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Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 15 (2004) 2951–2954

A straightforward synthesis of an aminocyclitol based on an enzymatic aldol reaction and a highly stereoselective intramolecular Henry reaction

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Received 30 April 2004; accepted 10 June 2004
Available online 29 July 2004

Abstract—The reactions of 4-nitroaldehydes **9** and **10** with dihydroxyacetonephosphate (DHAP) catalyzed by fructose-1,6-diphosphate aldolase from rabbit muscle were studied. Starting from **9** or **10**, only one main stereomer of nitrocyclitol **8** was isolated. A highly stereoselective intramolecular cyclization (Henry reaction or nitroaldol reaction) took place under acidic conditions during the aldolase catalyzed condensation and phytase catalyzed phosphate hydrolysis coupled step. The catalytic hydrogenation of nitrocyclitol **8** yielded aminocyclitol **7**, a valiolamine analogue. Its inhibition properties were evaluated towards five glycosidases.

1. Introduction

The design and synthesis of glycosidase inhibitors have attracted much interest because of their potential therapeutic applications. Glycosidase inhibitors can be used to treat diabetes, cancer, viral (HIV, influenza), and bacterial infections and act as insecticides.[1]

Owing to the protonation of their amino group at physiological pH, most of the inhibitors of the iminosugar and aminocarbasugar families are believed to mimic the transition state in the enzymatic glycoside hydrolysis step.[1e,2]

Aminocyclitols (aminocarbasugars) such as valiolamine **1**, validamine **2**, valienamine **3** (Fig. 1), and their analogues can be effective specific inhibitors of glycosidases involved in the intestinal degradation of carbohydrates.[3] Purely chemical strategies for aminocyclitol preparations have been extensively studied in recent years,[3,4] but to our knowledge chemo-enzymatic processes via the fructose-1,6-diphosphate aldolase have not been developed. To date, three fructose-1,6-diphosphate aldolase mediated syntheses of cyclitols have been described in the literature[5–7] but none of the structures

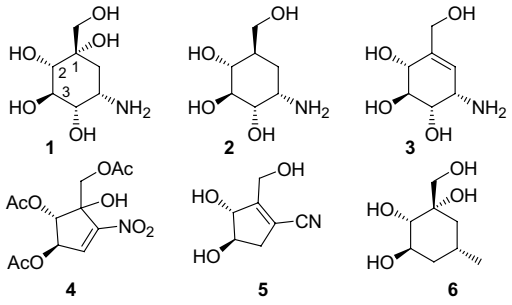

Figure 1.

were directly related to aminocyclohexitols. Wong et al. have synthesized nitrocyclopentitol **4**[5] and cyanopentitol **5**,[6] while Whitesides et al. have synthesized cyclohexitol **6**[7] (Fig. 1).

Herein we report the first one-pot chemo-enzymatic synthesis of nitrocyclohexitol **8** (Fig. 2). In this process, an

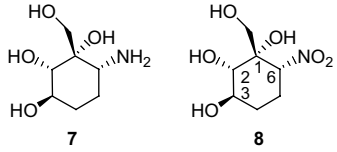

Figure 2.

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doi:10.1016/j.tetasy.2004.06.029

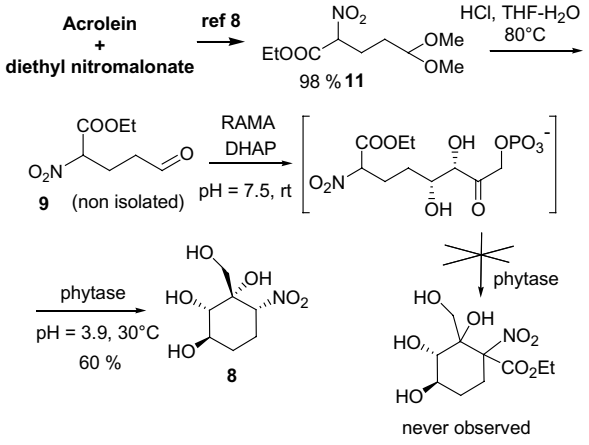
enantiomerically pure nitrocyclitol bearing four asymmetric centers is formed.

To access the target compound, our strategy used the condensation of DHAP catalyzed by fructose-1,6-diphosphate aldolase on a 4-nitroaldehyde. The enzyme controls the configuration of two stereocenters (2 and 3, Fig. 2), forming the C2–C3 bond.

The Henry reaction can take place between the aci-nitro and the carbonyl groups of the intermediate, thus forming the C1–C6 bond. Catalytic reduction of the nitro group yields the desired aminocyclohexitol (Fig. 2). This aminocyclitol **7** can be considered as a valiolamine analogue.

2. Results and discussion

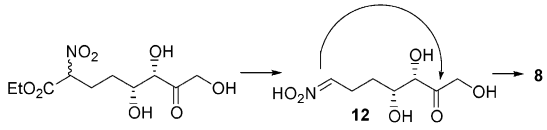
First we studied the reactivity of aldehyde **9** in an aldolase catalyzed condensation with DHAP. The carboxyl ester was assumed to facilitate the Henry reaction by increasing the acidity of the α proton. Accordingly, our synthetic pathway, (Scheme 1), started with the preparation of acetal 11 in two steps from acrolein and diethylnitromalonate in 98% overall yield.[8]



Scheme 1.

Acetal **11** was hydrolyzed to give the nitroester aldehyde **9** (quantitative from TLC). Without isolation, this compound,[9] was treated with rabbit muscle aldolase (RAMA) and DHAP[10] at pH 7.5. After the DHAP was consumed (followed by enzymatic assay),[11] the pH was adjusted to 3.9 and phytase[12] added to hydrolyze the phosphate group. The main product **8** was then isolated in 60% yield (from **11**). To our surprise, the cyclitol bearing the carboxylester group has never been isolated (Scheme 1).

To account for the formation of nitrocyclitol **8**, we hypothesize that the Henry reaction (nitroaldolization) occurs under acidic conditions (pH 3.9) at which acid phytase can operate optimally. Under these conditions, the intermediate probably evolves through a retro-Claisen step to give compound **12** (Scheme 2). An intramolecular cyclization involving the reaction between the newly formed aci-nitro group and the carbonyl function can then take place.



Scheme 2.

The fructose-1,6-dP aldolase is stereoselective and the stereocenter 2 is created with an (*S*)-configuration. Taking this into account, the NOEs observed between the ^{1}H NMR signals of H-2 ($\delta = 3.37$)/H-4ax ($\delta = 1.35$), H-2 ($\delta = 3.37$)/H-6 ($\delta = 4.82$), H-4ax ($\delta = 1.35$)/H-6 ($\delta = 4.82$) and H-5ax ($\delta = 2.46$)/H-3 ($\delta = 3.75$) and the coupling constants ($J_{H\text{-}2ax/H\text{-}3ax}$ 9.5 Hz; $J_{H\text{-}3ax/H\text{-}4ax}$ 12 Hz; $J_{H\text{-}4ax/5ax}$ 13.1 Hz; $J_{H5ax/H\text{-}6}$ 12.9 Hz), the configurations found for compound **8** were (1*S*,2*S*,3*R*,6*R*). All the substituents are in equatorial position on the cyclohexane ring (Fig. 3). The (2*S*,3*R*)-configurations are consistent with the usually observed diastereoselectivity of fructose-1,6-dP aldolase.

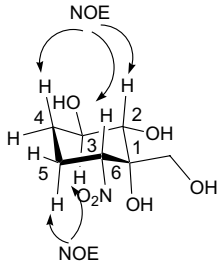
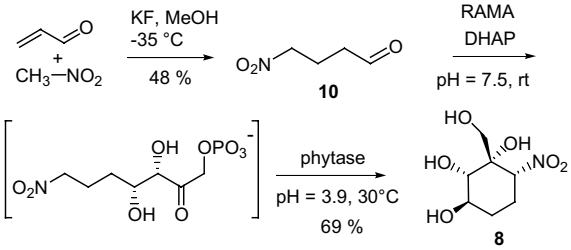


Figure 3.

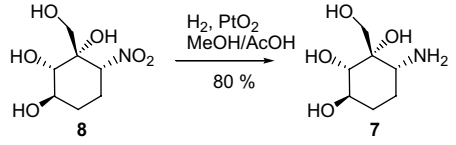
To confirm the intermediate formation of compound **12** before nitroaldolization, we synthesized nitroaldehyde **10**. As this aldehyde is also a RAMA substrate,[13] the same nitrocyclitol should be isolated. The synthesis of **10** has already been described[14] but none of the procedures were reproduced with satisfactory purity (polyalkylation occurred) or yield. We therefore developed our own procedure. Acrolein and excess nitromethane were mixed with KF as a mild base at −35 °C to control the monoalkylation (Scheme 3). On a gram scale, aldehyde **10** was isolated in 48% yield. It was treated with RAMA and DHAP at pH 7.5 with 10% DMSO



Scheme 3.

as co-solvent. After DHAP was consumed, the pH was adjusted to 3.9 and phytase added.

As expected, the same nitrocyclitol **8** was isolated in 69% yield. It is noteworthy, that the carboxyl ester group was not necessary for the intramolecular Henry reaction. As a consequence, the synthesis of **8** was simplified and gave a greater yield, (as aldehyde **10** is a better substrate than aldehyde **9**), in fewer steps. Finally, hydrogenation in the presence of PtO_2 as catalyst gave amine **7** in 80% yield (Scheme 4).



Scheme 4.

Compound **7** was then tested as an enzymatic inhibitor towards five commercially available glycosidases. Probably owing to the modification of the amine position and lack of one hydroxyl group, unlike valiolamine, our aminocyclitol was not active towards the glucosidases tested. It presented only a weak competitive inhibition toward β-galactosidase from *Aspergillus oryzae* ($K_i = 880\,\mu M$). It showed no inhibitory activity at 1 mM concentration towards any of the following enzymes: α-glucosidase from baker's yeast, β-glucosidase from almonds, α-galactosidase from green coffee beans, and α-mannosidase from Jack beans.

3. Conclusion

The work presented herein broadens the scope of the enzymatic aldol reaction with a short efficient synthesis of new nitro- and aminocyclitols. We have opened up a new route to valiolamine analogues, combining in one-pot the formation of two carbon–carbon bonds with high stereoselectivity. Four stereocenters were created and an enantiomerically pure compound isolated. The use of the above strategy for the preparation of aminocyclitols bearing more hydroxyl groups (at the 4 and/or 5 positions) and their inhibition properties is now being investigated.

4. Experimental

4.1. General

All the reagents and solvents were of commercial quality and purchased from Aldrich or Acros. Merck 60 F254 silica gel TLC plates and Merck 60/230-400 and 60/40-63 mesh silica gel for column chromatography were used. Optical rotations were measured with a Jasco Dip-370 polarimeter. IR spectra were recorded on an FT IR Perkin Elmer 881 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer in $CDCl_3$, D_2O, and CD_3OD, J values are given in Hz and δ in ppm. The inhibition constants (K_i) and the type of inhibition (competitive, noncompetitive, mixed) were determined from Hanes–Woolf plots. Fructose-1,6-diphosphate aldolase from rabbit muscle (RAMA; EC 4.1.2.13, suspension in ammonium sulfate) and phytase from *Aspergillus ficuum* (EC 3.1.3.8, crude) were from Sigma.

4.2. Ethyl 2-nitro-5-oxopentanoate 9

To a solution of ethyl 5,5-dimethoxy-2-nitropentanoate **11** (1.5 g, 6.37 mmol) in 18 mL THF/H_2O 1:9 was added 150 μL of concentrated HCl. The mixture was stirred at 80 °C for 3 h (total disappearance detected by TLC). The pH was adjusted to 7 with 3 M NaOH. The THF was then evaporated under vacuum to give 16 mL of a solution of **9**. For ^{13}C NMR, **9** was extracted with acetyl acetate and washed with water and brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum to give a pale yellow oil. ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.4 (C-5), 164.1 (C-1), 62.8 (C-6), 38.9 (C-2), 22.5 (C-4), 20.9 (C-3), 14.0 (C-7).

4.3. 4-Nitrobutanal 10

To a solution of nitromethane (200 mL, 3.7 mol, 50 equiv) and KF (13.83 g, 231.6 mmol, 3.1 equiv) previously dissolved in 100 mL MeOH was added dropwise a solution of acrolein (5 mL, 74 mmol) in 25 mL MeOH for 30 min at −35 °C. After stirring for 1.5 h at −35 °C, the mixture was treated with 500 mL AcOEt, and washed with water (2×200 mL) and brine (200 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated. The product was distilled under vacuum (0.1 mmHg, 100 °C), and 4-nitrobutanal isolated as a colorless oil in 48% yield. ^{13}C NMR (100 MHz, $CDCl_3$) δ 199.7 (C-1), 74.3 (C-4), 34 (C-2), 19.6 (C-3).

4.4. (1*S*,2*S*,3*R*,6*R*)-1-Hydroxymethyl-6-nitrocyclohexane-1,2,3-triol 8

4.4.1. Method 1. To the above solution of aldehyde **9** (16 mL, 6.37 mmol), was added 14.4 mL of DHAP (4.46 mmol). The pH was adjusted to 7.5 with NaOH (1 M). After degassing the solution with argon, 260 U of dialyzed aldolase (RAMA) was added. After 48 h, the mixture was extracted with AcOEt (3×20 mL). The water phase was then adjusted to pH 3.9 with HCl (1 M) and phytase (150 U) added. The mixture was stirred at 30 °C for 24 h. The water was removed under vacuum. Column chromatography (CH_2Cl_2/MeOH 90/10) yielded **8** (554 mg, 60% from DHAP) as a brown solid.

4.4.2. Method 2. Nitrobutanal **10** (400 mg, 3.4 mmol) was added to a solution of DHAP (2.2 mmol, 11 mL) in DMSO (3 mL) and water (20 mL). The pH was adjusted to 7.5 with NaOH (0.1M). After degassing with argon, 105 U of centrifuged RAMA was added. The reaction was stirred at 25 °C for 24 h. The mixture was then washed with AcOEt (1×20 mL) and AcOEt/cyclohexane 50:50 (2×20 mL). The pH was adjusted to 3.9 with HCl (0.1 M), after which 80 U of phytase was added and the reaction then stirred at 30 °C for 24 h.

Water was removed under vacuum. Column chromatography (CH_2Cl_2/MeOH 90/10 then 80/20) yielded **8** (287 mg, 69% from DHAP) as a brown solid. $[\alpha]_D^{25} = +21.2$ (*c* 2.5, MeOH). IR (film) 3367 (OH), 1553 (NO_2) cm^{-1}. ^1H NMR (400 MHz, CD^3OD) δ 4.82 (dd, 1H, H-6, $J_{6ax\text{-}5eq} = 4$, $J_{6ax\text{-}5ax} = 12.9$), 3.83 (d, 1H, H-7a, $J_{7a\text{-}7b} = 11$), 3.75 (ddd, 1H, H-3, $J_{3ax\text{-}4ax} = 12$, $J_{3ax\text{-}2ax} = 9.5$, $J_{3ax\text{-}4eq} = 4.7$), 3.39 (d, 1H, H-2, $J_{2x\text{-}3ax} = 9.5$), 3.35 (d, 1H, H-7b, $J_{7a\text{-}7b} = 11$), 2.44 (dddd, 1H, H-5ax, $J_{5ax\text{-}6ax} = 12.9$, $J_{5ax\text{-}4e} = 3.3$, $J_{5ax\text{-}x} = 13.1$, $J_{5ax-5eq} = 16$), 2.03 (m, 1H, H-5eq), 2.0 (m, 1H, H-4eq), 1.35 (dddd, 1H, H-4ax, $J_{4ax\text{-}4eq} = 16$, $J_{4ax\text{-}5ax} = 13.1$, $J_{4ax\text{-}3ax} = 12$, $J_{4ax\text{-}5} = 3.3$). ^{13}C NMR (100 MHz, CD_3OD) δ 87.2 (C-6), 78 (C-1), 75.7 (C-2), 71.4 (C-3), 62.4 (C-7), 30.3 (C-1), 25.4 (C-4). MS (CI/isobutane) *m/z* 208 $[(M+H)^+]$, 190 $[(MH-H_2O)^+]$. ESI-HRMS calcd for $(M+Na)^+$: 230.0641. Found: 230.0640.

4.5. (1*S*,2*S*,3*R*,6*R*)-6-Amino-1-hydroxymethyl-cyclohexane-1,2,3-triol 7

In a hydrogenation flask, nitrocyclitol **8** (287 mg, 1.4 mmol) was added to a solution of MeOH/AcOH 95:5 (40 mL) containing PtO_2 (85 mg). The mixture was hydrogenated in a Parr apparatus under 50 psi for 48 h at room temperature, and then ultrafiltered and concentrated. The crude amine was purified by cation exchange chromatography (Dowex 50×8, H^+ form, water then 1 M NH_4OH). Amine **7** was isolated in 80% yield (198 mg). $[\alpha]_D^{25} = -5.7$ (*c* 1.4, MeOH). IR (film) 3400–3300 cm^{-1} (OH, NH). ^1H NMR (400 MHz, CD_3OD) δ 3.77 (d AB, 1H, H-7a, J_{app} 11.2), 3.67 (ddd, 1H, H-3, $J_{3\text{-}4eq}$ 4.7, $J_{3\text{-}2}$ 11, $J_{3\text{-}4ax}$ 13), 3.64 (d AB, 1H, H7b, J_{app} 11.2), 3.28 (d, 1H, H-2, $J_{2\text{-}3}$ 11), 3.02 (dd, 1H, H-6, $J_{6\text{-}5eq}$ 5, $J_{6\text{-}5ax}$ 11), 1.92 (m, 1H, H4eq, *J* 4, 7, 10), 1.72 (m, 2H, H5), 1.32 (m, 1H, H4ax, *J* 5, 13, 17). ^{13}C NMR (100 MHz, CD_3OD) δ 75.8 (C-2), 74.9 (C-1), 70.9 (C-3), 64.9 (C-7), 53.9 (C-6), 31.3 (C-4), 25.3 (C-5). MS (CI/methane) *m/z* 178 $[(M+H)^+]$, 160 $[(MH-H_2O)^+]$, 142 $[(MH-2H_2O)^+]$. Anal. Calcd for $C_7H_{15}NO_4$: C, 47.45; H, 8.53; N, 7.90. Found: C, 47.47; H, 8.72; N, 7.64.

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