BIOORGANIC CHEMISTRY 20, 173–179 (1992)

The Enzymatic Synthesis of 1,5-Dideoxy-1,5-diimino-D-talitol and 1-Deoxygalactostatin Using Fuculose-1-phosphate Aldolase[1]

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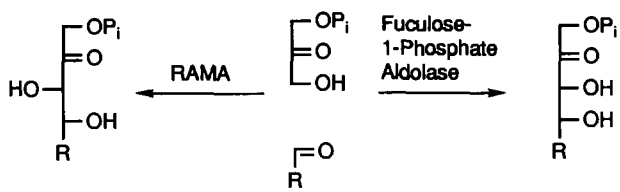
Received January 13, 1992

A new polyhydroxylated piperidine derivative of D-*talo* configuration, 1,5-dideoxy-1,5-imino-D-talitol **(2)**, and 1-deoxygalactostatin **1** have been prepared using fuculose-1-phosphate aldolase (EC 4.1.2.17). The 6-azido-1,3,4,5-tetrahydroxyhexan-2-one, formed by the enzyme-catalyzed condensation of 3-azido-2-hydroxypropanal and dihydroxyacetone-phosphate, is reduced stereospecifically to the corresponding polyhydroxylated piperidine derivative with hydrogen.

INTRODUCTION

Polyhydroxylated piperidine derivatives are interesting because many of them inhibit glycosyl hydrolases (glycosidases) (*1*), and because they have antiviral (*2–4*), antitumor (*5, 6*), and antibiotic activity (*7, 8*). This paper demonstrates the utility of a recently isolated fuculose-1-phosphate aldolase in synthesizing polyhydroxylated piperidine derivatives (*9–11*). Fuculose-1-phosphate aldolase catalyzes the aldol condensation between aldehydes and dihydroxyacetone phosphate, DHAP, to form 2-keto-sugars with 3(*R*), 4(*R*) stereochemistry at the newly



formed centers (*9*). This stereochemistry is different at the C-3 center from the product of aldol condensation catalyzed by the more commonly used rabbit muscle aldolase (RAMA) (*12*). We chose 1-deoxygalactostatin (D-galacto-1-deoxynojirimycin, 1,5-dideoxy-1,5-imino-galactitol) and a new compound, 1,5-dideoxy-1,5-

[1] After this work was completed, a paper describing results very similar to our own appeared: Liu, K. K.-C., Kajimoto, T., Chen, L., Zhong, Z., Ichikawa, Y., Wong, C.-H. (1991) *J. Org. Chem.* **56,** 6280–6289.

0045-2068/92 $5.00

imino-D-talitol, as synthetic targets because of the importance of this class of compounds (vide supra) and because their synthesis was simple and economical using enzymes (*13, 14*). 1-Deoxygalactostatin and 1,5-dideoxy-1,5-imino-L-talitol, the enantiomer of 1,5-dideoxy-1,5-imino-D-talitol, inhibit galactosidases (K_i down to 1.6 nM) and fucosidases (K_i down to 10 μM), respectively (*1, 15*).

Several groups have described the synthesis of 1-deoxygalactostatin (*16–19*) and 1,5-dideoxy-1,5-imino-L-talitol (*15*) starting from carbohydrates. Preparation of 1-deoxygalactostatin from tartaric acid (*20*) and by reduction of galactostatin isolated from natural sources are also known (*21*). Wong and co-workers have recently synthesized 1-deoxygalactostatin in an approach similar to that described here using fuculose-1-phosphate aldolase (*11*). Wong used racemic 3-azido-2-hydroxypropanal and DHAP as substrates for fuculose-1-phosphate aldolase. Fuculose-1-phosphate aldolase reacted selectively with the *S*-isomer of the aldehyde to give 6-azido-6-deoxy-L-*lyxo*-hex-2-ulose 1-phosphate in approximately 20% yield. Cleavage of the phosphate group by acid phosphatase followed by the reduction of the azido-sugar with hydrogen resulted in 1-deoxygalactostatin.

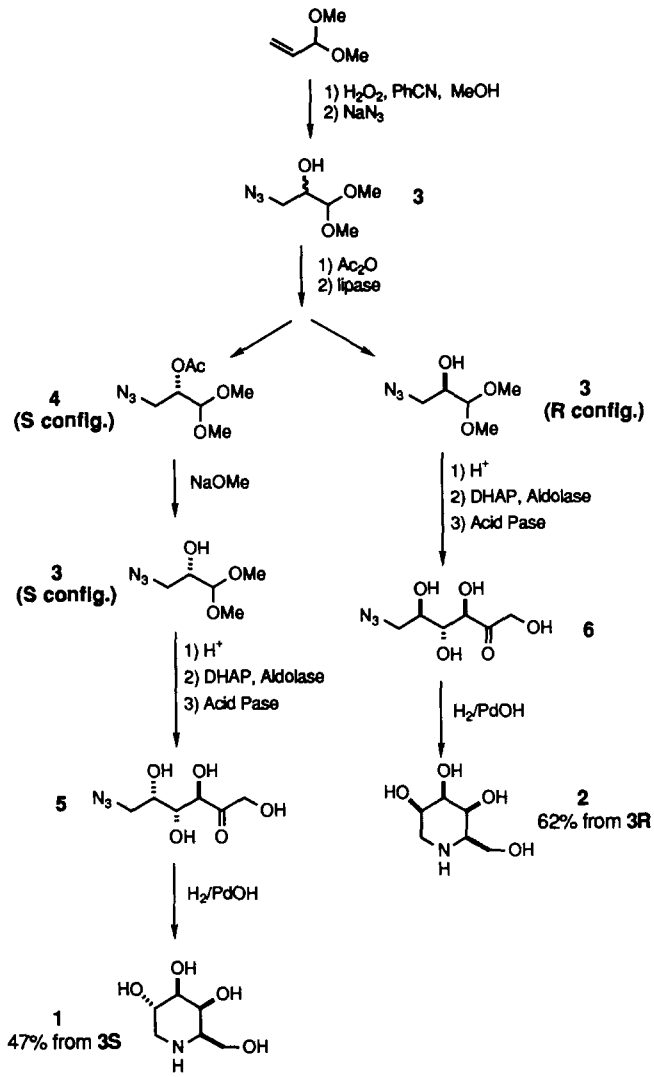
RESULTS AND DISCUSSION

Scheme 1 shows the synthesis of 1-deoxygalactostatin, **1**, and 1,5-dideoxy-1,5-imino-D-talitol, **2**. Acrolein dimethyl acetal was oxidized to glycidal dimethyl acetal. The epoxide of glycidal dimethyl acetal was reacted with sodium azide and gave 3-azido-2-hydroxypropanal dimethyl acetal with an overall yield of 37%. The alcohol was acetylated and the acetate resolved using lipase following the procedure of Wong (*13*). Resolved aldehyde was allowed to react with DHAP with catalysis by fuculose-1-phosphate aldolase to stereoselectively make the corresponding 6-azido-3(*R*),4(*R*),5(*S* or *R*)-trihydroxyhexan-2-one 1-phosphate. The phosphate group was hydrolyzed with acid phosphatase to the corresponding 6-azido-1,3(*R*),4(*R*),5(*S* or *R*)-tetrahydroxyhexan-2-one, **5** or **6**. The azide was reduced with H_2 to the amine, the amine formed a Schiff's base with the ketone *in situ*, and the imine was reduced with H_2, from the face opposite the axial hydroxyl group, with high stereoselectively (>10 : 1) to the corresponding piperidine derivative, **1** or **2**. 1-Deoxygalactostatin and 1,5-dideoxy-1,5-imino-D-talitol were thus synthesized in 47 and 62% overall yield, respectively, based on resolved 3-azido-2-hydroxypropanal dimethyl acetal.

EXPERIMENTAL SECTION

General Methods

Starting materials were commercial products obtained from Aldrich. DHAP was synthesized according to the procedure of Effenberger *et al.* (*14*). Fuculose-1-phosphate aldolase was isolated according to the procedure of Ozaki *et al.* (*9*). NMR spectra were recorded in $CDCl_3$ or D_2O. Chemical shifts are reported in δ



SCHEME 1.

(ppm) using $CHCl_3$ (7.24) or HOD (4.80) as an internal standard. ^{13}C NMR shifts were recorded in ppm using $CDCl_3$ as an internal standard or dioxane as an external standard. Elemental analyses were performed by Oneida Research Services.

Compound Synthesis

3-Azido-2-hydroxypropanal dimethyl acetal (**3**). The procedure is similar to that reported by Wong for the diethyl acetal (*13*). Acrolein dimethyl acetal (45 ml, 0.38 mol), benzonitrile (30 ml), and hydrogen peroxide (30% soln, 32 ml) were added

to a stirred suspension of potassium carbonate (4.53 g, 0.045 mmol) in methanol (200 ml). After 14 h at 40°C, benzonitrile (11 ml) and H_2O_2 (20 ml) were added. After 24 h at 40°C, H_2O_2 (10 ml) was added. After 36 h at 40°C, the solution was cooled to 20°C and sodium azide (52 g, 0.80 mol) was added. The pH was then maintained at 7.5 with 2 N H_2SO_4 for the next 36 h. After evaporating the methanol, the solution was partitioned between water (150 ml) and CH_2Cl_2 (200 ml). The aqueous layer was extracted with CH_2Cl_2 (2 × 200 ml). The combined organic phase was extracted with brine (150 ml), dried ($MgSO_4$), filtered, and concentrated at aspirator pressure. Hexane was added and the solution was filtered and concentrated at aspirator pressure. The resulting liquid was distilled (bp 75–80°C, 1 Torr) and purified by chromatography on silica gel (eluent: hexane/ethyl acetate 3/1) to provide 22.5 g (37% yield) of 3-azido-2-hydroxypropanal. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.28 (d, J = 6.3 Hz, 1H), 3.75 (m, 1H), 3.44 (dd, J = 12.8, 3.3 Hz, 1H), 3.44 (s, 3H), 3.41 (s, 3H), 3.31 (dd, J = 12.9, 6.0 Hz, 1H), 2.53 (broad s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) 104.48, 70.76, 55.69, 55.10, 52.21 ppm. *Anal.* Calcd. for $C_5H_{11}N_3O_3$: C 45.78; H 6.91; N 10.68. Found: C 45.53; H 6.97; N 10.54.

3-Azido-2-acetoxypropanal dimethyl acetal (**4**). The acetal **3** (3.23 g, 20 mmol), acetic anhydride (2.5 ml), and pyridine (2.5 ml) were stirred at 20°C for 48 h. Methanol (1.0 ml) was added and the solution was partitioned between ethyl acetate (15 ml) and water (10 ml). The organic layer was extracted with 10 ml of H_2O, 1 N HCl (2 × 10 ml), sat aq $NaHCO_3$ (10 ml), and brine (10 ml), dried ($MgSO_4$), filtered, and concentrated *in vacuo* to provide 3.64 g (90% yield) of 3-azido-2-acetoxypropanal dimethyl acetal. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.05 (td, J = 5.6, 4.2 Hz, 1H), 4.43 (d, J = 5.7 Hz, 1 H), 3.47 (m, 2H), 3.41 (s, 3H), 3.38 (s, 3H), 2.12 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 170.05, 102.57, 71.29, 55.82, 54.47, 50.22, 20.89 ppm.

Resolution of 3-azido-2-acetoxypropanal dimethyl acetal. The resolution was carried out in a manner similar to that reported previously (*13*). The acetate **4** (4.07 g, 20 mmol) and lipoprotein lipase (200 mg, 160,000 U) were dissolved in 200 ml of potassium phosphate buffer (50 mM, pH 7.0). The solution was maintained at pH 7.1 until 26 ml of 0.25 N NaOH had been added. The solution was then extracted with ethyl acetate (3 × 300 ml). The combined organic phase was dried ($MgSO_4$), filtered, concentrated at aspirator pressure, and purified by chromatography on silica gel (eluent: hexane/ethyl acetate 5/1 to 2/1) to provide 934 mg (5.8 mmol) of the alcohol **3** (95% ee, *R*, vide infra) and 2.39 g (11.8 mmol) of the acetate **4**. The leftover acetate **4** (2.39 g, 11.8 mmol) was then resubmitted to the above procedure with lipase to provide 1.24 g (6.2 mmol) of the acetate **4** (>98% ee, *S*, vide infra). The acetate was dissolved in 5 ml of methanol containing 5 mg of sodium methoxide. After 16 h, the solution was concentrated at aspirator pressure and purified by chromatography on silica gel (eluent: hexane/ethyl acetate 3/1 to 1/1) to provide 862 mg (5.4 mmol) of the alcohol **3** (>98% ee, *S*, vide infra). The % ee of each alcohol was determined by making a Mosher's ester.

6-Azido-6-deoxy-L-lyxo-hex-2-ulose (**5**). The acetal **3** (342 mg, 2.1 mmol, *S* configuration), water (5.0 ml), and concn HCl (150 μl) were mixed together and heated at 45°C for 10 h before being cooled to 4°C. DHAP (18 ml, 2.0 mmol) was added and the solution was adjusted to pH 7.0. Fuculose-1-phosphate aldolase (40 U)

was added. After 48 h at 4°C, barium chloride ($BaCl_2 \cdot 2H_2O$, 1.0 g, 4.4 mmol) and acetone (50 ml) were added. After 24 h more at 4°C, the solution was centrifuged at 1000 rpm for 30 min. The supernatant was separated by decantation and discarded, and the solid was acidified by stirring with Dowex 50W-X8. The acidified solution was adjusted to pH 5.0 with 1 N NaOH and diluted to 200 ml. Acid phosphatase (300 U) was added. After 48 h at 20°C, the solution was partially concentrated *in vacuo* and purified by chromatography on AG-1-X8 (35 ml, HCO_3 form, eluent: water), Dowex 50W-X8 (20 ml, eluent: water), and then silica gel (eluent: methanol/CH_2Cl_2 1 : 7 going to 1 : 1) to provide 245 mg of 6-azido-6-deoxy-D-*lyxo*-hex-2-ulose. Crude 6-azido-6-deoxy-L-*lyxo*-hex-2-ulose-1-phosphate [a and b refer to the two anomers (1 : 3 ratio in the ^1H NMR spectrum)]: ^{13}C NMR (100 MHz, D_2O) 105.64 (d, J = 8.0 Hz, C-2a), 102.94 (d, J = 9.0 Hz, C-2b), 79.35 (b), 78.87 (a), 76.92 (a), 72.01 (b), 71.71 (a, b), 66.70 (d, J = 4.3 Hz, 1b), 66.19 (d, J = 4.3 Hz, 1a), 51.94 (6b), 51.66 (6a) ppm. 6-Azido-6-deoxy-L-*lyxo*-hex-2-ulose [a and b refer to the two anomers (1 : 3 ratio in the ^1H NMR spectrum)]: ^{13}C NMR (100 MHz, D_2O) 106.15 (2a), 103.71 (2b), 79.42 (b), 78.62 (a), 77.87 (a), 72.25 (a), 71.92 (b), 71.41 (a), 63.46 (1b), 63.22 (1a), 51.99 (6b), 51.29 (6a) ppm.

1-Deoxygalactostatin (**1**). 6-Azido-6-deoxy-L-*lyxo*-hex-2-ulose (120 mg) and palladium hydroxide (25 mg, 20% on carbon, <50% water) were added to a mixture of ethanol (25 ml) and H_2O (25 ml). The solution was hydrogenated at 1200 psi for 24 h, filtered through Celite, and concentrated *in vacuo*. The residue was dissolved in water and purified by chromatography (AG1-X8, H^+ form, 10 ml, eluent: 1 M NH_4OH) to provide 80 mg (0.49 mmol, 47% yield from the acetal) of 1-deoxygalactostatin. ^1H NMR (400 MHz, D_2O) δ 3.97 (dd, J = 2.9, 1.1 Hz, H-4), 3.71 (td, J = 10.4, 5.2 Hz, H-2), 3.60 (dd, J = 11.2, 6.7 Hz, H-6a), 3.55 (dd, J = 11.2, 6.7 Hz, H-6b), 3.44 (dd, J = 9.7, 3.2 Hz, H-3), 3.08 (dd, J = 12.6, 5.3 Hz, H-1a), 2.71 (td, J = 6.7, 1.1 Hz, H-5), 2.35 (dd, J = 12.6, 10.9 Hz, H-1b); ^{13}C NMR (100 MHz, D_2O) 75.86, 70.06, 68.97, 62.21, 59.66, 49.87 ppm. $[\alpha]_D^{298}$ +44.6° (c 1.1., H_2O) [lit. (*20*) $[\alpha]_D^{298}$ +52.6° (c 1.3, H_2O)].

6-Azido-6-deoxy-ribo-hex-2-ulose (**6**). The acetal **3** (487 mg, 3.0 mmol, *R* configuration), water (7.5 ml), and concn HCl (150 μl) were mixed together and heated at 45°C for 11 h before being cooled to 4°C. DHAP (36 ml, 4.0 mmol) was added and the solution was adjusted to pH 7.0. Fuculose-1-phosphate aldolase (40 U) was added and the reaction mixture was left at 4°C for 30 h. The resulting solution was adjusted to pH 4.8 with phosphoric acid, warmed to 20°C, and diluted with water (100 ml). Acid phosphatase (70 U) was added. After 48 h, the solution was partially concentrated *in vacuo* and purified by chromatography on AG-1-X8 (35 ml, HCO_3 form, eluent: water), Dowex 50W-X8 (20 ml, eluent: water), and then silica gel (eluent: methanol/CH_2Cl_2 1 : 7 going to 1 : 1) to provide 575 mg of 6-azido-6-deoxy-D-*ribo*-hex-2-ulose (75% pure, main contaminant is dihydroxyacetone). Crude 6-azido-6-deoxy-*ribo*-hex-2-ulose-1-phosphate [a and b refer to the two anomers (3 : 7 ratio in the ^1H NMR spectrum)]: ^{13}C NMR (100 MHz, D_2O) 106.33 (J = 9.7 Hz, C-2a), 103.77 (J = 9.8 Hz, C-2b), 82.10 (b), 81.98 (a), 75.29 (a), 72.37 (a), 71.97 (b), 71.66 (b), 67.39 (J = 4.8 Hz, 1b), 66.17 (J = 4.7 Hz, 1a), 53.81 (6b), 52.72 (6a) ppm. 6-Azido-6-deoxy-*ribo*-hex-2-ulose [a and b refer to the two anomers (1 : 2 ratio in the ^1H NMR spectrum)]: ^{13}C NMR (100 MHz, D_2O) 106.96 (2a), 104.55

(2b), 81.92 (a), 81.82 (b), 75.46 (a), 72.68 (b), 72.01 (a), 71.30 (b), 64.31 (1b), 63.20 (1a), 53.87 (6a), 52.78 (6b) ppm.

1,5-Dideoxy-1,5-imino-D-talitol (**2**). 6-Azido-6-deoxy-D-*ribo*-hex-2-ulose (575 mg) and palladium hydroxide (50 mg, 20% on carbon, <50% water) were added to a mixture of methanol (100 ml) and H_2O (25 ml). The solution was hydrogenated at 1200 psi for 24 h, filtered through Celite, and concentrated *in vacuo*. The residue was dissolved in water and purified by chromatography (AG1-X8, H^+ form, 10 ml, eluent: 1 M NH_4OH) to provide 303 mg (1.86 mmol, 62% yield from the acetal) of 1,5-dideoxy-1,5-imino-D-talitol. ^1H NMR (400 MHz, D_2O) δ 3.98 (m, 2H), 3.69 (m, 3H), 3.15 (dd, J = 14.2, 2.6 Hz, 1H), 2.82 (m, 2H); ^{13}C NMR (100 MHz, D_2O) 70.38, 70.13, 69.97, 62.19, 59.79, 49.75 ppm. Acidification with DCl resulted in a spectrum indistinguishable from that reported previously for 1,5-dideoxy-1,5-imino-L-talitol (*15*). The compound was characterized as the peracetylated sugar (Ac_2O/pyridine). The two rotomers about the amide bond are referred to as a and b (0.55 : 0.45 ratio). ^1H NMR (400 MHz, C_6D_6, 298 K) δ 5.73 (t, J = 2.5 Hz, H-3a), 5.71 (t, J = 2.6 Hz, H-3b), 5.57 (ddd, J = 10.2, 6.5, 2.5 Hz, H-5b), 4.96 (dd, J = 13.2, 5.4 Hz, H-1a), 4.92 (dd, J = 11.9, 10.5 Hz, H-6b), 4.84 (dd, J = 6.7, 2.7 Hz, H-4b), 4.74 (ddd, J = 11.6, 5.4, 2.7 Hz, H-2a), 4.70 (dd, J = 12.0, 10.1 Hz, H-6a), 4.64 (dd, J = 6.4, 2.8 Hz, H-4a), 4.37 (ddd, J = 11.3, 5.2, 2.8 Hz, H-2b), 4.32 – 4.28 (m, H-5a), 4.29 (dd, J = 11.8, 2.7 Hz, H-6b), 4.20 (dd, J = 12.1, 2.6 Hz, H-6a), 3.46 (dd, J = 13.5, 5.3 Hz, H-1b), 3.31 (dd, J = 13.5, 11.3 Hz, H-1b), 2.90 (t, J = 12.4 Hz, H-1a), 1.93 (s, 3H), 1.79 (s, 3H), 1.76 (s, 3H), 1.65 (s, 3H), 1.63 (s, 3H), 1.60 (s, 6H), 1.56 (s, 3H), 1.56 (s, 3H), 1.54 (s, 3H); ^{13}C NMR (100 MHz, C_6D_6, 298 K) 68.75, 68.72, 68.19, 67.30, 67.14, 66.34, 60.19, 59.70, 54.02, 48.99, 39.36, 33.99, 21.56, 21.26, 20.52, 20.26, 20.22, 20.11, 20.05, 20.02 ppm. ^1H NMR (400 MHz, C_6D_6, 353 K) δ 5.67 (t, J = 2.6 Hz, 1H), 4.78 (br s, 1H), 4.72 (br s, 1H), 4.61 (br s, 1H), 4.28 (dd, J = 11.9, 2.8 Hz, 1H), 3.10 (br s, 1H), 1.87 (br s, 3H), 1.75 (s, 3H), 1.69 (br s, 3H), 1.64 (s, 3H), 1.62 (s, 3H). *Anal.* Calcd for $C_{16}H_{23}NO_9$: C 51.47; H 6.21; N 3.75. Found: C 51.45; H 6.17; N 3.65.

ACKNOWLEDGMENT

This work was supported by the National Institutes of Health Grant GM 30367. W.J.L. was supported by a NSERCC Centennial Award.

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8. These polyhydroxylated piperidine derivatives probably inhibit glycosidases by interacting reversibly with a carboxylate residue that is important for enzymatic activity (See Karplus, M., and Post, C. B. (1986) *J. Am. Chem. Soc.* **108,** 1317) (*1*). The inhibition constants of the polyhydroxylated piperidine derivatives are typically in the micromolar to nanomolar range (*1*). Several of these derivatives (castanospermine, *N*-butyldeoxynojirimycin, 1-deoxynojirimycin, *N*-methyl-1-deoxyfuconojirimycin) have anti-HIV activity (*2–4*). Anti-HIV activity may result from the inhibition of glycosidases that selectively modify polysaccharides on viral proteins and thus inhibit syncytium formation (*4*). Castanospermine and swainosine have shown anti-tumor activity (*5*). The action of this class of compounds as inhibitors of glycosidases may also be useful in model studies of genetic diseases resulting from misfunctioning glycosidases (See Stansbbury, J. B., Wyngaarden, J. B., Fredrickson, D. S., Goldstein, J. L., and Brown, M. S. (Eds.) (1983) The Metabolic Basis of Inherited Diseases, 5th ed., McGraw/Hill, New York.
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