3D Printed mini reactor-tunned chemoenzymatic synthesis of C14 and C16 Macrolides

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Abstract

Herein is reported a flow system based on a 3D Printed mini reactor developed for enzymatic platform to synthesize key intermediates using alcohol dehydrogenase extracted from the root of *Daucus carota* and laccase. For the laccase oxidation system, TEMPO was used as mediator for the oxidation of substituted furans catalyzed by laccase from *Aspergillus oryzae*. For immobilization, calcium alginate microspheres were prepared as solid support.

Key words: furan, bioreductases, minireactor, laccase, oxidation

Introduction

Pyrenophorin [1] and Chlonostachdinone [2] are C14 and C16 biologically active macrolides (Figure 1). These compounds possesses microbicidal, bactericidal and herbicidal action, in addition to a potent inhibition growth of a wide range of grasses (1), (2). Due to its biological importance as well as potential application, the present work aims to develop a flow system based on enzymatic platform to synthesize key intermediates by using alcohol dehydrogenase from the root of *Daucus carota* and laccase from *Aspergilus oryzae.* Thus, the aim was to obtain advanced intermediates and access to all isomers of the target compounds. Of particular interest, we decided to use a 3D printed mini reactor and compare its results with conventional conditions.

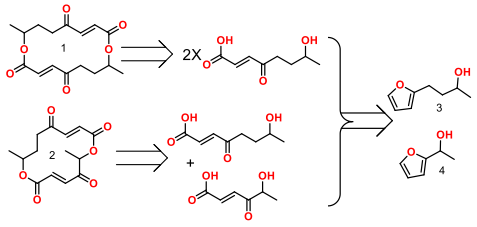


Figure 1. Retrosynthesis os Pyrenophorin (1) and Chlonostachdinone (2)

Experimental

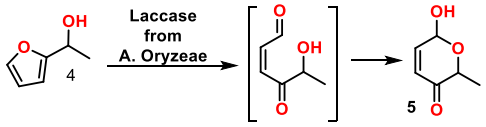
The preparation of racemic alcohols 3 and 4 started by reacting ketones with $NaBH_4$ while for the synthesis of chiral substrates alcohol dehydrogenase extracted from the root of *Daucus carota* were used. Thus, freshly pureed *Daucus carota* roots were immobilized in calcium alginate microspheres for the formation of immobilized beads (3).

Both the alcohols, racemic and chiral, were subjected for oxidation of furan ring using the enzyme Laccase and TEMPO as mediator, followed by an Achmatowicz rearrangement of dihydropyranone intermediates. The flow reactions were performed in a 27 cm long and 3 mm inner diameter hose connected to a packed-bed bioreactor connected to a peristaltic pump (AC-2110 II Perista® Pump).

Results and Discussion

Asymmetric bioreduction of ketones to furnish **3** and **4** were succeeded by immobilized *D. carota* cells. Immobilization ensured high cell concentrations and cell reuse. Continuous production of chiral alcohols in the 3D printed reactor achieved chiral alcohols with high conversion rate (>99%) (1,4 g/L substrate) in 12 h of substrates residence in a packed-bed bioreactor. The reactions are 3 times faster if compared with reported methods under conventional conditions (72h to 96h) (4).

Likewise, the Achmatowicz reaction between alcohol 4 and Laccase proved to be promising leading to the quantitative formation of lactol 5 (Scheme 1).



Scheme 1. Laccase-catalyzed oxidative ring opening reaction of 4 followed by Achmatowicz rearrangment

Conclusion

Our results showed that production of chiral alcohols conducted in a packed-bed bioreactor using *D. carota* roots as biocatalysts leaded to the formation of desired compounds in good yields. Actually, we are scaling up the process in order to produce 3 and 4 in gram scale, which is important for industrial purpose. In the same way, laccase/TEMPO system is under study for exploring its capacity in being supported in microspheres and perform laccase promiscuity in furan substrates in others 3D printed mini reactors.

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