Short Communication

Original and Efficient Synthesis of D-Cycloserine

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A simple pathway for the preparation of D-cycloserine is presented. The intermediates and D-cycloserine were characterized by FT-IR, ^{1}H-NMR spectra and elemental analysis. D-Cycloserine can inhibit the growth of *Mycobacterium tuberculosis* and can be used as a second-line drug for the treatment of tuberculosis, especially for the use in developing countries.

Keywords: D-Cycloserine / Hydroxylamine hydrochloride / D-Serine / Synthesis / Thionyl chloride

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Introduction

D-Cycloserine or D-4-amino-3-isoxazolidinone (Fig. 1a) is an analogue of the amino acid D-alanine; it inhibits the enzymes D-alanine racemase and D-alanine synthetase [1, 2]. The most important property of D-cycloserine is the inhibition of the growth of *Mycobacterium tuberculosis*. In general, it is more effective against Gram-positive than Gram-negative bacteria [3]. However, it is seldom used in the management of tuberculosis due to the toxicity associated with effective dosages. Nevertheless, the worldwide resurgence of tuberculosis, the emergence of multiply drug-resistant tuberculosis, and the problematic use of available drugs required to treat these infections, have resulted in the application of D-cycloserine as a second-line drug for the treatment of tuberculosis [4].

Numerous studies have been focused on the synthesis of the D-cycloserine. It can be produced by *Streptomyces garyphalus* and *Streptomyces orchidaceus* [5–7]. But this drug can also be obtained by chemical synthesis [6, 8, 9].

Our aim was to prepare D-cycloserine by an original and efficient method. By using this method, the synthetic route for D-cycloserine was shorter and had a good yield.

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Results and discussion

In this paper, D-cycloserine was obtained by using an original and efficient method outlined in Scheme 1. D-2-Amino-3-chloropropionic acid chloride **1** was prepared by the reaction of 2.5 equiv. excess of thionyl chloride and 1 equiv. of D-serine (Fig. 1b) in chloroform at room temperature for 2 h and under reflux for 4 h. This pathway is an original part of our research. Using this method, the chlorination and chloroformylation reaction of D-serine can synchronously take place. Meanwhile, the amidogen of D-serine was protected by the dry HCl.

D-2-Amino-3-chloropropionohydroxamic acid **2** was obtained by the reaction of 1.2 equiv. excess of hydroxylamine hydrochloride and 1 equiv. of intermediate **1** with a yield of 86% for intermediate **2**, much higher than in methods reported previously.

During the experiment, we found that the temperature, the volume of the water, and the pH were very important for the last step. If the temperature is low, the reaction speed was very slow, especially at the end of the reaction. However, if the temperature is high, not only the hydroxylamine hydrochloride easily decomposed, but also the intermediate **2** was easy hydrolyzed or eliminated from the reaction. Though hydroxylamine hydrochloride is unstable in water, using water as the reaction solvent was feasible as can be demonstrated by the characterizing result of the product. However, the volume of the water was to be observed critically. Because the hydroxylamine hydrochloride is an alkalescent base, the pH of the mixture should be controlled in the appropriate range. After repeating the experiment, the best reaction conditions are described in this paper.

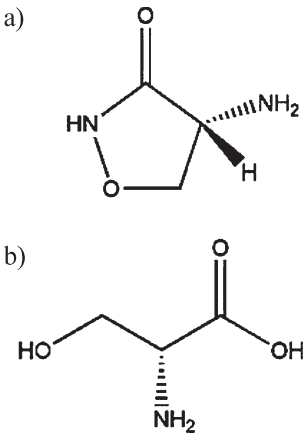


Figure 1. Chemical structure of: (a) D-cycloserine and (b) D-serine.

Conclusion

An original and efficient method for the synthesis of D-cycloserine is reported. Our synthesis is a very simple two-step procedure; all intermediates are stable, readily crystalline compounds and the yields are very satisfactory.

Experimental

Material and physical measurements

All reagents were obtained from commercial suppliers and were used without further purification. Solvents used for the reactions were purified and dried by conventional methods.

Melting points were taken on a SGW X-4 melting point apparatus (Jinghe, Shanghai, China). ^{1}H-NMR spectra was obtained at 400 MHz using a Bruker Avance 400 NMR (Bruker Bioscience, USA) with tetramethylsilane (TMS, $\delta = 0$ ppm) as internal standard. The infrared spectra were determined on a Bruker Tensor 27 FT-IR spectrometer (Bruker, Ettlingen, Germany) using KBr pellets. Elemental analysis was performed by Heraeus Rapid Analyser (Heraeus, Germany).

Synthesis

D-2-Amino-3-chloropropionic acid chloride ***1***

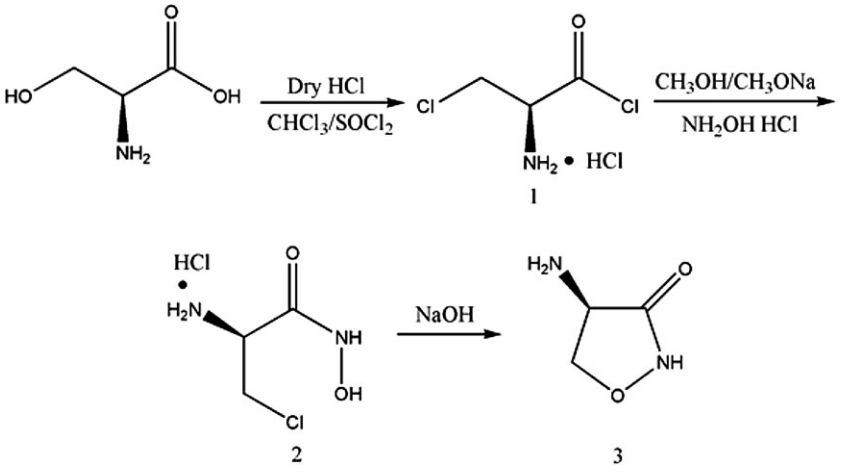
D-Serine (2.10 g, 0.02 mol) was dissolved in chloroform (40 mL) and dry HCl was added to the mixture for 10 min. Then, the mixture was cooled to 0°C and thionyl chloride (5.95 g, 0.05 mol) was added dropwise into the mixture within 1 h. The mixture was stirred at room temperature for 2 h and then under reflux for 4 h. The reaction mixture was cooled subsequently and the solid was removed by filtration. The solid was washed with chloroform and dried *in vacuo*. Then, a white powder (3.21 g) was obtained. Yield: 90%; m. p.: 128–130°C; IR (KBr, cm^{-1}): 3445, 2643, 1750, 1518, 1250, 792; ^{1}H-NMR (400 MHz, D_2O) δ: 4.30 (m, 1H, –CH–), 4.11 (m, 1H, $J = 12.6$ Hz, $-CH_2-$), 4.01 (m, 1H, $J = 12.6$ Hz, $-CH_2-$) ppm. Anal. calcd. for $C_3H_6NOCl_3$: C, 20.19; H, 3.39; N, 7.85. Found: C, 20.18; H, 3.41; N, 3.36.

D-2-Amino-3-chloropropionohydroxamic acid ***2***

To a cooled solution of hydroxylamine hydrochloride (2.08 g, 0.03 mol) in absolute ethanol (20 mL) 20 mL of 0.91 N sodium methoxide (0.03 mol) in methanol were added. The mixture was cooled to 0°C, the sodium chloride was filtered, and a solution intermediate **1** (3.57 g, 0.02 mol) in absolute ethanol (20 mL) was added to the filtrate. This solution was cooled to 0°C and 15 mL of 0.91 N sodium methoxide (0.03 mol) were added slowly. The solution was concentrated *in vacuo* at room temperature to about 45 mL. The water (20 mL) was added to the solution, 40 mL of 0.46 N hydrochloric acid (0.02 mol) were added dropwise and the product was crystallized. The product was filtered and dried *in vacuo*, then 2.94 g solid was obtained. Yield: 84%; m. p.: 191–193°C; IR (KBr, cm^{-1}): 3445 2643, 1750, 1540, 1518, 1250, 792; ^{1}H-NMR (400 MHz, D_2O) δ: 4.30 (d, 1H, –CH–), 4.11 (d, 1H, $J = 12.6$ Hz, $-CH_2-$), 4.01 (s, 1H, $J = 12.6$ Hz, $-CH_2-$) ppm. Anal. calcd. for $C_3H_8N_2O_2Cl_2$: C, 20.59; H, 4.61; N, 16.01. Found: C, 20.56; H, 4.62; N, 16.00.

D-Cycloserine or D-4-amino-3-isoxazolidone ***3***

A solution of 3.5 g (0.02 mol) of intermediate **2** in 20 mL of water was stirred and cooled. The mixture's pH was adjusted to 11 by an aqueous sodium hydroxide solution (30% wt). The mixture was stirred at 25°C for 2 h. Then 50 mL of 1:1



Scheme 1. Synthesis of D-cycloserine.

ethanol/isopropyl alcohol was added. The precipitated salts were filtered and the filtrate was cooled to 5°C. To the cold well-stirred solution sufficient glacial acetic acid was added dropwise over a 35-min period to bring the alcoholic solution to pH 6.0. The crystalline precipitate was filtered, washed twice with 1:1 ethanol/isopropyl alcohol and twice with ether. Then, 1.02 g colorless crystals of D-4-amino-3-isoxazolidone were obtained. Yield: 50%; m. p.: 153–155°C; FT-IR (KBr, cm^{-1}): 3445, 2950, 1630, 1405; ^{1}H-NMR (400 MHz, D_2O) δ: 3.75 (m, 1H, $-CH_2-$), 3.77 (m, 1H, $-CH_2-$), 4.42 (m, 1H, $-CH-$) ppm. Anal. calcd. for $C_3H_6N_2O_2$: C, 35.29; H, 5.92; N, 27.45. Found: C, 35.31; H, 5.91; N, 27.42.

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