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Chemoenzymatic One-pot Synthesis of Cefazolin from Cephalosporin C in Fully Aqueous Medium, Involving Three Consecutive Biotransformations Catalyzed by D-Aminoacid Oxidase, Glutaryl Acylase and Penicillin G Acylase

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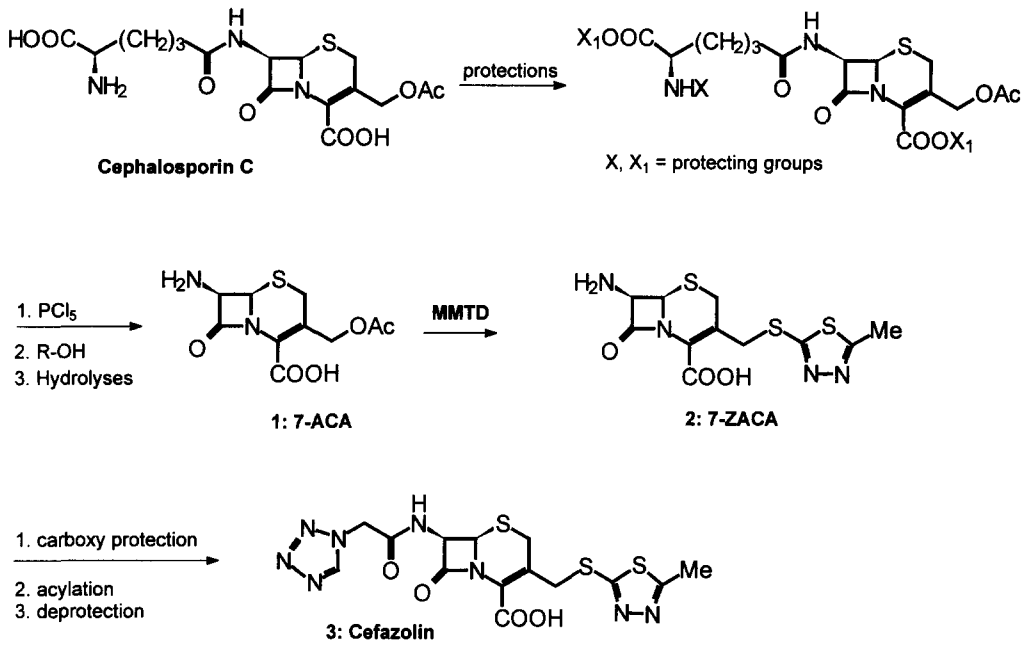
Abstract: A new chemoenzymatic synthesis of Cefazolin through the correct assembly of three biotransformations catalysed by D-aminoacid oxidase, glutaryl acylase and penicillin G acylase is described. This multienzymatic synthesis has been performed from the natural Cephalosporin C in fully aqueous medium without intermediate purification stages. Almost quantitative yields have been achieved in all the enzymatic reactions. © 1997 Elsevier Science Ltd.

Cefazolin **3** is a β-lactamic antibiotic of clinical relevance,[1] currently synthesized[2,3] from Cephalosporin C. The chemical cleavage of C 7-side chain gives 7-aminocephalosporanic acid **1** (7-ACA), which is reacted with 2-mercapto-5-methylthiadiazole (MMTD) to give the 7-amino-3-(5-methyl-1,3,4-thiadiazol-2-yl)thiomethyl-3-cephem-4-carboxilic acid **2** (7-ZACA).[3] The final chemical acylation of the 7-amino group gives Cefazolin **3** (Scheme 1). This synthesis presents some drawbacks: the high sensitivity of the β-lactamic ring to acid and alkaline media requires the use of easily removable protecting groups and very low reaction temperature during the acylation (-40°C), while the drastic reaction condition required in the 3'-acetoxy displacement with MMTD cause partial hydrolysis of β-lactamic ring resulting in reduced yield. According to **scheme 1** two intermediate purification stages are required to separate the 7-ACA **1** and the β-lactamic nucleus **2** from by-products formed during the chemical deacylation of Cephalosporin C and the 3'-acetoxy displacement. Moreover the use of toxic reagents for the chemical activation of the acyl donor carboxylic group, and the use of chlorinated solvents, pose problems of environmental impact.

In order to avoid the above mentioned problems, the use of enzymatic catalysts such as D-aminoacid oxidase (DAO) and glutaryl acylase (GA) for Cephalosporin C hydrolysis,[4] and penicillin G acylase (PGA), for β-lactamic nuclei acylations,[5] could be considered of great interest.

Scheme 1



This letter reports a new chemoenzymatic synthesis of Cefazolin **3** from the natural Cephalosporin C, through the "*correct assembly*" of three biotransformations in full aqueous medium without intermediate purification stages. This "*one pot*" synthesis has been performed by enzymatic deacylation of Cephalosporin C, catalyzed by DAO and GA, and further PGA catalyzed acylation of 7-ACA **1**. Almost quantitative yields have been achieved in all the enzymatic reactions performed, and the obtained 7-[(1*H*-tetrazol-1-yl)-acetamido]-3-acetoxymethyl-Δ^3-cephem-4-carboxilic acid **5** was then used as an intermediate for Cefazolin **3** synthesis by 3'-acetoxy group displacement with MMTD (Scheme 2).

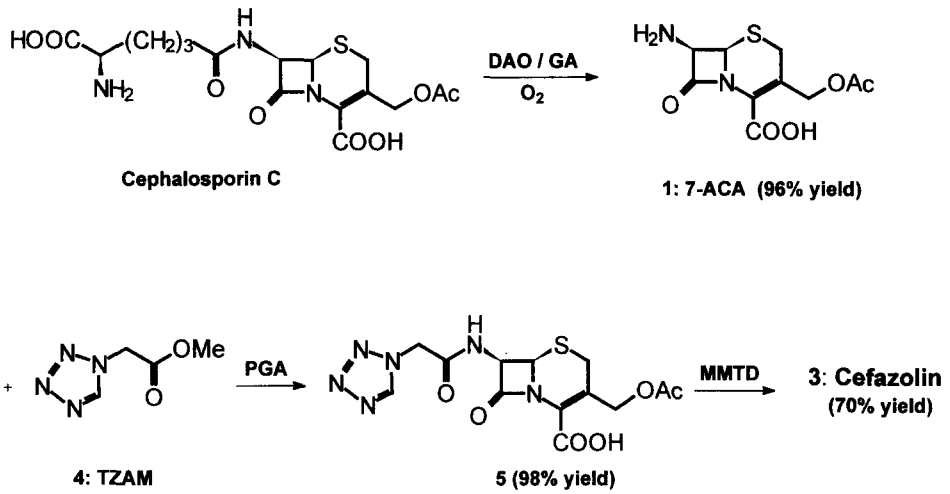
The enzymatic deacylation of Cephalosporin C was carried out in the same reaction vessel by the simultaneous use of immobilized DAO from *Trigonopsis variabilis* and GA from *Acetobacter* sp, under a continuous flow of O_2. DAO is known[4] to catalyze the oxidative deamination of the α-aminoadipic side chain of Cephalosporin C to give the α-ketoadipic derivative. The decarboxylation in the presence of O_2 gives the glutaryl analogous, which is then deacylated by GA to obtain 7-ACA **1**.

By using a pH value of 8 the high activity and stability of the enzyme derivatives employed allowed the transformation of high concentration (50 mM) of Cephalosporine C into 7-ACA **1** (96% yield) in only 2.5 hours.

The PGA catalyzed acylation, to obtain compound **5**, was directly performed on the solution of the crude 7-ACA **1** (50 mM), obtained by simple filtration of the enzyme derivatives of DAO and GA. Almost quantitative yields were obtained carrying out the reaction at pH 6.5 and 4°C, using the immobilized PGA isolated from a mutated strain of *Escherichia Coli* ATCC 11105[6] and a 3:1 molar ratio of tetrazolylacetic acid methyl ester **4** (TZAM).

Final displacement of the 3-acetoxy group with MMTD was directly performed heating to 65°C the aqueous solution of crude **5** obtained by filtration of the PGA enzyme derivative. This step was not optimized and Cefazolin **3** was isolated in 70% yield.

Scheme 2



This synthetic approach presents several practical advantages if compared with conventional chemical processes. The use of toxic reagents and chlorinated solvents is avoided, while the substrate specificity and chemoselectivity of the ustilized enzymes make unnecessary any reactive groups protections and reduce the purification stages required. The substrate specificity of PGA allowed the acylation of 7-ACA **1** with TZAM **4** to be performed avoiding the purification from the glutaric acid produced during the Cephalosporin C deacylation. This bicarboxylic acid was in fact not recognized as inhibitor or substrate by the enzyme. Moreover the high PGA activity showed in mild reaction conditions reduces the problem of the β-lactamic ring stability and avoids the use of the low temperature currently utilized in the conventional chemical processes.

The results obtained in the DAO and GA immobilization study,[7] in the Cephalosporin C enzymatic hydrolysis optimization,[7] as well as in the PGA catalyzed acylation, will be the matter of forthcoming papers.

The results reported, as well as the possibility to perform 3'-acetoxy group displacement with different nucleophiles, make the proposed chemoenzymatic process a really interesting general approach to the synthesis of 3'-functionalized cephalosporines.

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6. The utilized PGA was isolated from a strain of *Escherichia coli* ATCC 11105 of industrial provenience (Antibioticos S.A. Leon, Spain), produced from *E. coli* ATCC 11105 submitted to conventional mutagenesis (e.g., chemical), in order to obtain an hyper-producer strain, and stabilized by covalent multiple-point attachment on activated agarose beads performed as previously described: Guisán J.M.; Fernández-Lafuente, R.; Rodriguez, V.; Bastida, A.; Blanco, R.M.. and Alvaro, G. in *Stability and Stabilization of Enzymes*; van den Tweel, W.J.J.; Harder, A. and Buitelaar, R.M., Editors (Elsevier Science Publishers, The Netherlands) **1993** pp. 55. (b) Alvaro, G.; Fernández-Lafuente, R.; Blanco R.M. and Guisán J.M *Appl. Biochem. Biotechnol.* **1990**, *26*, 181. (c) Guisan, J.M. *Enzyme Microb. Technol.* **1988**, *10*, 375.
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