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Chemoenzymatic Approaches to the Synthesis of the Calcimimetic Agent Cinacalcet Employing Transaminases and Ketoreductases

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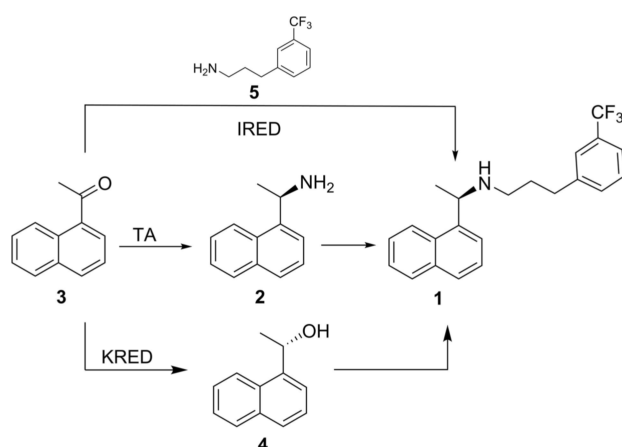
Abstract: Several chemoenzymatic routes have been explored for the preparation of cinacalcet, a calcimimetic agent. Transaminases (TAs) and ketoreductases (KREDs) turned out to be useful biocatalysts for the preparation of key optically active precursors. Thus, the asymmetric amination of 1-acetonaphthone yielded an enantiopure (*R*)-amine, which can be alkylated in one step to yield cinacalcet. Alternatively, the bioreduction of the same ketone resulted in an enantiopure (*S*)-alcohol, which was easily converted into the previous (*R*)-amine. In addition, the reduction was efficiently performed with the KRED and its cofactor co-immobilized on the same porous surface. This self-sufficient heterogeneous biocatalyst presented an accumulated total turnover number (TTN) for the cofactor of 675 after 5 consecutive operational cycles. Finally, in a preparative scale synthesis the TA-based approach was performed in aqueous medium and led to enantiopure cinacalcet in two steps and 50% overall yield.

Keywords: Cinacalcet; Transaminases; Ketoreductases; Asymmetric synthesis; Biocatalysis

1 Introduction

Cinacalcet (**1**), chemically *N*-[(1*R*)-1-(naphthyl)ethyl]-3-[3-(trifluoromethyl)-phenyl]propane-1-amine,[1] is a calcimimetic approved for the treatment of secondary hyperparathyroidism in patients with chronic kidney disease (CKD) receiving dialysis and for the treatment of hypercalcaemia associated to parathyroid carcinoma.[2] The plethora of reported syntheses are based mainly on the coupling of a chiral precursor, namely (*R*)-(+)-1-(1-naphthyl)ethylamine (**2**), with an achiral moiety by different strategies: e.g. amide or imine formation followed by reduction, or nucleophilic substitution.[3] The key chiral amine was accessed by classical or enzymatic resolutions which suffer from the inherent 50% maximum yield[4] or by several asymmetric synthetic approaches in organic media.[5] The excellent properties displayed by enzymes in terms of selectivity and reactivity under mild reaction conditions make enzymatic catalysis an attractive alternative for the production of building blocks for pharmaceuticals.[6] Thus, taking the advances in the

present biocatalytic toolbox into consideration as well as the structural features of cinacalcet, we devised three feasible chemoenzymatic approaches from 1-acetonaphthone (**3**, Scheme 1). Specifically, the asymmetric step would be catalyzed by different enzymes: a) a direct amination of **3** with 3-(3-(trifluoromethyl)phenyl)propan-1-amine (**5**) mediated by imine reductases (IREDs), to provide **1** in a single step; b) an asymmetric amination of **3** using transaminases (TAs) to yield the primary amine (*R*)-**2**; c) an asymmetric reduction catalyzed by ketoreductases (KREDs). Then, in the last two approaches the selective follow-up chemistry of the resulting optically active compounds would enable facile entry to cinacalcet according to reported methods. Herein, we report our findings on the development of novel chemoenzymatic routes towards this drug.



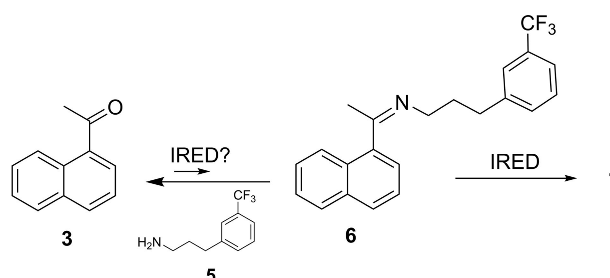
Scheme 1. Chemoenzymatic roadmap towards cinacalcet (**1**).

2 Results and Discussion

2.1 IRED-Catalyzed Reductive Amination of 3

Traditionally, chiral amines have been prepared enzymatically from racemates and by means of a kinetic resolution based on lipase-catalyzed aminolysis or hydrolysis reactions.[7] However, in the last decade other enzymatic approaches converting prochiral carbonyl compounds into amines have emerged as a valuable alternative: i) TAs transfer a primary amino group from a donor substrate to an acceptor compound mediated by pyridoxal-5-phosphate (PLP);[8] ii) amino dehydrogenases (AmDHs) catalyze the NAD(P)H-dependent reductive amination of carbonyl compounds with ammonia;[9] iii) IREDs and its recently discovered subclass reductive aminases (RedAms) accept secondary linear and cyclic amino donors and give entry to secondary and tertiary amines which are not accessible by using the previous enzymes.[10]

Initially, we focused on the most straightforward approach to cinacalcet, namely the IRED-catalyzed reductive amination of **3**. To this end, we used the commercially available amine **5** and screened a set of IREDs overexpressed in *E. coli* from the Greifswald University collection (Scheme 2).[11] Unfortunately, and despite using a high excess of amine (50 equivalents), this combination of substrates did not yield detectable product after 48 h. In view of this, we were interested whether the ketone **3** or the amine **5** was causing the limitation. Actually, whereas ketones of comparable size to **3** have been successfully converted by IREDs, **5** might be a challenging amino donor since it is by far bigger than reported examples.[10c,d] Besides, a drawback could arise from the unfavorable equilibrium of imine formation in water for **3** even though some substantial evidences have recently supported the imine formation in the active site of some IREDs with a selection of ketone and amine partners (Scheme 2).[10d] Further attempts performed with the chemically synthesized imine **6** and IREDs did not show conversion in any case.



Scheme 2. Proposed IRED-catalyzed synthesis of **1**.

2.2 Transaminase Catalyzed Amination of 3

Given the disappointing results displayed by IREDs, we turned our attention to the amination of **3** employing TAs which would enable the primary amine **2** in 100% yield,[12] one step away from cinacalcet. Thus, **3** was screened with a series of 28 commercially available TAs (Codex® Amine Transaminase Screening Kit) and also with several TAs overexpressed in *E. coli*.

In a first set of experiments **3** was assayed at a 20 mM substrate concentration with the commercial kit of TAs at 45 °C in 100 mM phosphate buffer pH 9.0, supplemented with PLP (1 mM) and isopropylamine (*i*PrNH$_2$, 1 M). Additionally, 10% v/v of DMSO was added due to the solubility issues of **3**. Under these conditions, some of the TAs were very active and reached high conversions after 24 h. More interestingly, almost all of them displayed perfect asymmetry in the amination of the carbonyl group,

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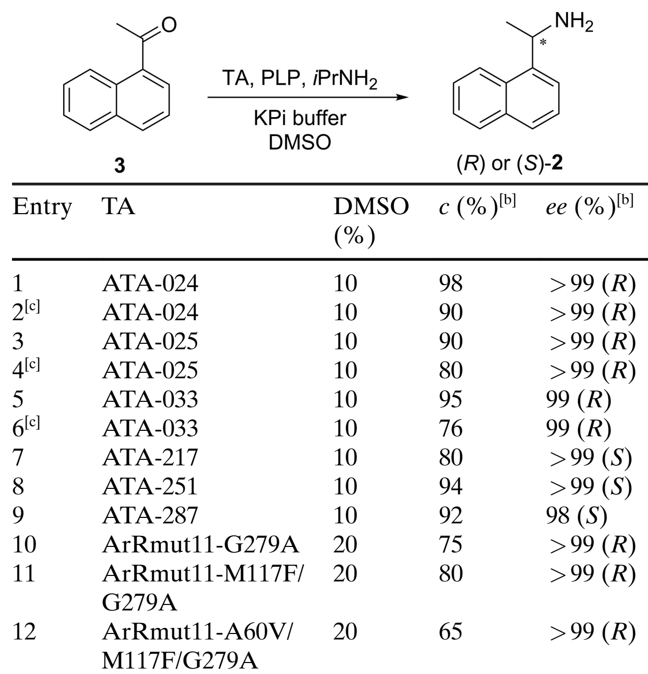
leading to the target (*R*)-**2** with >99% *ee* and also its counterpart (*S*)-**2** depending on the catalyst. Table 1 shows selected results of this screening.[13] Further attempts at higher substrate concentration (50 mM) resulted in lower conversion, as typically observed with TAs (entries 2, 4 and 6). Concerning the TAs overexpressed in *E. coli*, only variants of the *R*-selective ArRmut11[14] emerged as active biocatalysts.

Table 1. Asymmetric amination of **3** employing ATAs.[a]



Entry	TA	DMSO (%)	*c* (%)[b]	*ee* (%)[b]
1	ATA-024	10	98	>99 (*R*)
2[c]	ATA-024	10	90	>99 (*R*)
3	ATA-025	10	90	>99 (*R*)
4[c]	ATA-025	10	80	>99 (*R*)
5	ATA-033	10	95	99 (*R*)
6[c]	ATA-033	10	76	99 (*R*)
7	ATA-217	10	80	>99 (*S*)
8	ATA-251	10	94	>99 (*S*)
9	ATA-287	10	92	98 (*S*)
10	ArRmut11-G279A	20	75	>99 (*R*)
11	ArRmut11-M117F/G279A	20	80	>99 (*R*)
12	ArRmut11-A60V/M117F/G279A	20	65	>99 (*R*)

[a] Reaction conditions for entries 1–9: **3** (20 mM) in 100 mM KPi pH 9.0 (500 µL, 1 mM PLP, 1 M *i*PrNH$_2$), TA (1 mg), DMSO (10% v/v), 24 h at 45 °C and 250 rpm; Entries 10–12: **3** (50 mM) in 100 mM KPi pH 9.0 (1 mL, 1 mM PLP, 500 mM *i*PrNH$_2$), TA (20 mg lyophilized cells), DMSO (20% v/v), 48 h at 45 °C and 800 rpm.
[b] Measured by HPLC.
[c] [**3**]: 50 mM.

Originally, ArRmut11 has been specifically evolved starting from a wild type enzyme[15] for ketones bearing two bulky substituents on an industrial setting.[14] Just recently, the introduction of further mutations reducing the size of one of the two binding pockets while maintaining the exceptionally high activity and stability of ArRmut11 has been reported.[16] Thus, we envisioned variants ArRmut11-G279A, ArRmut11-M117F/G279A and ArRmut11-A60V/M117F/G279A to be convenient catalysts for the transformation of ketone **3**. They were used as lyophilized cells under slightly different reaction conditions, namely 45 °C, 800 rpm, 100 mM phosphate buffer pH 9.0 (1 mM PLP, 500 mM *i*PrNH$_2$) and 20% v/v DMSO. Interestingly, the substrate concentration could be increased to 50 mM. Again, **3** was aminated with perfect enantioselectivity, (*R*)-**2** being obtained in enantiopure form in all cases (entries 10–12). Likewise, the conversion after 48 h was up to 80%. Further attempts at higher concentrations such as 100 mM and 200 mM resulted in a drastic drop in conversion.

In view of the excellent stereoselectivity displayed by the ArRmut11 variants, we took the challenge of optimizing the biotransformation heading for a process scale up in a manufacture setting. Hence, aiming at a conversion enhancement, we tested other amino donors such as D-alanine, benzylamine and (*R*)-α-methylbenzylamine (MBA) in a high molar excess since the thermodynamic equilibrium is strongly shifted towards **3**. Table 2 collects the conversion measured at different reaction times with these reagents. In no case traces of the (*S*)-enantiomer were detected by HPLC, which demonstrates the perfect selectivity exerted by the TAs. On the other hand, D-alanine was not a suitable amino donor for these enzymes, with conversions up to 5% due to thermodynamics as one possible reason. Concerning the other reagents, the three ArRmut11 variants led to similar conversions with values around 80%. Therefore, and attending to its lower price and facility to be removed, we opted for implementing the amination based on *i*PrNH$_2$ in the synthetic scheme.

Table 2. Optimization of the amino donor in the amination of **3** with variants from ArRmut11.[a]

Entry	TA	Amino donor	Conversion (%)[b] 5 h	24 h	48 h
1	ArRmut11-G279A	*i*PrNH$_2$	50	67	75
2	ArRmut11-M117F/G279A	*i*PrNH$_2$	50	67	80
3	ArRmut11-A60V/M117F/G279A	*i*PrNH$_2$	4	62	65
4	ArRmut11-M117F/G279A	D-alanine	1	5	5
5	ArRmut11-A60 V/M117F/G279A	D-alanine	3	3	2
6	ArRmut11-M117F/G279A	BnNH$_2$	37	64	63
7	ArRmut11-A60V/M117F/G279A	BnNH$_2$	51	72	78
8	ArRmut11-M117F/G279A	(*R*)-MBA	35	64	84
9	ArRmut11-A60V/M117F/G279A	(*R*)-MBA	43	65	84

[a] Reaction conditions: **3** (50 mM) in 100 mM KPi pH 9.0 (1 mL, 1 mM PLP, 500 mM amino donor), TA (20 mg lyophilized cells), DMSO (20% v/v), 48 h at 45 °C and 800 rpm.
[b] Determined by HPLC. The *ee* was >99% in all cases for (*R*)-**2**.

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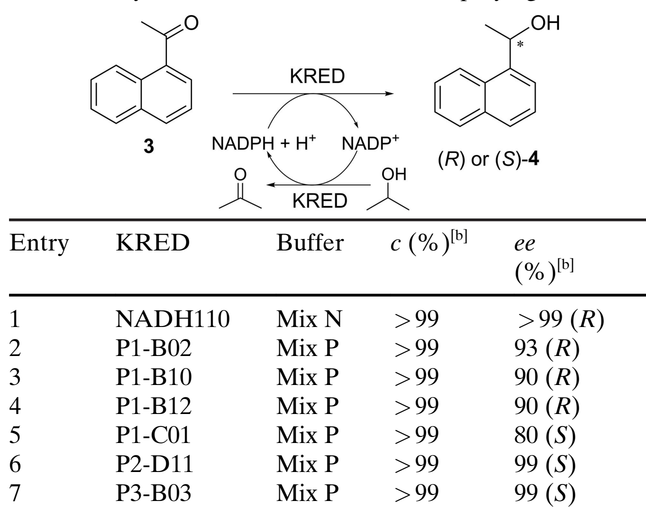
2.3 KRED-Catalyzed Bioreduction of 3

The last approach displayed in Scheme 1 towards cinacalcet consisted in a stereoselective reduction of **3** to yield (*S*)-1-(1-naphthyl)ethanol [(*S*)-**4**].[17] For this, the panel of engineered KREDs from Codexis was tested employing glucose/glucose dehydrogenase (GDH) or isopropanol (IPA) for cofactor recycling. Initially, the screening was performed at 27 mM substrate concentration with a phosphate buffer pH 7.0, IPA (15% v/v) and DMSO (5% v/v). The reaction mixtures were incubated at 30 °C and 250 rpm during 24 h. From the series of KREDs contained in the kit, it was possible to identify biocatalysts affording both enantiomers of **4** with complete conversion and excellent enantioselectivity (Table 3). Actually, the (*S*)-enantiomer of **4** is the target compound for our synthetic purposes towards **1**, as discussed later.

Table 3. Asymmetric bioreduction of **3** employing KREDs.[a]



Entry	KRED	Buffer	*c* (%)[b]	*ee* (%)[b]
1	NADH110	Mix N	> 99	> 99 (*R*)
2	P1-B02	Mix P	> 99	93 (*R*)
3	P1-B10	Mix P	> 99	90 (*R*)
4	P1-B12	Mix P	> 99	90 (*R*)
5	P1-C01	Mix P	> 99	80 (*S*)
6	P2-D11	Mix P	> 99	99 (*S*)
7	P3-B03	Mix P	> 99	99 (*S*)

[a] Reaction conditions for entry 1: **3** (27 mM), Mix N pH 7.0 [900 μL, 250 mM KPi, 2 mM $MgSO_4$, 1.1 mM $NADP^+$, 1.1 mM NAD^+, 80 mM D-glucose, 10 U/mL GDH], KRED (2 mg), *i*-PrOH (165 μL), DMSO (55 μL), 24 h at 30 °C and 250 rpm. For entries 2–7, identical conditions but using Mix P: 125 mM KPi, 1.25 mM $MgSO_4$, 1.0 mM $NADP^+$, pH 7.0.
[b] Determined by HPLC.

Very recently the co-immobilization of a ketoreductase and NADPH on agarose beads activated with tertiary amine groups (AG-DEAE) has been reported. The resulting catalyst, defined as self-sufficient heterogeneous biocatalyst, was able to catalyze asymmetric reductions without exogenous cofactor addition.[18] Moreover, it could be successfully re-used several batch operational cycles and operated in a plug-flow reactor. Seeing as the exquisite selectivity and conversion displayed by the KREDs towards **3**, we envisaged to integrate this technology into the present synthetic approach.[19] Actually, many promising enzymatic processes are often hampered by their cofactor dependency that requires very expensive reagents, endangering the economic viability.[20] With these premises, KRED–P2-D11 was immobilized on porous agarose beads activated with diethyl-aminoethyl groups (AG-DEAE) (Table 4). 96% of the offered enzyme was immobilized preserving 100% of its specific activity. The high expressed activity upon the immobilization is an excellent result compared to those reported for other KREDs.[18,21] Under the operational conditions tested in Table 3, the immobilized enzyme was 9 times more stable than its soluble counterpart (Table 4). Both immobilized and soluble enzyme underwent a first-order inactivation with residual activity under those inactivation conditions (Supporting information, Figure S1).

Table 4. Immobilization and stability parameters of KRED P2-D11 on AG-DEAE.

	Soluble	Immobilized
Load ($mg \times g_{carrier}^{-1}$)	–	0.96
Immobilization yield (%)[a]	–	96
Specific Activity ($U \times mg^{-1}$)	2.26	2.25
Expressed Activity (%)[b]	–	105 ± 10
Inactivation constant (h^{-1}) [c]	0.33	0.07
Half-life time (h)[c]	4.47	40.2
Stabilization factor[d]	–	9

[a] Immobilization yield (ψ) = (immobilized $mg_{KRED} \times g^{-1}_{carrier}$/ offered $mg_{KRED} \times g^{-1}_{carrier}$) × 100.
[b] Expressed activity (%) = (Specific activity of immobilized KRED/Specific activity of soluble KRED) × 100. The specific activity was calculated with 1 mM ketone **3**, 0.25 mM NADPH and 9% Acetonitrile at 25 °C and pH 7.
[c] Stability parameters were calculated by non-linear fitting of inactivation time-courses under 17% IPA, 4.9% DMSO at pH 7 and 30 °C (See supporting information Fig. S1).
[d] Stabilization factor = Half-life time immobilized enzyme/ Half-life time soluble enzyme.

The high activity and stability of the immobilized KRED P2-D11 encouraged us to co-immobilize higher KRED loads (6.5 $mg \times g_{carrier}^{-1}$) with NADPH (10 μmol of NADPH per gram of carrier). Then, the resulting biocatalyst was tested for the reduction of **3** using 3% of catalyst load and IPA as sacrificing reductant. We found a conversion of > 99% after 24 h preserving the enantioselectivity of the KRED [> 99% *ee* for (*S*)-**4**] without exogenous supply of NADPH. Moreover, this self-sufficient biocatalyst was reused up in 5 consecutive cycles without losing effectiveness (Table 5) with a catalyst load decreased by a factor of 9 (0.195 mg_{KRED}/mL) regarding to the free enzyme (1.79 mg_{KRED}/mL).

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The recyclability of the biocatalyst significantly increased the total turnover number (TTN) of both enzyme and cofactor. Hence, the immobilized NADPH accumulated a TTN of 450 whereas the KRED did 3600. On the other hand, we studied the continuous asymmetric reduction of **3** in a bed-packed flow reactor using the self-sufficient heterogeneous biocatalyst. Unfortunately, the systems failed observing conversions <5% at 50–200 $\mu L \times min^{-1}$ flow rates. Unlike the enzyme of the original report, namely KRED P1-A04,[18] KRED P2-D11 immobilized on the same matrix (AG-DEAE) was inefficient recycling the immobilized redox cofactor which burdened the continuous production of (*S*)-**4** (see the Supporting Information for more details).

Table 5. Recycling of KRED P2-D11 and NADPH co-immobilized on AG-DEAE for the reduction of **3**.[a]

Entry	Cycle	*t* (h)	*c* (%)[b]	*ee* (%)[b]
1	1	24	>99	>99 (*S*)
2	2	24	>99	>99 (*S*)
3	3	24	>99	>99 (*S*)
4	4	24	>99	>99 (*S*)
5	5	24	>99	>99 (*S*)

[a] Reaction conditions: **3** (27 mM) and 1 mM $MgCl_2$ were incubated in 1.0 mL of 17% IPA in 10 mM Tris-HCl at pH 7 and 25 °C with 30 mg of AG-DEAE–P2-D11 (6.5 mg_{KRED} and 10 $\mu mol_{NADPH}/g_{carrier}$) and maintained under gentle rotational agitation (50 rpm).
[b] Determined by HPLC.

2.4 Chemoenzymatic Synthesis of Cinacalcet

The synthetic sequences towards **1** are depicted in Scheme 3. The TA-based approach (route A) started with the amination of **3** with *i*$PrNH_2$ catalyzed by ArRmut11-M117F/G279A (Table 2, entry 2). This biotransformation was scaled up to 100 mg with **3** at 50 mM substrate concentration to render enantiopure (*R*)-**2** in 77% yield. Then, and after extractive work up, this amine was directly alkylated in toluene with 3-[3-(trifluoromethyl)phenyl]propan-1-ol (**7**) mediated by the iridium catalyst $[Cp^*IrCl_2]_2$ (1 mol%).[22] Thus, **1** was isolated after filtration through silica gel with a yield of 60% for the overall two-step sequence.

Encouraged by this result, we took the challenge of developing a one-pot sequential synthesis of cinacalcet in aqueous medium. Actually, a related catalyst $[Cp^*IrI_2]_2$ was reported to promote the alkylation of equimolar amounts of amine and alcohol in water.[23] Hence, a first issue was confirming that $[Cp^*IrCl_2]_2$ catalyzes the coupling of **2** and **7** in refluxing water. Pleasantly, the process took place with high yield and water as the only by-product released. Accordingly, the amination of **3** catalyzed by ArRmut11-M117F/G279A was performed again as described above. Next, the reaction mixture containing enantiopure (*R*)-**2** was evaporated to dryness to remove the excess of *i*$PrNH_2$, preventing the consumption of alcohol **7** by the amino donor. Then, the resulting crude product was suspended in water and treated with **7** and catalyst $[Cp^*IrCl_2]_2$ (1 mol%) at reflux. Unfortunately, no product was detected after 12 h which suggested metal inhibition by the enzyme or its cofactor. A further attempt consisted in testing commercially available TAs instead of lyophilized whole cells to simplify the enzymatic milieu. However, the iridium catalyst was totally inhibited again, precluding the one-pot process.[24] Nevertheless, the only required setting to solve this drawback was removing the insolubles (e.g., precipitated protein) by centrifugation after the enzymatic step, and adding to the supernatant both $[Cp^*IrCl_2]_2$ and **7**. Under this experimental setting, enantiopure **1** was produced with an overall yield of 50%.

Concerning the KRED-based approach (route B), the first step was affording the enantiopure alcohol (*S*)-**4** with complete conversion by using one of the two last enzymes depicted in Table 3 (entries 6–7). Then, and following a patented procedure (route B1), the hydroxyl group was treated with mesyl chloride to make it a good leaving group for a further nucleophilic substitution.[25] However, 1-(1-chloroethyl)naphthalene (**8**) was isolated in racemic form instead of the expected mesyl derivative. Similarly, the employment of tosyl chloride led to identical outcome. In this context, a study a few years ago showed that the treatment of electron withdrawing group-substituted benzyl alcohols with tosyl chloride renders chloride derivatives instead of the expected tosylates.[26] On the other hand, the fact of obtaining racemic species reveals the formation of a carbocation, via S_N1 mechanism, which would be stabilized by the phenyl ring and reacted with Cl^- to form racemic **8**. In a further attempt, the employment of $AgNO_3$ as a scavenger of chloride ions enabled to obtain the mesylate **9** (route B2). Subsequent nucleophilic substitution with the amine **10** gave entry to **1** although as a racemate. This suggests the lability of the mesyl derivative and the inconvenience of this methodology. Finally, the use of a base such as DBU in the presence of diphenyl phosphorazidate (DPPA) suppressed the S_N1 chemistry and rendered the azide (*R*)-**9** of opposite stereochemistry with a very slight erosion of the enantiomeric purity (97% *ee*, route B3).[27] Then, $LiAlH_4$ reduction provided the amine (*R*)-**2** which was converted into **1** as described above. The overall yield of the formal total synthesis of **1** was 65% after four steps. Comparing both chemoenzymatic approaches, route A (TA-based approach) involves two less steps and does not require chromatographic purification. Likewise, and from an environmental point of view, it

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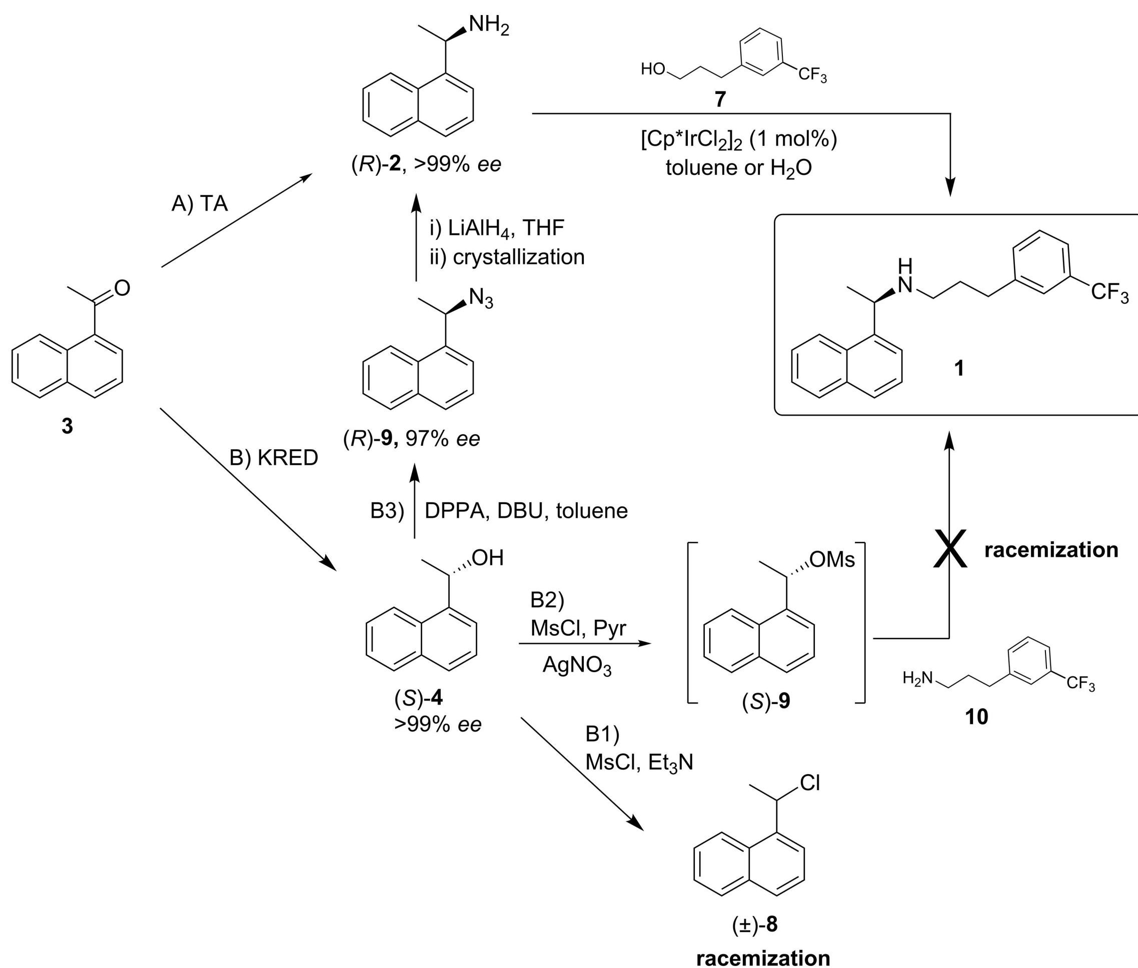

Scheme 3. Chemoenzymatic synthetic approaches towards **1**.

avoids the employment of hazardous reagents such as DPPA and is entirely performed in aqueous medium.

3 Conclusion

Two chemoenzymatic syntheses towards cinacalcet, the only approved drug acting as calcimimetic, have been developed. In the first route, the key asymmetric step was an amination of the ketone **3** employing TAs. Further iridium-catalyzed alkylation of the resulting enantiopure amine (*R*)-**2** led to cinacalcet in 50% overall yield for the two-step sequence. Notably, both steps were run in aqueous medium and the process did not require purification or isolation of any intermediates. On the other hand, the bioreduction of the same ketone by KREDs provided enantiopure alcohol (*S*)-**4** quantitatively. Then, further follow-up chemistry enabled the amine (*R*)-**2** via S_N2 inversion with DPPA and further reduction of the resulting azide, to complete the synthetic scheme towards cinacalcet in four steps and 65% overall yield. Despite the second route requiring more steps, the KRED was successfully co-immobilized with NADPH giving the possibility to be re-used in 5 batch operational cycles without adding of exogenous NADPH.

Experimental Section

General Remarks

Kits of TAs and KREDs were purchased from Codexis. The cultures for overexpression of the IREDs were inoculated from *E. coli* BL21 (DE3) glycerol stocks from Greifswald University containing the respective IREDs in a pET 22b(+) vector. The standard procedure for expression was followed as described in ref. 11a. TA variants from ArRmut11 were overexpressed in *E. coli* and used as lyophilized cells (for more details about these TAs, see ref. 16). For the enzymatic reactions, commercially available solvents were used. Thin-layer chromatography was performed on precoated TLC plates of Merck silica gel $60F_{254}$, using potassium permanganate as developing reagent. For column chromatography, Merck silica gel 60 (particle size, 40–63 μm) was used. ^{1}H NMR and proton-decoupled ^{13}C NMR spectra were obtained using a 300 MHz spectrometer using the δ scale

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(ppm) for chemical shifts; calibration was made on the $CDCl_3$ (^{13}C; 76.95 ppm) or the residual $CHCl_3$ (^{1}H; 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, 18 µm, 4.6×50 mm, Agilent). HPLC analyses to determine *ee* were performed on a Hewlett Packard 1100 LC liquid chromatograph, using normal phase columns (Chiralcel OJ–H and Chiralpak IA).

Amination of 3 Employing TAs from the Codexis' Kit (Analytical Scale)

Reactions were carried out in a 2.0 mL Eppendorf tube. The corresponding TA (1.0 mg) was added to 500 µL of 100 mM KH_2PO_4 buffer pH 9.0 containing *i*$PrNH_2$ (1 M) and the cofactor PLP (1.0 mM). Then, a solution of **3** (20 mM) in DMSO (50 µL) was added and the resulting mixture was shaken at 250 rpm and 45 °C for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL of Milli-Q water and analyzed by achiral reverse phase with previous centrifuging and filtering of the sample. Then, the reaction was quenched by addition of 100 µL of aqueous 10 N NaOH and extracted with ethyl acetate (2×500 µL). The organic layers were separated by centrifugation (90 s, 13000 rpm), combined, and finally dried over Na_2SO_4. Then, the *ee* of the amine was determined by chiral normal phase.

Amination of 3 Employing Variant ArRmut11-M117F/G279A (Preparative Scale)

Ketone **3** (100 mg, 0.588 mmol) was placed in a bottom round flask containing 10.0 mL of 100 mM KH_2PO_4 buffer (pH 9, 0.5 M *i*$PrNH_2$, 1 mM PLP). Then, lyophilized whole cells of ArRmut11-M117F/G279A (200 mg) were added and the resulting suspension magnetically stirred at 800 rpm and 45 °C for 72 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL of Milli-Q water and analyzed by achiral reverse phase with previous centrifuging and filtering of the sample. After that time the reaction was basified with a saturated solution of Na_2CO_3 (5 mL) and extracted with ethyl acetate (2×5 mL). The organic layers were separated by centrifugation (90 s, 13000 rpm), combined, and finally dried over Na_2SO_4. Then, the resulting amine (*R*)-**2** was submitted to the next synthetic step without further purification (Scheme 3, route A). Eventually, for an analytical sample the amine **2** was purified by flash chromatography of by acid-base extraction (78 mg, 77% yield). The *ee* of (*R*)-**2** was determined by chiral normal phase (>99%).

Bioreduction of 3 Employing KREDs from the Codexis' Kit (Analytical Scale)

In a 2.0 mL Eppendorf tube, KRED (2 mg), **3** (27 mM), DMSO (55 µL) and *i*-PrOH (165 µL) were added to 900 µL of 125 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. The reaction was shaken at 250 rpm and 30 °C for 24 h. To determine the conversion, 10 µL of the mixture were diluted with 90 µL of Milli-Q water and analyzed by achiral reverse phase with previous centrifuging and filtering of the sample Then the mixture was extracted with ethyl acetate (2×500 µL), the organic layers separated by centrifugation (90 sec, 13000 rpm), combined and dried over Na_2SO_4. Then, the *ee* was measured by chiral normal phase.

Immobilization of, Thermal Stability and Activity Assay of KRED P2-D11 Immobilized on Ag-DEAE

The protein immobilization was carried out with 1 g of AG-DEAE incubated with 10 mL of 0.1–0.65 mg/mL KRED P2-D11 in 10 mM sodium phosphate pH 7.0 for 1 h at room temperature. Then, 1 g of AG-DEAE immobilizing KRED was incubated with 10 mL of 1 mM NADPH in 10 mM Tris-HCl at pH 7.0. The operational stability was determined by incubating soluble and immobilized KRED under the reaction conditions (1 mM $MgCl_2$, 4.9% DMSO and 17% IPA in a 10 mM tris-HCl at pH 7.0) at 30 °C. Samples were withdrawn at different incubation times and the residual enzymatic activity was spectrophotometrically determined. For this purpose, the activity of KRED was measured by monitoring the absorbance at 340 nm along the time in a Varioskan™ Flash Multimode Reader (Thermo Scientific) using 96-well plates. Briefly, 200 µL of a reaction mixture containing 1 mM of **3**, 1 mM $MgCl_2$, 0.25 mM of NADH and 9% Acetonitrile in 10 mM sodium phosphate buffer (pH 7.0) was incubated with 20 µL of enzymatic solution or suspension at 25 °C under orbital shaking integrated into the reader. One unit of activity was defined as the amount of enzyme that was required to either reduce or oxidize 1 µmol of NADPH at 25 °C and pH 7.0.

Bioreduction of 3 in Batch Employing an Immobilized KRED

In a micro Bio-spinTM chromatographic column (BIO-RAD), a reaction mixture containing **3** (27 mM) and 1.0 mL of 17% IPA in 10 mM Tris-HCl and 1 mM $MgCl_2$ at pH 7.0 was incubated with 30 mg of self-sufficient heterogeneous biocatalysts (6.5 $mg_{KRED\ P2-D11}$ and 10 $\mu mol_{NADPH}/g_{carrier}$) and maintained under gentle rotational agitation (50 rpm) at 25 °C. As control reaction, same amount of KRED immobilized on AG-DEAE and soluble and exogenous NADPH were mixed in the same conditions. The reaction was stopped by vacuum filtration and the conversion and *ee* determined as described above. Next, the immobilized biocatalyst was washed with 10 volumes of 10 mM Tris-HCl at pH 7.0 to be re-used in the next batch cycle.

Bioreduction of 3 in Continuous-flow Mode Employing an Immobilized KRED

1 g of self-sufficient heterogeneous biocatalyst (5.85 $mg_{KRED\ P2-D11}$ and 10 $\mu mol_{NADPH}/g_{carrier}$) were packed into a column (2×0.4 cm) and connected to a flow system driven by a syringe pump. A reaction mixture containing 27 mM of **3**, 1 mM $MgCl_2$, 4.9%

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DMSO and 17% IPA in a 10 mM tris-HCl buffer solution at pH 7.0 passed through the column at different flow rates (50–200 $\mu L \times min^{-1}$) and 25 °C. Different samples were collected from the outlet of the system and analyzed by HPLC.

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