(2 × CH_3); EIHRMS 713.6925 (M^+, $C_{21}H_{11}O_6{}^{79}Br_3{}^{81}Br^{37}Cl$, ΔM −0.6 mmu).

Rubrolide C diacetate (11): obtained as a colorless solid; IR (CH_2Cl_2) ν_{max} 2925, 2854, 1775, 1503, 1458, 1371, 1213, 1200, 1180, 1168, 1065, 912 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.00 (s, H2″/H6″), 7.50 (d, J = 8.5 Hz, H2′/H6′), 7.28 (d, J = 8.5 Hz, H3′/H5′), 6.26 (s, H2), 6.01 (s, H5), 2.36 (s, 4′-OAc), 2.40 (s, 4″-OAc); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.1 (C), 167.8 (C), 167.1 (C), 157.5 (C), 152.5 (C), 149.2 (C), 146.4 (C), 133.9 (2 × CH), 133.5 (C), 129.7 (2 × CH), 127.4 (C), 122.6 (2 × CH), 118.1 (2 × C), 115.7 (C), 109.7 (C), 21.1 (CH_3), 20.5 (CH_3); EIHRMS 479.9024 (M^+ − C_2H_2O, $C_{19}H_{12}O_5{}^{79}Br^{81}Br$, ΔM −0.9 mmu).

Rubrolide D diacetate (12): obtained as a colorless solid; IR (CH_2Cl_2) ν_{max} 1766, 1506, 1455, 1370, 1217, 1198, 1183, 1169, 946 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table II; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table III; EIMS, m/z (rel intensity) 524 (3.6), 522 (7.1), 520 (3.7), 482 (37.6), 480 (74.6), 478 (37.2), 440 (51.0), 438 (100.0), 436 (51.7), 382 (2.4), 358 (3.1), 330 (2.2), 301 (5.3), 274 (5.0), 250 (17.6), 221 (7.7), 134 (36.7), 43 (91.0), 32 (81.4); EIHRMS 521.9132 (M^+, $C_{21}H_{14}O_6{}^{79}Br^{81}Br$, ΔM −0.7 mmu).

Rubrolide E diacetate (13): obtained as a colorless solid; IR (CH_2Cl_2) ν_{max} 2927, 2854, 1764, 1609, 1501, 1370, 1201, 1166, 1085, 1016, 960, 915 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table II; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table III; EILRMS, m/z (rel intensity) 364 (15.0), 322 (72.3), 280 (100.0), 252 (5.2), 224 (7.4), 134 (38.1), 133 (35.2), 77 (18.3), 43 (87.8); EIHRMS 364.0945 (M^+, $C_{21}H_{16}O_6$, ΔM −0.2 mmu).

Rubrolide G triacetate (14): obtained as a colorless solid; $[\alpha]_D$ 0° ($CHCl_3$, c 0.3); IR (film) ν_{max} 1778, 1459, 1370, 1183, 1034, 909 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table II; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table III; EILRMS, m/z (rel intensity) 702 (3.8), 700 (13.4), 698 (19.7), 696 (13.2), 694 (3.3), 660 (1.2), 658 (4.1), 656 (6.2), 654 (4.3), 652 (1.3), 642 (19.5), 640 (58.7), 638 (78.9), 636 (55.2), 634 (13.0), 600 (21.1), 598 (58.0), 596 (75.4), 594 (47.7), 592 (11.6), 482 (16.9), 480 (34.7), 478 (17.5), 440 (20.2), 438 (40.3), 436 (21.2), 349 (11.4), 334 (10.0), 332 (15.6), 330 (7.9), 310 938.7), 308 (76.9), 306 (39.8), 278 (12.4), 276 (24.2), 274 (13.0), 267 (53.3), 265 (100.0), 263 (53.0), 185 (12.4), 43 (29.9); EIHRMS 739.7532 (M^+, $C_{23}H_{16}O_8{}^{79}Br_2{}^{81}Br_2$, ΔM −0.9 mmu).

Rubrolide H triacetate (15): obtained as a colorless solid; $[\alpha]_D$ 0° ($CHCl_3$, c 0.4); IR (film) ν_{max} 1798, 1776, 1543, 1459, 1371, 1182, 1066, 992, 908, cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), see Table II; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table III; EILRMS, m/z (rel intensity) 776 (0.4), 774 (0.4), 740 (0.6), 738 (6.4), 736 (25.2), 734 (52.2), 732 (100.0), 730 (37.6), 692 (14.4), 690 (15.5), 688 (8.2), 686 (1.9), 634 (27.3), 632 (61.6), 630 (72.1), 628 943.0), 626 (10.6), 384 (6.5), 382 (8.1), 312 (18.1), 310 (72.5), 308 (100.0), 306 (45.0), 267 (56.4), 265 (76.0), 263 (35.0), 185 (11.1), 80 (20.4), 43 (37.6), 32 (87.9); EIHRMS 773.7158 (M^+, $C_{23}H_{15}O_8{}^{79}Br_2{}^{81}Br_2{}^{35}Cl$, ΔM 0.6 mmu).

Methylation of Rubrolides G and H. An acetone solution containing a mixture of rubrolides G and H (≈1 mg in 0.3 mL) was mixed with an ethereal solution of CH_2N_2 (0.2 mmol in 5 mL) and stirred at rt for 15 h. After evaporation of the solvent, the mixture was chromatographed on TLC (silica gel, MeOH/CH_2Cl_2 1:99) followed by HPLC (silica gel, EtOAc/hexane 1:3) to give two pure compounds, trimethylrubrolide G 17 (≈0.5 mg) and 18 (≈0.5 mg). **Trimethylrubrolide G (17)**: colorless solid; IR (CH_2Cl_2) ν_{max} 2926, 2854, 1719, 1712, 1655, 1560, 993 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.44 (s, 2 H), 7.34 (s, 2 H), 6.13 (s, 1 H), 3.91 (s, 3 H), 3.88 (s, 2 H), 3.86 (s, 3 H), 3.82 (s, 3 H); EILRMS, m/z (rel intensity) 660 (1.4), 658 (4.0), 656 (5.8), 654 (3.8), 652 (1.5), 628 (5.6), 626 (16.3), 624 (22.1), 622 (14.1), 620 (3.6), 379 (43.4), 377 (86.0), 375 (43.0), 351 (21.1), 349 (34.0), 291 (19.3), 281 (15.2), 279 (30.0), 277 (18.8), 270 (11.8), 268 (12.3), 183 (5.3), 154 (8.2). 18: colorless solid; IR ($CHCl_2$) ν_{max} 2949, 2923, 1736, 1729, 1605, 1472, 1423, 1211, 1177, 991, 909 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.60 (s, 2 H), 7.22 (s, 2 H), 5.05 (dd, J = 18.3, 4.2 Hz), 4.61 (s, J = 18.2, 8.6 Hz), 4.15 (d, J = 17.1 Hz), 3.97 (d, J = 17.1 Hz), 3.90 (s, 3 H), 3.85 (s, 3 H), 3.71 (s, 3 H), 3.14 (dd, J = 8.6, 4.2 Hz); EILRMS, m/z (rel intensity) 700 (0.3), 698 (0.5), 696 (0.4), 694 (0.1), 674 (4.3), 672 (16.3), 670 (24.9), 668 (17.3), 666 (5.0), 615 (2.2), 613 (8.1), 611 (12.6), 609 (8.6), 607 (2.6), 393 (25.7), 391 (51.1), 389 (25.5), 365 (16.6), 363 (33.0), 361 (19.4), 284 (30.1), 282 (32.7), 281 (19.9), 279 (40.2), 277 (21.3), 225 (9.5), 223 (8.2), 203 (15.1).

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Supplementary Material Available: NMR spectra for compounds 1, 2, 3, 6, 12, 13, 14, and 15 and HETCOR and FLOCK spectra for 1 (17 pages). Ordering information is given on any current masthead page.

Use of Dihydroxyacetone Phosphate Dependent Aldolases in the Synthesis of Deoxyazasugars[1]

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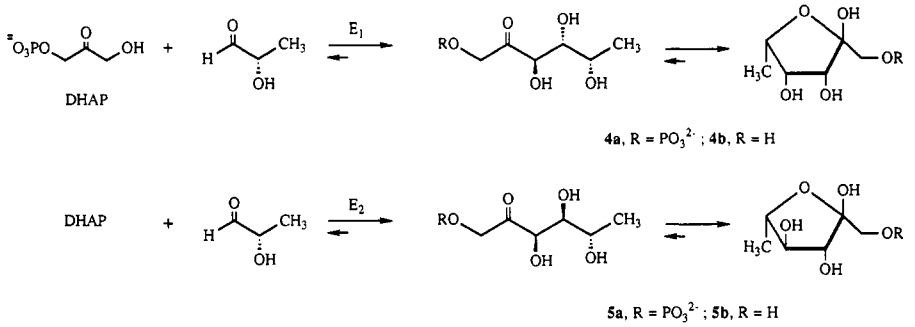
The use of fructose-1,6-diphosphate (FDP), fuculose-1-phosphate (Fuc-1-P) and rhamnulose-1-phosphate (Rham-1-P) aldolases in organic synthesis is described. Fuc-1-P, Rham-1-P, and their phosphate-free species have been prepared and characterized. Both Fuc-1-P and Rham-1-P aldolases accept 3-azido-2-hydroxypropanal as a substrate to form L-ω-azidoketose phosphates, which upon dephosphorylation and hydrogenolysis on Pd/C, gave 1-deoxyazasugars structurally related to D-galactose and L-mannose. Hydrogenolysis of the enzyme products azidoketose 1-phosphates, however, gave 1,6-dideoxyazasugars structurally related to 6-deoxygalactose and L-rhamnose. Explanations for the stereoselectivity in the hydrogenolysis reactions were provided. Similarly, FDP aldolase catalyzed the aldol condensation reaction with 2-azido-3-hydroxypropanal to afford a new synthesis of 2(*R*),5(*S*)-bis(hydroxymethyl)-3(*R*),4(*R*)-dihydroxypyrrolidine, a potent inhibitor of a number of glycosidases. A new empirical formula is developed to relate the inhibition constants and inhibitor binding for α- and β-glucosidases.

Naturally occurring and synthetic azasugars[2,3] and their derivatives are useful inhibitors of enzymes associated with carbohydrate processing. Synthesis of azasugars based on fructose-1,6-diphosphate (FDP) aldolase (EC 4.1.2.3) has

0022-3263/91/1956-6280$02.50/0

Scheme I[a]



[a] E_1, fuculose-1-phosphate aldolase; E_2: rhamnulose-1-phosphate aldolase.

only recently been developed and has proven to be a promising approach.[4] Since more than 20 aldolases with different substrate specificity and stereospecificity are available and the enzymes generally exhibit a broad acceptor specificity,[5] enzymatic aldol reactions with azido aldehydes followed by catalytic reductive amination is considered to be a general and effective route to a number of deoxyazasugars.

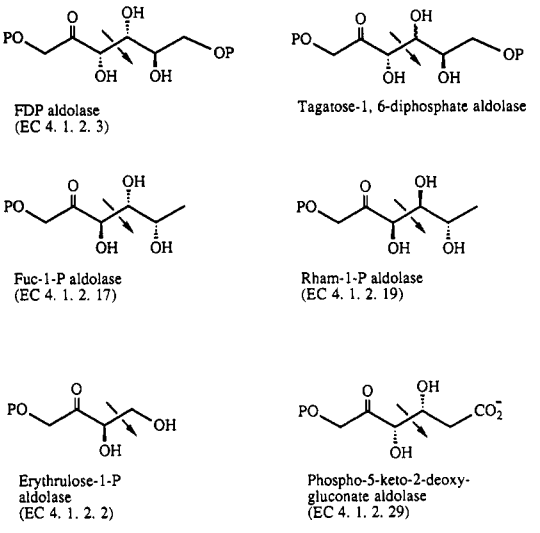


Figure 1. Natural substrates for DHAP-dependent aldolases. Arrows indicate the bond cleaved or formed.

Six dihydroxyacetone phosphate (DHAP) dependent aldolases have been isolated. The natural substrate specificity and stereospecificity of each of these enzymes are indicated in Figure 1.[6] All these enzymes show a high degree of enantio- and diastereoselectivity in the reversible aldol reaction except tagatose-1,6-diphosphate aldolase, which cannot discriminate the diastereotopic π-faces of the natural acceptor D-glyceraldehyde 3-phosphate. Of these DHAP-dependent aldolases, FDP aldolase from rabbit muscle[5] (a Schiff base forming enzyme) or *Escherichia coli*[4c] (a Zn^{2+} enzyme) has been studied the most. Fuculose-1-phosphate (Fuc-1-P) aldolase (EC 4.1.2.17) and rhamnulose-1-phosphate (Rham-1-P) aldolase (EC 4.1.2.19) have been investigated to some extent.[6] We here report

(1) Supported by the NIH (GM44154).

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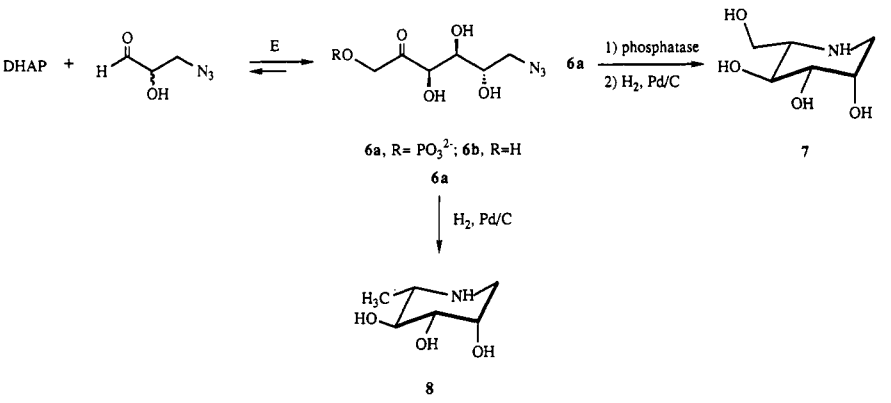
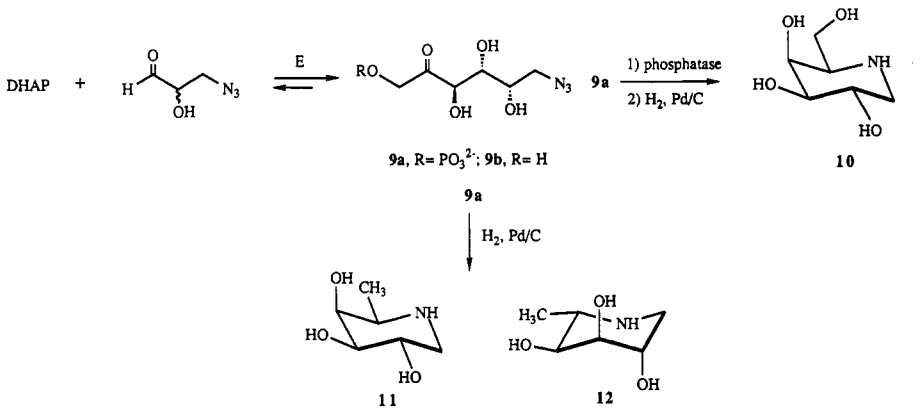
Scheme II[a]



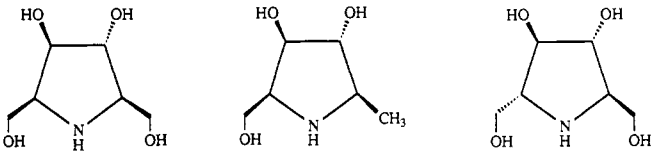
[a] E, rhamnulose-1-phosphate aldolase.

Scheme III[a]



[a] E, fuculose-1-phosphate aldolase.

the use of Fuc-1-P aldolase and Rham-1-P aldolase, respectively, in the synthesis of Fuc-1-P, Rham-1-P, and their phosphate-free species and the enzymatic synthesis of 1-deoxyazasugars via reaction with 3-azido-2-hydroxypropanal followed by dephosphorylation and Pd-mediated reductive amination. The phosphate group of the enzyme products can be reductively cleaved in the Pd-mediated reductive amination, providing a new method for the synthesis of dideoxyazasugars. Both enzymatic reactions and reductive aminations were conducted in aqueous solution without protection of the functional groups, and isolation of products was very straightfoward.[4,7] We also report the synthesis of 2(*R*),5(*S*)-bis(hydroxymethyl)-3-(*R*),4(*R*)-dihydroxypyrrolidine (**1**) and its 2(*R*)-methyl derivative **2** via a thermodynamic FDP aldolase reaction with racemic 2-azido-3-hydroxypropanal and reductive amination. Compound **1** is the 5-epimer of the natural product **3**.[8] It exhibits a broad spectrum of inhibition against glycosidases with K_i in the range of μM. With this inhibitor, a new empirical formula has been developed to relate the inhibition constants and inhibitor binding contributed from conformation, charge, and topographical orientation.



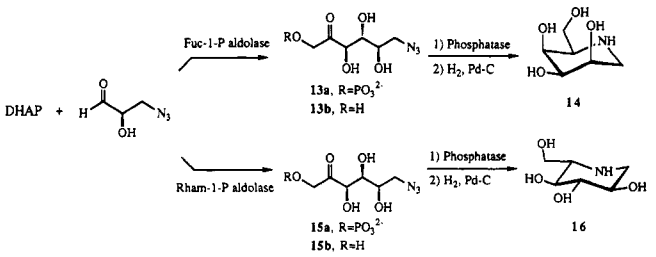
Synthesis with Rham-1-P and Fuc-1-P Aldolases

Rham-1-P and Fuc-1-P aldolases catalyze aldol condensations to form two new stereogenic centers with 3*R*,4*S* and 3*R*,4*R* configurations, respectively. We first carried out aldol condensations using natural substrates (Scheme I) for the synthesis of Fuc-1-P (**4a**) and Rham-1-P (**5a**) based on Fuc-1-P and Rham-1-P aldolase, respectively. The phosphate groups were then enzymatically removed with phosphatase to give fuculose (**4b**) and rhamnulose (**5b**), respectively. Unnatural aldehydes were also used as substrates. Thus, with the use of 3 equiv of racemic 3-azido-2-hydroxypropanal[4c] as substrate (Schemes II and III), compounds **6a** and **9a** were obtained selectively in ~50% yield from Rham-1-P aldolase and Fuc-1-P aldolase reactions, respectively. It is worth noting that both Rham-1-P and Fuc-1-P aldolases accept the *S* aldehyde as substrate, whereas FDP-aldolase is selective for the *R* enantiomer.[4a,b] Pd-mediated reductive amination of **6a** and **9a** gave **8** and a 1:1 mixture of **11** and **12**, respectively, each in approximately 90% yield. Reductive amination of the phosphate-free **6b** and **9b**, however, gave **7** and **10**,

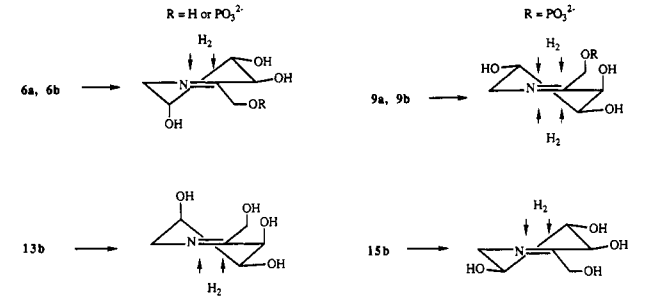
(7) For a preliminary study, see: Kajimoto, T.; Liu, K. K.-C.; Chen, L.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 6678. (Compound **4b** should be L-1-deoxynojirimycin. The configuration at C-5 was mistakenly assigned.)

(8) Isolated from certain legumes (Welter, A.; Dardenne, G.; Marlier, M.; Casimir, J. *Phytochemistry* **1976**, *25*, 747). For the synthesis of **3**, see: Fleet, G. L. L.; Smith, P. W. *Tetrahedron Lett.* **1985**, *26*, 1469. Shing, T. K. M. *J. Chem. Soc., Chem. Commun.* **1987**, 262. Dureault, A.; Portal, M.; Depezay, J. C. *Synlett* **1991**, *4*, 225. Card, P. J.; Hitz, W. D. *J. Org. Chem.* **1985**, *50*, 891. For inhibition studies, see: Fleet, G. W. J.; Nicholas, S. J.; Smith, P. W.; Evans, S. V.; Fellows, L. E.; Nash, R. J. *Tetrahedron Lett.* **1985**, *26*, 3127. For a practical chemical synthesis of **1** from 5-keto-D-fructose, see: Reitz, A. B.; Baxter, E. W. *Tetrahedron Lett.* **1990**, *31*, 6777.

Scheme IV

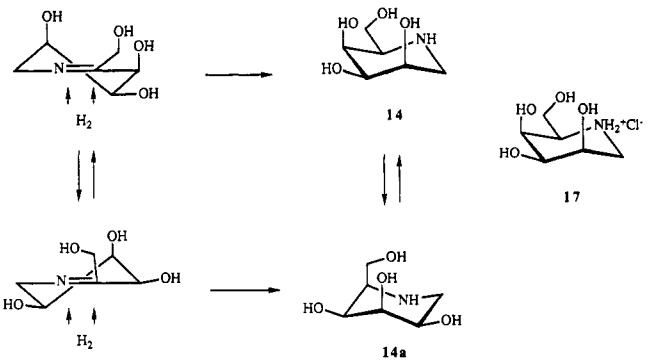


Scheme V

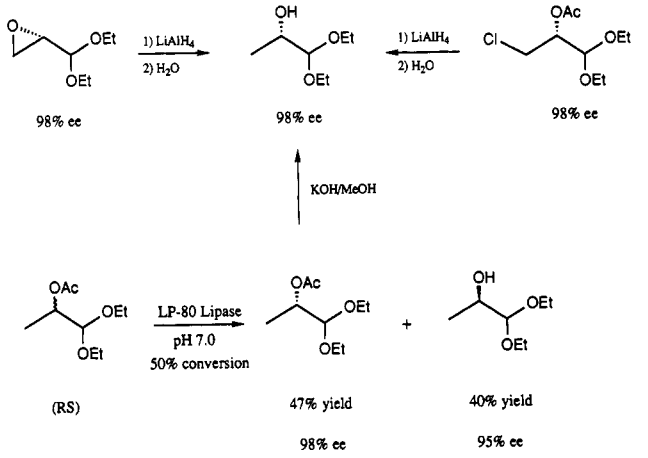


respectively, each also in approximately 90% yield. Reaction of DHAP with (*R*)-3-azido-2-hydroxypropanal by Fuc-1-P aldolase and Rham-1-P aldolase gave **13a** and **15a**, respectively, which upon removal of the phosphate group followed by reductive amination generated **14** and **16** exclusively (Scheme IV). The reductive aminations of the phosphate-free azidoketoses are all diastereoselective. It appears that hydrogens are delivered to the imine intermediate from the face opposite to axial OH-group(s) (Scheme V), (e.g., reactions with **6**, **9** and **13**) with the exception of **9a** from which a 1:1 mixture of 5-epimers was generated. It seems that in the latter case steric effect is as significant as the torsional strain effect (i.e., hydrogens would attack the top face to form a trans C_4–C_5 product) as in the case of **15b** → **16**. This situation was only observed in the reductive amination of **9a**. One explanation is that the phosphate group was reductively cleaved prior to the reduction of imine, and, due to the smaller size of CH_3 compared to CH_2OH, torsional strain becomes insignificant in the imine reduction. It should be noted that all the imine intermediates presented in Scheme V are the proposed half-chair conformations which lead to the final products upon reductive amination. An additional finding is that the $A_{1,2}$ strain (e.g. reaction with **6** or **15b**) does not seems to significantly affect the stereochemical course of the reduction. Of particular interest is the reductive amination of **13b**. The ^1H NMR spectrum of the hydrochloride salt of the product, **14**, suggests that the molecule exists exclusively as conformer **14** ($^4C_1$) instead of **14a** ($^1C_4$). The protons of H-2, -3, and -4 had small coupling constants in the range of 2–3 Hz. The imine intermediate in this reduction could be the less stable one which gave **14** upon reduction, or the more stable one which gave **14a** and then isomerized to **14** (Scheme VI). It is noted, however, that the NMR spectrum is based on the hydrochloride salt **17** instead of the neutral species, which showed very poor resolution at 500 MHz. All the conformations (either $^4C_1$ or $^1C_4$) and relative configurations were determined by NMR on the basis of the coupling constants, NOE data, and selective proton decoupling. Compounds **7**, **8**, **11**, **12**, and **14** prepared in this study are new and characterized. Compound **10** (D-1-deoxygalactojirimycin) was reported[3i,w,x] and our NMR data were consistent with the reported values. Compound **7** is the enantiomer of D-1-deoxymannojirimycin (it may be called L-1-deoxymannojirimycin). Compound **8** is an azasugar structurally related to rhamnose (it can be called 1-deoxyrhamnojirimycin). It may have potential value as selective antimicrobial agent or as herbicide as rhamnose is often found in microorganisms or plants, but not in animals or humans. Compounds **11**, **12**, **14**, and **16** are structurally related to 6-deoxy-D-galactose, 6-deoxy-L-altrose, D-talose, and L-glucose, respectively; they may be named D-1,6-dideoxygalactojirimycin, L-1,6-dideoxyaltojirimycin, D-1-deoxytalojirimycin, and L-1-deoxynojirimycin, respectively. Evaluation of these azasugars as glycosidase inhibitors is in progress.

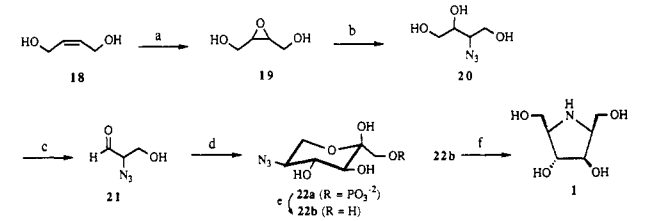
Scheme VI



Scheme VII



Scheme VIII. Preparation of 2(*R*),5(*S*)-Bis(hydroxymethyl)-3(*R*),4(*R*)-dihydroxypyrrolidine (1)[a]



[a] (a) MCPBA, CH_3CN (92%, ref 9); (b) NaN_3 (5 equiv), NH_4Cl (5 equiv), MeOH/H_2O = 8:1, reflux overnight (90%); (c) $NaIO_4$ (1.1 equiv) 0 °C, 5 min; (d) DHAP (0.5 equiv)/FDP aldolase, 2 days; (e) acid phosphatase, pH 4.7, 37 °C, 36 h (78% yield from **18**; (f) H_2/Pd 50 psi, 1 day (97%).

Synthesis of 1 via FDP Aldolase

As shown in Scheme VIII, 2-azido-3-hydroxypropanal (**21**) was generated in situ with periodate cleavage of 2-azido-2-deoxythreitol (**20**), which was prepared from nucleophilic ring opening of *cis*-2,3-epoxy-1,4-butanediol (**19**) with sodium azide (90%) (Scheme VIII). After removing

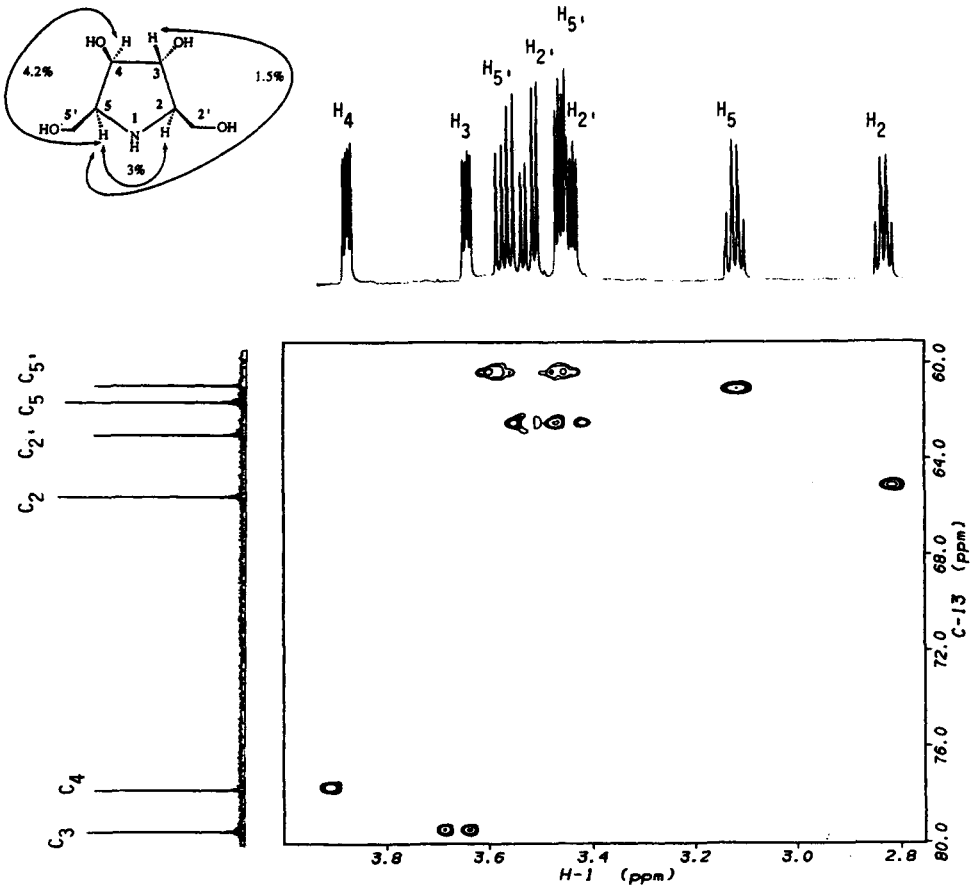


Figure 2. ^1H–^{13}C heterocorrelation 2D spectrum of 1. One-dimensional ^1H (top) and ^{13}C NMR (left) spectra are included. Insert is NOE.

the barium salt of periodate, the solution was adjusted to pH 7, then DHAP (0.5 equiv) and FDP aldolase (from rabbit muscle, 500 U) were added to the solution, and the mixture was slowly stirred for 2 days. The product was recovered and subjected to enzymatic hydrolysis of the phosphate moiety with acid phosphatase, as described previously,[4] to yield 5-azido-5-deoxy-L-*xylo*-hexulose (**22b**) (78% yield, based on DHAP); $[\alpha]^{23}_D$ −54.12° (*c* 3.64, MeOH). Compound **22b** was reductively aminated under hydrogen to furnish **1** as a viscid liquid; $[\alpha]^{23}_D$ +12.75° (*c* 4, H_2O) (97%). Direct hydrogenolysis of the phosphate product (**22a**), however, gave the 2(*R*)-methyl derivative **2** in 78% yield.

The stereochemistry of the C-2 center of compound **1** was determined to have a cis relation to H-5 based on a stronger NOE effect between H-2 and H-5 than H-3 and H-5 (Figure 2). A complete assignment of **1** was established on the basis of selective proton decoupling, NOE and coupling constants, and the ^1H–^{13}C heterocorrelation 2D spectrum as indicated in Figure 2. Compound **1** was then evaluated as an inhibitor of several glycosidases. The results (see Figure 3 and Table I) indicate that it is a potent inhibitor of α-glucosidase and β-glucosidase with K_i in the range of 10^{-6} M. It also inhibits α-galactosidase and α-mannosidase with K_i of 5×10^{-5} M and 3.1×10^{-3} M, respectively. This broad spectrum of inhibition is similar to that observed for a glucose-based amidinium ion, which inhibits β-glucosidase, β-galactosidase, and α-mannosidase at 8–10 μM,[9] and can be explained on the basis of the active-site model,[4f] which indicates that compounds mimicking the flattened-chair transition state of glycosidic cleavage and with a positive charge character will have a broad spectrum of inhibition. We propose that compound **1** may be protonated and possesses an envelope conformation for binding to the active site (Figure 4). With this new inhibitor, a new empirical formula relating the overall dissociation energies to inhibition constants is developed on the basis of the method described previously[4f] (Figure 5). This formula may be useful for the prediction of inhibition constants for new inhibitors.

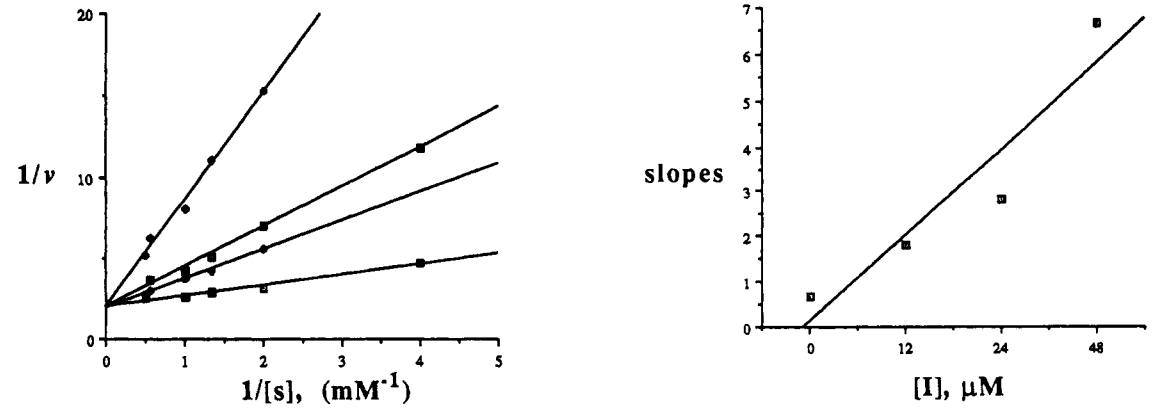
It is worth noting that the hydrogenolysis reaction is stereoselective and follows the same pattern as observed previously,[4c,f,7] i.e., it favors the product with a trans relation between C_2 and C_3. The major advantages illustrated in the synthesis of **1** are that the preparation of racemic azidoaldehyde **21** is very straightforward (no column chromatography or distillation is required) and that the enzymatic reaction is enantio- and diastereose-

Table I. Inhibition of Glycosidases (see Figure 4)

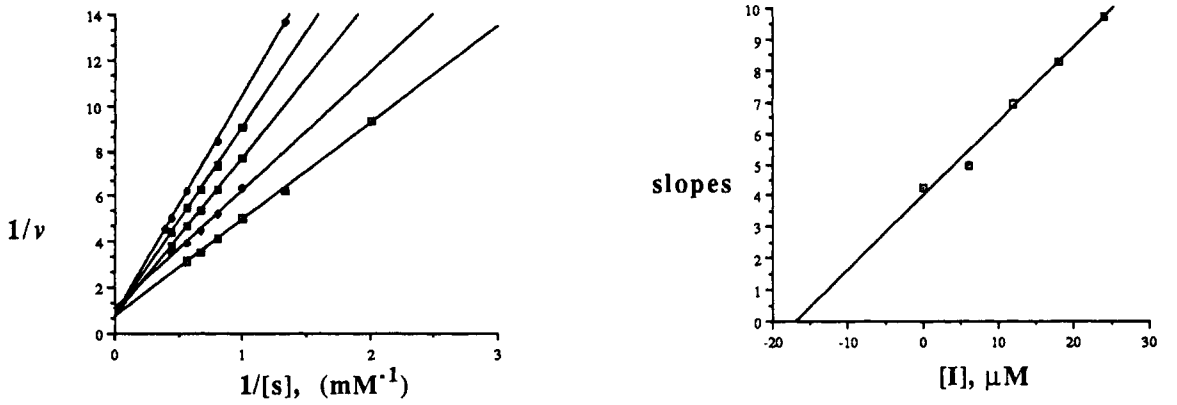
enzyme	K_i (M),[a] A	K_i (M), B	K_i (M),[b] C
α-glucosidase (Brewer's yeast)	ND[c]	2.8×10^{-6}	3.3×10^{-6}
β-glucosidase (almond)	$(8 \pm 5) \times 10^{-6}$	1.9×10^{-5}	7.8×10^{-6}
α-galactosidase (green coffee bean)	$(8 \pm 5) \times 10^{-6}$	5.0×10^{-5}	no inhibition
β-galactosidase (*E. coli*)	ND	no inhibition at 1 mM	no inhibition
α-mannosidase (Jake bean)	9×10^{-6}	3.1×10^{-3}	no inhibition
β-xylosidase (*A. niger*)	ND	ND	2.5×10^{-4}

[a] Tong, M. K.; Papandreon, G.; Ganem, B. *J. Am. Chem. Soc.* **1990**, *112*, 6137. [b] Fleet, G. W. J.; Nicholas, S. J.; Smith, P. W.; Evans, S. V.; Fellows, L. E.; Nash, R. J. *Tetrahedron Lett.* **1985**, *26*, 3127. [c] Not determined.

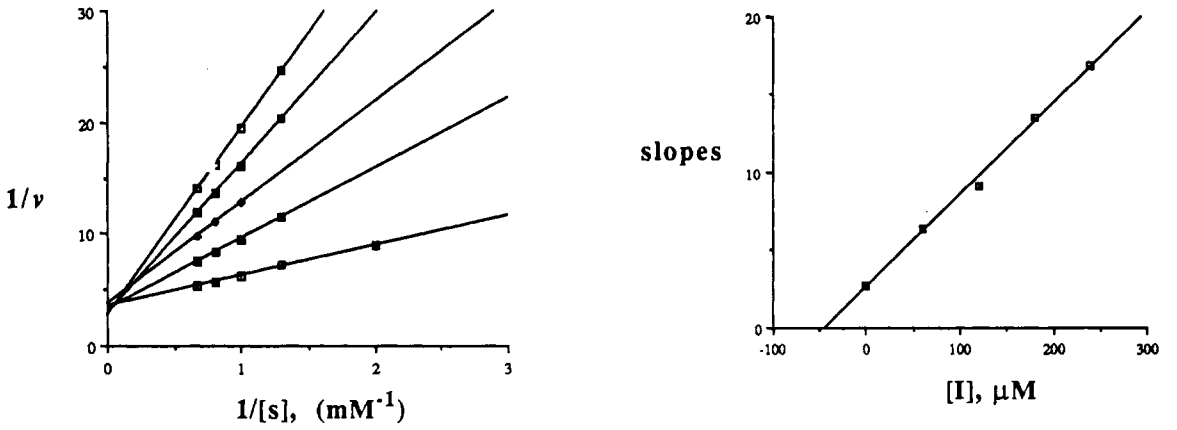
(9) Tong, M. K.; Papandreou, G.; Ganem, B. *J. Am. Chem. Soc.* **1990**, *112*, 6137.



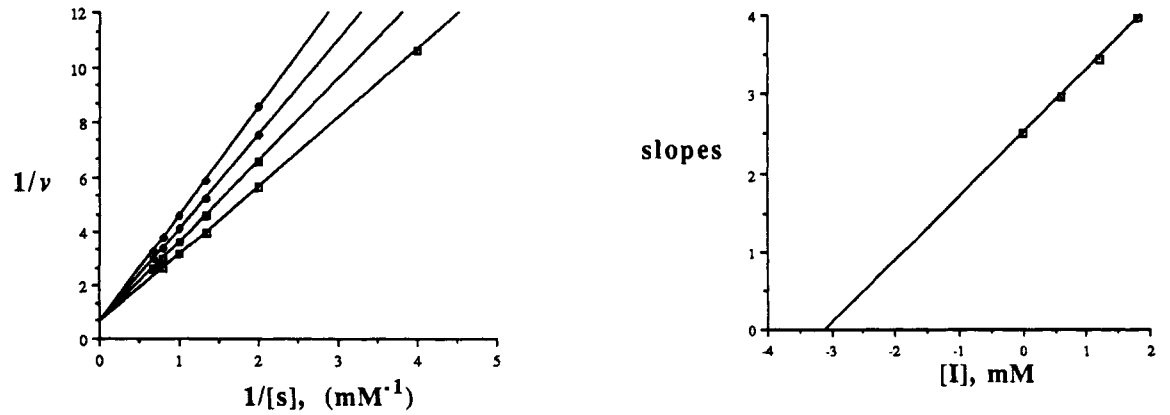
Inhibition of α-Glucosidase (Brewers Yeast)



Inhibition of β-Glucosidase (Almond)



Inhibition of α-Galactosidase (Green Coffee Beans)



Inhibition of α-Mannosidase (Jack Beans)

Figure 3. Lineweaver–Burk plot for the determination of inhibition constant (K_i).

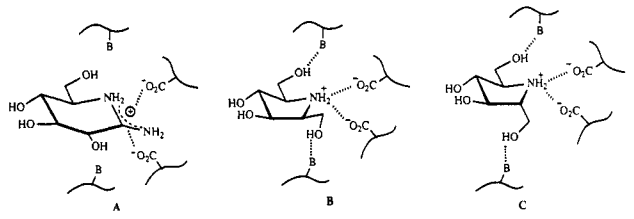


Figure 4. Proposed mode of binding of 1 to the active site as compared to the binding of the glucose-based amidinium ion.

lective. The overall yield is >60% based on 18. The phosphate group makes isolation of the aldolase product very straightforward, and hydrogenolysis of the azide-containing sugar phosphate provides a new route to dideoxy azasugars.

Discussion

In summary, this study demonstrates that azidoketoses prepared from aldolase reactions can be converted to six-membered-ring azasugars via a diastereoselective Pd-mediated reductive amination. It appears to be general that this combined chemical and enzymatic approach is a very effective way for the construction of piperidines and pyrrolidines structurally related to many natural and unnatural monosaccharides. It is interesting that in a kinetic process, the diastereoselectivity of these aldol reactions is completely controlled by the enzyme, not by the chiral aldehyde substrate. For example (Figure 6), the Cram–Felkin mode of attack[10] on α-hydroxy aldehydes was observed in FDP aldolase and Rham-1-P aldolase reactions with good substrates (i.e. D aldehydes for FDP aldolase and L aldehydes for Rham-1-P aldolase), and the anti-Cram–Felkin mode of attack was observed in reactions with the corresponding enantiomeric aldehydes that are weak substrates. On the contrary, Fuc-1-P aldolase reactions follow the anti-Cram–Felkin mode for good aldehyde substrates (i.e. the L isomers) and Cram–Felkin mode for weak substrates (the D isomers). Similarly, the Cram–Felkin mode was observed in 2-deoxyribose-5-phosphate aldolase catalyzed reactions.[7] The only exception is the sialic acid aldolase catalyzed reactions with D- and L-mannose and their 6-deoxy analogues where the stereoselectivity is completely controlled by the substrates, i.e., the anti-Cram–Felkin mode was observed with both enantiomeric aldehydes.[11]

In a thermodynamic process of FDP aldolase catalyzed reaction with an α-substituted β-hydroxy aldehyde, the aldol product tends to form a six-membered-ring hemiketal. In such as case, the L aldehyde is selectively transformed to the product as the α-substituent and will be in the relatively more stable equatorial positoins; this is also the case in the reaction with a β-substituted β-hydroxy aldehyde (e.g. β-hydroxybutanal)[12] (Figure 7).

Experimental Section

Syntheses of Fuculose 1-Phosphate (4a) and Rhamnulose 1-Phosphate (5a) and the Corresponding Phosphate-Free Ketoses 4b and 5b. To an aqueous solution (100 mL of L-lactaldehyde (0.79 mmol), prepared from D-threonine by reaction with ninhydrin[13] or via lipase reaction (Scheme VIII), were added DHAP (0.79 mmol) and Tris buffer (675 mM, KCl 750 mM, pH 7.5, 10 mL), and the pH was adjusted to 7.5 with 1 N NaOH. To this solution was added *E. coli* (K-40 or K-58, 0.2 g)[14] treated with lysozyme (from egg white, 3 mg) in Tris buffer (45 mM, KCl 50 mM, pH 7.5, 10 mL) for 1 h at 35 °C, and the mixture was stirred slowly until 90% of the DHAP was consumed. After the reaction, the solution was adjusted to pH 7.0, $BaCl_2 \cdot 2H_2O$ (600 mg, 2.5 mmol) was added, and the precipitate was removed by centrifugation. Acetone (twice the volume) was added to the supernatant. The mixture was kept in a refrigerator for 2 h, and the precipitate newly appeared was collected. To remove the barium ion, Dowex 50 (H^+) was added with stirring followed by filtration. Neutralization and lyopholization gave a ketose 1-phosphate sodium salt (ca. 180 mg). For the cleavage of the phosphate group, the filtrate was adjusted to pH 4.7 and mixed with acid phosphatase (from sweet potato, type V, 100 units) at 37 °C for 1 day. After being neutralized, the solution was lyophilized and the residue was purified by silica gel chromatography ($CHCl_3$/MeOH/H_2O = 7:3:0.3) to yield ketoses (ca. 40 mg, 30% overall).

L-Fuculofuranose 1-phosphate monosodium salt: ^{13}C NMR (D_2O) strong peaks at δ 14.6, 66.4 (d, J = 4.2 Hz), 72.3, 72.5, 76.3, 101.8 (d, J = 7.5 Hz), weak peaks at 14.2, 64.8 (d, J = 4.2 Hz), 74.3, 75.8, 78.5, 104.4 (d, J = 5.6); HRMS (M – H^+) calcd 266.0168, found 266.0172. Anal. Calcd for $C_6H_{12}O_7P$: C, 31.72; H, 5.29. Found: C, 31.70; H, 5.30.

L-Rhamnulofuranose 1-phosphate monosodium salt: ^{13}C NMR (D_2O) strong peaks at δ 19.2, 66.4 (d, J = 3.4 Hz), 76.3 (2 C), 79.4, 101.0 (d, J = 7.3 Hz), weak peaks at 18.1, 65.1 (d, J = 4.7 Hz), 78.0, 81.1, 82.6, 104.5 (d, J = 7.2); HRMS (M – H^+) calcd 266.0168, found 266,1076. Anal. Calcd for $C_6H_{12}O_7P$: C, 31.72; H, 5.29. Found: C, 31.74; H, 5.31.

L-Fuculofuranose (4b): $[\alpha]^{23}_D$ = 0° (*c* 1.9, H_2O); ^1H NMR (D_2O) strong peaks at δ 1.13 (3 H, d, J = 6.1 Hz, H-6), 3.36, 3.43 (each 1 H, d, J = 12.0 Hz, H-1), 3.97 (1 H, qd, J = 6.1, 3.4 Hz, H-5), 3.98 (1 H, dd, J = 4.5, 3.4 Hz, H-4), 4.10 (1 H, d, J = 4.5 Hz, H-3), weak peaks at 1.07 (3 H, d, J = 6.5 Hz, H-6), 3.42, 3.55 (each 1 H, d, J = 11.8 Hz, H-1), 3.98 (1 H, dd, J = 5.0, 3.5 Hz, H-4), 4.15 (1 H, d, J = 5.0 Hz, H-3), 4.20 (1 H, qd, J = 6.5, 3.5 Hz, H-5) [The ratio of strong to weak peaks was 2:1.]; ^{13}C NMR (D_2O) strong peaks at δ 15.0, 63.0, 71.5, 73.0, 76.9, 102.7, weak peaks at δ 14.0, 62.8, 73.3, 75.9, 79.0, 105.0; HRMS (M + Na^+) calcd 187.0582, found 187.0583. Anal. Calcd for $C_6H_{12}O_5$: C, 43.90; H, 7.32. Found: C, 43.91; H, 7.31.

L-Rhamnulofuranose (5b): $[\alpha]_D$ +9.4° (*c* 1.4, H_2O); ^1H NMR (D_2O) strong peaks at δ 1.17 (3 H, d, J = 6.2 Hz, H-6), 3.34, 3.42 (each 1 H, d, J = 12.0 Hz, H-1), 3.65 (1 H, qd, J = 6.2, 8.0 Hz, H-5), 3.74 (1 H, t, J = 8.0 Hz, H-4), 3.92 (1 H, d, J = 8.0 Hz, H-3), weak peaks at 1.15 (3 H, d, J = 6.8 Hz, H-6), 3.43, 3.49 (each 1 H, d, J = 12.0 Hz, H-1), 3.57 (1 H, dd, J = 6.0, 7.5 Hz, H-4), 3.90 (1 H, qd, J = 6.8, 7.5 Hz, H-5), 3.91 (1 H, d, J = 6.0 Hz, H-3) [The ratio of strong to weak peaks was 3:1.]; ^{13}C NMR (D_2O) strong peaks at δ 20.0, 63.8, 76.1, 77.4, 80.5, 102.1, weak peaks at 18.4, 63.9, 76.1, 82.3, 83.6, 104.8; HRMS (M + Cs^+) calcd 296.9739, found 296.9742. Anal. Calcd for $C_6H_{12}O_5$: C, 43.90; H, 7.32. Found: C, 43.88; H, 7.30.

Synthesis of 1-Deoxyazasugars 7, 10, and 14. To an aqueous solution of (*RS*)- or (*R*)-3-azido-2-hydroxypropanal, prepared by heating a suspension of 3-azido-2-hydroxypropanal diethyl acetal (1.1 g, 5.8 mmol)[4c] in pH 1.0 buffer (40 mL) at 45 °C for 12 h, were added DHAP (1.9 mmol) and Tris buffer (675 mM, KCl 750

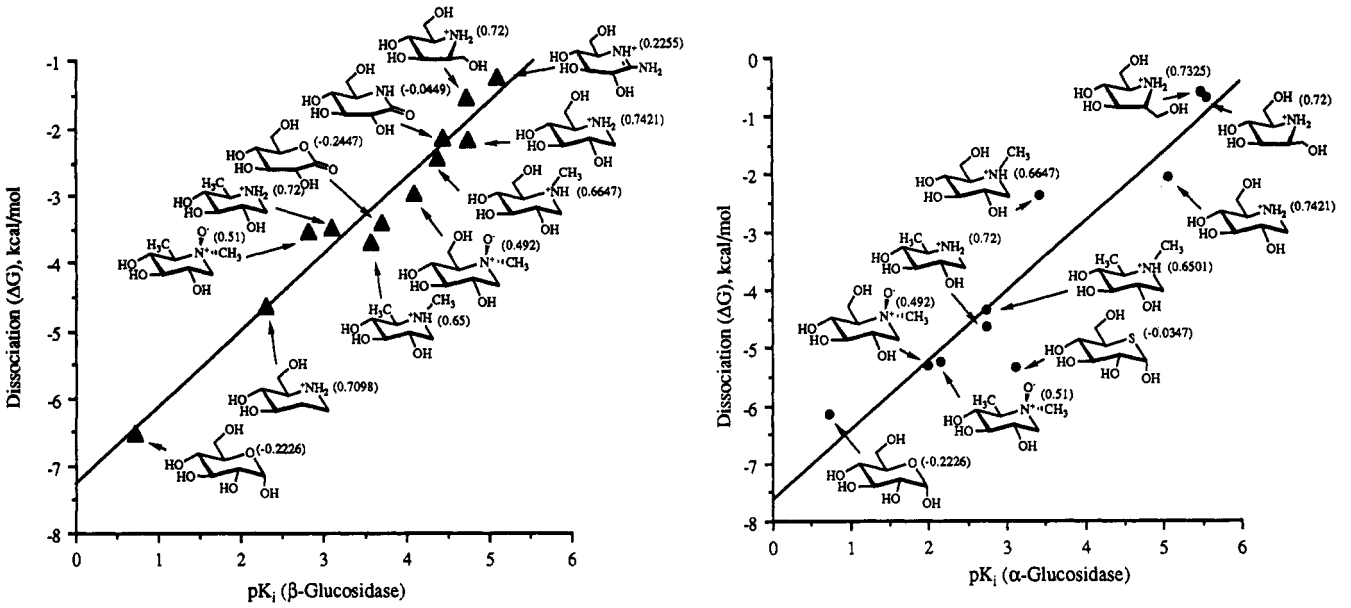
(10) Cram, D. J.; Abd Elhafez, F. A. *J. Am. Chem. Soc.* **1952**, *74*, 5828. Cherest, M.; Felkin, H.; Prudent, N. *Tetrahedron Lett.* **1968**, 2199. Anh, N. T. *Top. Curr. Chem.* **1980**, *88*, 145. For further investigations, see: Reetz, M. T. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 556. Lodge, E. P.; Heathcock, C. H. *J. Am. Chem. Soc.* **1987**, *109*, 3353. Bartlett, P. A. *Tetrahedron* **1980**, *45*, 2. Morrison, J. D.; Mosher, H. S. *Asymmetric Organic Reactions*; Prentice-Hall: Englewood Cliffs, NH, 1971; p 84. Eliel, E. L. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic: New York, 1983; Vol. 2, p 125.

(11) Gautheron-Le Narvor, C.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* In press.

(12) See ref 5b and Durrwachter, J. R.; Wong, C.-H. *J. Org. Chem.* **1988**, *53*, 4175. Liu, K. K.-C.; Pederson, R. L.; Wong, C.-H. *J. Chem. Soc., Perkin Trans. 1.* In press.

(13) Wong, C.-H.; Mazenod, F. P.; Whitesides, G. M. *J. Org. Chem.* **1983**, *48*, 3493.

(14) In this study, *E. coli* K40 was used as a source for Rham-1-P aldolase and *E. coli* K58 was used as a source for Fuc-1-P aldolase. The cells were treated with lysozyme (to release the enzyme) and used directed without further purification. For procedure, see: (a) Drueckhammer, D. G.; Durrwachter, J. R.; Pederson, R. L.; Crans, D. C.; Daniels, L.; Wong, C.-H. *J. Org. Chem.* **1989**, *54*, 70. (b) Fuc-1-P aldolase from *E. coli* has recently been cloned, overexpressed, and used in synthesis (Ozaki, A.; Toone, E. J.; von der Osten, C. H.; Sinskey, A. J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1990**, *112*, 4970). (c) For cloning and synthetic application of Fuc-1-P and Rham-1-P aldolases from *E. coli*, see: Feesner, W.-D.; Sinerious, G. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 555.



$\Delta G = \Delta G(\text{conformation}) + \Delta G(2\text{-OH}_{eq}) + \Delta G(6\text{-OH}_{eq}) + \Delta G(\text{charge})$
$= 2.596\ F(\text{conformation}) + 2.372\ F(2\text{-OH}) + 1.236\ F(6\text{-OH}) + 3.242\ F\ (\text{charge}) - 8.181$ for β-glucosidase and
$= 2.055\ F(\text{conformation}) + 0.766\ F(2\text{-OH}) + 2.219\ F(6\text{-OH}) + 4.252\ F\ (\text{charge}) - 8.181$ for α-glucosidase

where

F(conformation) = 1 if the inhibitor is in half-chair or envelope conformation and 0 if in chair conformation.

F(2-OH) = 1 for the presence of 2-OH$_{eq}$ and 0 for the lack of 2-OH$_{eq}$;
= 0.2 for the 2-CH$_2$OH group of the 5-membered-ring compounds.

F(6-OH) = 1 if 6-OH is free to be a H-bond donor (i.e. free of intramolecular H-bonding).
= 0.5 for those with 6-OH in weak intramolecular H-bond or for N-oxides which may have eletrostatic interaction with the COOH from the enzyme
= 0 for those without 6-OH or with strong intramolecular H-bond (Glc);

F(charge) = partial charge distribution of the ring heteroatom (in parenthesis) calculated by PM3. The values were taken as calculated except for the N-oxide compounds. In those cases, 50% of the calculated values were used due to possible shielding from the negatively charged oxygen atoms.

Figure 5. Linear relation of inhibition constants and inhibitor binding.

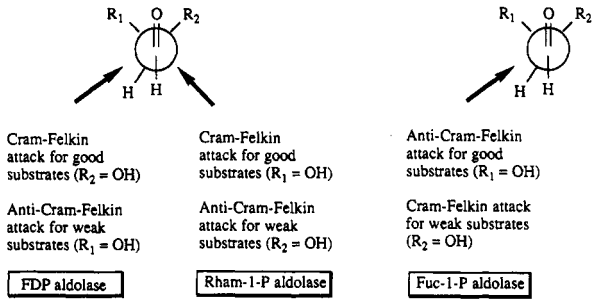


Figure 6. Kinetically controlled enzyme-catalyzed aldol reactions.

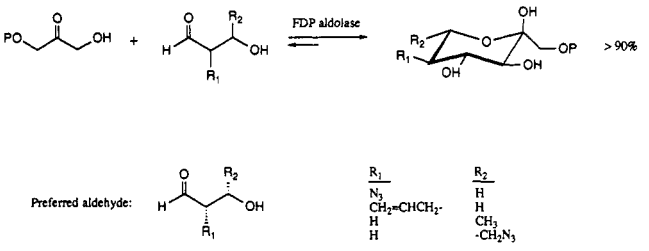


Figure 7. Thermodynamically controlled FDP aldolase catalyzed reactions.

mM, pH = 7.5, 5.0 mL), and the pH was adjusted to 7.5 with 1 N NaOH. To this solution was added the aldolase from *E. coli* (1 g) treated with lysozyme (from egg white, 10 mg) in Tris buffer (45 mM, KCl 50 mM, pH = 7.5, 20 mL) for 1 h at 35 °C, and the mixture was stirred slowly until 90% of DHAP was consumed.

After the reaction, the solution was adjusted to pH 7.0, Ba$Cl_2 \cdot 2H_2O$ (950 mg, 3.9 mmol) was added, and the precipitate was removed by centrifugation. Acetone (twice the volume) was added to the supernatant. To remove the barium ion, Dowex 50 (H^+) was added with stirring followed by filtration. The filtration was adjusted to pH 4.1, acid phosphatase (from sweet potato, type V, 600 U) was added, and the solution was stirred at 37 °C for 1 day. After being neutralized, the solution was lyophilized and the residue was purified by silica gel chromatography ($CHCl_3$/MeOH/H_2O = 8:2:0.1) to yield an azidoketose.

To a solution of this azidoketose in ethanol (30 mL) was added Pd–C (20 mg), and the mixture was hydrogenated under the pressure of 50 psi. After 1 day, the catalyst was filtered off and the filtrate was concentrated. The residue was purified by silica gel chromatography ($CHCl_3$/MeOH/H_2O = 6:4:1 ~ 5:5:2) to yield an azasugar.

Compound 14: prepared via **13b** (^{13}C NMR (CD_3OD) δ 53.3, 65.2, 71.8, 72.8, 82.0, 104.8)); $[\alpha]^{23}_D$ = −22.4° (*c* 1.6, MeOH); yield 20% (based on DHAP); ^{13}C NMR (D_2O) δ 49.3, 59.6, 61.4, 69.2, 69.4, 69.7; ^{1}H NMR (HCl salt, D_2O) δ 3.07 (1 H, dd, *J* = 1.76, 13.79 Hz, H-1a), 3.21 (1 H, bt, *J* = 1.40, 7.08 Hz, H-5), 3.32 (1 H, dd, *J* = 2.83, 13.8 Hz, H-1e), 3.66 (1 H, t, *J* = 3.32 Hz, H-3), 3.68 (2 H, d, *J* = 6.93 Hz, H-6), 3.96 (1 H, dt, *J* = 1.62, 3.23 Hz, H-4), 4.04 (1 H, ddt, *J* = 1.79, 3.07, 4.59 Hz, H-2); HRMS (M + H^+) calcd 164.0923, found 164.0923. Anal. Calcd for $C_6H_{13}O_4N$: C, 44.17; H, 7.98; N, 8.59. Found: C, 44.14; H, 7.95; N, 8.60.

Compound 7: prepared via **6b** (^{13}C NMR (CD_3OD) δ 54.6, 64.2, 76.8, 77.6, 81.1, 103.3)); $[\alpha]^{23}_D$ = +28.0° (*c* 1.0, MeOH); yield 40% (based on DHAP); ^{13}C NMR (D_2O) 48.6, 60.8, 60.9, 68.6, 69.5, 74.9; ^{1}H NMR δ 2.34 (1 H, dt, *J* = 9.5, 3.5 Hz, H-5), 2.62 (1 H, d, *J* = 14.2 Hz, H-1a), 2.86 (1 H, dd, *J* = 14.2, 2.4 Hz, H-1e), 3.41 (1 H, dd, *J* = 9.5, 3.0 Hz, H-3), 3.46 (1 H, t, *J* = 9.5 Hz, H-4), 3.62 (2 H, d, *J* = 3.5 Hz, H-6), 3.85 (1 H, bs, H-2); HRMS (M + H^+) calcd 164.0923, found 164.0926.

Compound 10: prepared via **9b** (^{13}C NMR (CD_3OD) δ 52.7, 66.7, 71.9, 72.8, 80.1, 104.4)); $[\alpha]^{23}_D$ = +31.8° (*c* 1.3, MeOH) (lit.[3w] $[\alpha]_D^{20}$ +54° (*c* 0.9, H_2O)); yield 20% (based on DHAP); ^{13}C NMR (D_2O) δ 49.1, 59.3, 61.5, 68.2, 69.4, 75.2; ^{1}H NMR (D_2O) δ 2.28 (1 H, t, *J* = 12.3 Hz, H-1a), 2.66 (1 H, td, *J* = 6.5, 1.6 Hz, H-5), 3.01 (1 H, dd, *J* = 12.3, 5.3 Hz, H-1e), 3.34 (1 H, dd, *J* = 9.7, 3.0 Hz,

H-3), 3.50 (2 H, m, H-6), 3.62 (1 H, ddd, J = 12.3, 9.7, 5.3 Hz, H-2), 3.87 (1 H, dd, J = 3.0, 1.6 Hz, H-4); HRMS (M + H^+) calcd 164.0923, found 164,0923.

Syntheses of 1,6-Dideoxyazasugars. Direct hydrogenation of ketose phosphate generates 1,6-dideoxyazasugars, which were purified by silica gel chromatography ($CHCl_3$/MeOH/H_2O = 6:4:0.8) from the residue. In the case of **11** the C-5 epimer was also generated with a ratio of 1:1 based on the shifts of methyl groups (δ 1.3, d).

8: ^1H NMR (D_2O) δ 1.00 (3 H, d, J = 6.5 Hz, 5-CH_3), 2.30 (1 H, m, H-5), 2.56 (1 H, d, J = 14.4 Hz, H-1a), 2.78 (1 H, dd, J = 14.4, 2.3 Hz, H-1e), 3.14 (1 H, t, J = 9.9 Hz, H-4), 3.35 (1 H, dd, J = 9.9, 2.9 Hz, H-3), 3.82 (1 H, bs, H-2); ^{13}C NMR (D_2O) δ 17.4, 48.6, 55.6, 69.8, 74.3, 74.5; HRMS (M + Cs^+) calcd 279.9950, found 279.9950.

11: $[\alpha]^{23}_D$ = +18.2° (c 1.1, MeOH); ^1H NMR (D_2O) δ 1.20 (3 H, d, J = 6.7 Hz, 5-CH_3), 2.71 (1 H, t, J = 12.0 Hz, H-1a), 3.30 (1 H, qd, J = 6.7, 1.5 Hz, H-5), 3.31 (1 H, dd, J = 12.0, 5.5 Hz, H-1e), 3.50 (1 H, dd, J = 9.7, 3.0 Hz, H-3), 3.87 (1 H, dd, J = 3.0, 1.5 Hz, H-4), 3.90 (1 H, ddd, J = 11.5, 9.5, 5.5 Hz, H-2); ^{13}C NMR (D_2O) δ 14.4, 46.5, 55.3, 64.8, 70.3, 73.5; HRMS (M + H^+) calcd 148.0974, found 148.0974.

Synthesis of (*S*)-Lactaldehyde Diethyl Acetal (Scheme VII). Method A. (*S*)-Glycidaldehyde diethyl acetal (34.1 mmol, >95% ee)[15] was reacted with lithium aluminum hydride (31.3 mmol) in ether according to the procedure described previously[16] to give to (*S*)-lactaldehyde diethyl acetal (30.2 mmol, 89% yield) in >95% ee: $[\alpha]^{25}_D$ = −8.7° (c 3.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 1.20 (d, J = 6.3 Hz, CH_3CH), 1.23 (t, J = 7.0 Hz, 3 H, CH_3CH_2), 1.25 (t, J = 7.0 Hz, 3 H, CH_3CH_2), 2.26 (br s, 1 H, OH), 3.58 (dq, J = 9.4, 7.0 Hz, 1 H, OCH_2), 3.59 (dq, J = 9.4, 7.0 Hz, 1 H, OCH_2), 3.73 (app quint, J = 6.3 Hz, 1 H, CHOH), 3.79 (dq, J = 9.4, 7.1 Hz, 1 H, OCH_2), 3.79 (dq, J = 9.4, 7.1 Hz, 1 H, OCH_2), 4.16 (J = 6.2 Hz, 1 H, $CH(OEt)_2$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 15.41 (2 CH_3CH_2), 17.44 (CH_3CH), 63.46, 63.57 (OCH_2), 68.02 (CHOH), 106.09 $CH(OEt)_2$; HRMS (M + Na^+) calcd 171.0997, found 171.0982. Anal. Calcd for $C_7H_{16}O_3$: C, 56.76; H, 10.81. Found: C, 56.77; H, 10.80.

Method B. Racemic lactaldehyde diethyl acetal prepared from racemic glycidaldehyde diethyl acetyl was acetylated with acetic anhydride in pyridine and the product (17.8 mmol) was subjected to immobilized LP-80 lipase catalyzed resolution (Scheme VII)[15] at pH 7.0. The reaction was stopped at 50% conversion, and the products were purified by flash column chromatography (1:11 → 1:3 EtOAc/hexane) to produce (*R*)-lactaldehyde diethyl acetal (1.03 g, 7 mmol, 40% yield, 95% ee) and (*S*)-lactaldehyde diethyl acetal *O*-acetate (1.51 g, 8 mmol, 45% yield, $[\alpha]^{25}_D$ = −25.9° (c 1.62, $CHCl_3$), 98% ee), which can be deprotected to (*S*)-lactaldehyde as described previously[4c,15] and used in situ in enzymatic aldol condensation.

Method C. Treatment of (*R*)-3-chloro-2-acetoxypropanal diethyl acetal[15] (1.003 g, 4.4 mmol) with $LiAlH_4$ (0.3 g, 7.52 mmol) in ether (30 mL) at 0 °C under N_2 gave (*S*)-lactaldehyde diethyl acetal (0.43 g, 66% yield, >95% ee) after quenching and workup. The enantiomeric purity of each isomer was determined on the basis of their (+)-MTPA ester.[17] The shifts at 4.48 and 4.40 for the *R* and *S* enantiomers, respectively, were used for the determination.

***cis*-2,3-Epoxy-1,4-butanediol (19).** Compound **19** was prepared according to the reported procedure[18] except that the reaction was carried out at room temperature for 36 h.

2-Azido-2-deoxythreitol (20). A solution containing **19** (1.82 g, 17.50 mmol), NaN_3 (5.68 g, 5 equiv), and NH_4Cl (4.68 g, 5 equiv) in 100 mL of MeOH and 12 mL of H_2O was refluxed for 24 h. The solvent was removed under reduced pressure; then EtOH was added and the precipitate was filtered off. The procedure was repeated several times to remove excess NaN_3 and NH_4Cl to obtain the title compound as a yellow liquid (90%): R_f = 0.28 (EtOAc 100%); IR (neat) 2109 cm^{-1} (N_3); ^1H NMR (CD_3COCD_3) δ 3.49 (1 H, m) 3.59 (3 H, m), 3.79 (5 H, m), 4.03 (1 H, t, J = 5.5 Hz), 4.19 (1 H, d, J = 5.5 Hz), 4.30 (1 H, t, J = 5.5 Hz); HRMS (M + H^+) calcd 148.0722, found 148.072. Anal. Calcd for $C_4H_9O_3N_3$: C, 32.65; H, 6.12; N, 32.65. Found: C, 32.66; H, 6.11; N, 32.66.

5-Azido-5-deoxy-L-*xylo*-hexulose (22b). A solution containing the compound prepared above (476 mg, 3.24 mmol) in 10 mL of H_2O was cooled to 0 °C and $NaIO_4$ (762 mg, 1.1 equiv) was added. After 10 min, the starting material disappeared completely and a new spot appeared according to TLC (R_f = 0.5, EtOAc). $BaCl_2 \cdot 2H_2O$ (870 mg, 1.1 equiv) was then added to the solution and the precipitate was filtered off. The solution was acidified to pH 1 with Dowex 50 (H^+). After filtration, the solution was adjusted to pH 7 with NaOH (10 N). DHAP (1.5 mmol) was then added and the solution was readjusted to pH 7 again with 10 N NaOH. To the solution, rabbit muscle FDP aldolase (500 U) was added and the solution was stirred slowly for 2 days. Enzymatic assay indicated that all of the DHAP had been consumed. The pH of the solution was adjusted to 4.7 with 2 N HCl. Acid phosphatase (400 U) was added and the mixture was incubated at 37 °C for 36 h. The solution was adjusted to pH 7 and lyophilized. The residue was treated with MeOH and filtered to remove the insoluble material; then MeOH was removed under reduced pressure. The crude product was purified by silica gel column chromatography ($CHCl_3$/MeOH = 6:1) to yield the title compound as the only product (240 mg, 78%, R_f = 0.45 ($CHCl_3$/MeOH = 4:1): $[\alpha]^{23}_D$ −54.12° (c 3.64, MeOH); ^1H NMR (D_2O) δ 3.32 (1 H, d, $J_{1,1'}$ = 11.5 Hz, H-1), 3.37 (1 H, d, $J_{3,4}$ = 9.5 Hz, H-3), 3.38 (1 H, ddd, $J_{4,5}$ = 9.5 Hz, $J_{5,6e}$ = 5 Hz, $J_{5,6a}$ = 11 Hz, H-5), 3.45 (1 H, t, $J_{6a,5} = J_{6a,6e}$ = 11 Hz, H-6a), 3.52 (1 H, d, $J_{1,1'}$ = 11.5 Hz, H-1), 3.57 (1 H, t, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 3.64 (1 H, dd, $J_{6e,6a}$ = 11 Hz, $J_{6e,5}$ = 5 Hz, H-6e); ^{13}C NMR (D_2O) δ 69.1, 71.6, 73.3, 80.3, 82.5, 107.6; HRMS (M + H^+) calcd 337.9753, found 337.9778. Anal. Calcd for $C_6H_{11}O_5N_3$: C, 35.12; H, 5.37; N, 39.02. Found: C, 35.11; H, 5.38; N, 39.03.

2(*R*),5(*S*)-Bis(hydroxymethyl)-3(*R*),4(*R*)-dihydroxypyrrolidine (1). A solution containing the compound prepared above (70 mg, 0.34 mmol) in 10 mL of H_2O was hydrogenated with 10 mg of 10% Pd/C under 50 psi of hydrogen for 1 day. The catalyst was removed by filtration and the filtrate was concentrated in vacuo and further purified with a BioGel P_2 column to yield compound **1** (50 mg, 90%): $[\alpha]^{23}_D$ +25.75° (c 4.00, H_2O); for NMR data, see Figure 2; HRMS (M + H^+) calcd 164.0923, found 164.0911. Anal. Calcd for $C_6H_{13}NO_4$: C, 44.17; H, 7.98; N, 8.59. Found: C, 44.19; H, 7.89; N, 8.60.

2(*R*)-Methyl-5(*S*)-(hydroxymethyl)-3(*R*),4(*R*)-dihydroxypyrrolidine (2). 5-Azido-5-deoxy-L-*xylo*-hexulose 1-phosphate was obtained by following the same aldol condensation procedure as described. The corresponding phosphate was isolated as barium salt by adding 2 equiv of $BaCl \cdot 2H_2O$ to the reaction mixture. The solution was maintained at −20 °C overnight. The precipitate was recovered and treated with Dowex 50 (H^+) in distilled water to remove barium cations. After filtration, the solution was adjusted to pH 7 and lyophilized to obtain **22a** (75%): ^1H NMR (D_2O) δ 3.13 (1 H, d, J = 9.5 Hz, H-3), 3.14 (1 H, ddd, J = 9.5, 5, 11 Hz, H-5), 3.20 (1 H, t, J = 11 Hz, H-6a), 3.31 (1 H, t, J = 9.5 Hz, H-4), 3.37 (1 H, dd, J = 6, 11 Hz, H-6e), 3.40–3.44 (2 H, m, 2 × H-1); ^{13}C NMR (D_2O) δ 61.78, 63.36, 67.35, 70.95, 97.67 (d, J = 9.5 Hz); HRMS (M − 4H^+ + 5Na^+) calcd 395.9540, found 395.9538.

A solution of **22a** (100 mg, 0.35 mmol) in 5 mL of water was hydrogenated with 20 mg of 10% Pd/C under 40 psi of hydrogen for 1 day. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The residue was chromatographed on a silica gel column (MeOH/$CHCl_3$/H_2O = 6:4:2) to yield the title compound (40 mg, 78% yield, 2*R*:2*S* ≈ 6:1): ^1H NMR (D_2O) δ 1.31 (3 H, d, J = 7 Hz, 2*R* CH_3), 1.27 (3 H, d, J = 6.5 Hz, 2*S* CH_3), 3.36 (1 H, m, H-2), 3.66 (1 H, m, H-5), 3.74–3.81 (2 H, m, 2 × H-5), 3.85 (1 H, m, H-3), 4.08 (1 H, dd, J = 2.5, 4.5 Hz, H-4); ^{13}C NMR (D2O) δ 16.58 (C-2′), 57.90 (C-5′), 61.50, 63.44, 75.62, 87.09; HRMS (M + H^+) calcd 148.0974, found 148.0974. Anal. Calcd for $C_6H_{13}NO_3$: C, 48.98; H, 8.84; N, 9.52. Found: C, 48.96; H, 8.85; N, 9.48.

Inhibition Study. Materials. All of the buffers, substrates and enzymes were purchased from Sigma and used directly. The

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following solutions were prepared for enzymatic assay: (1) PIPES–NaOAc buffer (0.01 M PIPES, 0.2 M NaOAc and 0.01 mM EDTA, pH 6.5). This buffer was prepared according to the literature procedure.[19] (2) α-D-Glucosidase: 1.5 mg of solid protein (70 U/mg) was dissolved in 1 mL of PIPES–NaOAc buffer solution and used for assay without dilution. (3) β-D-Glucosidase: the assay enzyme solution was prepared by dissolving 20 mg of solid protein (4.8 U/mg solid) in 6 mL of PIPES–NaOAc buffer solution. (4) α-D-Mannosidase: 5 mg of solid protein was suspended in 1 mL of 3 M $(NH_4)_2SO_4$ and 0.1 M ZnOAc. (5) α-D-Galactosidase: 5 U of α-galactosidase was dissolved in 2.2 mL of PIPES–NaOAc buffer solution. (6) β-D-Galactosidase: 0.5 mg of solid protein (345 U/mg) was dissolved in 1 mL of PIPES–NaOAc buffer solution.

General Procedure for Enzyme Assay. For each inhibitor, four or five inhibitor concentrations, ranging from 0 to 3 times K_i, were used to determine the K_i value. At each inhibitor concentration, five substrate concentrations were used to obtain a single Lineweaver–Burk plot (Figure 5). The amount of enzyme added in each assay was adjusted so that less than 10% of the substrate, with its lowest substrate concentration, would be consumed within 1 min. The assays were monitored at 400 nm for measuring the released *p*-nitrophenol group. The following example illustrates the procedure in detail.

To a 1-mL disposable cuvette was added 950 μL of NaOAc–PIPES buffer solution, 20 μL of inhibitor solution, and 20 μL of *p*-nitrophenol α-D-glucoside solution (25 mM in PIPES–NaOAc buffer, pH 6.5). The solution was well mixed and 20 μL of α-D-glucosidase solution was added to the cuvette to start the reaction. The reaction was monitored at 400 nm on a Beckman DU-70 spectrophotometer for 1 min and the initial hydrolysis rate was calculated. The same procedure was repeated with four other substrate concentrations. After all the initial rates were obtained, the corresponding Lineweaver–Burk plot at that inhibitor concentration was constructed.

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Supplementary Material Available: NMR spectra (^1H or ^{13}C) of **4a**, **4b**, **5a**, **5b**, **7**, **8**, **10**, **11**, and **17** (9 pages). Ordering information is given on any current masthead page.

C-Glycosides. 9. Stereospecific Synthesis of *C*-Glycosidic Spiroketal of the Papulacandins

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The reaction of ortho-lithiated triphenylmethyl benzyl ether with perbenzylated D-gluconolactone 1 followed by cyclization by $BF_3{\cdot}Et_2O$ provides a new stereospecific synthesis of *C*-glycosidic spiroketals. The structure of the peracetylated derivative was determined by X-ray diffraction. This methodology is applied to the synthesis of the spiroketal unit of papulacandins.

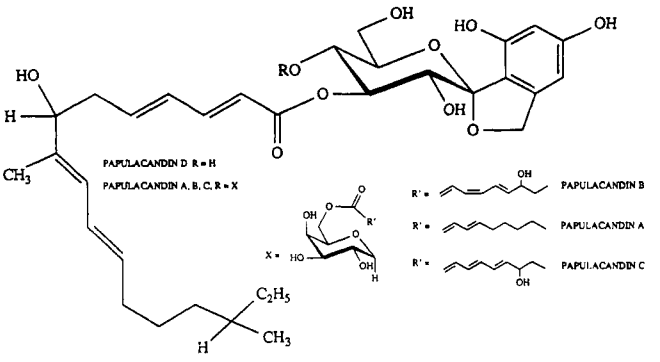
The papulacandins (A–D) are antifungal antibiotics that were isolated from a strain of *Papularia sphaerosperma*.[1] These four papulacandins are built on the same skeleton and differ only by substitution of one hydroxyl of papulacandin D by different groups (Chart I).

From a synthetic standpoint, the most interesting feature in these molecules represents the spiroketal unit involving a substituted aromatic ring β C–C linked to a glucopyranosyl moiety. Different approaches toward the synthesis of the spiroketal of papulacandin have been reported: lengthy partial synthesis of the racemic form[2] and multistep synthesis of the pure enantiomer from D-glucose.[3]

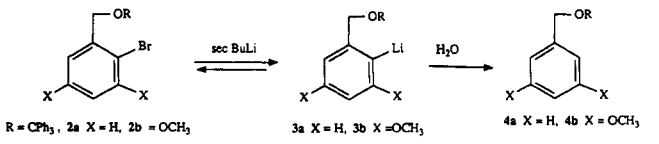
As a part of a continuing program of C–C bond formation at the anomeric center of a sugar moiety, we have initiated some studies in that area and preliminary results were published.[4]

In a previous work,[5] we demonstrated that aryl-β-D-*C*-glucosides could be prepared in a stereospecific manner by condensation of an aryllithium derivative with 2,3,4,6-tetra-*O*-benzyl-D-gluconolactone[6] (1) followed by reduction by triethylsilane in the presence of $BF_3{\cdot}Et_2O$. We also observed that, under these conditons, a 1,5-anhydro derivative was obtained from protected ribonolactone, indicating the participation of O-5 and the cleavage of *O*-trityl and *O*-silyl ethers.[7] So, we anticipated

Chart I



Scheme I



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