Tetrahedron 68 (2012) 7691–7694

Contents lists available at SciVerse ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Improved chemoenzymatic asymmetric synthesis of (*S*)-Rivastigmine

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ARTICLE INFO

Article history:
Received 25 February 2012
Received in revised form 21 May 2012
Accepted 8 June 2012
Available online 21 June 2012

Keywords:
Rivastigmine
Transaminase
Biocatalysis
Asymmetric synthesis

ABSTRACT

(*S*)-Rivastigmine [(*S*)-**1**] was obtained via a four-step synthesis using an asymmetric enzymatic transamination protocol as the key step. An early introduction of the carbamate pharmacophore side chain avoided the use of protective group strategies and hence led to a considerable shortcut. This strategy required a novel ω-transaminase from *Paracoccus denitrificans*, which could transform the highly polar key substrate 3-acetylphenyl ethyl(methyl)carbamate (**4**) to the corresponding amine (*S*)-**5** in 99% ee and >80% conversion.

1. Introduction

(*S*)-Rivastigmine {(*S*)-3-[1-(dimethylamino)-ethyl]phenyl ethyl(methyl)carbamate (**1**)} acts as potent cholinesterase inhibitor and represents one of the most potent agents for the treatment of Alzheimer's and Parkison's disease at early stages.[1,2] However, only the (*S*)-enantiomer exhibits the desired biological activity,[2] which intrinsically requires the drug to be administered in enantiomerically pure form (Fig. 1).

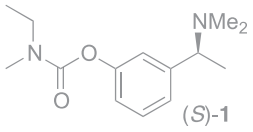

Fig. 1. (*S*)-Rivastigmine (**1**).

To date, several asymmetric methods have been developed for the preparation of enantiopure Rivastigmine (e.g., racemate resolution using chiral acids,[3] asymmetric addition of organozinc species onto imines using transition metal catalysis,[4a] diastereoselective reductive amination[4b] or lipase-catalyzed (dynamic) kinetic resolution of a hydroxy-precursor[5]). We have recently reported an asymmetric total synthesis of Rivastigmine by forming the chiral amine moiety via enzymatic amination of the corresponding ketone employing ω-transaminases (ω-TAs).[6] The latter enzymes transfer the amine functionality from an amino acid (e.g., alanine) onto a ketone. The main obstacle to overcome is the equilibrium of this reaction, which is far on the alanine/ketone side.[7] In this context, various approaches have been undertaken, mainly via removal of the formed co-product pyruvate, e.g., through reduction (using lactate dehydrogenase) or decarboxylation (catalysed by pyruvate decarboxylase).[8–10] The most attractive alternative represents a formal reductive amination, where alanine is recycled from pyruvate using an amino acid dehydrogenase together with an appropriate NADH-recycling system and ammonia as amine donor.[10,11]

2. Results and discussion

During our previous study, we were unable to identify an ω-TA, which would transform the 'ideal' ketone precursor **4** bearing the polar carbamate pharmacophor at the *m*-phenolic group at satisfying conversions of >30% (Table 1). The problem was circumvented by a cumbersome protection–deprotection strategy using a MOM-analog. However, the rapid growth of readily available ω-TAs[12,13] prompted us to search for a suitable alternative enzyme. Thus, ω-TAs from *Paracoccus dentrificans* (Pd-ωTA),[12] *Pseudomonas putida* KT2440 [Pp-ωTA_1 (Gen PP5182) and Pp-ωTA_2 (Gen PP2180)],[14] *Chromobacterium violaceum* DSM 30191 (CV-ω-TA),[15] *Bacillus megaterium* SC6394 (BM-ω-TA),[16] *Arthrobacter* sp. CNB05-01 (ArS-ω-TA)[17] and *Vibrio fluvialis* (Vf-ω-TA) as well as two commercially available ω-TAs (ATA-113, ATA-114, Codexis) were

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0040-4020/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tet.2012.06.031

Table 1
Transamination of ketone **4** to (*S*)-**5**

Entry	ω-TA	Conv. [%][a]	ee [%][b]
1	BM-ω-TA[c]	4	n.d.
2	CV-ω-TA[c]	8	n.d.
3	ATA 113[d]	7	n.d.
4	ATA 114[d]	9	n.d.
5	Pp-ω-TA_1[c]	7	n.d.
6	ArS-ω-TA[c]	27	77
7	Vf-ω-TA[d]	29	99
8	Pp-ω-TA_2[c]	35	98
9	Pd-ω-TA[c]	83 (76)	99 (99)

Reaction conditions: Substrate **4** (50 mM, 11 mg, 0.05 mmol), phosphate buffer (1.0 mL, 100 mM, pH 7.0, 1 mM pyridoxal 5′-phosphate), L-alanine (250 mM, 22 mg, 0.25 mmol), ω-TA (10 mg of crude enzyme preparation, or 20 mg of lyophilized whole *E. coli* host cells containing overexpressed ω-TA), LDH mix (30 mg/mL, contains LDH, GDH, glucose, NAD^+), shaking at 30 °C and 120 rpm for 24 h.

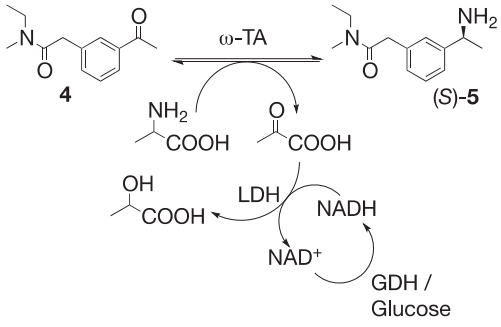
[a] Conversions were determined via GC–MS peak area integration; isolated yield and ee of prep-scale experiment are given in brackets.

[b] Ee was determined via HPLC-UV after derivatisation (for details see experimental section).

[c] Lyophilized whole cells of *E. coli* BL21(DE3) containing overexpressed ω-TA.

[d] Lyophilized crude enzyme preparation.

investigated. For shifting the equilibrium towards the desired amine **5**, the co-product pyruvate was removed via reduction to the lactate using lactate dehydrogenase in presence of glucose dehydrogenase (GDH)/glucose NADH-recycling (Scheme 1).[6,9]



ω-TA = ω-Transaminase, GDH = glucose dehydrogenase, LDH = lactate dehydrogenase

Scheme 1. Asymmetric enzymatic transamination of Rivastigmine key precursor **4**.

Most of the ω-TAs showed trace activities with substrate **4** (entries 1–5), also (*S*)-selective ω-TAs from *Arthrobacter* sp. and *V. fluvialis* gave modest, but synthetically insufficient conversions of ~30% (entries 6 and 7). The latter is presumably caused by the high polarity of the carbamate group, which is heavily hydrated in the aqueous reaction medium. On the contrary, the results from the novel ω-TAs were encouraging: Although Pp-ωTA_2 was only slightly better than Vf-ω-TA (entry 8), ω-TA from *Paracoccus denitrificans* (Pd-ωTA), which shows a remarkably high sequence identity of 94% to Vf-ω-TA, exhibited dramatically increased activities leading to >80% conversion (entry 9).

For reason of comparison, the pyruvate–lactate reduction system was exchanged with alanine dehydrogenase in combination with the formate dehydrogenase/formate NADH-recycling system, which allows the regeneration of L-alanine at the expense of cheap ammonium formate.[11] However, the conversion dropped to 29% in comparison to the LDH system.

For the total synthesis of (*S*)-Rivastigmine (**1**) the biotransformation was performed on a preparative scale, which gave amine (*S*)-**5** in 76% isolated yield and 99% e.e. Reductive amination of the latter with formaldehyde in the presence of sodium triacetoxyborohydride gave (*S*)-Rivastigmine (**1**) quantitatively, leading to an isolated overall yield of 66% over four steps (Scheme 2).

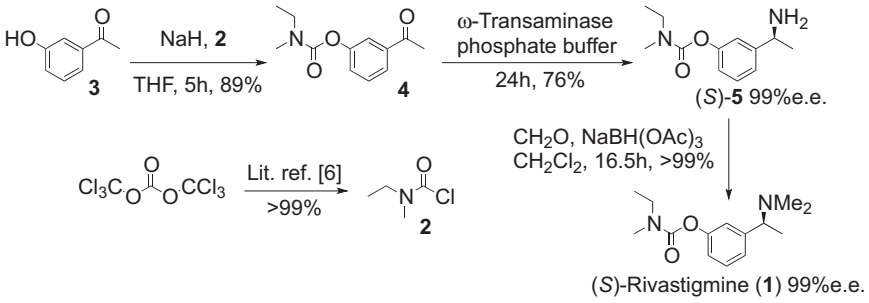
3. Conclusion

In conclusion, we have shown that ω-transaminase from *Paracoccus denitrificans* (Pd-ωTA) shows a unique ability to accept the precursor substrate **4** bearing the highly polar carbamate pharmacophore side chain of Rivastigmine. This protocol eliminates the use of protective group strategies and gives the target compound (*S*)-**1** in 66% overall yield via four steps, which (to the best of our knowledge) depicts the shortest route to enantiopure Rivastigmine reported to date.

4. Experimental

4.1. General

All chemicals were purchased from Sigma Aldrich or Acros Organics and were used as received. All solvents were purchased from Roth. Dry THF was freshly distilled from sodium/benzophenone. All moisture sensitive reactions were operated using standard Schlenk techniques with dry argon. Biocatalytic reactions and rehydration of enzymes were accomplished in a HT Infors Unitron AJ 260 shaker at 120 rpm and 30 °C (horizontal position). Centrifugation was done at 13,000 rpm in a Heraeus Biofuge pico or at 4000 rpm in a Heraeus Biofuge primo. Derivatisation of amines was performed in an Eppendorf thermomixer comfort. NMR spectra were recorded on a Bruker NMR unit at 300 (^{1}H) and 75 (^{13}C) MHz, shifts are given in parts per million and coupling constants (*J*) are given in Hertz. All GC–MS measurements were carried out with an Agilent 7890A GC system, equipped with an Agilent 5975C mass-selective detector (electron impact, 70 eV) and a HP-5-MS column (30 m×0.25 mm×0.25 μm film) using He as carrier gas at a flow of 0.55 mL/min. The following temperature program was used in all GC–MS measurements: initial temperature 100 °C, hold for 0.5 min, 10 °C/min, to 300 °C. High resolution mass spectra were recorded on a Waters Synapt HDMS Q-TOF mass spectrometer (ESI



Scheme 2. Chemoenzymatic asymmetric synthesis of (*S*)-Rivastigmine (**1**).