J = 1, 7 Hz, 1 H), 2.34 (dddd, J = 10, 10, 6.5, 1 Hz, 1 H), 2.16–0.96 (series of m, 13 H), 1.24 (d, J = 7 Hz, 3 H), 1.03 (d, J = 6 Hz, 3 H), 0.92 (s, 3 H); ^{13}C NMR (75 MHz) δ 93.9 (s), 92.8 (s), 80.1 (d), 76.7 (d), 72.4 (d), 72.1 (d), 56.7 (d), 56.6 (d), 53.7 (d), 52.1 (d), 50.9 (d), 49.4 (s), 49.3 (d), 47.3 (s), 39.9 (d), 39.4 (d), 34.7 (t), 34.0 (t), 33.5 (t), 32.7 (t), 26.2 (t), 25.4 (t), 25.2 (t), 25.1 (t), 24.5 (t), 24.4 (t), 19.4 (q), 18.8 (2 C, q), 17.6 (q), 17.3 (q), 15.1 (q): MS m/z (M^+) calcd 250.1933, obsd 250.1918.

Ketone 34. Alcohols **33** (13.4 mg, 0.057 mmol) were subjected to oxidation with pyridinium chlorochromate (50 mg, 0.232 mmol) in dry CH_2Cl_2 (3 mL) and pyridine (1 drop) at room temperature for 3 h. The usual workup and purification (elution with PE–E (4:1)) afforded 8.4 mg (63%) of **34** as a colorless oil: IR (cm^{-1}) 1715; ^{1}H NMR δ (diastereomer A) 4.20 (br q, J = 7 Hz, 1 H), 2.54–2.32 (m, 2 H), 2.20–1.15 (series of m, 12 H), 1.20 (d, J = 7 Hz, 3 H), 1.09 (d, J = 6 Hz, 3 H), 1.02 (s, 3 H); (diastereomer B) 3.95 (dq, J = 1, 7 Hz, 1 H), 2.54–2.32 (m, 1 H), 2.20–1.15 (series of m, 13 H), 1.35 (d, J = 7 Hz, 3 H), 1.08 (d, J = 6 Hz, 3 H), 1.01 (s, 3 H); ^{13}C NMR (75 MHz) δ 208.7 (s), 208.3 (s), 94.8 (s), 93.7 (s), 81.6 (d), 78.1 (d), 61.6 (d), 59.4 (2 C, d), 59.1 (d), 53.9 (d), 52.8 (d), 50.5 (s), 48.9 (s), 41.4 (d), 40.9 (d), 36.3 (t), 35.7 (t), 29.8 (t), 29.4 (t), 26.7 (t), 25.9 (t), 25.5 (t), 25.2 (t), 25.0 (t), 24.3 (t), 19.5 (q), 19.4 (q), 19.1 (q), 17.1 (q), 17.0 (q), 15.3 (q); MS m/z (M^+) calcd 248.1776, obsd 248.1804.

Double-Bond Isomerization in 6. A solution of **6** in C_6D_6 was allowed to stand at room temperature for 7 days when clean isomerization to **35** was complete. Solvent evaporation provided for the quantitative isolation of **35**, a pale yellowish oil: ^{1}H NMR (C_6D_6) δ 6.00 (ddddd, J = 2, 2, 2, 2, 2 Hz, 1 H), 4.39–4.34 (m, 1 H), 2.54 (brdd, J = 7.5, 6.5 Hz, 1 H), 2.45–2.14 (m, 5 H), 1.95–1.75 (m, 3 H), 1.85 (dq, J = 1.5, 1 Hz, 3 H), 1.82 (ddd, J = 7.5, 7.5, 2 Hz, 1 H), 1.75–1.50 (m, 2 H), 1.54 (dq, J = 1, 1 Hz, 3 H), 1.21–1.11 (m, 1 H), 0.98 (d, J = 7 Hz, 3 H); ^{13}C NMR (75 MHz, C_6D_6) δ 146.3 (s), 143.9 (s), 124.0 (d), 104.9 (s), 75.5 (d), 51.0 (d), 44.1 (d), 33.3 (2 C, t), 32.6 (t), 31.2 (d), 28.7 (t), 23.7 (t), 22.8 (q), 16.8 (q), 15.3 (q); MS m/z (M^+) calcd 232.1827, obsd 232.1851.

Claisen Rearrangement of 35. Vinyl allyl ether **35** (48 mg, 0.207 mmol) in dry CH_2Cl_2 (5 mL) was subjected to the usual rearrangement conditions (–78 to 20 °C, overnight) in the presence of (i-Bu)$_3$Al (0.70 mL of 1.0 M in toluene, 0.70 mmol). Workup and purification as before furnished 34.5 mg (71%) of **36** and 3.2 mg (7%) of **37**.

For 36: colorless oil; IR (cm^{-1}) 3550; ^{1}H NMR δ 5.51 (br s, 1 H), 3.87 (dq, J = 2, 6.5 Hz, 1 H), 2.92 (br d, J = 2 Hz, OH), 2.41–2.14 (m, 5 H), 1.85–1.45 (m, 8 H), 1.34 (dddd, J = 12, 11.5, 10.5, 7 Hz, 1 H), 1.10–0.98 (m, 1 H), 1.10 (d, J = 6.5 Hz, 3 H), 1.06 (d, J = 6.5 Hz, 3 H), 0.86 (s, 3 H); ^{13}C NMR (75 MHz) δ 142.6 (s), 123.4 (d), 73.9 (d), 46.7 (d), 46.6 (d), 41.4 (d), 40.9 (d), 40.1 (s), 32.5 (t), 30.2 (t), 28.5 (t), 26.1 (t), 24.8 (q), 23.8 (t), 21.5 (q), 17.6 (q); MS m/z (M^+) calcd 234.1984, obsd 234.1983.

For 37: colorless oil; ^{1}H NMR δ 5.31 (dddd, J = 2.5, 2.5, 2.5, 2.5 Hz, 1 H), 3.83 (q, J = 6.5 Hz, 1 H), 2.38–0.95 (series of m, 14 H), 1.15 (d, J = 6.5 Hz, 3 H), 1.00 (d, J = 6.5 Hz, 3 H), 0.98 (s, 3 H); ^{13}C NMR (75 MHz) δ 139.8 (s), 121.5 (d), 70.4 (d), 46.8 (d), 45.8 (d), 45.0 (d), 41.0 (s), 40.5 (d), 32.1 (t), 30.7 (t), 28.8 (t), 27.6 (t), 24.3 (t), 21.7 (q), 19.5 (q), 18.8 (q); MS m/z (M^+) calcd 234.1984, obsd 234.1980.

Ketone 38. A. Oxidation of 36. A 14-mg (0.060 mmol) sample of **36** was oxidized in the usual manner with pyridinium chlorochromate (50 mg, 0.232 mmol) in dry CH_2Cl_2 (3 mL) containing pyridine (1 drop) at room temperature for 3 h. The usual workup and purification by filtration through silica gel (elution with PE–E (9:1)) afforded pure **38** (12.6 mg, 91%) as a colorless oil: IR (cm^{-1}) 1705; ^{1}H NMR δ 5.38–5.34 (m, 1 H), 2.36–2.23 (m, 3 H), 2.23–2.03 (m, 1 H), 2.08 (s, 3 H), 1.88–1.00 (series of m, 10 H), 1.20 (s, 3 H), 1.04 (d, J = 7 Hz, 3 H); ^{13}C NMR (75 MHz) δ 212.3 (s), 140.9 (s), 119.8 (d), 53.0 (s), 46.7 (d), 45.5 (d), 44.0 (d), 41.9 (d), 33.0 (t), 31.2 (t), 27.7 (t), 27.3 (q), 27.2 (t), 24.2 (t), 22.5 (q), 20.9 (q).

Ketone **38** was obtained by analogous oxidation of **37** as seen by appropriate TLC and high-field ^{1}H NMR comparisons.

Epoxidation of 36. A solution of **36** (24 mg, 0.102 mmol) in dry C_6H_6 (3 mL) was treated with purified m-CPBA (30 mg, 0.174 mmol) at 20 °C overnight. The usual workup and purification (elution with PE–E (3:2)) gave 18.4 mg (72%) of **39** as a colorless crystalline solid (from PE): mp 150–151 °C; IR (cm^{-1}) 3635, 3500–3350 ^{1}H NMR δ 3.90 (q, J = 6.5 Hz, 1 H), 3.82 (br d, J = 6 Hz, 1 H), 2.46 (br dd, J = 8.5, 8.5 Hz, 1 H), 2.30–2.15 (m, 1 H), 2.10–1.08 (series of m, 12 H), 1.15 (d, J = 6.5 Hz, 3 H), 1.02 (d, J = 7 Hz, 3 H), 0.86 (s, 3 H); ^{13}C NMR (75 MHz) δ 94.2 (s), 81.4 (d), 71.8 (d), 48.0 (d), 45.4 (2C, d + s), 45.2 (d), 34.5 (t), 34.0 (t), 33.5 (t), 28.4 (t), 24.4 (t), 24.1 (t), 23.2 (q), 17.3 (q), 13.4 (q); MS m/z (M^+) calcd 250.1933, obsd 250.1975.

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Supplementary Material Available: Crystallographic details, crystallographic experimental, computer-generated drawings, tables of atomic positional and thermal parameters, bond distances and angles, and torsional angles (in selected compounds) for **11**, **24b**, and **39**; decoupling and NOE studies for **7a**, **14**, **15**, **18**, **21**, and **25**, and ^{1}H or ^{13}C spectra of those compounds for which elemental analyses are not available (56 pages). Ordering information is given on any current masthead page.

α-Amino Aldehyde Equivalents as Substrates for Rabbit Muscle Aldolase: Synthesis of 1,4-Dideoxy-D-arabinitol and 2(*R*),5(*R*)-Bis(hydroxymethyl)-3(*R*),4(*R*)-dihydroxypyrrolidine

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This work examined the application of rabbit muscle aldolase (RAMA) to stereospecific carbon–carbon bond formation in the preparation of carbohydrates containing amino groups. Several α-amino aldehyde equivalents were evaluated as substrates for RAMA and for their synthetic utility in transformations following the aldol reaction. This methodology is illustrated by the syntheses of the pyrrolidine alkaloids 1,4-dideoxy-D-arabinitol and 2(*R*),5(*R*)-bis(hydroxymethyl)-3(*R*),4(*R*)-dihydroxypyrrolidine. The kinetic resolution of racemic aldehydes by RAMA and mild methods for transforming the amino equivalents into the desired amines are discussed briefly.

Introduction

Polyhydroxylated amines have attracted attention for their activity as glycosidase inhibitors, with potential pharmaceutical applications as antibiotic and antitumor agents.[1] Pyrrolidines [e.g., swainsonine, 1,4-dideoxy-1,4-imino-D-arabinitol (1), and 2(*R*),5(*R*)-bis(hydroxymethyl)-3(*R*),4(*R*)-dihydroxypyrrolidine (2)] inhibit several

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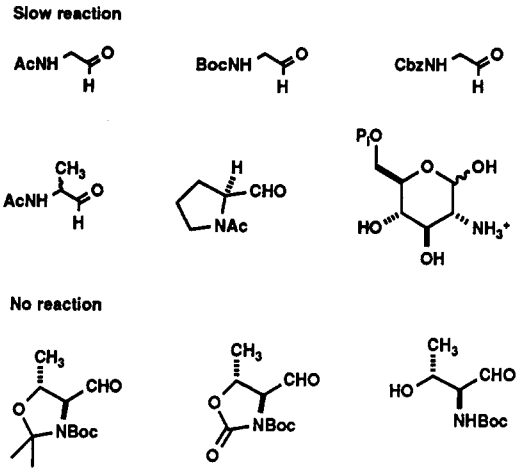
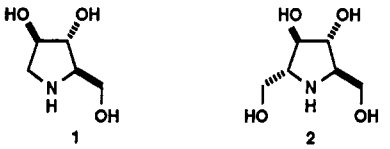
0022-3263/91/1956-3849$02.50/0

Scheme I. Equivalents of α-Amino Aldehydes That Are Known as Substrates for RAMA



α- and β-glucosidases and mannosidases with values of ID_{50} (the concentration for 50% inhibition) below 10 μM.[2-4] Piperidines (e.g., deoxynojirimicin and deoxymannojirimicin) exhibit similar activity.[2]



1 2

This work demonstrates the application of rabbit muscle aldolase (RAMA, EC 4.1.2.13, fructose-1,6-diphosphate aldolase) to the synthesis of polyhydroxylated amines. RAMA catalyzes the stereocontrolled aldol condensation of dihydroxyacetone phosphate (DHAP) with aldehydes and affords (3*S*,4*R*)-α-keto phosphates. The enzyme has been used previously to prepare polyhydroxylated compounds, including amino sugars.[5-11] The best method for incorporation of nitrogen in RAMA-catalyzed reactions has not, however, been systematically investigated. We have examined several aldehydes having protected nitrogen in the α-position, in order to establish effective strategies for their incorporation into the keto phosphate and for protection/deprotection in this position (eq 1).[12]

(1)

Previous studies have shown that a small number of α-amino aldehyde equivalents are substrates for RAMA (Scheme I),[5-7] but that their rates of reaction are too slow to be synthetically useful. Unprotected amino aldehydes are generally impractical as substrates because they are self-reactive. Preliminary data, along with a crystal structure at 2.7 Å, also suggest that a positively charged ammonium ion would bind poorly in the postively charged active site of RAMA.[5,13] Among the protected aldehydes, only the adduct formed from *N*-(carbobenzyloxy)aminoacetaldehyde and DHAP has been adequately characterized.[7] Other protected amino aldehydes have been tested with the enzyme and found not to be substrates, with only products of decomposition observed.[14]

A range of data suggests that RAMA prefers, as substrates, aldehydes that have small substituents in the α-position.[5] We hypothesized that small groups such as azide and formamide would be accepted by the enzyme at synthetically practical rates. The ease of preparation of the α-substituted aldehyde and the utility in synthesis of the aldol product derived from it are also important in determining usefulness in synthesis. Not only should the amino equivalent serve as a substrate for the enzyme with a useful rate, but it should also be readily transformed into the desired amine.

We chose two pyrrolidine alkaloids, 1,4-dideoxy-1,4-imino-D-arabinitol (1) and 2(*R*),5(*R*)-bis(hydroxymethyl)-3-(*R*),4(*R*)-dihydroxypyrrolidine (2) as the targets to test our synthetic methods. The syntheses of the piperidines deoxynojirimicin and deoxymannojirimicin using RAMA with β-azido aldehydes have been reported.[7-9,11] Pyrrolidine 1 has been previously synthesized enzymatically by using a thiamin-dependent transketolase to construct intermediate 4[9] or by using aldolases with *N*-(carbobenzyloxy)aminoacetaldehyde.[7,10] Here, we report an efficient route to the key intermediate 4 using RAMA. The C_2-symmetric six-carbon pyrrolidine 2 has been prepared previously from both D-fructose and L-sorbose by classical carbohydrate methods.[15] Our work showed that RAMA catalyzes the formation of the desired pyranose from achiral starting materials. The routes to 1 and 2 formally require no protection and deprotection steps (although an acetonide is formed en route to 2 to improve separation of two diastereomers by chromatography).

The kinetic resolution of α-hydroxy aldehydes by RAMA has been documented, but the utility and limitations of this method have not been well-defined.[5,8,11] We also wished to use the synthesis of 2 to explore the ability of RAMA to resolve racemic α-nitrogen-substituted aldehydes. In fact, the levels of diastereoselectivity observed were low, and the diastereomer required for the synthesis of 2 was the minor product. Nonetheless, this synthesis, in combination with various reductive strategies, illustrates an efficient methodology for stereospecific carbon–carbon bond formation in the preparation of polyhydroxylated amines.

Results

Preparation of Aldehydes. We have found that the ozonolysis of olefinic precursors generally provides the most convenient route to the aldehydes required as sub-

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(12) N* refers to a protected amino or an azido group.
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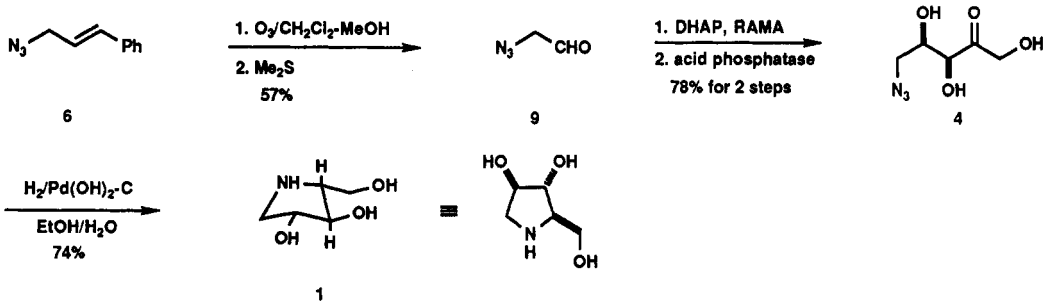
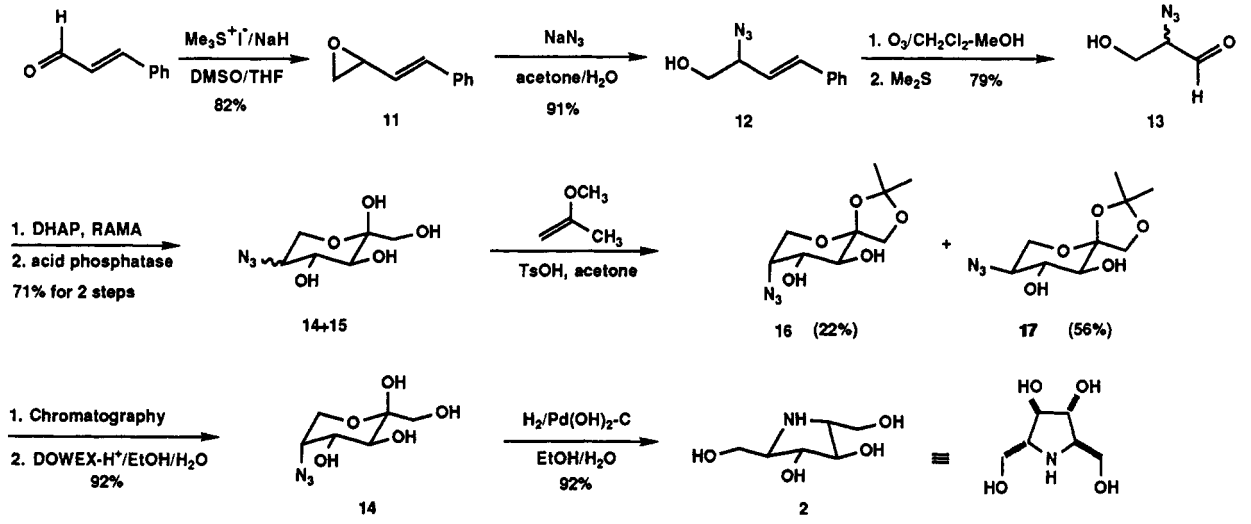
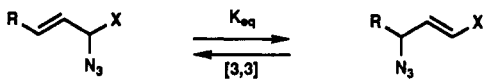
Scheme II. Synthesis of 1,4-Dideoxy-1,4-imino-D-arabinitol (1)



Scheme III. Synthesis of 2



strates for RAMA.[17] The ozonolysis of allylic azides, however, is complicated by the tendency of these olefins to exist as an equilibrium mixture of two isomers, interconverting by facile [3,3] sigmatropic shifts at room temperature.[18,19] Ozonolysis of this mixture generates four aldehydes. Conjugation of the allylic azide to a phenyl ring successfully shifted the equilibrium in favor of the desired alkene (eq 2). In these conjugated systems, only a single isomer was detected by ^1H NMR.



(2)

R	X	K_{eq}
H	CH_2OH	~1
Ph	H	>50
Ph	CH_2OH	>50

Kinetics. The kinetic data for simple α-substituted acetaldehydes confirmed that RAMA prefers sterically unhindered nitrogen equivalents in the aldehyde (Table I). The rates of the reactions were followed by monitoring the disappearance of DHAP versus time.[5] Both the azido- and N-formamido acetaldehydes reacted rapidly enough to be synthetically practical equivalents for preparative-scale reactions to afford the corresponding keto phosphates.

Table I. Relative Rates for the RAMA-Catalyzed Reaction of Aldehyde XCH_2CHO with DHAP

X[a]	V_{rel}
P_iOCH_2CHOH[b,c]	100
HCONH[b]	15
N_3[b]	10
CH_3CONH[b,d]	5
BocNH[d]	2
CbzNH[d,e]	1

[a] [Aldehyde] = 50 mM; [DHAP] = 45–50 mM; [RAMA] = 10 units/mL. [b] Reaction complete within 10 min. [c] D-Glyceraldehyde 3-phosphate. [d] Reference 5. [e] Reference 7.

Synthetic Applications. Although the formamide was kinetically superior to the azide as a substrate, the azide is more useful synthetically because it is more readily reduced to the desired amine. Using the azide as starting material, we developed syntheses of the pyrrolidine alkaloids 1 and 2 (Schemes II and III). ^1H and ^{13}C NMR spectra of the pyrrolidines corresponded to those previously reported.[20,21]

Azidoacetaldehyde (9) was obtained by the ozonolysis of cinnamyl azide. The RAMA-catalyzed reaction of azidoacetaldehyde (9) with DHAP, followed by dephosphorylation with acid phosphatase, afforded the key intermediate 4 in the synthesis of 1,4-dideoxy-1,4-D-arabinitol. Hydrogenation in the presence of palladium

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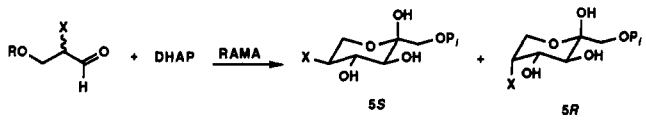
Table II. Kinetic Diastereoselectivity of RAMA



R	X	5S:5R
P_iO	HO	5:95[a]
HO	N_3	50:50[b]
HO	HCONH	75:25[b]
HO	CH_3CONH	70:30[b]

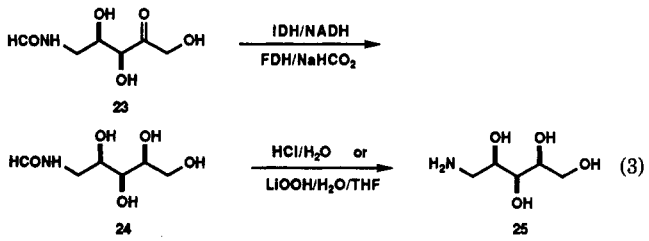
[a] The 5R diastereomer, D-fructose 1,6-diphosphate, exists primarily as the furanose in solution.[5] [b] As each reaction proceeded, the initial ratio of 5S:5R diastereomers increased due to variable rates of epimerization of aldehyde and equilibration toward a thermodynamic ratio of products.

hydroxide on carbon (Pearlman's catalyst) furnished the desired pyrrolidine in high yield, with less than 10% of the undesired epimer at C-2. This undesired epimer can be removed by recrystallization of the hydrochloride from methanol/ether.[9]

While the simple azidoacetaldehyde provided a straightforward route to its corresponding pyrrolidine, the analogous synthesis of **2** required a chemical separation of the 5R and 5S ketoses formed by the aldol condensation. Methylenation of *trans*-cinnamaldehyde with dimethylsulfonium methylide furnished the conjugated allylic epoxide **11**.[22] Nucleophilic opening of the conjugated allylic epoxide **11** with sodium azide to yield **12** and subsequent ozonolysis provided aldehyde **13** for the RAMA-catalyzed reaction with DHAP (prepared in situ from fructose-1,6-diphosphate and triose phosphate isomerase)[5] to produce a 1:1 mixture of C-5 epimers.[23] After dephosphorylation, conversion of the diastereomeric mixture to 1,2-acetonides facilitated chromatographic separation. Acid hydrolysis and reduction of the 5R ketose **14** cleanly afforded the C_2-symmetric pyrrolidine **2**.[16]

Diastereoselectivity. Introduction of nitrogen at a chiral α-center presented the possibility of kinetic resolution of the aldehyde by RAMA. A series of α-substituted β-hydroxypropanals were examined (Table II). The *N*-acylamino aldehydes were prepared from azide **12** by a three-step sequence of reduction with triphenylphosphine,[24] acylation, and ozonolysis. The ratios of 5R to 5S diastereomers were determined by ^1H and ^{31}P NMR spectroscopy. The configuration of the major diastereomer of each RAMA adduct was assigned according to ^1H NMR coupling constants of each pyranose following dephosphorylation. While the enzyme showed no kinetic diastereoselectivity between the (2R)- and (2S)-azido-3-hydroxypropanals, RAMA did exhibit a preference for the 2S configuration of the amides. The 2S aldehyde yielded the all-equatorial, thermodynamically preferred pyranose. In contrast, the natural substrate D-glyceraldehyde 3-phosphate has the R configuration, affording D-fructose 1,6-diphosphate. Although one might postulate a steric explanation for selectivity, a shift to the larger acetamide did not result in a significant increase in kinetic diastereoselectivity beyond that achieved with the formamide. At higher percentages of conversion of aldehyde, the analysis became complicated by the variable rates of epimerization and equilibration for each of the three substrates. Although RAMA exhibited no kinetic selectivity for aldehyde **13**, in preparative-scale reactions, partial equilibration occurred in favor of the 5S diastereomer. Useful ratios (>4:1) of 5S to 5R epimers can be achieved by increasing the concentration of enzyme and prolonging reaction times.

Deprotection of the Protected Amine. While the azide may be reduced to the amine by a variety of mild reagents including hydrogen, phosphines, and thiols,[25] the simple amides require more strenuous deprotection conditions. Using *N*-formyl-1-amino-1-deoxy-L-xylitol (**24**) (prepared by reduction of the corresponding xylulose **23** with L-iditol dehydrogenase and formate dehydrogenase as an NADH regeneration system)[26] as a model compound, we surveyed various conditions for deprotecting the amide to afford the amino polyol **25** (eq 3). (It was necessary to use **24** rather than **23** because **23** was too reactive to survive some of the reaction conditions surveyed.)



(3)

Treatment of the amide with aqueous acid at pH 1 at room temperature for 36 h cleanly yields polyol **25**, whose ^1H NMR spectrum was indistinguishable from that of the D enantiomer.[27] At pH 3, the same amide is stable for over 24 h. Oxidative cleavage in 10% aqueous hydrogen peroxide at 60 °C for 24 h afforded only a 50% yield of the desired compound, along with both starting material and products of decomposition.[28] These oxidative conditions, however, failed to cleave *N*-formylethanolamine. Nucleophilic cleavage with lithium hydroperoxide in aqueous THF for 18 h furnished **25** as the free amine in modest yield (47%) following purification by ion-exchange chromatography.[29] Under these mildly basic conditions, D-mannose epimerizes to D-glucose less than 10%.[30] Hydrogenolysis of the amide **23** in acetic acid at 1200 psi directly afforded pyrrolidine **1** in 30% yield, along with products of decomposition.[31] Although amide **23** was successfully cleaved, the imine resulting from treatment with lithium hydroperoxide could not be isolated from the reaction mixture. The results of this brief survey suggest that, for systems that can withstand strongly acidic conditions, aqueous acid provides a convenient method for deprotection of simple amides. For more sensitive compounds, cleavage with lithium hydroperoxide serves as a

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(30) Lithium hydroperoxide in aqueous THF catalyzed the isomerization of mannose to glucose and fructose with a half-life of 69 h. In pure aqueous solution, hydroperoxide degrades carbohydrates to formic acid. Isbell, H. S.; Frush, H. L.; Martin, E. T. *Carbohydr. Res.* **1973**, *26*, 287. Isbell, H. S.; Czubarow, P. *Carbohydr. Res.* **1990**, *203*, 287. This degradative pathway was not observed when THF was used as a cosolvent.
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milder mode of deprotection.

Discussion

The sterically unhindered azido and *N*-formylamino aldehydes display a marked kinetic advantage over analogues having larger N-protecting groups in the RAMA-catalyzed aldol condensation. Improved yields reflect this kinetic advantage. Accompanying this rate enhancement, however, is a decrease in the kinetic diastereoselectivity in analogous chiral aldehydes by the enzyme. In the series of α-substituted β-hydroxypropanals, the kinetic selectivity shifted from the *R* configuration of the natural substrate to the thermodynamically favored *S* configuration. While thermodynamic selectivity may be improved in principle by increasing the size of the α-substituent, this selectivity is ultimately limited by the enzyme's kinetic preference for small, polar substituents.

The simplicity and convenience of the azide as the amino equivalent have been demonstrated by the efficient syntheses of 1,4-dideoxy-1,4-imino-D-arabinitol and 2-(*R*),5(*R*)-bis(hydroxymethyl)-3(*R*),4(*R*)-dihydroxypyrrolidine. Although the enzyme failed to resolve aldehyde **13** kinetically, a simple separation provided access to both diastereomers in reasonable yields. The yield of the thermodynamically preferred diastereomer can be markedly improved under equilibrating conditions.

We have extended the well-established carbon–carbon bond forming strategy of rabbit muscle aldolase to substrates bearing α-amino equivalents on the aldehyde reactant. Our methodology, in combination with aldolases having other stereochemistry,[32] provides rapid entry into a series of novel amino sugars.

Experimental Section

General. NMR chemical shifts are reported relative to acetone and $CHCl_3$ (^1H) and CH_3OH, DMSO-d_6, $CDCl_3$ (^{13}C) as internal standards and phosphoric acid as an external standard (^{31}P). The enzymes rabbit muscle aldolase (EC 4.1.2.13), alcohol dehydrogenase (EC 1.1.1.1), α-glycerophosphate dehydrogenase (EC 1.1.1.8), L-iditol (polyol) dehydrogenase (EC 1.1.1.14), triosephosphate isomerase (EC 5.3.1.1), and acid phosphatase (EC 3.1.3.2) were purchased from Sigma. Formate dehydrogenase (EC 1.2.1.2) was purchased from Boehringer Mannheim. Enzyme units are defined in micromoles/minute for the natural substrates. Ethyl formate was supplied by MCB Chemical. All other reagents were obtained from Aldrich. Dihydroxyacetone phosphate was prepared by following the method of Effenberger and Straub.[33]

***N*-Formylallylamine (5).** Allylamine (8.57 g, 0.15 mol) was refluxed with ethyl formate (13.3 mL, 0.17 mol) for 1 h.[34] The volatiles were removed in vacuo. The residue was distilled to afford 11.04 g (86%) of the amide **5** as a clear, colorless liquid: bp 63–5 °C (0.4 mm); ^1H NMR (250 MHz, $CDCl_3$) δ 8.15 (s, 1 H, NC*H*O), 6.24 (br s, 1 H, N*H*), 5.85–5.72 (m, 1 H, *H*C=CH_2), 5.23–5.08 (m, 2 H, HC=C*H*$_2$), 3.86 (m, 2 H, C*H*$_2$); ^{13}C NMR (63.0 MHz, $CDCl_3$) δ 161.1, 133.0, 115.4, 39.8; IR (neat) 1666, 1534 cm^{-1}. Anal. Calcd for C_4H_7NO: C, 56.45; H, 8.29; N, 16.46. Found: C, 55.96; H, 8.23; N, 15.94.

Cinnamyl Azide (6). Sodium azide (0.94 g, 14.5 mmol) in water (5 mL) was gently refluxed with cinnamyl chloride (1.02 g, 6.48 mmol) in acetone (10 mL) for 1 h. The acetone was removed in vacuo. Water (15 mL) was added, and the mixture was extracted with methylene chloride (3 × 25 mL). The organic extracts were dried with Na_2SO_4, filtered, and concentrated to yield a yellow liquid. The crude azide was purified by flash chromatography (silica, 18:1 hexane/ethyl acetate) to afford 0.98 g (94%) of cinnamyl azide as a clear, colorless liquid, whose ^1H NMR spectrum was indistinguishable from that reported previously.[35]

***N*-Acetylallylamine (7).** The amide **7** was prepared from allylamine and acetic anhydride according to the method of Stille and Becker.[36]

***N*-Formamidoacetaldehyde (8), Azidoacetaldehyde (9), and *N*-Acetamidoacetaldehyde (10).** The aldehydes **8–10** were prepared by the ozonolysis of the corresponding olefins. The olefin was treated with ozone at −78 °C in a solution of 4:1 methylene chloride/methanol. After reduction with dimethyl sulfide (10 equiv), the aldehyde was purified by flash chromatography. **8**: (silica, CH_3CN); ^1H NMR (300 MHz, D_2O) δ 8.07 (s, 1 H, NC*H*O), 5.09 (t, 1 H, *J* = 5.3 Hz, C*H*$(OH)_2$), 3.32 (d, 2 H, *J* = 5.3 Hz, C*H*$_2$); ^{13}C NMR (100 MHz, D_2O) δ 165.6, 89.1, 44.5. **9**: (silica, 15:1 CH_2Cl_2/CH_3OH); ^1H NMR (500 MHz, D_2O) δ 5.15 (t, 1 H, *J* = 4.8 Hz, hydrate), 3.33 (d, 2 H, *J* = 4.8 Hz, C*H*$_2$N); ^{13}C NMR (CD_3OD, 100 MHz) δ 98.0, 55.6. **10** (silica, CH_3CN): spectral data were in agreement with that previously reported.[17]

Kinetic Assay of α-Substituted Acetaldehydes. The rate of the RAMA-catalyzed reaction with each aldehyde was followed by monitoring the loss of DHAP, as described by Bednarski et al.[5] The aldehyde concentration was determined by assay with alcohol dehydrogenase[37] and by ^1H NMR. The rate coefficients for the disappearance of DHAP were calculated for pseudo-first-order plots of ln [DHAP] versus time.

5-Azido-5-deoxy-D-xylulose 1-Phosphate (3). α-Azidoacetaldehyde (**9**) (273 mg, 3.21 mmol) was added in two portions to a solution of DHAP (1.54 mmol) in water (14 mL), adjusted to pH 6.8 with 1 N NaOH. The solution was purged with argon. The reaction was initiated with RAMA (100 units) and stirred at room temperature for 24 h. An aliquot (300 μL) was lyophilized for analysis. The remaining keto phosphate was dephosphorylated with acid phosphatase without purification: ^1H NMR (300 MHz, D_2O) δ 4.71 (dd, 1 H, *J* = 18.7 Hz, J_{P-H} = 6.3 Hz, H-1), 4.59 (dd, 1 H, *J* = 18.7 Hz, J_{P-H} = 6.7 Hz, H-1′), 4.49 (br s, 1 H, H-3), 4.27 (m, 1 H, H-4), 3.49–3.41 (m, 2 H, H-5 and H-5′); ^{31}P NMR (125 MHz, pH 6.4, H_2O) δ 2.76 (t, *J* = 6.5 Hz).

5-Azido-5-deoxy-D-xylulose (4). The solution containing the crude 5-azido keto phosphate **3** was adjusted to pH 4.8 with 1 N HCl. Acid phosphatase (76 units) was added, and the reaction mixture was stirred at room temperature. After 24 h, the hydrolysis of the phosphate ester was complete as determined by ^{31}P NMR. The reaction mixture was concentrated in vacuo, and the residue was purified directly by flash chromatography (silica, 4:1 CH_2Cl_2/CH_3OH) to yield 211 mg (78% overall from aldehyde **9**) of **4** as a yellow foam: ^1H NMR (500 MHz, D_2O) δ 4.65 (d, 1 H, *J* = 19.5 Hz, H-1), 4.54 (d, 1 H, *J* = 19.5 Hz, H-1′), 4.43 (d, *J* = 2.3 Hz, H-3), 4.21 (m, 1 H, *J* = 2.3, 5.3, 7.7 Hz, H-4), 3.52 (dd, 1 H, *J* = 7.7, 12.8 Hz, H-5), 3.47 (dd, 1 H, *J* = 5.3, 12.8 Hz, H-5′); ^{13}C NMR (125 MHz, D_2O) δ 213.4, 76.8, 71.6, 67.1, 53.5; IR (neat) 1729, 2108 cm^{-1}; HR-FABMS (MH^+) 176.0694.

1,4-Dideoxy-1,4-imino-D-arabinitol (1). A solution of azido ketose **4** (265 mg, 1.51 mmol) in 50% aqueous methanol (10 mL) was hydrogenated under 1200 psi at room temperature for 16 h in the presence of palladium hydroxide on carbon (110 mg, 12 mol %). The reaction mixture was filtered through Celite and concentrated in vacuo to yield 148 mg (74%) of pyrrolidine **1** as a clear, colorless oil, whose ^1H and ^{13}C NMR spectra were indistinguishable from those reported previously.[20]

(*E*)-1,2-Epoxy-4-phenyl-3-butene (11). Dimethylsulfonium methylide was prepared under nitrogen from sodium hydride and trimethylsulfonium iodide in DMSO and THF.[22] Sodium hydride (8.67 g, 0.22 mol, 60% mineral oil dispersion) was washed with petroleum ether (3 × 30 mL). The residual petroleum ether was removed under vacuum. Under nitrogen, dry THF (110 mL) and dry DMSO (110 mL) were added and the reaction mixture was cooled in an ice/salt bath. A solution of trimethylsulfonium iodide (42.43 g, 0.21 mol) in DMSO (160 mL) was added by cannula over 5 min. After the addition was complete, *trans*-cinnamaldehyde

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(9.25 g, 70 mmol) was added in one portion. The reaction mixture was stirred at 0 °C for 30 min and at room temperature for an additional 40 min. The reaction mixture was slowly quenched with 400 mL of water and ice and extracted with methylene chloride (3 × 300 mL). The combined organic extracts were washed with water (2 × 300 mL), dried with potassium carbonate, filtered, and concentrated to yield a brown liquid. Distillation afforded 22.76 g (82%) of the epoxide as a pale yellow liquid: bp 67–70 °C (0.2 mm); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43–7.24 (m, 5 H, aromatic), 6.80 (d, 1 H, J = 16.0 Hz, PhC*H*=C), 5.87 (dd, J = 16.0, 8.0 Hz, PhCH=C*H*), 3.50 (m, 1 H, C*H*O), 3.04 (dd, 1 H, J = 5.2, 4.0 Hz, C*H*HO) 2.76 (dd, J = 5.2, 2.6 Hz, CH*H*O); ^{13}C NMR (75 MHz, $CDCl_3$) δ 134.4, 128.6, 128.0, 127.0, 126.4, 52.5, 49.1. Anal. Calcd for $C_8H_{10}O$: C, 82.16; H, 6.89. Found: C, 81.66; H, 6.86.

(*E*)-2-Azido-4-phenyl-3-buten-1-ol (12). A solution of the epoxide 11 (4.05 g, 27.7 mmol) and sodium azide (3.63 g, 55.8 mmol) in acetone (40 mL) and water (20 mL) was refluxed gently for 2 h. The reaction mixture was acidified by the addition of ammonium chloride (1.0 g) and stirred for an additional 10 min at room temperature. Water (10 mL) was added, and the acetone was removed in vacuo. The aqueous residue was extracted with methylene chloride (3 × 50 mL), dried with Na_2SO_4, filtered, and concentrated. The crude azido alcohol 12 was purified by flash chromatography (silica, 5:1 hexane/ethyl acetate) to yield 4.79 g (91%) of a clear, yellow liquid: ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.24–7.41 (m, 5 H, aromatic), 6.71 (d, 1 H, J = 15.9 Hz, H-4), 6.12 (dd, 1 H, J = 15.9, 8.1 Hz, H-3), 4.24 (m, 1 H, H-2), 3.67 (m, 2 H, H-1 and H-1′), 1.86 (dd, 1 H, J = 5.8, 7.2 Hz, O*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.6, 135.3, 128.7, 128.5, 126.7, 122.9, 66.3, 65.0); IR 3380, 2109 cm^{-1}. Anal. Calcd for $C_{10}H_{11}N_3O$: C, 63.48; H, 5.86; N, 22.22. Found: C, 63.15; H, 5.91; N, 22.52.

(±)-3-Hydroxy-2-azidopropanal (13). The azido alcohol 12 (2.11 g, 11.1 mmol) was treated with ozone in methylene chloride (15 mL) and methanol (15 mL) at −78 °C until a blue color persisted. The reaction mixture was purged with nitrogen. Dimethyl sulfide (12 mL) was added and the reaction mixture allowed to warm to room temperature. Water (6 mL) was added, and the mixture was stirred overnight at room temperature. Following removal of the volatiles in vacuo, the residue was purified by flash chromatography (silica, 3:1 hexane/ether, 1:1 hexane/ether, ether) to afford 1.10 g (79%) of the aldehyde as a clear, colorless oil: ^{1}H NMR (400 MHz, D_2O) δ 5.01 (d, 1 H, J = 5.2 Hz, H-1), 3.77 (dd, 1 H, J = 3.6, 11.9 Hz, H-3), 3.60 (dd, 1 H, J = 7.6, 11.9 Hz, H-3′), 3.51 (ddd, 1 H, J = 3.6, 5.2, 7.6, Hz, H-2); ^{13}C NMR (100 MHz, D_2O) δ 90.2, 68.3, 61.7.

5-Azido-5-deoxy-D-fructose (14) and 5-Azido-5-deoxy-L-sorbose (15). A solution of aldehyde 13 (589 mg, 4.43 mmol) and fructose 1,6-diphosphate (1.18 g, 2.88 mmol, sodium salt) in water (35 mL) was adjusted to pH 7.0 with 1 N NaOH. RAMA (150 units) and triose phosphate isomerase (500 units) were added and the reaction mixture was allowed to stand at room temperature for 22 h. The pH was adjusted to 5.2 with 1 N HCl. Acid phosphatase (100 units) was added and the mixture stirred at room temperature for 12 h. The pH was readjusted to 7.0 with 1 N NaOH, and the reaction mixture was applied directly onto 20 mL of AG-1X8 (HCO_3^-) ion-exchange resin and eluted with water (80 mL). The fractions containing 14 and 15 were combined, applied to 25 mL of AG-50W-X8 (H^+), and eluted with water (75 mL). The aqueous fractions were combined and concentrated in vacuo. The crude pyranoses were purified by flash chromatography (silica, acetone) to yield 649 mg (71%) of a clear, pale yellow oil, which crystallized upon standing.

5-Azido-5-deoxy-D-fructose 1,2-Acetonide (16) and 5-Azido-5-deoxy-L-sorbose 1,2-Acetonide (17). The mixture of 14 and 15 (1.03 g, 5.02 mmol) was stirred with 2-methoxypropene (1.5 mL) and *p*-toluenesulfonic acid (10 mg) in acetone (40 mL) at room temperature for 2 h. The reaction mixture was concentrated in vacuo and purified by flash chromatography (silica, 1:1 ether/hexane to 4:1 ether/hexane) to afford 272 mg (22%) of 16 as a white solid and 693 mg (56%) of 17 as a white solid. 16: R_f = 0.17 (4:1 ether/hexane); mp 114 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.17 (d, J = 8.9 Hz, H-1), 4.01 (d, J = 8.9 Hz, H-1′), 3.95 (dd, J = 1.6, 12.6 Hz, H-6), 3.93 (m, J = 1.6, 1.8, 3.6 Hz, H-5), 3.90 (ddd, J = 3.6, 4.1, 9.4 Hz, H-4), 3.76 (dd, J = 1.8, 12.6 Hz, H-6′), 3.70 (dd, J = 9.4, 10.7 Hz, H-3), 2.49 (d, J = 4.1, OH), 1.72 (d, J = 10.7 Hz, OH), 1.48 (s, CH_3), 1.42 (s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 112.2, 105.5, 72.2, 71.9, 69.3, 62.0, 61.6, 26.3, 26.2. Anal. Calcd for $C_9H_{15}N_3O_5$: C, 44.08; H, 6.17; N, 17.16. Found: C, 43.88; H, 6.18; N, 17.09. 17: R_f = 0.30 (4:1 ether/hexane); mp 110 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.14 (d, J = 8.8 Hz, H-1), 3.95 (d, J = 8.8 Hz, H-1′), 3.72 (dd, J = 4.5, 10.2 Hz, H-6), 3.65 (ddd, J = 2.6, 9.0, 9.2 Hz, H-4), 3.56 (dd, J = 10.2, 11.1 Hz, H-6′), 3.49 (m, J = 4.5, 9.0, 11.1 Hz, H-5), 3.39 (dd, J = 9.2, 10.5 Hz, H-3), 2.95 (d, J = 2.6 Hz, OH), 2.01 (d, J = 10.5 Hz, OH), 1.47 (s, CH_3), 1.42 (s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 112.4, 104.9, 75.1, 71.9, 71.8, 61.3, 60.8, 26.4, 26.3. Anal. Calcd for $C_9H_{15}N_3O_5$: C, 44.08; H, 6.17; N, 17.16. Found: C, 43.94; H, 5.97; N, 17.09.

5-Azido-5-deoxy-D-fructose (14). 5-Azido-5-deoxy-D-fructose 1,2-acetonide (16) (482 mg, 1.91 mmol) was hydrolyzed in water (25 mL) and ethanol (10 mL) with DOWEX-50W (H^+, 5 mL) at 70 °C for 4 h. The solution was filtered and the resin washed with an additional 30 mL of water. The aqueous extracts were concentrated and purified by flash chromatography (silica, acetone) to yield 369 mg (92%) of 14 as a white, crystalline solid: ^{1}H NMR (400 MHz, D_2O) δ 3.96 (dd, J = 3.9, 9.8 Hz, H-4), 3.93 (dd, J = 1.6, 12.9 Hz, H-6), 3.88 (m, J = 1.6, 1.8, 3.9 Hz, H-5), 3.64 (dd, J = 1.8, 12.9 Hz, H-6′), 3.62 (d, J = 9.8 Hz, H-3), 3.54 (d, J = 11.8 Hz, H-1), 3.38 (d, J = 11.8 Hz, H-1′); ^{13}C NMR (100 MHz, D_2O) δ 99.2, 70.8, 68.8, 64.7, 63.7, 61.9.

5-Azido-5-deoxy-L-sorbose (15). 5-Azido-5-deoxy-L-sorbose 1,2-acetonide (17) (100 mg, 0.41 mmol) was hydrolyzed in water (8 mL) and ethanol (5 mL) with DOWEX-50W (H^+, 4 mL) at 70 °C for 5 h. The solution was filtered and the resin washed with an additional 15 mL of water. The aqueous extracts were concentrated and purified by flash chromatography (silica, acetone) to yield 65 mg (77%) of 15 as a white, crystalline solid: ^{1}H NMR (500 MHz, D_2O) δ 3.67 (dd, J = 5.3, 10.9 Hz, H-6), 3.60 (dd, J = 9.4, 9.6 Hz, H-4), 3.55 (d, J = 11.8 Hz, H-1), 3.48 (dd, J = 10.9, 11.4 Hz, H-6′), 3.42 (m, J = 5.3, 9.4, 11.4 Hz, H-5), 3.41 (d, J = 9.6 Hz, H-3), 3.35 (d, J = 11.8 hz, H-1′); ^{13}C NMR (100 MHz, D_2O) δ 98.9, 73.8, 71.5, 64.4, 62.4, 61.0; HR-FABMS (MNa^+) 228.0592.

2(*R*),5(*R*)-Bis(hydroxymethyl)-3(*R*),4(*R*)-dihydroxypyrrolidine (2). 5-Azido-5-deoxy-D-fructose (14) (369 mg, 1.80 mmol) was hydrogenated in 50% aqueous ethanol (50 mL) at 1200 psi in the presence of palladium hydroxide on carbon (360 mg, 20 mol %) at room temperature for 14 h. The reaction mixture was filtered twice through Celite and concentrated in vacuo to afford 271 mg (92%) of 2 as a clear, colorless oil, which crystallized as needles upon standing and whose ^{1}H and ^{13}C NMR data were indistinguishable from those previously reported.[16,21]

(*E*)-2-Amino-4-phenyl-3-buten-1-ol (18). To a solution of the azido alcohol 12 (1.00 g, 5.23 mmol) in THF (20 mL) was slowly added triphenylphosphine (1.51 g, 5.75 mmol) in THF (30 mL). The reaction mixture was stirred overnight at room temperature. Water (5 mL) was added, and the solution was stirred for an additional 24 h at room temperature. The THF was removed in vacuo. Ether (30 mL) was added, and the organic fraction was extracted with 5% HCl (3 × 30 mL). The aqueous extracts were combined, and the pH was adjusted to 13 with 1.0 N NaOH until a white precipitate formed. The aqueous fraction was reextracted with methylene chloride (3 × 40 mL). The organic extracts were dried with Na_2SO_4, filtered, and concentrated to yield 0.78 g (91%) of a white solid. The crude amino alcohol was recrystallized from ether to yield white crystalline plates: mp 70–71 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36–7.20 (m, 5 H, aromatic), 6.56 (d, 1 H, J = 16.0 Hz, H-4), 6.15 (dd, 1 H, J = 16.0, 5.7 Hz, H-3), 3.67 (dd, 1 H, J = 10.3, 4.5 Hz, H-1), 3.62 (m, 1 H, H-2), 3.43 (dd, 1 H, J = 10.3, 7.6 Hz, H-1′), 2.54 (br s, 3 H, O*H*, NH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 136.6, 131.0, 130.1, 128.6, 127.7, 126.5, 66.3, 55.4. Anal. Calcd for $C_{10}H_{13}NO$: C, 73.59; H, 8.03; N, 8.59. Found: C, 73.36; H, 8.07; N, 8.35.

***N*-Formyl-2-amino-4-phenyl-3-buten-1-ol (19).** A suspension of amino alcohol 18 (355 mg, 2.18 mmol) in ethyl formate (10 mL, excess) was refluxed for 90 min.[34] The volatiles were removed in vacuo, and the residue was purified by flash chromatography (silica, ethyl acetate, 1:1 ethyl acetate/acetone) to yield 283 mg (68%) of a white solid, which upon recrystallization from a mixture of hexane and ethanol afforded a white crystalline solid: mp 89–91 °C; R_f = 0.29 (1:1 ethyl acetate/acetone); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (d, J = 8.3 Hz, NH), 8.10 (s, CHO), 7.41–7.20 (m, aromatic), 6.49 (d, J = 16.1 Hz, H-4), 6.27 (dd, J = 5.8, 16.1

Hz, H-3), 4.93 (br s, OH), 4.53 (m, J = 5.8, 8.3 Hz, H-2), 3.47 (dd, H-1 and H-1′); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.7, 136.6, 129.9, 128.6, 128.4, 127.4, 126.2, 63.5, 51.4. Anal. Calcd for $C_{11}H_{13}NO_2$: C, 69.09; H, 6.85; N, 7.33. Found: C, 68.96; H, 6.88; N, 7.05.

***N*-Acetyl-2-amino-4-phenyl-3-buten-1-ol (20).** The amino alcohol **18** (326 mg, 2.00 mmol) was stirred with acetic anhydride (210 μL, 2.25 mmol) in dry pyridine (12 mL) at room temperature for 12 h. Toluene (3 × 15 mL) was added, and the reaction mixture was concentrated in vacuo. The residue was purified by flash chromatography (silica, ethyl acetate, 4:1 ethyl acetate/acetone) to afford 394 mg (96%) of a white crystalline solid: mp 146–147 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.91 (d, 1 H, J = 8.2 Hz, NH), 7.39–7.20 (m, 5 H, aromatic), 6.46 (d, 1 H, J = 16.1 Hz, PhCH), 6.25 (dd, 1 H, J = 5.9, 16.1 Hz, PhCH=CH), 4.83 (t, 1 H, J = 5.7 Hz, OH), 4.44 (m, 1 H, J = 5.9, 8.2 Hz, CHN), 3.44 (m, 1 H, J = 5.7 Hz, CH_2O); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.6, 136.7, 129.6, 129.0, 128.6, 127.4, 126.1, 63.6, 52.7, 22.8. Anal. Calcd for $C_{12}H_{15}NO_2$: C, 70.22; H, 7.37; N, 6.83. Found: C, 69.78; H, 7.43; N, 6.68.

Aldehydes 21 and 22. Olefins **19** and **20** were treated with ozone at −78 °C in 1:1 methanol/methylene chloride as described earlier. The aldehydes were purified by flash chromatography. **21** (silica, 7:1 methylene chloride/methanol): ^{1}H NMR (500 MHz, D_2O) major rotamer δ 8.13 (s, NCHO), 5.07 (d, J = 4.7 Hz, hydrate), 3.98 (m, J = 4.6, 4.7, 6.8 Hz, CHN), 3.73 (dd, J = 4.6, 11.8 Hz, CHHO), 3.62 (dd, J = 6.8, 11.8 Hz, CHHO); minor rotamer δ 7.97 (s, NCHO), 5.02 (d, J = 4.9 Hz, hydrate), 3.76 (dd, J = 4.1, 11.8 Hz, CHHO), 3.60 (dd, J = 8.0, 11.8 Hz, CHHO) 3.47 (m, J = 4.1, 4.9, 8.0 Hz, CHN); ^{13}C NMR (100 MHz, D_2O) δ 165.7, 89.5, 61.1, 55.7. **22** (silica, 8:1 methylene chloride/methanol): ^{1}H NMR (500 MHz, D_2O) δ 5.04 (d, J = 4.8 Hz, hydrate), 3.89 (m, J = 4.4, 4.8, 6.9 Hz, CHN), 3.71 (dd, J = 4.4, 11.7 Hz, CHHO), 3.60 (dd, J = 6.9, 11.7 Hz, CHHO), 2.00 (s, CH_3); ^{13}C NMR (100 MHz, D_2O) δ 175.7, 89.8, 61.2, 57.0, 23.1.

Determination of Diastereoselectivity by ^{1}H and ^{31}P NMR Spectroscopy. The reactions of aldehydes **13**, **21**, and **22** with DHAP in the presence of RAMA were followed by ^{1}H (500 MHz) and ^{31}P (202 MHz) NMR. To a freshly prepared solution of DHAP (hydrolyzed in D_2O and adjusted to pD 6 with NaOD) was added a solution of aldehyde in D_2O. The concentrations were adjusted to approximately 50 mM DHAP and 200 mM aldehyde. Each reaction mixture was characterized by NMR at t = 0. The reaction was initiated with the addition of RAMA (10–20 units), and the ratio of products was determined as a function of time. After all the DHAP had been consumed, additional DHAP was added to convert all remaining aldehyde to products. The stereochemistry of the major diastereomer in each reaction was assigned by ^{1}H NMR after dephosphorylation with acid phosphatase.

***N*-Formyl-5-deoxy-5-amino-D-xylulose (23).** Aldehyde **8** (522 mg, 6.0 mmol) was stirred with DHAP (4.0 mmol) in water (40 mL), adjusted to pH 6.4 with 1 N NaOH. RAMA (100 units) was added and the reaction mixture incubated at room temperature for 24 h. The pH was lowered to 4.8 with 1 N HCl. Acid phosphatase (120 units) was added, and the mixture was stirred at room temperature for 48 h. The solution was concentrated in vacuo to afford a yellow semisolid, which was redissolved in water (3 mL), applied to a column of AG-501-X8 ion-exchange resin (mixed bed, 20 mL), and eluted with water. The fractions were pooled and concentrated in vacuo to afford 565 mg (80%) of a yellow oil as a mixture of the desired ketose and its hydrate: ^{1}H NMR (500 MHz, D_2O) ketone δ 8.31 (s, NCHO), 4.58 (d, J = 19.4 Hz, H-1), 4.48 (d, J = 19.4 Hz, H-1′), 4.36 (d, J = 2.2 Hz, H-3), 4.12 (ddd, J = 2.2, 5.3, 8.1 Hz, H-4), 3.46 (dd, J = 5.3, 13.8 Hz, H-5), 3.32 (dd, J = 8.1, 13.8 Hz, H-5′); hydrate δ 8.37 (d, J = 1.2 Hz, NCHO), 4.24 (ddd, J = 7.0, 7.1, 7.5 Hz, H-4), 3.96 (d, J = 7.1 Hz, H-3), 3.88 (dd, J = 7.5, 12.2 Hz, H-5), 3.80 (d, J = 12.4 Hz, H-1), 3.74 (d, J = 12.4 Hz, H-1′), 3.03 (ddd, J = 1.2, 7.0, 12.2 Hz, H-5′); ^{13}C NMR (100 MHz, D_2O) hydrate δ 165.2, 89.7, 76.8, 71.7, 63.1, 47.8.

***N*-Formyl-1-amino-1-deoxy-L-xylitol (24).** Ketose **23** (216 mg, 1.22 mmol) was dissolved in a solution of sodium formate (250 mg, 3.68 mmol) and NADH (12 mg, 0.017 mmol, trisodium salt) in water (20 mL). The reaction mixture was purged with argon for 15 min before the addition of enzyme. L-Iditol dehydrogenase (10 units) and formate dehydrogenase (10 units) were added, and the reaction mixture was allowed to stand at room temperature for 48 h. The reaction mixture was purified by desalting through AG-501-X8 (mixed bed). The fractions containing polyol **24** were pooled and concentrated to yield 119 mg (54%) of a yellow oil: ^{1}H NMR (500 MHz, D_2O) δ 7.96 (s, NCHO), 3.72 (m, J = 4.0, 4.4, 8.0 Hz, H-2), 3.67 (m, J = 4.3, 4.8, 6.8 Hz, H-4), 3.56 (dd, J = 4.3, 11.7 Hz, H-5), 3.48 (dd, J = 6.8, 11.7 Hz, H-5′), 3.44 (dd, J = 4.0, 4.8 Hz, H-3), 3.34 (dd, J = 4.4, 14.0 Hz, H-1), 3.21 (dd, J = 8.0, 14.0 Hz, H-1′); ^{13}C NMR (100 MHz, D_2O) δ 165.7, 73.0, 72.4, 70.9, 63.6, 41.8; HR-FABMS (MH^+) 180.0877.

1-Amino-1-deoxy-L-xylitol (25). Amide **24** (55 mg, 0.31 mmol) was dissolved in hydrogen peroxide (0.5 mL, 30%) and THF (6 mL). Lithium hydroxide (52 mg, 1.24 mmol) in water (1.5 mL) was added and the mixture stirred at room temperature for 18 h. After the reaction mixture was cooled in an ice bath, saturated aqueous sodium bisulfite was added dropwise until the mixture no longer showed a positive response with starch–iodide paper. The reaction mixture was concentrated to remove the THF. The aqueous residue was purified by ion-exchange chromatography (AG-50W-X8, H^+) and eluted with a gradient of ammonium hydroxide (0.5–3.0 M). The fractions were concentrated and dried under vacuum to yield 22 mg (47%) of the free amine of 1-amino-1-deoxy-L-xylitol as a clear, colorless oil. The ^{1}H NMR spectrum of its hydrochloride was identical with that reported for the D enantiomer.[27] ^{1}H NMR analysis of the crude reaction mixture indicated that only the desired product was present; a significant loss of yield was incurred upon purification.

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