TWO-STEP BIOCATALYTIC SYNTHESIS OF (1*S*)-NOR(PSEUDO)EPHEDRINE USING IMMOBILIZED ENZYMES

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Nor(pseudo)ephedrines (N(P)Es) are vicinal amino alcohols with sympathomimetic activity which have a plethora of applications, especially in the pharmaceutical field. Isolation of N(P)Es in high yield and optical purity from natural sources is not viable on a large scale. Similarly, chemical asymmetric syntheses of NEs and NPEs involve tedious multi-step procedures, thus making it cumbersome to achieve high yield and optical purity simultaneously.[1]

The synthesis of 1(*S*)-N(P)Es (**Figure 1**) and their analogues by a two-step biocatalytic cascade has been recently proposed by some of the Authors. This cascade consists of an acyloin condensation catalysed by the (*S*)-selective acetoin:dichlorophenolindophenol oxidoreductase (Ao:DCPIP OR) from *Bacillus licheniformis*, followed by the transamination mediated by an amine transaminase ((*S*)- or (*R*)-ATA).[2] The use of free enzymes, however, presents several limitations in terms of enzyme reuse and downstream process, especially in the perspective of a large-scale application.

In this study, we successfully immobilized both Ao:DCPIP OR and ATAs and used them in the biosynthetic cascade to N(P)Es. Immobilization yield, activity recovery, and stability of the immobilized enzymes both during the reaction (enzyme recycling) and under storage (shelf-life) were assessed. The two-step biotransformation for the synthesis of (1*S*,2*S*)-NPE and (1*S*,2*R*)-NE (**Figure 1**) using immobilized enzymes was performed on a semi-preparative scale with good to excellent yield and optical purity.[3]

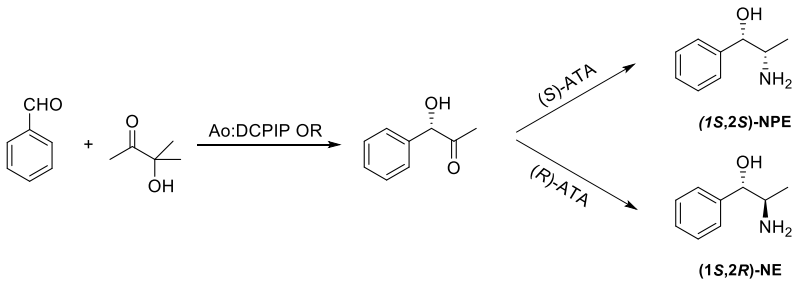

Figure 1: Biocatalysed stereoselective synthesis of (1*S*)-nor(pseudo)ephedrine.

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[2] Fracchiolla, N.; Patti, S.; Sangalli, F.; Monti, D.; Presini, F.; Giovannini, P. P.; Parmeggiani, F.; Brenna, E.; Tessaro, D.; Ferrandi, E. E., Insight into the stereoselective synthesis of (1*S*)-nor(pseudo)ephedrine analogues by a two-steps biocatalytic process. *ChemCatChem* **2024**, *16*, e202301199.
[3] Patti, S.; Magrini Alunno, I.; Semproli, R.; Tessaro, D.; Monti, D.; Riva, S.; Ubiali, D.; Ferrandi, E. E. Two-step biocatalytic synthesis of (1*S*)-nor(pseudo)ephedrine using immobilized enzymes. Manuscript in preparation.