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Journal of Molecular Catalysis B: Enzymatic 1 (1996) 161–164

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

Enzymatic synthesis of 2-amino-3-hydroxy-1,6-hexanedicarboxylic acid using serinehydroxymethyltransferase

M.J. Miller *, S.K. Richardson

Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556, USA

Received 27 September 1995; accepted 4 October 1995

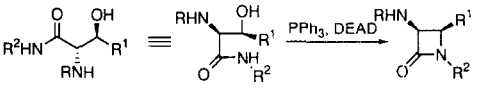
Abstract

The scale up of earlier work from these laboratories using the enzyme serinehydroxymethyltransferase has resulted in the use of this enzyme for the synthesis of 2-amino-3-hydroxy-1,6-hexanedicarboxylic acid (**7**) on a preparative scale. This compound, which has been barely described in the literature, is potentially useful for the synthesis of carbocyclic β-lactams and carbocyclic nucleosides.

Keywords: Serinehydroxymethyltransferase; Amino acids; Aldol reaction; β-Lactams

1. Introduction

Among the known procedures for the preparation of azetidinones, ring closure from N-1 to C-4 has attracted significant interest [1] mimicking, as it does, the biosynthetic route to penicillins [2]. Recently, it has been demonstrated that β-hydroxy amides [3,4], β-hydroxy acylhydrazines [5] and β-hydroxy hydroxamic acids [6] can undergo just such a conversion, under Mitsunobu conditions, to give β-lactams via intramolecular nucleophilic attack at the C-4 position (Eq. 1).



(1)

A number of published examples indicate that this is an efficient procedure which tolerates additional functionality at the α and more remote positions. In order to fully exploit this transformation for the synthesis of β-lactams, an efficient, stereoselective synthesis of L-*erythro* α-amino β-hydroxycarboxylic acids is required. One approach, reported from these

* Corresponding author. Tel: (+1-219)6317571, Fax: (+1-219)6316652, e-mail: marvin.j.miller.2@nd.edu

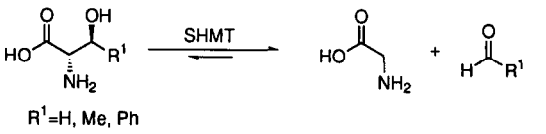
SSDI 1381-1177(95)00022-4

laboratories, which successfully fulfills this criterion, albeit in racemic form, relies on the aldol reaction between an oxazolinone protected glycine equivalent and an appropriate aldehyde. The product (**3**) has been further elaborated to a carbacephalosporin (Scheme 1) [7,6]. Although the potential of this route and of the adduct (**3**) for the synthesis of β-lactams and carbocyclic nucleoesides [8] is obvious, the development of a procedure requiring the use of fewer protecting groups would be a significant improvement.

2. Results and discussion

Unlike many chemosynthetic reactions, enzyme catalysed reactions occur under neutral conditions with naturally occurring, often commercially available, substrates to give multifunctional products without the need for protecting groups. Many enzymes have been shown to be capable of accepting substrates other than those required for physiological reasons, and the number of enzymes which can be used by the organic chemist on a preparative scale is increasing rapidly [9–11]. Thus, an enzyme synthesis of α-amino β-hydroxycarboxylic acids capable of producing compounds analogous to (**3**), would have the advantage of occurring under mild conditions and eliminate some of the protection/deprotection steps.

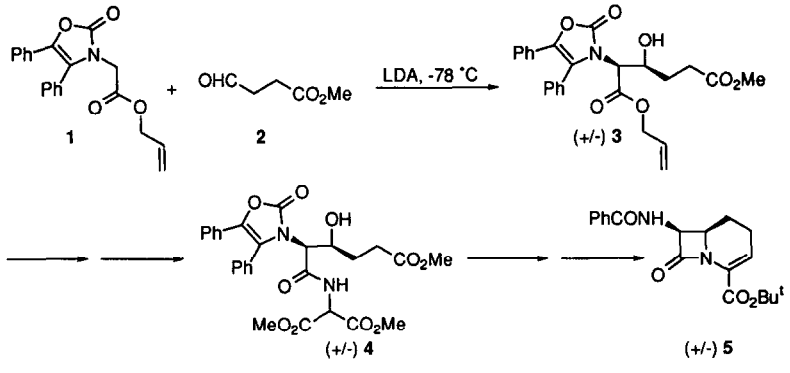
One enzyme with the potential to assist in achieving this objective is serinehydroxymethyltransferase (SHMT) which is found in a variety of bacteria, plants and higher organisms, and whose biological role is the interconversion of serine to glycine and formaldehyde (Eq. 2) [12].



(2)

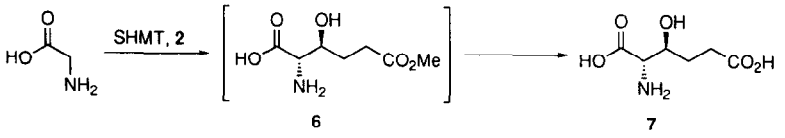
Other equilibria, e.g. that between acetaldehyde and threonine and between benzaldehyde and β-phenylserine (Eq. 2), are also established in the presence of SHMT indicating some tolerance for larger groups at the β position. However, for our purposes it is important to note that, under normal conditons, the equilibrium for SHMT favours the retroaldol reaction. As might be expected for an enzyme catalysed reaction, there is usually a high level of stereoselectivity for a single isomer of the α-amino β-hydroxycarboxylic acid. Thus, the rate of the retroaldol reaction of L-*allo* threonine is appreciably faster than that for any of the other threonine isomers in the presence of SHMT. This makes the use of this enzyme particularly attractive for our work as an enantioselective synthesis of the biologically active configuration of the β-lactams requires the L-*erythro* isomer of the precursor α-amino β-hydroxycarboxylic acids (Eq. 1 cf. Eq. 2).

A prior publication from these laboratories



Scheme 1.

has, in fact, already extended the range of aldehydes known to act as substrates for SHMT, and also showed that the SHMT catalysed aldol reaction between glycine and the aldehyde (**2**) can be used to prepare (**6**) stereoselectively (Eq. 3).



6 7

(3)

Under the reaction conditions (**6**), a highly simplified analogue of the adduct (**3**) used earlier (Scheme 1), was transformed to a more polar species believed to be diacid (**7**) [13,7]. More recently, we have demonstrated that the yield of (**7**) is apparently enhanced in the presence of DMSO as a cosolvent [14]. These earlier studies were performed on an analytical scale and the formation of the intermediate (**6**) confirmed by comparison with an authentic sample prepared chemosynthetically [13,7]. This publication describes the initial progress in our attempts to scale up this reaction, and the first isolation and characterisation of the product (**7**).

The procedure used earlier for the analytical scale reaction was repeated, with appropriate scale up, as described in the experimental section and in the previous report [13,7]. After a total of 35 h at 30°C, HPLC analysis of the crude reaction mixture indicated that none of the initial product (**6**) remained and the reaction mixture was composed of a mixture of glycine and the more polar product (**7**) (60% of the final reaction mixture). This solution was immediately lyophilised for storage.

Preliminary experiments had indicated that attempts to purify the enzyme mixture using a strongly basic anion ion exchange resin had failed to effect significant separation between glycine and (**7**). This was attributed to the presence of a large concentration of phosphate buffer which overwhelms the capacity of the resin to separate products of similar pK_a. Attempts to overcome this problem by precipitation of at least some of the phosphate with barium were not immediately successful. Fortunately, changing to a less basic resin (BioRad AG3X-4A) appeared to enhance the resolution between the two compounds and a product was obtained, analysis of which by ^{1}H NMR indicated that it was a 10:1 mixture of (**7**) and glycine. The authenticity of (**7**) was confirmed by a combination of spectral techniques with no evidence for the presence of other diastereoisomers.

In conclusion, we have achieved a synthesis of the title compound (**7**) in unprotected form from glycine and the readily available aldehyde (**2**). Although, as yet, the isolated yield is not as high as indicated by HPLC analysis of the crude reaction mixture, it is anticipated that improvements in the efficiency of the isolation will overcome this. In addition, attempts to increase the conversion by the use of DMSO as a cosolvent [14] or by the use of excess glycine as has been reported recently [15–17], are logical extensions of this project and will be the result of further studies.

3. Experimental section

Reagents were commercially available unless otherwise stated and were used as received without further purification. Rabbit liver SHMT, cloned into *Escherichia coli*, was supplied by Eli Lilly and Co., Indianapolis. Methyl succinate semialdehyde (**2**) was prepared according to a previously published procedure [6]. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Plus 300 spectrometer in D2O using

dioxan as the internal reference. Mass spectra were obtained using AEI Scientific Apparatus MS 902, Du Pont DP 102 and Finnigan MAT Model 8430 spectrometers. HPLC was performed on an ISCO HPLC system (pump model no. 2350, gradient programmer model no. 2360 and fluorescence detector model no. FL-2) using a C-18 reverse phase column. Flow rates, eluent gradients and derivatisation procedures were as previously described [13].

3.1. 2-Amino-3-hydroxy-1,6-hexanedicaboxylic acid (7)

A mixture of SHMT (45 mg), glycine (230 mg. 17 mM), **2** (370 mg, 17 mM) and pyridoxyl 5-phosphate monohydrate (4 mg, 80 mM) were dissolved in phosphate buffer (185 ml, pH = 7.3, 10 mM) and incubated at 30°C for 35 h in the dark. At the end of that time the solution was lyophilised. BioRad AG3X-4A (120 g) ion exchange resin was prepared as follows: wash with water until the eluent was clear, 0.5 M HCl for 30 min, water until the eluent was neutral, 0.5 M NaOH for 30 min and then water until the eluent was neutral. The lyophilised reaction mixture was dissolved in a minimal amount of water and loaded onto the resin which was eluted with water (500 ml) then acetic acid (500 ml at 0.02 M, then the same volume in 0.02 increments from 0.02 M to 0.16 M and 0.05 M increments from 0.2 M to 0.55 M) collecting 125–250 ml fractions. Those fractions enriched in the product **7** (0.35–0.45 M AcOH) were lyophilised to give 35 mg of (**7**) (containing 10% glycine). ^{1}H NMR δ ppm 3.86–3.92 (m, ^{1}H, C*H*OH), 3.64 (d J = 6.5 Hz, 1H, C*H*NH_2, 2.19–2.38 (m, 2H, CH_2CHOH), 1.57–1.64 (m, 2H, CH_2CO_2H). ^{13}C NMR 28.83, 31.20, 59.29, 66.50, 171.45, 178.77. HRMS calcd for $C_6H_{12}NO_5$ (MH^+) 178.0715, found 178.0753.

Acknowledgements

We gratefully acknowledge the NIH for financial support and Eli Lilly and Co., Indianapolis for additional funding and a generous gift of SHMT. The use of the NMR facilities at the Lizzadro Magnetic Research Center at the University of Notre Dame is appreciated. We also thank John Belletini for assistance in acquiring NMR spectra.

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