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Note

# Enzymatic synthesis of D-glucosaminic acid from D-glucosamine

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**Abstract**—D-Glucosaminic acid (2-amino-2-deoxy-D-gluconic acid), a component of bacterial lipopolysaccharides and a chiral synthon, is easily prepared on a multigram scale by air oxidation of D-glucosamine (2-amino-2-deoxy-D-glucose) catalysed by glucose oxidase.


*Keywords:* D-Glucosaminic acid; D-Glucosamine; Glucose oxidase

D-Glucosaminic acid (2-amino-2-deoxy-D-gluconic acid, **1**) has recently been identified as an unusual component of *Rhizobium leguminosarum* lipopolysaccharide[1] (in most other bacterial species, D-glucosamine is present in these structures). The biosynthesis of this amino acid is being studied, and most probably involves oxidation of D-glucosamine (2-amino-2-deoxy-D-glucose) by a specific membrane oxidase.[2]

D-Glucosaminic acid is also a member of the 'chiral pool' and has been used as a starting material for the asymmetric synthesis of various amino acids: 4,5,6-trihydroxynorleucine,[3] L-β-hydroxyhistidine,[4] and several glycosidase inhibitors, like (+)-castanospermine, (+)-6-epicastanospermine,[5] (+)-2-epideoxymannojirimycine[6] and a polyhydroxypyrrolidine.[7]

The properties of **1** as a cation coordinating agent has also been widely studied.[8–10] Subsequently, it has been investigated for potential use in the chromatography of charged biomolecules[11] and as a complex with platinum for cancer therapy.[12]

The classical synthesis of **1** from D-glucosamine involves the use of mercuric acetate as an oxidant, followed by a treatment with hydrogen sulfide, in a totally non-ecological process.[13] We propose here a new efficient, facile and environmentally safe preparation of D-glucosaminic acid by use of molecular oxygen as an oxidant and glucose oxidase as a catalyst.

Glucose oxidase (EC 1.1.3.4) from *Aspergillus niger* has been considered to be a very specific enzyme, acting on glucose as its only substrate. For that reason, it is widely used in commercial kits for routine enzymatic determination of glucose in biological fluids.[14] This analytical utilisation needs only relatively small amounts of enzyme. The production of glucose oxidase on the industrial scale now permits its use in preparation of hundreds of kilograms of gluconic acid from glucose for use in the food industry.[15] Chemical and enzymatic preparation of aldonic acids has been recently reviewed.[16,17]

It was established a long time ago that glucose oxidase accepts D-glucosamine only as a poor substrate, with a maximal catalytic efficiency of 2% as compared to that on D-glucose.[18] However, by using larger amounts of enzyme and prolonged reaction times, we are able to prepare several grams of D-glucosaminic acid from D-glucosamine hydrochloride in good yield (Scheme 1).

Catalase (EC 1.11.1.6) was added to the reaction medium in order to decompose the hydrogen peroxide formed as a byproduct, which is known to have a deleterious effect on glucose oxidase. The pH was maintained constant at pH 7 by continuous addition of sodium hydroxide, preferably by use of a pH-stat. Not surprisingly, the reaction rate was pH dependent,

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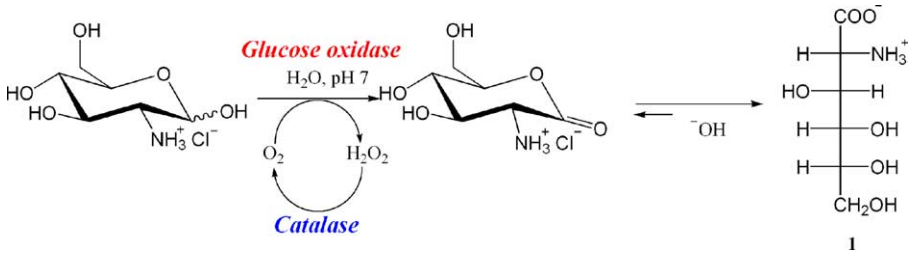


**Scheme 1.** Synthesis of D-glucosaminic acid from D-glucosamine catalysed by glucose oxidase and catalase.

indicating that the actual substrate of glucose oxidase must be the free amine (Fig. 1). However, to avoid degradation of D-glucosamine by too alkaline a medium, and to work within the range of optimal pH of glucose oxidase (known to be pH 5–7), we chose to limit the pH at 7. The theoretical amount of sodium hydroxide was consumed after 72 h at room temperature, and the reaction mixture appeared milky, as a result of **1** having partially precipitated. After concentration and cooling, pure **1** was collected by a simple filtration in a yield of 76% relative to the starting D-glucosamine. A complete recovery of the product could be obtained by percolating the reaction medium through a column of Dowex 50 [pyridinium$^+$], followed by passage through a column of Dowex 1 [AcO$^-$], followed by evaporation of the aqueous mixture.

In conclusion, we devised a very simple preparative synthesis of D-glucosaminic acid from D-glucosamine, taking advantage of the availability of a cheap and robust industrial enzyme.

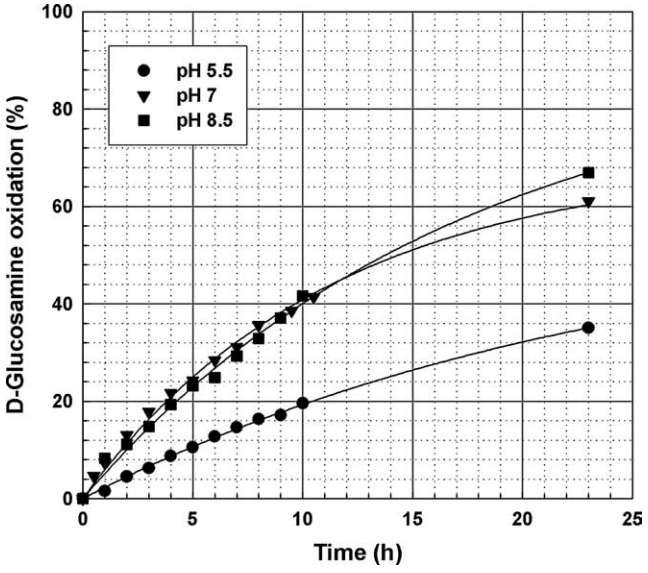


**Figure 1.** pH dependence of D-glucosamine oxidation catalysed by glucose oxidase and catalase.

## 1. Experimental

### 1.1. Enzymes

Glucose oxidase from *A. niger* (Gluzyme®, from Novozymes) had a specific activity of 2 U/mg. Catalase (Catazyme®, from Novozymes) had a specific activity of 25 kU/mL.

### 1.2. Procedure

D-Glucosamine hydrochloride (3 g, 14 mmol) was dissolved in 30 mL of water, and the pH was adjusted to 7 with 1 M NaOH. Glucose oxidase (250 mg, 500 U) and 1 mL of catalase (25 kU) were added. The mixture was vigorously stirred under air, and the pH was kept constant at 7 by means of a pH-stat adding continuously 1 M NaOH. After 72 h, the mixture was concentrated under reduced pressure to about 10 mL and cooled in ice, and the D-glucosaminic acid that formed was recovered by filtration (2.07 g, 76%); mp 260 °C (dec), $[\alpha]_D$ −15 (*c* 4, 2.5% aq HCl); lit: $[\alpha]_D$ −14 (*c* 1.64, 2.5% aq HCl),[19] $^{13}$C NMR (200 MHz, $D_2O$): $\delta$ 173.49, 73.52, 71.59, 68.1, 63.57, 59.13; lit: $\delta$ 173.5, 73.7, 71.7, 68.2, 63.7, 59.3.[20]

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