

## ENZYMATIC ESTERIFICATION OF LACTONES IN AQUEOUS BUFFER

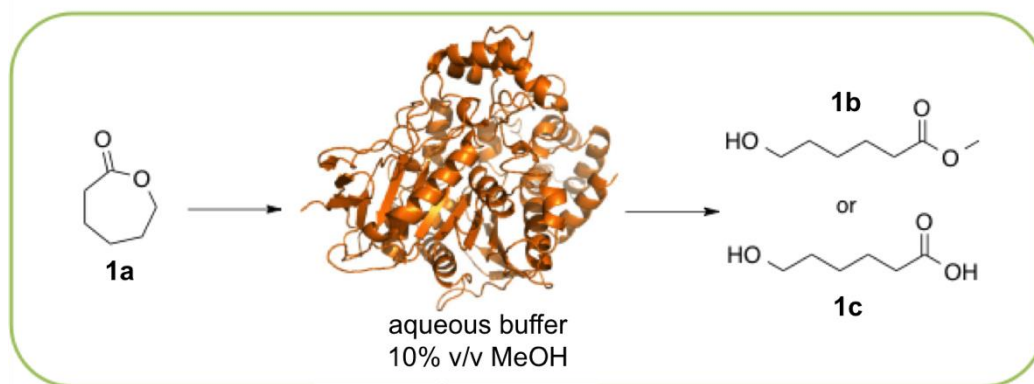
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Opening lactones with alcohols to yield esters can traditionally be achieved by acid or base catalysis. The produced esters are intermediates for a vast variety of reactions [1] and can also be used for polymerization reactions like for polycaprolactone.

Recently a biocatalytic cascade was reported for the transformation of cyclohexanol to the nylon-6 monomer [2]. A key step was the *in-situ* capping strategy of the carboxylic functionality by opening  $\epsilon$ -caprolactone (1a) with methanol to methyl-6-hydroxyhexanoate (1b) employing a crude preparation of horse liver esterase (HLE). The conversion was performed in aqueous buffer in the presence of 10% v/v methanol.

We successfully expressed three HLE isoenzymes with high sequence identity (93 - 96%) and a hydrolase from *Bacillus subtilis* in *E. coli*. The three HLE isoenzymes showed different optimal reaction conditions and one of the enzymes favored the formation of the carboxylic acid over ester formation, while the other two enzymes favored the ester formation employing  $\epsilon$ -caprolactone (1a) as substrate. The hydrolase from *Bacillus subtilis* esterified and hydrolyzed  $\epsilon$ -caprolactone (1a) at comparable rate. However, the hydrolase from *Bacillus subtilis* was not capable of hydrolyzing methyl-6-hydroxyhexanoate (1b), which was in contrast to the HLE isoenzymes. Crystal structures of the HLE isoenzymes could lead to more information about potential tunnels what might explain the preference of alcohols over water as nucleophile and *vice versa*.



[1] S.E. Choi, M.K.H. Pflum, Bioorg. Med. Chem. Lett., 22 (2012) 7084-7086

[2] J.H. Sattler, M. Fuchs, F.G. Mutti, B. Grischek, P. Engel, J. Pfeffer, J.M. Woodley, W. Kroutil, Angew. Chem. Int. Ed., 52; (2014), 14153-14157