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Gluconobacter mediated synthesis of amino sugars

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ABSTRACT

An easy and convenient chemo-enzymatic method for preparation of 1-deoxygalactonojirimycin and 1-deoxynojirimycin hydrochlorides has been demonstrated. All the compounds prepared have been characterized by MASS, ^{1}H NMR, ^{13}C NMR and SOR.

1. Introduction

Nojirimycins were first isolated in 1966 from the fermentation broths of several *Streptomyces* strains [1], and are reported to be potent β-glycosidase inhibitors [2]. Their glycosidase inhibitory potential has been realized in the treatment of type II diabetes mellitus [3] and lysosomal storage disorders [4]. 1-Deoxygalactonojirimycin is reported to be a potent galactosidase inhibitor [5]. The deoxynojirimycin family of iminosugars comprises a six-carbon skeleton with different configurations at carbon atoms C-2, C-3, C-4, and C-5. The first synthesis of deoxynojirimycin was reported in 1967 by Paulsen et al. [6]. Since then different diastereomers have been synthesized [7–12]. However all these methods involve long synthetic routes and complex chemical compounds. Considering the potential of 1-deoxygalactonojirimycin as galactosidase inhibitor a simple and efficient method for its preparation would be highly invaluable. Another iminosugar which has been of biological importance is 1-deoxynojirimycin hydrochloride. There are several methods reported for the preparation of 1-deoxynojirmycin [13,14]. Preparation of deoxynojirmycin using *N*-formyl amino sorbitol is the most convenient and efficient of all the reported methods [15]. However preparation of deoxynojirimycin using this route results in the formation of 1-deoxygalactonojirimycin as an impurity in the range of 0.25–0.60%. Herein we developed a chemo-enzymatic method for preparation of 1-deoxygalactonojirimycin hydrochloride. The synthetic approach for preparation of 1-deoxygalctonojirimycin involves conversion of D-galactose to galactamine which is then formylated using methyl formate, oxidized using *Gluconobacter oxydans* DSM 2003, cyclized and reduced using sodium hydroxide, sodium borohydride to give 1-deoxygalactonojirimycin hydrochloride (Scheme 1). The same method has been deployed for preparation of 1-deoxynojirimycin hydrochloride with final purification in 2-methoxy ethanol to yield the compound with purity greater than 99.5%. *G. oxydans* DSM 2003 cell paste can be prepared by a method given in literature [16] and they are reported to carry out several biotransformation reactions [17–22]

2. Experimental

2.1. Analytical methods

2.1.1. HPLC

2.1.1.1. HPLC method for isolated 1-amino-1-deoxy-galactitol. High performance liquid chromatography analysis was performed on Waters alliance 2695 high performance liquid chromatography instrument connected with 2414 refractive index detector using synergi polar RP 80A column (4.0 μm particle size, 250 mm × 4.6 mm length) eluted with isocratic mobile phase

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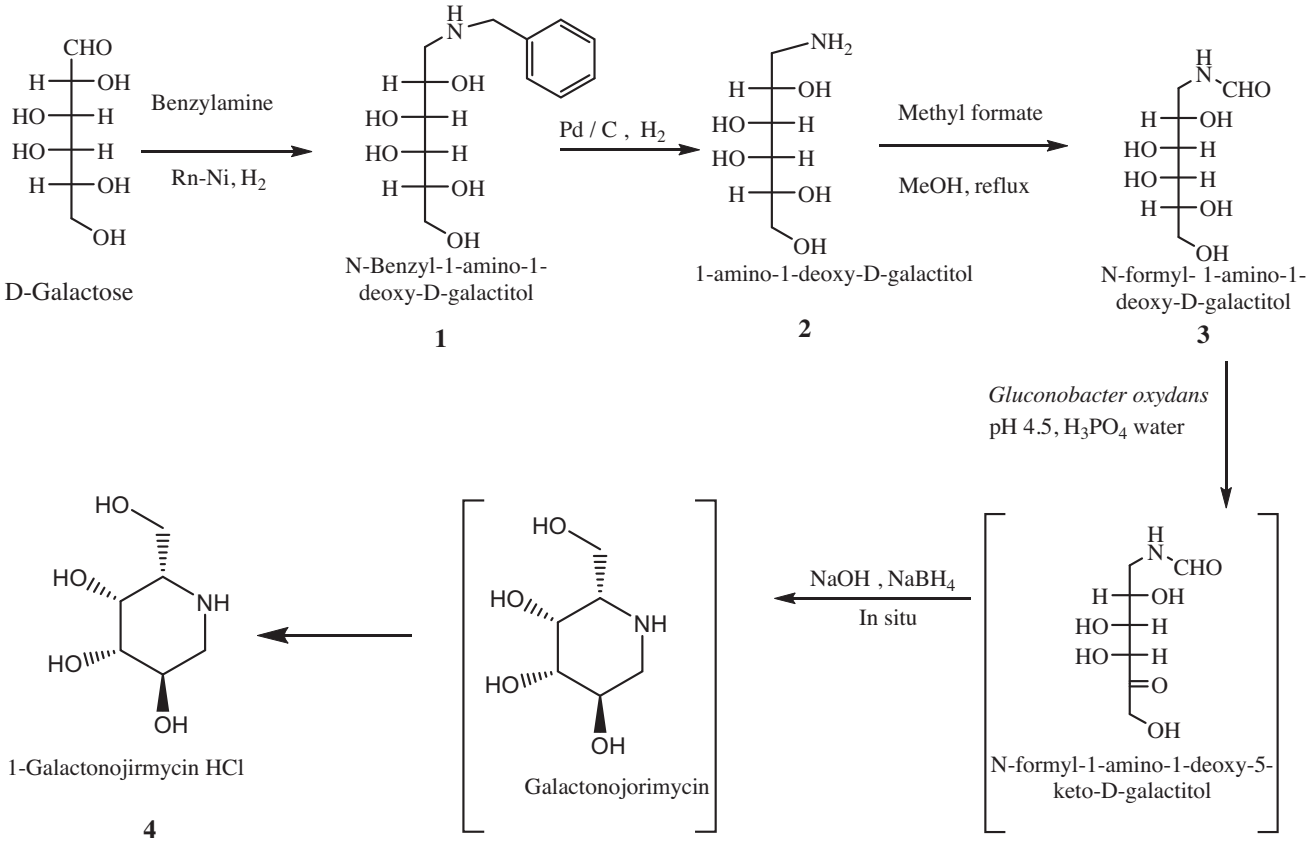

Scheme 1. Synthetic scheme for preparation of 1-deoxygalactonojirimycin hydrochloride.

system 60% acetonitrile in 0.154% ammonium acetate solution at a flow rate of 0.5 mL/min with column oven temperature at 40 °C and detector oven temperature 40 °C. The retention time of both 1-amino-1-deoxy-1-galactitol and 1-amino-1-deoxy-glucitol was found to be 8.51 min.

2.1.1.2. HPLC method for monitoring the reaction towards the formation of N-benzyl-1-amino-1-deoxy-galactitol. High performance liquid chromatography analysis was performed on Waters alliance 2695 high performance liquid chromatography instrument connected with 2414 refractive index detector using Inertsil amino column (5.0 μm particle size, 250 mm × 4.6 mm length) eluted with isocratic mobile phase system containing 25:75 (v/v) 0.272% potassium di hydrogen phosphate pH 6.5 and acetonitrile at a flow rate of 1.0 mL/min with column oven temperature at 40 °C and detector oven temperature 40 °C. The retention time of both N-benzylated-1-amino-1-deoxy-1-galactitol and N-benzyl-1-amino-1-deoxy-glucitol was found to be 22.34 min.

2.1.1.3. HPLC method for monitoring the reaction towards the formation of N-formyl 1-amino-1-deoxy-galactitol. High performance liquid chromatography analysis was performed on Waters alliance 2695 High performance liquid chromatography instrument connected with 2414 refractive index detector using synergi polar RP 80A column (4.0 μm particle size, 250 mm × 4.6 mm length) eluted with isocratic mobile phase system 60% acetonitrile in 0.154% ammonium acetate solution at a flow rate of 0.5 mL/min with column oven temperature at 40 °C and detector oven temperature 40 °C. The retention time of N-formyl 1-amino-1-deoxy-glucitol and N-formyl 1-amino-1-deoxy-galactitol was found to be 4.7 min.

2.1.1.4. HPLC method for monitoring the reaction towards deoxynojirimycin hydrochloride/deoxygalactonojirimycin. High performance liquid chromatography analysis was performed on Waters alliance 2695 High performance liquid chromatography instrument connected with 2487 UV detector using Insertil Amino column (5.0 μm particle size, 250 mm × 4.6 mm length) eluted with isocratic mobile phase system 70% acetonitrile in 0.154% ammonium acetate solution at a flow rate of 1.2 mL/min with column oven temperature at 40 °C and detector oven temperature 40 °C. This method was used for 1-deoxynojirimycin, 1-deoxynojirimycin HCl and 1-deoxygalactonojirimycin HCl. The retention time of deoxynojirimycin, deoxynojirimycin HCl was found to be about 10 min. The retention time of 1-deoxygalactonojirimycin was found to be about 15 min.

2.1.2. NMR spectroscopy

The ^{1}H NMR and ^{13}C NMR spectra was recorded in DMSO-d_6 on a Bruker Avance 300 spectrometer. The chemical shifts are reported in δ ppm relative to TMS (δ 0.00) and DMSO-d_6 as internal standards respectively.

2.1.3. Mass spectrometry

Electron Spray Ionization-Mass spectra (ESI-MS) of isolated compounds were measured using Agilent 1100 LC/MSD Trap SL instrument.

2.1.4. Infra-red spectroscopy

IR spectra of the isolated compounds were measure using 1% KBr pellet method in PERKIN ELMER FT-IR spectrometer.