; and then
   Step B: stereoselectively elongating the aldol intermediate of said Step A with a nucleophilic donor by means of an aldolase-catalyzed aldol addition reaction for producing the carbohydrate.

2. A method for synthesizing a carbohydrate as in claim 1 wherein:
   in said Step B, the aldolase being selected from the group consisting of fructose 1,6-diphosphate aldolase, L-rhamnulose 1-phosphate (Rha 1-P) aldolase, L-fucolose 1-phosphate (Fuc 1-P) aldolase, or tagatose 1,6-diphosphate (Tag) aldolase.

3. A method for synthesizing a carbohydrate as in claim 1 wherein:
   Step A includes the following substeps:
   Substep A(1): providing an alkenal as the alkene; then
   Substep A(2): protecting the alkenal of said Substep A(1) by conversion to an olefinic acetal; then
   Substep A(3): converting the olefinic acetal of said Substep A(2) to a dihydroxy acetal by means of the osmium-catalyzed asymmetric dihydroxylation (AD) reaction; and then
   Substep A(4): deprotecting the dihydroxy acetal
of said Substep A(3) by conversion to the aldol intermediate.

4. A method for synthesizing a carbohydrate as in claim 3 wherein:
   in said Step B, the aldolase being selected from the group consisting of fructose 1,6-
   diphosphate (FDP) aldolase, L-rhamnulose 1-
   phosphate (Rha 1-P) aldolase, L-fuculose 1-
   phosphate (Fuc 1-P) aldolase, and tagatose 1,6-diphosphate (Tag) aldolase.

5. A method for synthesizing a carbohydrate as in claim 4 comprising the following further step after said Step B:
   Step C: removing phosphate groups from the carbohydrate produced in said Step B by acid
   phosphatase-catalyzed phosphate hydrolysis.

6. A method for synthesizing a carbohydrate as in claim 3 wherein:
   in said Substep A(1), the alkenal is an α,β-
   alkenal; and
   in said Substep A(4), the aldol intermediate is an α,β-dihydroxy aldol.

7. A method for synthesizing a carbohydrate as in claim 3 wherein:
   in said Substep A(3), the osmium-catalyzed asymmetric dihydroxylation (AD) reaction
   employing a catalytic mixture selected from a group consisting essentially of an AD-mix-
   α and an AD-mix-β, the AD-mix-α including potassium osmate \( \{K_2O_{2}(OH)_4\} \) and a ligand
   selected from the group consisting of
(DHQ)$_2$-PHAL, as defined in Figure 1, and (DHQ)$_2$-PYR, as defined in Figure 1, the AD-mix-$B$ including potassium osmate \( \{K_2O\text{SO}_4(OH)_4\} \) and a ligand selected from the group consisting of (DHQD)$_2$-PHAL, as defined in Figure 1, and (DHQD)$_2$-PYR, as defined in Figure 1.

8. A method for synthesizing a carbohydrate as in claim 3 wherein:

- in said substep A(2), the olefinic acetal including a 1,2-benzenedimethanol acetal protecting group.

9. A method for synthesizing a carbohydrate as in claim 8 wherein:

- in said substep A(4), the 1,2-benzenedimethanol acetal protecting group being removed from the dihydroxy acetal by a method selected from the group consisting of acid catalyzed hydrolysis and Pd(II)O-catalyzed hydrogenolysis.

10. A method for synthesizing a carbohydrate as in claim 1 wherein:

Step A including the following substeps:

1. Substep A(1): providing a polyolefin as the alkene; then

2. Substep A(2): converting the polyolefin of said Substep A(1) to a dihydroxy olefin by means of the osmium-catalyzed asymmetric dihydroxylation (AD) reaction; and then

3. Substep A(3): converting the dihydroxy olefin of said Substep A(3) to the aldol intermediate.
11. A method for synthesizing a carbohydrate as in claim 10 wherein:
   in said Step B, the aldolase being selected from the group consisting of fructose 1,6-
   diphosphate (FDP) aldolase, L-rhamnulose 1-
   phosphate (Rha 1-P) aldolase, L-fuculose 1-
   phosphate (Fuc 1-P) aldolase, and tagatose 1,6-diphosphate (Tag) aldolase.

12. A method for synthesizing a carbohydrate as in claim 11 comprising the following further step after said Step B:
   Step C: removing phosphate groups from the carbohydrate produced in said Step B by acid
   phosphatase-catalyzed phosphate hydrolysis.

13. A method for synthesizing a carbohydrate as in claim 10 wherein:
   in said Substep A(1), the polyolefin is a diolefin; and
   in said Substep A(3), the dihydroxy olefin of said Substep A(3) is converted to the aldol
   intermediate by a method selected from the group consisting of ozonization and
   periodation.

14. A method for synthesizing a carbohydrate as in claim 10 wherein:
   in said Substep A(2), the osmium-catalyzed asymmetric dihydroxylation (AD) reaction
   employing a catalytic mixture selected from a group consisting essentially of an AD-mix-
   α and an AD-mix-β, the AD-mix-α including potassium osmate \( \{K_2OsO_4(OH)_4\} \) and a ligand
   selected from the group consisting of
(DHQ)₂-PHAL, as defined in Figure 1, and (DHQ)₂-PYR, as defined in Figure 1, the AD-mix-β including potassium osmate \( \{K_2OsO_2(OH)_4\} \) and a ligand selected from the group consisting of (DHQD)₂-PHAL, as defined in Figure 1, and (DHQD)₂-PYR, as defined in Figure 1.

15. A method for synthesizing a carbohydrate comprising the following steps:

Step A: stereoselectively converting an \( \alpha,\beta \)-alkenal to an \( \alpha,\beta \)-aldol intermediate by means of an osmium-catalyzed asymmetric dihydroxylation (AD) reaction according to the following substeps:

Substep A(1): providing an \( \alpha,\beta \)-alkenal; then

Substep A(2): protecting the \( \alpha,\beta \)-alkenal of said Substep A(1) by conversion to an \( \alpha,\beta \)-olefinic acetal having a 1,2-benzenedimethanol acetal protecting group; then

Substep A(3): converting the \( \alpha,\beta \)-olefinic acetal of said Substep A(2) to an \( \alpha,\beta \)-dihydroxy acetal by means of the osmium-catalyzed asymmetric dihydroxylation (AD) reaction employing a catalytic mixture selected from a group consisting essentially of an AD-mix-α and an AD-mix-β, the AD-mix-α including potassium osmate \( \{K_2OsO_2(OH)_4\} \) and a ligand selected from the group consisting of (DHQ)₂-PHAL, as defined in Figure 1, and (DHQ)₂-PYR, as defined in Figure 1, the AD-mix-β including potassium osmate \( \{K_2OsO_2(OH)_4\} \) and a ligand selected from the group consisting of (DHQD)₂-PHAL, as defined in Figure 1, and (DHQD)₂-PYR, as defined in
Figure 1; then
Substep A(4): deprotecting the $\alpha,\beta$-dihydroxy acetal of said Substep A(3) by removal of the 1,2-benzenedimethanol acetal protecting group and conversion to the $\alpha,\beta$-aldol intermediate; then
Step B: stereoselectively elongating the $\alpha,\beta$-aldol intermediate of said Step A with a nucleophilic donor by means of an aldolase-catalyzed aldol addition reaction for producing the carbohydrate, the aldolase being selected from the group consisting of fructose 1,6-diphosphate (FDP) aldolase, L-rhamnulose 1-phosphate (Rha 1-P) aldolase, L-fuculose 1-phosphate (Fuc 1-P) aldolase, or tagatose 1,6-diphosphate (Tag) aldolase; and then
Step C: removing phosphate groups from the carbohydrate produced in said Step B by acid phosphatase-catalyzed phosphate hydrolysis.

16. A method for synthesizing a carbohydrate as described in claim 15 wherein:
   in said Substep A(1), attaching the $\alpha,\beta$-alkenal to a solid phase support.

17. A method for synthesizing a carbohydrate comprising the following steps:
   Step A: stereoselectively converting a polyolefin to an aldol intermediate by means of an osmium-catalyzed asymmetric dihydroxylation (AD) reaction according to the following substeps:
       Substep A(1): providing a polyolefin as the alkene; then
       Substep A(2): converting the polyolefin of
said Substep A(1) to a dihydroxy olefin by means of the osmium-catalyzed asymmetric dihydroxylation (AD) reaction; and then

Substep A(3): converting the dihydroxy olefin of said Substep A(3) to the aldol intermediate by a method selected from the group consisting of ozonization and periodation;

Step B: stereoselectively elongating the aldol intermediate of said Step A with a nucleophilic donor by means of an aldolase-catalyzed aldol addition reaction for producing the carbohydrate, the aldolase being selected from the group consisting of fructose 1,6-diphosphate (FDP) aldolase, L-rhamnulose 1-phosphate (Rha 1-P) aldolase, L-fuculose 1-phosphate (Fuc 1-P) aldolase, or tagatose 1,6-diphosphate (Tag) aldolase; and then

Step C: removing phosphate groups from the carbohydrate produced in said Step B by acid phosphatase-catalyzed phosphate hydrolysis.

18. A method for synthesizing a carbohydrate as described in claim 17 wherein:
   in said Substep A(1), attaching the polyolefin to a solid phase support.
FIG. 2

Pd(OH)$_2$/H$_2$. 25°C, 2d
or 1.0 buffer
pH 65°C, 12h

2a (90%, >95% e.e.)
2b (95%, 82% e.e.)
2c (91%, >95% e.e.)

3a (93%, >95% e.e.)
3b (100%, 79% e.e.)
3c (87%, >95% e.e.)

AD-mix-β, 25°C, 2d
AD-mix-α, 25°C, 2d

After recrystallization from benzene

RS

R$_L$

O

H

la (R = H)
lb (R = Me)
lc (R = Ph)
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6): Please See Extra Sheet.
US CL: Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>US, A, 5,143,831 (WONG ET AL) 01 SEPTEMBER 1992, see col. 1 and col. 4.</td>
<td>1-18</td>
</tr>
<tr>
<td>Y</td>
<td>US, A, 5,162,554 (SHARPLESS ET AL) 10 NOVEMBER 1992, see whole document.</td>
<td>1-9, 15, 16</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:
  "A" document defining the general state of the art which is not considered to be of particular relevance
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Date of the actual completion of the international search: 14 FEBRUARY 1995
Date of mailing of the international search report: 01 MAR 1995

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks
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Form PCT/ISA/210 (second sheet) (July 1992)
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):
C12P 19/02, 19/00

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :
435/105, 72

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.
435/105, 72; 536/124; 549/34, 214, 229, 230, 350

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):
APS, CHEMICAL ABSTRACTS; search terms: asymmetric dihydroxylation?, stereoselect?, carbohydrate, sugar
saccharide, aldolase, chiral