Carbohydrate Research, 239 (1993) 155–166
Elsevier Science Publishers B.V., Amsterdam

A chemoenzymic synthesis of 1,5-dideoxy-1,5-imino-L-mannitol and -L-rhamnitol and investigation of their effects on glycosidases

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(Received March 12th, 1992; accepted July 22nd, 1992)

ABSTRACT

1,5-Dideoxy-1,5-imino-L-rhamnitol (**3**) and 1,5-dideoxy-1,5-imino-L-mannitol (**2**), designed as inhibitors of α-L-rhamnosidase, have been synthesised from dihydroxyacetone phosphate and 3-azido-2-hydroxypropanal with the aid of partially purified *E. coli* L-rhamnulose 1-phosphate aldolase free of D-fructose 1,6-bisphosphate aldolase activity. Inhibitory effects of compounds **2**, **3**, 1,5-dideoxy-1,5-imino-D-mannitol, and 1,5-dideoxy-1,5-imino-D-glucitol (**12**) were tested on selected glycosidases. It has been found that compound **3** is a good inhibitor of α-L-rhamnosidase as well as α-L-fucosidases from bovine kidney and epididymis, **2** is a potent inhibitor of α-galactosidase and a moderate inhibitor of α-L-rhamnosidase, and **12** is a good inhibitor of α-galactosidase and α-L-rhamnosidase.

INTRODUCTION

L-Rhamnose (6-deoxy-L-mannose, **1**) is widely distributed in nature as a constituent of some glycosides, e.g., hesperidin and naringin, and of various polysaccharides in plant tissues and bacterial cell walls. α-L-Rhamnosidases from various sources are known to release L-rhamnose from glycosides. These enzymes have been widely employed in the food industry to debitter fruit juices[1] and for structure elucidation of complex molecules containing L-rhamnose[2]. It has been found that L-rhamnose and D-glucose are poor competitive inhibitors of α-L-rhamnosidase[3]. Azasugars are known to be a class of potent inhibitors of various glycosidases[4], but their effects on rhamnosidases have not been reported. Therefore, we undertook the synthesis of 1,5-dideoxy-1,5-imino-L-mannitol (**2**) and 1,5-dideoxy-1,5-imino-L-rhamnitol (**3**), two compounds structurally related to L-rhamnose, and investigated their effects on α-L-rhamnosidase and on other gly-

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cosidases. Compound **2** has been prepared from D-gulonolactone by Fleet et al.[5] but the effects of this compound on glycosidases have not been reported **.

Most synthetic sequences that have been developed for the preparation of azasugars have relied on the use of carbohydrates to supply the required chiral centers[8]. However, chemoenzymic syntheses based on the use of aldolases[9] require fewer synthetic steps, while allowing a compound with a particular stereochemistry to be generated by selection of the appropriate aldolase. In particular, the commercial D-fructose 1,6-bisphosphate aldolase (EC 4.1.2.13) has been intensively studied as a catalyst in carbohydrate synthesis[9]. Examination of the stereodirective properties of the four known hexose phosphate aldolases reveals that the configurations at C-3 and C-4 in the two target compounds **2** and **3** may be obtained by utilizing L-rhamnulose 1-phosphate aldolase[11] (EC 4.1.2.19) to condense dihydroxyacetone phosphate (**4**) and 3-azido-2-hydroxypropanal (**5**). In this paper we describe the purification of L-rhamnulose 1-phosphate aldolase, the synthesis of **2** and **3** utilizing this D-fructose 1,6-bisphosphate aldolase-free enzyme as a catalyst in the aldol addition step, and an investigation of the inhibitory effects of **2** and **3** on selected glycosidases.

RESULTS AND DISCUSSION

Purification of L-*rhamnulose 1-phosphate aldolase.*—Since a mixture of D-fructose 1,6-bisphosphate aldolase and L-rhamnulose 1-phosphate aldolase would generate enantiomeric products from compounds **4** and **5**[12], it was essential to remove any contaminating D-fructose 1,6-bisphosphate aldolase in order to get the desired isomers **6** or **7**. There are at least two D-fructose 1,6-bisphosphate aldolases in wild type *E. coli* strains[13,14], and cells grown on pyruvate or other carbon sources which can generate energy without D-fructose 1,6-bisphosphate aldolase activity still require this aldolase for the biosynthesis of pentoses. Even after considerable purification of L-rhamnulose 1-phosphate aldolase from *E. coli* strain K-40 grown on L-rhamnose as the sole carbon source, Chiu and Feingold[15] detected D-fructose 1,6-bisphosphate aldolase in enzyme preparations. We have also detected fructose 1,6-bisphosphate aldolase activity in the crude extracts of our *E. coli* strain similarly grown. We obtained complex reaction mixtures when these extracts were used for reaction of **4** and **5** (for syntheses see below). The activity of D-fructose 1,6-bisphosphate aldolase (0.32 units/mg protein) in the cell

** While our work was nearing completion, Wong and associates[6,7] independently reported the synthesis of **2** and **3** using a crude extract obtained from the cells of *E. coli* strain K-40. However D-fructose 1,6-bisphosphate aldolase should be present in the crude extract from this strain and therefore, it is unclear how contamination by this enzyme was avoided. In addition, very recently Wong and associates[10] reported that **3** and **2** are very poor inhibitors of bovine kidney α-L-fucosidase (K_i 1 and 90 mM, respectively). In contrast, our experiments presented in this report indicate that both **3** and **2** are powerful inhibitors of α-L-fucosidases. The utilization of crude cell extracts, leading to possible contamination of L-rhamnulose 1-phosphate aldolase by D-fructose 1,6-bisphosphate aldolase, may possibly explain these inconsistencies.

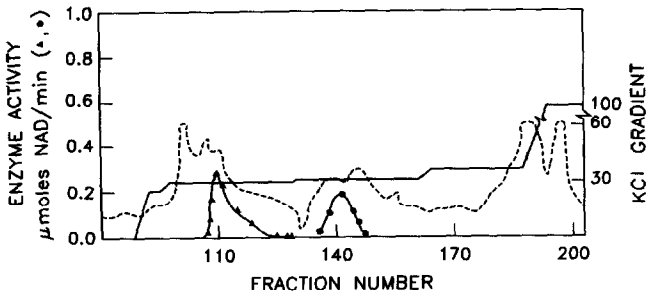


Fig. 1. Protein elution profile at 280 nm (— — —) and fractionation of D-fructose 1,6-bisphosphate aldolase (▲) and L-rhamnulose 1-phosphate aldolase (●) activities with a FPLC (Fast Protein Liquid Chromatography) system. KCl gradient (———).

homogenate from *E. coli* after a $MnCl_2$ precipitation step was more than that of the L-rhamnulose 1-phosphate aldolase (0.18 units/mg protein). Although most of the D-fructose 1,6-bisphosphate aldolase was present in the supernatant after the second ammonium sulphate fractionation (50% saturation), the precipitate also contained considerable amounts of this enzyme. However, the two aldolases were well separated by chromatography (Fig. 1 and Table I), and the L-rhamnulose 1-phosphate aldolase fraction was free from any detectable D-fructose 1,6-bisphosphate aldolase activity. Contamination by L-fuculose 1-phosphate aldolase in the enzyme preparation was ruled out, as only L-rhamnulose [6-deoxy-L-*arabino*-hexulose], but no L-fuculose [6-deoxy-L-*lyxo*-hexulose], was detected after dephosporylation of the adduct formed from **4** and L-lactaldehyde. When the concentration of aldehyde **5** was increased to more than 3 mM, azasugar formation was inhibited, perhaps due to inactivation of the enzyme by the aldehyde. The aldehyde was therefore added at low concentrations at various time intervals (see Experimental).

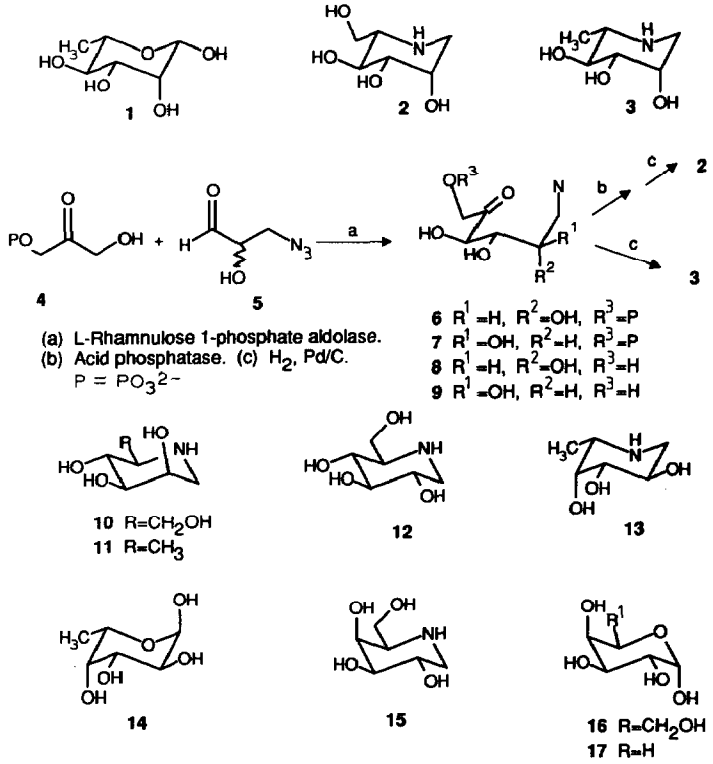
Synthesis.—Dihydroxyacetone phosphate (**4**) was generated from 2,5-diethoxy-2,5-bis(phosphonoxymethyl)-1,4-dioxane[16]. D,L-3-Azido-2-hydroxypropanal (**5**) was made by acidic hydrolysis of the corresponding diethyl acetal[17]. The aldol addition of **4** and **5** catalysed by partially purified L-rhamnulose 1-phosphate aldolase was found to give the highest yield when the reaction was carried out under sterile and dilute conditions. Dephosphorylation of a mixture of phosphates **6** and **7** with acid phosphatase (EC 3.1.3.2) gave readily separable **8** and **9** in a ratio of 6:1. Wong

TABLE I

Purification of L-rhamnulose 1-phosphate aldolase

Step [a]	Specific activity (units/mg protein)	Total activity (units)
$MnCl_2$	0.18	88
$(NH_4)_2SO_4$ (40–50%)	0.67	52
Q-Sepharose (FPLC)	2.33	7

[a] Enzyme activity in the crude extracts was not measured because of high NADH oxidase activity.



and associates[18] have shown that hydrogenolysis of 6-azido-6-deoxy-D-fructose and 6-azido-6-deoxy-D-fructose 1-phosphate diastereoselectively gave 1,5-dideoxy-1,5-imino-D-mannitol (**10**) and 1,5-dideoxy-1,5-imino-D-rhamnitol (**11**), respectively. Since compound **8** is enantiomeric to 6-azido-6-deoxy-D-fructose, hydrogenolysis of **8** should yield **2**. In fact hydrogenolysis of **8** furnished **2** and no other diastereomer was detected. Similarly, direct hydrogenolysis of phosphate **6** afforded solely compound **3**.

The absolute configuration of compound **2** was assigned by comparing the ^1H and ^{13}C NMR spectra and optical rotation of this compound with those of authentic 1,5-dideoxy-1,5-imino-D-mannitol (**10**) prepared according to Straub et al.[19]. Compound **2** showed identical NMR spectra but opposite optical rotation to **10**. Since compound **3** was obtained from the same intermediate (**6**) as compound **2**, the configurations at C-2, C-3, and C-4 of **3** should be identical to those of **2**. Then, the L-configuration at C-5 in **3** was confirmed by the value (9.5 Hz) of the H-4–H-5 coupling constant, which shows these two atoms to be trans–axially disposed. Signal assignments were made with the aid of a ^1H–^1H COSY spectrum.

Effects of compounds **2**, **3**, **10** *and* **12** *on glycosidases.*—The effects of **2** and **3**, as well as **10** and **12** for comparison purposes, on various glycosidases are summarized in Table II.

TABLE II

Inhibition of selected glycosidases by azasugars at a fixed concentration

Enzyme	Residual enzyme activity (%) in the presence of 1.25 mM azasugar [a]				Buffer
	2	10	12	3	
α-Galactosidase (EC 3.2.1.22, *Aspergillus niger*)	0	100	0	60	MES (100 mM, pH 4.5)
α-L-Fucosidase (EC 3.2.1.51, bovine epididymis)	36	3	100	0	Phosphate (50 mM, pH 6.7)
α-L-Rhamnosidase [b] (EC 3.2.1.40, *Penicillium decumbens*)	42	86	11	16	MES (100 mM, pH 5.0)
β-Galactosidase (EC 3.2.1.23, *E. coli*)	88	ND [c]	ND	96	Phosphate (50 mM, pH 6.7)
β-Glucosidase (EC 3.2.1.21, almonds)	84	ND	ND	88	MES (100 mM, pH 4.8)
α-Glucosidase (EC 3.2.1.20, yeast)	80	ND	ND	100	Phosphate (50 mM, pH 6.7)
β-Mannosidase (EC 3.2.1.25, snail)	64	ND	ND	98	MES (100 mM, pH 4.5)
α-Mannosidase (EC 3.2.1.24, jack beans)	77	ND	ND	45	MES (100 mM, pH 4.5)
Amyloglucosidase [d] (Glucan 1,4-α-glucosidase) (EC 3.2.1.3, *A. niger*)	ND	ND	ND	25	MES (100 mM, pH 5.0)
α-L-Rhamnosidase [e] (EC 3.2.1.40, *A. niger*)	47	ND	ND	20	MES (100 mM, pH 5.5)

[a] All values are % of the activity shown by a control without inhibitor. [b] Activity present in naringinase[33]. [c] Not determined. [d] 1.81 μmol *p*NP-α-glucopyranoside was used in the assay[34]. [e] Activity present in hesperidinase after the removal of sucrose from the commercial enzyme by dialysis.

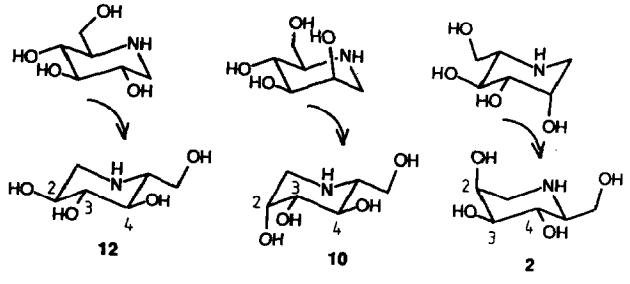
At a concentration of 1.25 mM, compound **3** exhibited moderate inhibition of amyloglucosidase as well as the α-L-rhamnosidase activity in naringinase and hesperidinase, and completely inhibited α-L-fucosidase. Compound **3** is a competitive inhibitor of α-L-fucosidase from bovine epididymis (Fig. 2a) and bovine kidney (plot not shown), with K_i values of 1.8 and 2.2 μM, respectively. The more potent inhibition by **13** (K_i 4.8 nM)[20], compared to **3**, of α-L-fucosidase from bovine kidney suggests the importance of the proper stereochemistry at C-2 and C-4 in these inhibitors in relation to L-fucose (6-deoxy-L-galactose, **14**). Compound **3** also inhibits α-galactosidase (plot not shown) and the α-L-rhamnosidase in naringinase competitively (Fig. 2b) with K_i values of 202 and 34 μM, respectively.

Compound **2** inhibited α-galactosidase competitively (Fig. 2c) with a K_i value of 8.2 μM, while compound **10** (enantiomer of **2**) showed no inhibition of the α-galactosidase (Table II). α-L-Fucosidase (bovine epididymis) and α-L-rhamnosidase in naringinase are also competitively inhibited by **2** with K_i values of 469 and 288 μM, respectively.

Compound **15** is known to be a potent inhibitor of α-D-galactosidase from green coffee-bean (K_i 1.6 nM)[21]. In comparison, compound **2** (K_i, 8.2 μM) is three orders of magnitude weaker but still and effective inhibitor. The activity of **2** against α-galactosidase could be rationalized from the similarity in the orientation of the OH groups at C-2, C-3, and C-4 in **2** to those at C-4, C-3, and C-2, respectively, in D-galactose (**16**). It is also appropriate to note that L-arabinose (**17**) is a weak inhibitor of α-galactosidase from various sources, including the enzyme from *A. niger*[22]. Comparison of the inhibitory effects of **2** and **3** on the galactosidase reveals that alteration of the hydroxymethyl group on C-5 in **2** to a methyl group in **3** drastically reduces inhibitory activity towards this enzyme.

Compound **2** is a much poorer inhibitor (K_i 469 and 213 μM, respectively) of the α-L-fucosidases of bovine epididymis and kidney than **3** (K_i 1.8 and 2.2 μM, respectively). These results demonstrate that the α-L-fucosidase active site can discriminate by over two orders of magnitude between inhibitors differing by methyl vs. hydroxymethyl functionality. Winchester et al.[23] have suggested that the minimum structural requirement for inhibition of α-L-fucosidase is satisfied when the configurations at C-2, C-3, and C-4 of the azasugar correspond to those of L-fucose (**14**). However, our results show that opposite configurations at C-2 and C-4 in **3** as compared to **13** did not totally abolish inhibition, but did results in a potency three orders of magnitude lower than that of **13**.

Compound **2** is a moderate inhibitor of α-L-rhamnosidase and this is in accord with the report that this enzyme can hydrolyse L-mannosides[24]. The inhibition of α-L-rhamnosidase by compound **2** was less than that of compound **3**, evidently because of the primary hydroxyl group in the former. Interestingly, D-azasugar **12** inhibits α-L-rhamnosidase as effectively as **3** (K_i 32 and 34 μM, respectively), but compound **10** does not inhibit this enzyme. This may be explained by reorienting the structures of **12** and **10** to reveal that the configurations at C-2 and C-3 in **12** are correct relative to C-4 and C-3 in L-rhamnose (**1**), but only C-3 of **10** is correct



if one assumes a similar binding mode for **12**, **10**, and L-rhamnose. This implies that a structural requirement for an azasugar to inhibit α-L-rhamnosidase is the correct configuration at both C-3 and C-4 with respect to L-rhamnose.

The results presented in this paper demonstrate that D-glycoside hydrolases as well as L-glycoside hydrolases can be substantially inhibited by L-aminosugars, and that these compounds can be used as complements to their D-analogues to further increase our understanding of glycohydrolytic enzymes. Although one can predict inhibition patterns based on structural similarity towards the substrate in the case of D-sugars, the same rationale cannot be simply applied to the L-sugars as inhibitors of D-glycoside hydrolases. The fact that **2** and **3** are inhibitors of α-L-rhamnosidase should be useful in extending our knowledge of the structure–activity relationships of L-glycoside hydrolases.

Fleet et al.[25] have reported that compound **2** has no anti-HIV activity, but have suggested that inhibitors of α-L-fucosidase have potential anti-HIV activity. Hence, such properties of compound **3** may be worthy of investigation.

EXPERIMENTAL

General.—Optical rotations were measured with a JASCO DIP-360 digital polarimeter. The NMR spectra were recorded with a Bruker AC-200 spectrometer. Chemical shifts for ^{13}C are reported as δ values referenced to external sodium 3-(trimethylsilyl)-1-propanesulfonate; ^{1}H chemical shifts of samples in D_2O were measured from HDO at δ 4.63. A Cary 3 UV-visible spectrophotometer was used for all absorbance measurements.

Unless otherwise noted, enzymes and substrates were obtained from Sigma, St. Louis, MO. Compounds **10** and **12** were purchased from Toronto Research Chemicals Inc., Toronto, ON, and other chemicals were supplied by Aldrich.

Enzyme assays.—L-Rhamnulose 1-phosphate was chemically synthesised as reported previously[26], or by enzymically phosphorylating L-rhamnulose, using L-rhamnulokinase (EC 2.7.1.5) from an L-rhamnulose 1-phosphate aldolase-negative *E. coli* strain (ECL716)[27], following the method described by Chiu and Feingold[28]. L-Rhamnulose 1-phosphate aldolase activity was measured by determining the rate of dihydroxyacetone phosphate formation from L-rhamnulose 1-phosphate, as previously described by Chiu and Feingold[15]. One unit of enzyme activity produces 1 μmol of dihydroxyacetone phosphate per min at 37°C. The rate of dihydroxyacetone phosphate formation was measured by reducing it to glycerol 3-phosphate by NADH in the glycerol 3-phosphate dehydrogenase (EC 1.1.1.8) reaction. D-Fructose 1,6-bisphosphate aldolase activity was measured in a similar manner by substitution of D-fructose 1,6-bisphosphate for L-rhamnulose 1-phosphate and omitting KCl from the reaction mixture. Since crude cell-free extracts of *E. coli* contain high levels of NADH oxidase, it was not possible to measure aldolase activities in this preparation[15].

Purification of L-rhamnulose 1-phosphate aldolase.—L-Rhamnulose 1-phosphate

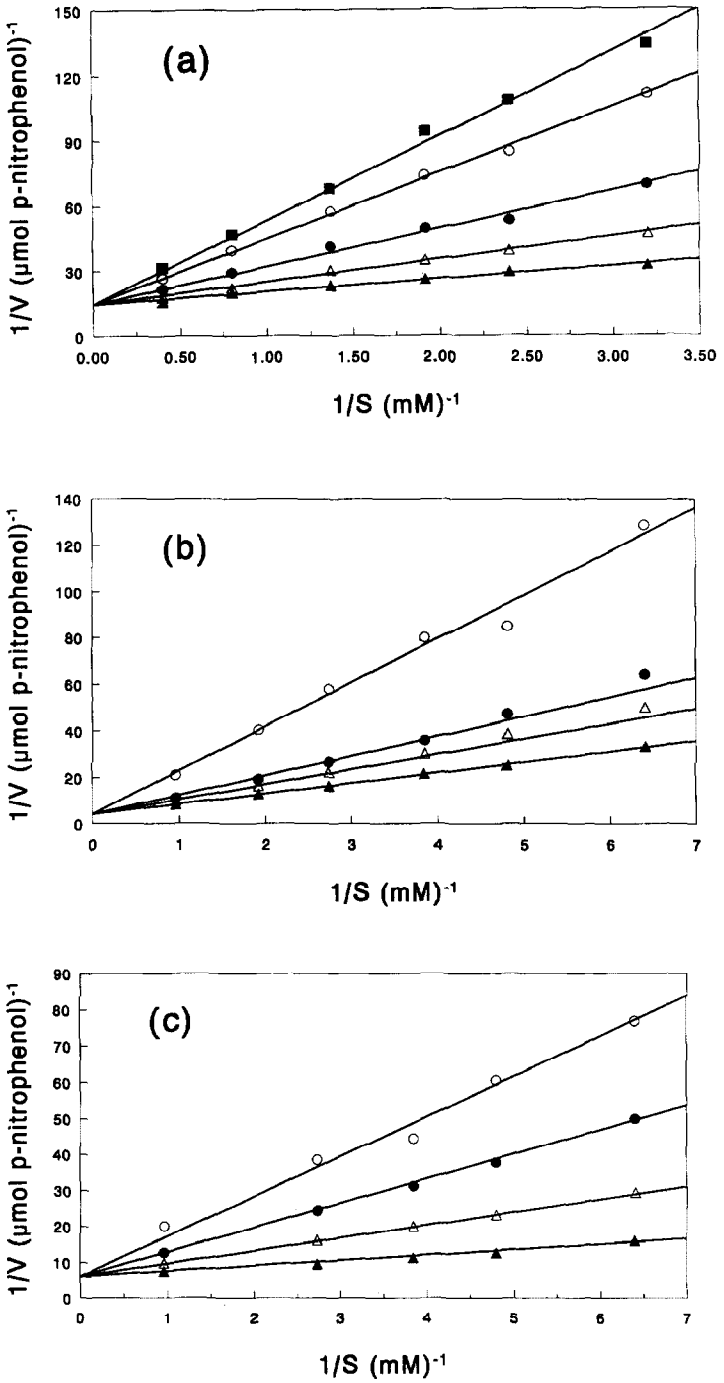


Fig. 2. Lineweaver–Burk plots showing competitive inhibition. (a) Incubation of α-L-fucosidase from bovine epididymis in the presence of: 0.0 (▲); 5.0 (△); 10.0 (●); 15.0 (○); and 20.0 μM (■) of compound 3. (b) α-L-Rhamnosidase in naringinase with 0.0 (▲); 16.7 (△); 33.3 (●); and 100.0 μM (○) of compound 3. (c) α-Galactosidase with 0.0 (▲); 8.3 (△); 20.8 (●); and 41.6 μM (○) of compound 2.

aldolase was purified from *E. coli* (ATCC 8739). The cells were grown in 20-L fermenters (Chemap 2000) in 15 L of medium[29] containing L-rhamnose (5 g/L) as the sole carbon source. Fermenter settings were: aeration, 16 L/min (0.8 vvm); agitation, 400 rpm; temperature, 37°C; pH maintained at 7; antifoam used. Cells were harvested after 9 h by centrifugation using a Sharples centrifuge (yield 6 g/L). The pellet was washed with sodium phosphate buffer (50 mM, pH 7.0) and recentrifuged (4000*g*), and the resulting pellet was frozen at −20°C. Frozen cells (45 g) were mixed with sodium phosphate buffer (0.2 M, pH 7.0, 45 mL) and glass beads (225 g, 0.01–0.11 mm, B. Braun, Melsungen AG, Melsungen, Germany) and homogenised in a Braun homogeniser (30 s, 4 cycles each with 1 min intermittent cooling on ice). All subsequent operations were carried out at 4°C.

The homogenate was diluted to 85 mL, centrifuged (30000*g*) for 20 min, and the supernatant (80 mL) containing the crude enzyme was recovered. Manganese chloride (0.3 M, 6.5 mL) was added to the supernatant with stirring and the mixture was left in an ice bath overnight. The precipitated nucleoprotein was removed by centrifugation (30000*g*) for 10 min, and mercaptoethanol was added to the recovered supernatant to a final concentration of 10 mM. Solid ammonium sulphate (18.5 g) was then added with stirring to the supernatant, the resulting precipitate was collected by centrifugation (30000*g*) for 30 min and discarded. Additional ammonium sulphate (4.75 g) was added to the supernatant and the precipitate was recovered by centrifugation. The precipitate was dissolved in 10 mL of triethanolamine buffer (TEM buffer, 20 mM, pH 7.5, containing 10 mM mercaptoethanol). During the two ammonium sulfate steps, the pH of the solution was maintained at 7.0 using N NH_4OH.

The enzyme in TEM buffer was dialysed against the same buffer (3 × 1 L, 3 h each), loaded on a Q-Sepharose fast-flow column (28 × 1 cm, Pharmacia), and fractionated by FPLC (fast protein liquid chromatography, Pharmacia) using a gradient of 0–1 M KCl in TEM buffer containing 10 mM mercaptoethanol, at a flow rate of 0.5 mL/min. Fractions of 0.5 mL were collected. Proteins emerging up to 0.3 M KCl were discarded (the KCl concentration was held at 0.3 M till the D-fructose 1,6-bisphosphate aldolase was completely eluted). Fractions eluted at 0.33 M KCl contained L-rhamnulose 1-phosphate aldolase free of any D-fructose 1,6-bisphosphate aldolase activity. These fractions were pooled and used for the aldol addition reaction (Table I).

Synthesis of 1,5-dideoxy-1,5-imino-L-mannitol (**2**).—3-Azido-2-hydroxypropionaldehyde diethyl acetal (1.04 g, 5.5 mmol) in 0.1 M HCl (10 mL) was stirred at 45–50°C for 6 h (hydrolysis monitored by TLC on silica gel, 1:10 MeOH–CH_2Cl_2) to give the corresponding aldehyde **5**. The resulting solution was adjusted to pH 7.0 with N NaOH. Dihydroxyacetone phosphate (**4**, 5.0 mmol) was prepared according to Effenberger and Straub[16] and the solution was adjusted to pH 7.0 with N NaOH. To a sterile mM $ZnCl_2$ solution (600 mL), L-rhamnulose 1-phosphate aldolase (sterilised through a 0.2-μm filter, 10 units) was added, followed by the addition of filter-sterilised solutions of **4** and **5** at 3 h intervals (11 additions,

0.5 mmol **4** and 0.45 mmol **5** in each addition) over a total incubation period of 66 h. All additions were made under sterile conditions and the reaction was carried out in a closed bottle at room temperature. The pH of the reaction mixture during the incubation period remained at 7.0 ± 0.3. Sugar formation was monitored using the cysteine–carbazole reaction[30]. At the end of the incubation, the precipitate formed was removed by centrifugation (10000*g*). The supernatant was lyophilised and the residue was dissolved in water (50 mL). The pH of the solution was adjusted to 7 with N NaOH, and M $BaCl_2$ (12 mL) was added. The mixture was kept at 0°C for 0.5 h and the precipitate was removed by centrifugation (10000*g*). An equal volume of abs. EtOH was then added to the supernatant and the mixture was kept at 0°C for 3 h. The precipitate was collected by centrifugation (10000*g*), washed with abs. EtOH (5 mL), and dried under vacuum to give the barium salts of **6** and **7** as a white powder (0.67 g, 32%).

The barium salts were dissolved in distilled water (15 mL) and stirred in the presence of DOWEX 50 W-X8 H^+ (5 mL) for 0.5 h. The resin was removed by filtration. The filtrate was adjusted to pH 4.5 with N NaOH followed by the addition of acid phosphatase (EC 3.1.3.2, Boehringer Mannheim, 20 units) and the resulting mixture was incubated for 12 h at 35°C. The solution was adjusted to pH 7 with $Ba(OH)_2$ (satd) and lyophilised to dryness. The residue was triturated with 1:1 EtOAc–MeOH (10 mL) and the insoluble material was removed by filtration. The filtrate was concentrated under vacuum, and the residual oil was dissolved in water (2 mL) and passed by gravity through a resin column (40 × 1 cm, 200–400 mesh Bio-Rad AG 1-X8 HCO_2^-, water) to give a mixture of diastereomers **8** and **9** (160 mg, 52%).

The two diastereomers were separated by flash chromatography (silica gel, 1:5 MeOH–CH_2Cl_2). In comparison with their authentic enantiomers prepared using D-fructose 1-phosphate aldolase according to Straub et al.[19], the first fraction (20 mg) was identified as **9** and the second fraction (118 mg) as **8**. The compound **8** (99 mg, 0.48 mmol) was dissolved in 1:1 MeOH–water (40 mL) and the solution was shaken in the presence of 10% Pd/C (30 mg) under H_2 (60 psi) at room temperature for 24 h. The Pd/C was removed by filtration through Celite, the filtrate was concentrated to 2 mL under vacuum, and chromatographed (40 × 1 cm gravity column, 200–400 mesh Bio-Rad AG 1-X8 HCO_2^-, water). The fractions containing product were collected and lyophilised to yield **2** (79 mg, 100%) as a white powder; $[\alpha]_D^{22}$ + 26.6° (*c* 0.56, H_2O); lit.[5] $[\alpha]_D^{20}$ + 12.6° for the hydrochloride; ^1H NMR (200 MHz, D_2O): δ 2.56 (ddd, 1 H, $J_{4,5}$ 9.6, $J_{5,6a}$ 3.5, $J_{5,6b}$ 5.0 Hz, H-5), 2.77 (dd, 1 H, $J_{1eq,2}$ 1.5, $J_{1eq,1ax}$ 14.1 Hz, H-1*eq*), 3.00 (dd, 1 H, $J_{1ax,2}$ 2.8 Hz, H-1*ax*), 3.45 (dd, 1 H, $J_{2,3}$ 2.9, $J_{3,4}$ 9.6 Hz, H-3), 3.55 (dd, 1 H, H-4), 3.67 (AB of ABX, $J_{6a,6b}$ 12.0 Hz, H-6), 3.93 (m, 1 H, H-2); ^{13}C NMR (50.3 MHz, D_2O): δ 50.6, 62.4, 63.1, 70.1, 70.7, 76.4; FABMS: *m/z* 164 (M + H). Anal. Calcd for $C_6H_{13}NO_4$: C, 44.16; H, 8.03; N, 8.58. Found: C, 44.20; H, 8.32; N, 8.74.

*1,5-Dideoxy-1,5-imino-*L*-rhamnitol* (**3**).—A solution of **6** and **7** (248 mg, 0.59 mmol) in water (100 mL) was shaken under H_2 (60 psi) in the presence of Pd/C

(500 mg) at room temperature for 48 h. The Pd/C was removed by filtration through Celite and the filtrate was lyophilised to dryness. The residue was passed by gravity through a resin column (40 × 1 cm, 200–400 mesh AG1-X8 HCO_2^-, water). The fractions containing the desired compound were lyophilised to dryness and further purified by flash chromatography (70–230 mesh silica gel, 1:3 water–MeOH) to give the final product **3** (49 mg, 56%) as a white powder, $[\alpha]_D^{20}$ + 59.14° (*c* 0.32, H_2O); ^1H NMR (200 MHz, D_2O): δ 1.15 (d, 3 H, $J_{5,6}$ 6.4 Hz, Me), 2.47 (dq, 1 H, $J_{4,5}$ 9.5 Hz, H-5), 2.72 (dd, 1 H, $J_{1ax,1eq}$ 14.4, $J_{1ax,2}$ 1.5 Hz, H-1*ax*), 2.94 (dd, 1 H, $J_{1eq,2}$ 2.7, H-1*eq*), 3.30 (dd, 1 H, $J_{3,4}$ 9.6 Hz, H-4), 3.50 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-3), 3.97 (m, 1 H, H-2); ^{13}C NMR (50.3 MHz, D_2O): δ 19.5, 50.9, 57.9, 71.8, 76.3, 76.7; HRMS: *m/z* calcd, 1.47.0896; found, 147.0884. Anal. Calcd for $C_6H_{13}NO_3 \cdot 0.4H_2O$: C, 46.64; H, 9.07; N, 9.52. Found: C, 46.83; H, 9.16; N, 9.19.

Inhibition.—The activities of all glycosidases, except β-galactosidase, were determined by the estimation of *p*-nitrophenol liberated by hydrolysis of the corresponding *p*-nitrophenyl glycosides. β-Galactosidase activity was estimated by monitoring the release of *o*-nitrophenol from *o*-nitrophenyl β-galactoside. Unless otherwise specified, all enzyme assays were conducted as described by Evans et al.[31,32] with minor modifications. A typical assay mixture contained 0.5 μmol of the nitrophenyl glycoside and the appropriate amount of enzyme in a final volume of 0.6 mL. Where appropriate, azasugars were included in the assay mixtures. Incubations were conducted at room temperature for 15 min before 0.5 N NaOH (0.4 mL) was added to terminate the reaction. Absorbance measurements at 410 nm were performed immediately after the addition of the base. Other conditions, specific for each enzyme, are indicated in Table II. Enzymes were used as obtained commercially, unless otherwise specified.

ACKNOWLEDGMENTS

We thank the Natural Sciences and Engineering Research Council of Canada for financial assistance and Y.-M. Chen and E.C.C. Lin for *E. coli* strain ECL716. Technical assistance from J. Sprenger is also acknowledged.

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