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Deracemization of Mexiletine Biocatalyzed by ω-Transaminases

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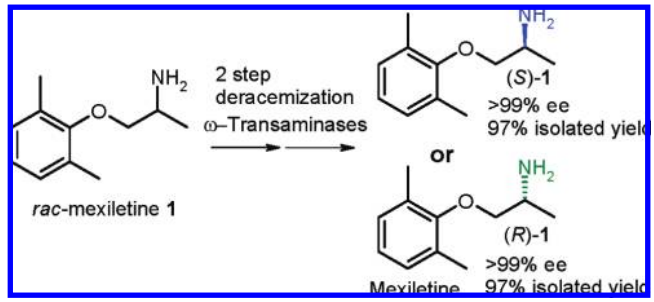
ABSTRACT



(*S*)- as well as (*R*)-mexiletine [1-(2,6-dimethylphenoxy)-2-propanamine], a chiral orally effective antiarrhythmic agent, was prepared by deracemization starting from the commercially available racemic amine using ω-transaminases in up to >99% ee and conversion with 97% isolated yield by a one-pot two-step procedure. The absolute configuration could be easily switched to the other enantiomer, just by switching the order of the applied transaminases. The cosubstrate pyruvate needed in the first oxidative step was recycled by using an amino acid oxidase.

Mexiletine **1** is a therapeutically relevant chiral amine that is clinically used as an antiarrhythmic, antimyotonic, and analgesic oral drug in its racemic form.[1−3] Activity studies in vivo[4] and in vitro[5] of pharmacologically active mexiletine indicate that its (*R*)-enantiomer binds preferentially to the cardiac sodium channels. In addition, (*R*)-mexiletine is also more active than (*S*)-mexiletine on native skeletal fibers.[6] The use of mexiletine as a racemate in the treatment of neuromuscular disorders is limited due to its possible side effects.[7] Therefore the development of methods to access enantiomerically pure mexiletine is desirable.

Preparation of both enantiomers of mexiletine has previously been reported by several groups.[8−14] Generally, the methods involved resolution of racemic intermediates,

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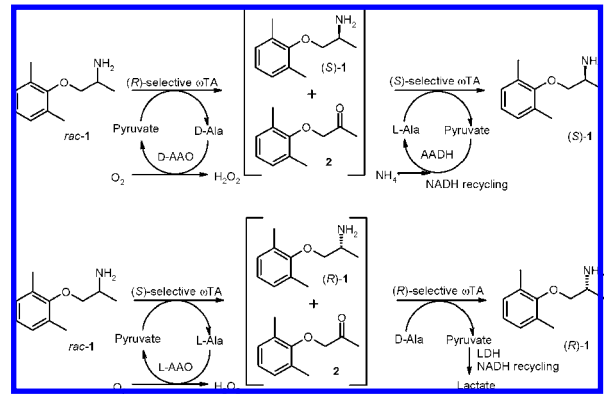
enzymatic hydrolysis of an *N*-acyl derivative,[10,11] or multistep stereospecific procedures.[12] Flippin and co-workers[13] reported a convenient procedure for the preparation of stereoisomers of mexiletine, but the scope of products was limited by the availability of chiral substrates and chromium tricarbonyl complexes of aryl halides. Recently, optically enriched (*R*)- and (*S*)-mexiletine was prepared by using asymmetric reduction of oxime ethers and *O*-benzyl oximes with borane-based catalysts.[14]

Enzymes such as ω-transaminases have emerged as viable biocatalysts for chiral amine preparation.[15] ω-Transaminases (ωTAs) can be used in two complementary ways, either (i) in kinetic resolution of racemic amines with pyruvate as an amine group acceptor or (ii) in the asymmetric synthesis from ketones.[16] We wish to report herein an deracemization protocol for the synthesis of both enantiomers of mexiletine employing ω-transaminases. Deracemization of amines via dynamic kinetic resolution and cyclic deracemization are well described.[17] The deracemization protocol here is based on a two-step one-pot process that consists of (i) a kinetic resolution and (ii) a stereoselective amination employing ω-transaminases (Scheme 1). Notably, the cosubstrate needed in the first oxidation step (pyruvate) is recycled by an amino acid oxidase[18] (AAO). The advantage is that the formed D-Ala or L-Ala in the kinetic resolution in the first step does not interfere with the second step, where alanine of opposite configuration is required. The deracemization approach avoids the limitation of a kinetic resolution (50% of conversion), thus leading to a theoretically quantitative yield of optically pure amine starting with racemic amines by using only a catalytic amount of amine acceptor pyruvate.

Testing first the kinetic resolution for racemic mexiletine, three commercial ω-transaminases (ATA-113, ATA-117,[19] and from *Vibrio fluvialis* Vf-TA[20]) and four transaminases described in the literature (from *Bacillus megaterium* BM-ωTA,[15e] *Alcaligenes denitrificans* AD-ωTA,[21] *Chromobacterium violaceum CV-ωTA,*[22] and a mutant termed CNB05-01 originating from an *Arthrobacter sp* ArS-ωTA[23]) were chosen as catalysts (Table 1). The four transaminases from literature were used as a lyophilized whole-cell system overexpressed in *E. coli*. The ω-transaminases catalyzed this reaction efficiently giving the amine with up to >99% ee with excellent enantioselectivity (E > 200). The important point, however, was that transaminases ATA-113, Vf-ATA, BM-ωTA, AD-ωTA, CV-ωTA, and ArS-ωTA showed (*S*)-preference while ATA-117 displayed (*R*)-preference, thus enantiocomplementary enzymes were available. This is actually the precondition for the complete deracemization sequence.

Scheme 1. Deracemization of *rac*-Mexiletine by a One-Pot Two-Step Synthesis Procedure



In the kinetic resolution above a stoichiometric quantity of pyruvate was applied, which could result in inhibition and leads to accumulation of alanine. To avoid this, in situ oxidation of alanine back to pyruvate by amino acid oxidase (AAO) at the expense of molecular oxygen was considered.[24]

To find the most efficient system for the kinetic resolution of *rac*-**1** at 50 mM substrate concentration three enantiocomplementary amino acid oxidases (one D-AAO from porcine kidney[25] and two L-AAO from *Crotalus adamanteus* and *Crotalus atrox*)[26] were tested with the most interesting transaminases employing only a catalytic amount of pyruvate (2 mM; 4 mol %) (Table 2). For instance, *rac*-Mexiletine was successfully resolved by ATA-117, giving the (*S*)-amine with >99% ee at 53% conversion. When (*S*)-selective ω-TAs

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Table 1. Kinetic Resolution of *rac*-**1** Catalyzed by ω-Transaminases Employing a Stoichiometric Amount of Pyruvate

entry	ωTA[a]	convn (%)[b]	ee (%)[c]	E[d]
1	117	55	>99 (*S*)	>200[e]
2	113	56	>99 (*R*)	>200[e]
3	Vf	74	>99 (*R*)	>200
4	BM	56	>99 (*R*)	>200
5	AD	45	50 (*R*)	7
6	CV	50	>99 (*R*)	>200
7	ArS	55	>99 (*R*)	>200

[a] Reaction conditions: *rac*- **1** (50 mM), sodium pyruvate (50 mM), and ω-transaminase (6 mg commercial enzyme preparation or 20 mg of *E. coli*/transaminase cells), phosphate buffer (100 mM, pH 7.0, 1 mM PLP), shaking at 30 °C for 24 h. [b] Determined by GC. [c] Determined by GC on a chiral phase. [d] Enantioselectivity was calculated from ee and conversion. [e] Comparable results have been reported previously.[15f]

were used the opposite enantiomer of mexiletine was obtained with up to >99% ee.

Having identified the suitable conditions for the kinetic resolution of *rac*-mexiletine (**1**) we turned our attention to couple the kinetic resolution with the stereoselective amination to achieve deracemization via a stepwise reaction sequence without separation of intermediates.

Table 2. Kinetic Resolution of *rac*-**1** Catalyzed by a ω-Transaminase/Amino Acid Oxidase System

entry	ωTA[a]	convn (%)[b]	ee (%)[c]	E
1	117	53	>99 (*S*)	>200
2	113	55	>99 (*R*)	>200
3	113[d]	40	66 (*R*)	7.5
4	Vf	48	97 (*R*)	>200
5	CV	51	>99 (*R*)	>200

[a] Reaction conditions: *rac*-**1** (50 mM), sodium pyruvate (2 mM), L- or D-AAO (15 mg, 4.5 U and 20 U, respectively), ω-TA (6 mg of crude enzyme or 20 mg of *E. coli*/transaminase cells), phosphate buffer (100 mM, pH 7.0, 1 mM PLP), shaking at 30 °C for 24 h. [b] Determined by GC. [c] Determined by GC analysis on a chiral phase. [d] L-AAO from *Crotalus atrox* (15 mg, 3 U).

Shifting the ketone−amine equilibrium to the amine side is a challenge when employing ω-transaminases and alanine.[15d] To shift the equilibrium the side product pyruvate was removed by using two complementary systems: (i) reduction by alanine dehydrogenase (AADH) and therefore recycling of the cosubstrate alanine in a coupled reaction system and (ii) reduction to lactate, using lactate dehydrogenase (LDH) (Scheme 1).[16]

The reaction sequence was performed in the way that after the kinetic resolution the second ω-transaminase with opposite stereopreference was added together with the corresponding alanine enantiomer. To avoid interference from using stereocomplementary ω-transaminases, a heat treatment was performed prior to the second step. Thus after the kinetic resolution, the sample was kept at 75 °C for 30 min, before the enzyme required for the second step was added.

Table 3. Synthesis of Optically Pure Mexiletine via Bioderacemization Protocol Employing ω-Transaminases

entry	ωTA[1]/ωTA[2] [a]	convn (%)[b]	ee (%)[c]
1	117/CV	97	>99 (*S*)
2	117/BM	77	95 (*S*)
3	117/113	98	>99 (*S*)
4	117/Vf	>99	96 (*S*)
5	CV/117	>99	>99 (*R*)
6	113/117	97	>99 (*R*)

[a] Order of adding ω-transaminase.; In the case of ATA-117 D-Ala was used in the amination reaction and L-Ala for (*S*)-selective ω-TAs. [b] Determined by GC. [c] Determined by GC analysis on a chiral phase.

Depending on the order of the ω-transaminases subjected, the (*R*)- as well as the (*S*)-enantiomer was accessible with up to >99% ee and excellent conversion >99% (Table 3).

Having optimized process conditions, a preparative transformation of 100 mg of racemic mexiletine (**1**) at 28 mM substrate concentration yielded (*S*)- or (*R*)-**1** with complete conversion after 48 h with >99% ee and 97% isolated yield.

In summary, a one-pot two-step deracemization sequence leading to both enantiomers of optically pure mexiletine via kinetic resolution combined with amino acid oxidase and subsequent stereoselective amination catalyzed by enantiocomplementary ω-transaminases was described. For the kinetic resolution only a catalytic amount of pyruvate was required due to in situ recycling by the amino acid oxidase.

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Supporting Information Available: Experimental details and achiral and chiral GC data. This material is available free of charge via the Internet at http://pubs.acs.org.

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