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Asymmetric synthesis of an enantiomerically pure rivastigmine intermediate using ketoreductase

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ABSTRACT

A novel chemo-enzymatic synthesis of (*S*)-rivastigmine using ketoreductases with NADH/NADPH as the proton donor has been demonstrated. An exclusive enzymatic process has been developed by exploring the possible ketoreductases by screening to identify enzymes useful for the preparation of the (*S*)-isomer intermediate, and scaling up of the enzymatic process for the production of an adequate, enantiomerically pure precursor of rivastigmine and for the total synthesis of (*S*)-rivastigmine.

1. Introduction

Rivastigmine {(*S*)-3-[1-(dimethylamino)-ethyl]phenyl ethyl-(methyl)carbamate}[1] is an active pharmaceutical ingredient which inhibits acetylcholinesterase enzymes present in the nervous system. It also helps in improving cognition, coordination for routine functions for patients with mild to moderate Alzheimer disease[2] and treatment of dementia caused by Parkinson's disease[3] and Lewy body.[4]

Early researchers synthesized (*S*)-rivastigmine by the resolution of racemic rivastigmine or an intermediate by a catalytic asymmetric hydrogen transfer based reaction using transition metals and chiral organic ligands.[5–18] Recently, researchers have reported on the synthesis of rivastigmine via a chemoenzymatic route using lipases[19–21] as well as transaminases.[22] However, there are various drawbacks for these methods such as complex operations, loss of yield, metal impurities in final API, multiple purification procedures in the lipase catalyzed resolution, three enzyme systems in the transaminase reactions, and the need for multiple crystallization steps with chiral acids.

Herein we report a practical and industrially scalable chemoenzymatic approach using ketoreductases toward the synthesis of (*S*)-rivastigmine.[23] Various references are available for stereoselective reduction reactions catalyzed by ketoreductases or alcohol dehydrogenases.[24–39] This procedure includes an enzyme catalyzed stereoselective reduction as the key step where ketoreductases undergo stereoselective reduction by utilizing the proton from NADH/NADPH while glucose dehydrogenase regenerates the NADH/NADPH from D-glucose to gluconic acid, thus shifting the equilibrium of the reaction to the formation of the alcohol. This process breaks the limitation of obtaining 50% maximum yield of (*S*)-rivastigmine.

2. Result and discussion

2.1. Synthesis

Our synthetic route (Scheme 1) involved the condensation of 3-hydroxy acetopheneone with ethyl methyl carbomyl chloride to give **1** which in turn was reduced stereoselectively to **2** using KRED which was then followed by bromination to give **3**; finally condensation with dimethylamine gave (*S*)-rivastigmine free base **4**.

2.2. Screening for the keto reductase

For the initial screening of the reduction of *N*-ethyl methyl carbomoyl acetophenone **1**, an ES-KRED 8000 sceening kit containing 80 enzymes (Table 1) was used. The screening medium containing 5 mg of each Keto reductase enzyme in 2 mL of phosphate buffer pH 7.0 (containing 5 mg NADPH/NADH, 330 mM D-glucose, 2 U/mL glucose dehydrogenase CDX901) in a 2 mL screw capped vial. A solution of *N*-ethyl methyl carbomyl acetophenone in dimethyl sulfoxide (10 mg in 0.1 mL) was then added. The mixture was incubated over a eppendorf thermomixture comfort at 30 °C at 1000 rpm for 24 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the product was subjected to chiral HPLC analysis of the alcohol enantiomer. All ketoreductases showed 100% conversion as indicated by RP-HPLC with the formation of the (*S*)-alcohol (99% ee) as indicated by chiral HPLC.

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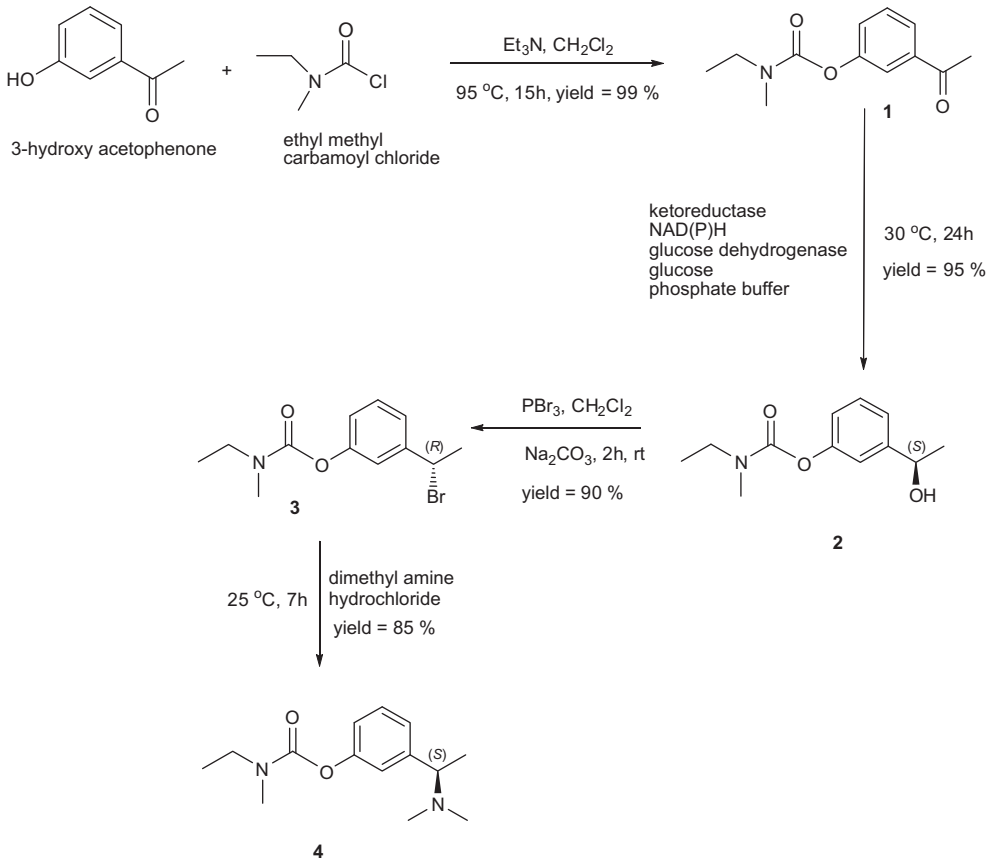

Scheme 1. Synthesis of (*S*)-rivastigmine from 3-hydroxy acetopheneone.

2.3. Reaction profile

In the presence of ES-KRED 119, the conversion showed an increase in the formation of **2** from **1** over 0–24 h (Fig. 1). The rate of reduction was found to be 0.0021 mol h^{-1} g^{-1}.

2.4. Effect of the enzyme concentration

A maximum conversion was achieved at 10% of ES-KRED-119 at 0.045 mol of *N*-ethyl methyl carbomyl acetophenone, 10% NADPH, 10% glucose dehydrogenase CDX901 and 119% D-glucose for incubations up to 24 h (Table 2). A 1–5% of ES-KRED 119 took more than 48 h to complete the reaction. In the case of glucose dehydrogenase CDX 901, maximum conversion was achieved at 10% of enzyme concentration.

2.5. Effect of the NADPH concentration

A maximum conversion was achieved at 10% of NADPH at 0.045 mol *N*-ethyl methyl carbomyl acetophenone, 10% ES-KRED-119, 10% glucose dehydrogenase CDX901 and 119% D-glucose for incubations up to 24 h (Table 2).

2.6. Effect of the buffer

For the optimization of the reaction with respect to buffer pH and molar concentration (Table 3 and 4), the complete reaction was achieved at 20 mL of 0.25 M phosphate buffer pH 6.8–7.0 containing *N*-ethyl methyl carbomyl acetophenone (0.0045 mol), 0.1 g of ES-KRED 119, 0.1 g of NAD, 1.19 g of D-glucose, 0.1 g of glucose dehydrogenase CDX901, 20% DMSO (Table 3). In all cases, the ee remained constantly perfect (>99%).

2.7. Effect of the co-solvents

A perfect stereoselectivity of more than >99% ee was observed with or without DMSO as the co-solvent. DMF showed 97.97% ee while methanol showed 84.58% conversion with 86.32% ee of the (*S*)-isomer. The formation of more of the (*R*)-isomer (85.5% ee) was observed when ethanol was used as the co-solvent (Table 5).

2.8. Effect of temperature

A decrease in stereoselectivity (96.3% ee) and % conversion (70.0%) was observed when the temperature was increased from 30 to 40 °C (Table 6). This decrease in activity was due to the denaturation of the enzymes[40] as well as the degradation of NADPH.[41] The stereoselectivity can in theory, either increase or decrease when the temperature is varied. The decrease in stereoselectivity of the enzyme may be due to a change in the steric requirement and Van der Waals interactions of the substrate binding at the active site.[42–44]

3. Conclusion

N-Ethyl methyl carbomyl acetophenone, synthesized by the condensation of *N*-ethyl methyl carbomyl chloride with 3-hydroxy acetophenone, has been screened for stereoselective reduction by the enzymes ES-KRED, NAD/NADPH, D-glucose and glucose dehydrogenase. ES-KRED 119 taken for optimization used 10% of