Enzymatic kinetic resolution of methyl 2-methyl-4-oxopentanoate

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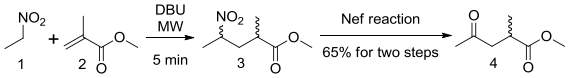
INTRODUCTION

The stereoselectivity of enzyme catalyzed reactions can be tuned by changing reaction conditions such as pH, temperature and with addition of co-solvents. Other alternatives for the modulation of the affinity and specificity between enzyme and extraneous organic compounds have been developed[1]. In special, addition of salts can promote changes in enzyme solvatation which leads to changes in its interaction with the substrate[2]. However, this approach is scarcely considered in biocatalytic reactions for preparative purposes. Herein, we describe the enzymatic kinetic resolution of methyl (*RS*)-2-methyl-4-oxopentanoate by a series of changes in the medium with merit to salt effect.

RESULTS AND DISCUSSION

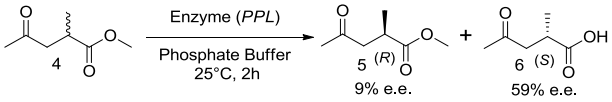
The starting material **4** was synthesized as follows in Scheme 1.[3]

Scheme 1: Synthesis of keto-ester **4**.



To find a suitable enzyme for kinetic resolution, **4** was then screened with different hydrolases. Albeit modest, *Porcine pancreas lipase* (*PPL*) has showed better performance among eleven enzymes tested. (Scheme 2).

Scheme 2: Enzymatic hydrolysis of keto-ester 4 by *PPL*.



From this point, changes in pH, temperature, addition of co-solvent and salt addition were applied looking for the improvement of enantioselectivity and conversion rate. Salt addition afforded the best results (Table2).

Table 2: Salt effect in the enzymatic hydrolysis of 4 by *PPL*

Salt (concentration/media ionic strength)	ee_a (%)	Conv. (%)	E
-	59	10	4.3
KF (1.6 M/3.6M)	3	3	1.1
NaCl (1.6 M/3.6M)	60	10	4.3
Na_2SO_4 (1.1 M/3.6M)	68	13	5.9
Na_2SO_4 (1.6 M/4.2M)	80	22	11.3

Conditions: Phosphate buffer 0.1M (5.0 mL/pH=7.2), temp.=25ºC, substrate=50.0 mg, PPL=25.0 mg, reaction time= 2 hours.

Also, it was observed that reaction time was an important parameter (Table 3).

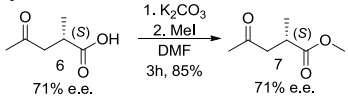
Table 3: Enzymatic hydrolysis of keto ester **4**

Reaction time	ee_e (%)	ee_a (%)	Conv. (%)	E
4 hours	60	84	42	19
6 hours	73	80	48	20
8 hours	88	77	52	22
10 hours	>99	71	56	23

Conditions: phosphate buffer 0.1 M (5.0 mL / pH=7.2, Na_2SO_4= 1.6 M), substrate = 25.0 mg, enzyme = 12.5 mg.

After 10 hours, the methyl (*R*)-2-methyl-4-oxopentanoate (**5**) was recovered in >99% e.e. and (*R*)-2-methyl-4-oxopentanoic acid (**6**) 71% e.e. was subjected to a esterification reaction (Scheme 3).

Scheme 3: Recycle of keto acid **6**.



Then, the compound **7** was subjected to a enzymatic hydrolysis under previous conditions affording (*R*)-2-methyl-4-oxopentanoic acid (**6**) with >99% e.e. after 6 hours of reaction.

Table 4: Enzymatic hydrolysis of **7** by *PPL*.

Reaction time	ee_e (%)	ee_a (%)	Conv. (%)	E
2 hours	56	>99	64	>200
4 hours	34	>99	74	>200
6 hours	2	>99	98	>200
7 hours	24	97	-	71
8 hours	30	95	-	51

Conditions: phosphate buffer 0.1 M (5.0 mL / pH=7.2, Na_2SO_4= 1.6 M), substrate = 25.0 mg, enzyme = 12.5 mg.

CONCLUSION

The present data shows that salt affects the PPL stereoselectivity in ester hydrolysis. These effects are not widely explored to improve biocatalyzed reactions and should be considered as important approach to reach better enantiomeric excess.

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