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Chemoenzymatic Synthesis of Sertraline

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Abstract: A chemoenzymatic approach has been developed for the preparation of sertraline, an established anti-depressant drug. Ketoreductases (KREDs) were employed to yield a key chiral precursor. The bioreduction of the racemic tetralone exhibited excellent enantioselectivity (>99% *ee*) and diastereomeric ratio (99:1) at 29% conversion (the maximum theoretical yield is 50%) after 7 hours. The resulting (*S*,*S*)-alcohol was efficiently oxidized to an enantiopure (*S*)-ketone, an immediate precursor of sertraline, by using sodium hypochlorite as oxidant and 2-azaadamantane *N*-oxyl (AZADO) as organocatalyst. Alternative routes aiming at the direct biocatatalytic amination using imine reductases and transaminases were unsuccessful.

Introduction

(*S*,*S*)-sertraline (**1**), (1*S*,4*S*)-4-(3,4-dichlorophenyl)-*N*-methyl-1,2,3,4-tetrahydronaphthalen-1-amine, is the active pharmaceutical ingredient (API) in Zoloft® (Pfizer), a drug for the treatment of depression and anxiety.[1] The implemented industrial process of **1** relies on a resolution of the racemic tetralone precursor followed by diastereoselective reductive amination.[2] With its two stereocenters, **1** is a challenging target, and in addition, the rather low yield of the established methodology, triggered us to seek new synthetic routes. The excellent properties displayed by enzymes in terms of selectivity and reactivity under mild reaction conditions make biocatalysis an attractive alternative for the production of enantiopure building blocks and pharmaceuticals.[3] Interestingly, the plethora of reported syntheses of **1** includes one chemoenzymatic approach based on a stereoselective enzyme- and ruthenium-catalyzed dynamic kinetic asymmetric transformation (DYKAT) employing *Candida antarctica* lipase B.[4] Very recently, Pfizer unveiled an imine reductase (IRED) capable of producing sertraline by diastereoselective reduction of the enantiopure (*S*)-*N*-methylimine precursor.[5] Having both these precedents and the current biocatalytic toolbox in mind, we envisaged alternative chemoenzymatic approaches from the commercially available racemic tetralone precursor **2** (Scheme 1). Given the structural features of **1**, the inherent enantiopreference of an enzyme ideally should set both stereocenters of the molecule at once. For this, we devised an approach starting from **2** based on ketoreductases (KREDs), according to Scheme 1.

Results and Discussion

Asymmetric bioreduction of *rac*-**2** catalyzed by KREDs was explored to yield the chiral alcohol **4** intermediate, followed by re-oxidation and selective amination to yield (*S*,*S*)-**1** (Scheme 1).[6,7] Ideally a KRED would be found that displays 4S-selectivity of **2**. This however means that inherently the maximum theoretical yield for the target (*S*,*S*)-**4**, or alternatively (*R*,*S*)-**4** is 50%, as it is assumed that no spontaneous racemization of **2** takes place. Accordingly, two different outcomes would be anticipated for our synthetic purposes: On the one hand, a diastereo- and enantioselective bioreduction in which only one enantiomer of the ketone reacts (50% conversion), namely (*S*)-**2**, leading to a 1:1 mixture of the target alcohol (*S*,*S*)-**4** or (*R*,*S*)-**4** and remaining ketone (*R*)-**2** (Scheme 2) or,

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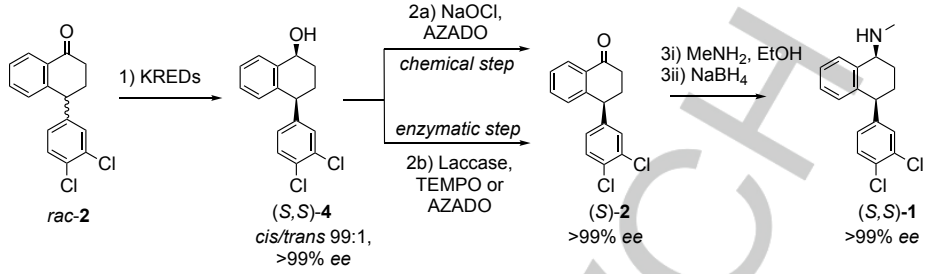
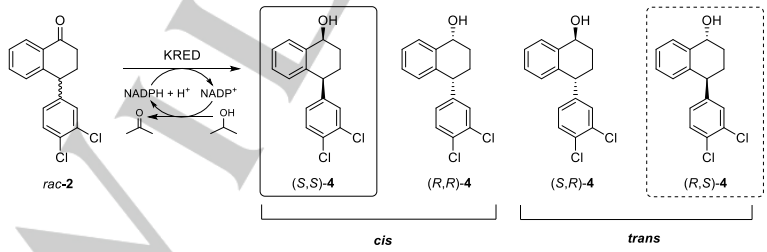


Scheme 1. Synthesis of the API Sertraline (*S,S*-**1**). The first step 1) is a KRED–catalysed selective bioreduction to the alcohol precursor (*S,S*)-**4**. The second step can either be achieved chemically 2a) by NaOCl/AZADO or enzymatically 2b) by *Trametes versicolor* laccase/AZADO. Step 3) comprises of a direct amination employing methylamine followed by reduction with $NaBH_4$.

on the other hand, an enantio- but non-diastereoselective process in which all the ketone is converted (100% conversion), leading to a diastereoisomeric mixture of alcohols (*S,S*)-**4** and (*S,R*)-**4**. Taking the further purification into account, the first scenario would be preferable due to the different polarity of ketone and alcohol. The initial screening for the bioreduction of *rac*-**2** was performed using NADPH-dependent KREDs from the Codex® KRED Screening Kit (standard conditions: 30 ºC and pH 7.5) with propan-2-ol for cofactor recycling and as co-solvent. Upon these conditions and after 24 h, half of the enzymes in the kit were very active and reached conversions higher than 90% (Supporting Information). Regarding the enantioselectivity, some KREDs displayed excellent selectivity towards the target *cis*-enantiomer (*S,S*)-**4** with *ee* up to 99%. However, none of the enzymes displayed diastereoselectivity and a 1:1 *cis*:*trans* ratio of **4** was obtained in all cases (Scheme 2). Further optimization was aimed at finding conditions in which KREDs are also diastereoselective (Table 1). For this, five KREDs from the initial screening emerged as promising candidates, namely P1-B02, P1-B05, P1-B10, P1-B12 and P1-A12 (Table 1, entry 6-10), with regards to *de* and *ee* after pH optimization (pH 5-10; full results in Supporting Information). As deduced from Table 1, the overall conversion decreased at pH 10 (9.0 for P1-A12) for all KREDs (entries 6-10). More interestingly, P1-B10, P1-B12 and P1-A12 additionally exhibited a remarkable increase in selectivity towards one of the desired isomers - (*S,S*)-**4** - (entries 8-10) with a *cis*:*trans* ratio up to 9:1. Accordingly, the specific conversion towards (*S,S*)-**4** turned out to be higher despite a lower overall conversion for those variants. Likewise, a temperature screen was conducted with the pH being kept at pH 7.5. At 50 ºC the overall conversion decreased although the diastereo- and enantioselectivity of the process was improved (entries 11-13). In particular, P1-B10 and P1-B12 led to (*S,S*)-**4** with *cis*:*trans* ratios of 10:1 and 16:1, respectively, with enantiomeric excess of the *cis* isomers of >99 % (entries 11-12).



Scheme 2. Bioreduction of racemic tetralone (*rac*-**2**) by NADPH-dependent KREDs. The four possible diastereomers are shown with the target alcohol isomers (*S,S*)-**4** (continuous) and (*R,S*)-**4** (dashed) framed. The co-factor NADPH is self-regenerated by the KREDs using 2-propanol as the sacrificial co-substrate.

Table 1. Optimisation of the KRED bioreduction of *rac*-**2** to (*S,S*)-**4**.[a]

	Entry	KRED	[**2**] (mM)	pH	T (°C)	conversion (%)[b]	*cis:trans*[b]	*ee* (%)[b]	
								cis	*trans*
initial screen	1	P1-B02	20	7.5	30	92	1:1	85 (*S,S*)	80 (*S,R*)
	2	P1-B05	20	7.5	30	40	1:1	99 (*S,S*)	87 (*S,R*)
	3	P1-B10	20	7.5	30	80	1:1	93 (*S,S*)	85 (*S,R*)
	4	P1-B12	20	7.5	30	74	1:1	89 (*S,S*)	65 (*S,R*)
	5	P1-A12	20	7.5	30	32	1:1	96 (*S,S*)	88 (*S,R*)
pH screen	6	P1-B02	7	10	30	87	1:1	80 (*S,S*)	69 (*S,R*)
	7	P1-B05	7	10	30	27	1:1	99 (*S,S*)	79 (*S,R*)
	8	P1-B10	7	10	30	29	9:1	95 (*S,S*)	44 (*S,R*)
	9	P1-B12	7	10	30	41	9:1	96 (*S,S*)	43 (*S,R*)
	10	P1-A12	7	9	30	26	3:1	98 (*S,S*)	87 (*S,R*)
temper-ature screen	11	P1-B10	7	7.5	50	29	10:1	>99 (*S,S*)	66 (*S,R*)[c]
	12	P1-B12	7	7.5	50	39	16:1	>99 (*S,S*)	54 (*S,R*)[c]
	13	P1-A12	7	7.5	50	15	1:4.5	95 (*S,S*)	95 (*S,R*)
combination of conditions & scale-up	14	P1-B10	55	7.5	50	43	9:1	98 (*S,S*)	57 (*R,R*)
	15	P1-B12	55	7.5	50	34	4:1	96 (*S,S*)	52 (*R,S*)
	16	P1-B12	55	9	50	34	16:1	99 (*S,S*)	18 (*S,R*)
	17	P1-B12	7	10	50	29[d]	99:1[d]	>99 (*S,S*) [d]	n.d.

[a] Reaction conditions: **2** (variable concentration), KRED (100% w/w), KPi buffer 125 mM pH 7.5 (1.25 mM $MgSO_4$, 1 mM NADPH), DMSO (5% v/v), *i*-PrOH (15% v/v), 250 rpm. [b] conversion, *cis:trans* ratio and *ee* were determined simultaneously by chiral HPLC analysis at 24 h reaction time. [c]due to low concentrations high standard deviations in this value. [d] Conversion and stereoselectivity determined at 7 h reaction time.

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Next, to get more insight into the process, we tested higher substrate concentrations. A higher loading of *rac*-**2** such as 55 mM produced a slight decrease of diastereomeric ratio and *ee* (entries 14-16). Finally, the synergistic effect of higher temperature and pH, namely 50 ºC and pH 10, was evaluated for KRED-P1-B12 at 7 mM and 55 mM (entries 16-17). At the lower concentration and after 7 h only *cis*-(*S*,*S*)-**4** was formed in >99% *ee* and 29% conversion (entry 17).

After separating the resulting enantiopure alcohol (*S*,*S*)-**4** from the remaining *R*-ketone by flash chromatography, next step consists of re-oxidizing this alcohol to the ketone (*S*)-**2**. Two catalytic methods proved to be effective. The enzymatic oxidation employing the laccase from *Trametes versicolor* with either TEMPO ((2,2,6,6-tetramethylpiperidin-1-yl)oxyl) or AZADO (2-azaadamantane-*N*-oxyl) as mediator reached full conversion in 16 h at room temperature.[8],[9] Alternatively, the combination of sodium hypochlorite (NaOCl) as oxidant and AZADO as organocatalyst (0.1 mol-%) in a biphasic medium (buffer:trifluorotoluene) oxidized quantitatively (*S*,*S*)-**4** to ketone (*S*)-**2** in 1 h at room temperature (95% yield).[10] In the last step the methylamine group is selectively added in quantitative yield following a reported synthetic procedure consisting of the imine formation followed by Raney-Ni-catalyzed hydrogenation,[11] the target compound Sertraline, (*S*,*S*)-**1** being obtained in 16% overall yield and >99% *ee*.

We have also explored alternative routes aiming at the direct biocatatalytic amination of *rac*-**2** using imine reductases and transaminases but these routes were unsuccessful (Supporting Information).

Conclusions

A chemoenzymatic route towards sertraline has been explored from a racemic ketone precursor providing an alternative route to the existing processes. In a KRED-based approach, the stereoselectivity could be optimized to 99:1 *cis*:*trans* ratio and >99% *ee* of the desired alcohol stereoisomer (*S*,*S*)-**4**, which was obtained at 29% conversion. The improvement could be achieved solely by condition optimization without resorting to protein engineering methods. Further organo-catalytic oxidation with a NaOCl/AZADO system provided nearly quantitatively the corresponding enantiopure ketone (*S*)-**2** within 1 h at 0.1 mol-% mediator loading. Finally, reaction with methylamine and selective chemical hydrogenation gave the final product. Overall, this new three-step chemo-enzymatic route yielded the final product (*S*,*S*)-**1** in 16% overall yield from the racemic ketone.

Experimental Section

General Remarks

A kit of KREDs was purchased from Codexis. All other commercial chemicals and enzymes were purchased from Sigma Aldrich. For the biotransformations, commercially available solvents were used. Thin-layer chromatography was performed on precoated TLC plates of Merck silica gel 60F254, using potassium permanganate as developing reagent. For column chromatography, Merck silica gel 60 (particle size, 40 - 63 µm) was used. 1H NMR and proton-decoupled 13C NMR spectra were obtained using a 400 MHz NMR spectrometer using the δ scale (ppm) for chemical shifts; calibration was made on the CDCl3 (13C; 76.95 ppm) or the residual CHCl3 (1H; 7.26 ppm) signals. HPLC analyses to determine degree of conversions were carried out in an Agilent RR1200 HPLC system, using a reversed phase column (Zorbax Eclipse XDB-C18, RR, 18µm, 4.6 x 50 mm, Agilent). HPLC analyses to determine *ee* were performed on a Hewlett Packard 1100 LC liquid chromatograph, using normal phase columns (Daicel Chiralcel OD-H and Daicel Chiralpak OJ-H).

Bioreduction of **2** employing KREDs

Initial screening: In a 1.5 mL Eppendorf tube, KRED (1 mg), *rac*-**2** (20 mM), DMSO (50 µL or as indicated) and *i*-PrOH (200 µL or as indicated) were added to 900 µL of 100 mM KH_2PO_4 buffer (1.25 mM $MgSO_4$, 1 mM $NADP^+$) pH 7.0 or 900 µL of 250 mM KH_2PO_4 buffer (2 mM $MgSO_4$, 1.1 mM $NADP^+$, 80 mM D-glucose, 10 U/mL glucose dehydrogenase) pH 7.0 in the case of KRED-101, KRED-119, KRED-130, KRED-NADH-101 and KRED-NADH-110 (or as specified in the SI). The reaction was shaken at 250 rpm and 30 ºC for 24 h. To determine the conversion, 10 µL of the mixture was diluted with 90 µL of Milli-Q water and analyzed by achiral reverse phase HPLC with previous centrifuging and filtering of the sample. Then the mixture was extracted with ethyl acetate (3x500 µL), and the *ee* and *de* were measured by HPLC in chiral normal phase.

Oxidation of (*S*,*S*)-**4** to (*S*)-**2**

Chemical oxidation

To a solution of (*S*,*S*)-**4** (20 mg, 0.08 mmol) and AZADO (0.1 mol-%) in $PhCF_3$ (150 µL), a 0.40 M solution of NaOCl (pH = 8.9; 1.0-1.4 equiv) was added. The mixture was vigorously stirred at room temperature during 1 h. Once the starting material disappeared (TLC control), the reaction mixture was extracted with ethyl acetate (3 × 600 µL). ^{1}H-NMR analysis of the crude product showed the corresponding ketone (*S*)-**2** in high purity.

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Enzymatic oxidation

To a solution of (*S*,*S*)-**4** (15 mg, 0.05 mmol) in citrate buffer pH 5.5 (900 µL) and DMSO (100 µL) was added TEMPO or AZADO (10 mol-%) and the mixture was stirred vigorously in an open-to-air vial at 20 ºC for 16 h. The reaction mixture was extracted with ethyl acetate (3 × 600 µL). ^{1}H-NMR analysis of the crude product showed the corresponding ketone (*S*)-**2** in high purity.

*Sertraline [(S,S)-**1**]*

(*S*)-**2** (30 mg, 0.10 mmol) was dissolved in 3.0 mL of a methylamine solution (2.0 M in methanol) and the mixture refluxed during 6 h. After this time, evaporation to dryness gave access to the corresponding *N*-methylimine. Without further purification, this intermediate was dissolved in methanol (2.0 mL) and reduced by conventional treatment with $NaBH_4$ (10 mg, 0.15 mmol). After purification by flash chromatography, (*S*,*S*)-**1** was obtained in excellent yield (28 mg, 92%).

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Layout 1:

FULL PAPER

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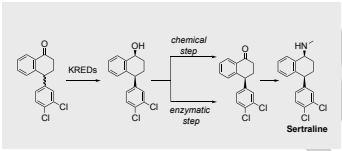

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