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One-step asymmetric synthesis of (*R*)- and (*S*)-rasagiline by reductive amination applying imine reductases†‡

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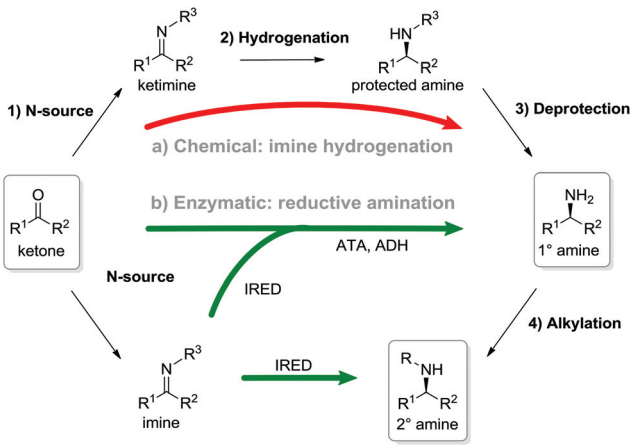
Imine reductases (IREDs) show great potential as catalysts for reductive amination of ketones to produce chiral secondary amines. In this work, we explored this potential and synthesized the pharmaceutically relevant (*R*)-rasagiline in high yields (up to 81%) and good enantiomeric excess (up to 90% ee) from the ketone precursor. This one-step approach in aqueous medium represents the shortest synthesis route from achiral starting materials. Furthermore, we demonstrate for the first time that tertiary amines also can be accessed by this route, which provides new opportunities for eco-friendly enzymatic asymmetric syntheses of these important molecules.

Introduction

Chiral amines are key components of a broad spectrum of pharmaceuticals and agrochemicals, and widely used in chemistry, *e.g.* as chiral auxiliaries and ligands.[1,2] Numerous synthesis approaches to optically pure amines have been developed.[1] In view of efficiency and atom economy, asymmetric syntheses starting from prochiral ketones as precursors are the preferred choice. Classical transition metal-catalyzed enamine or imine hydrogenation methods require 2–4 steps for synthesizing primary amines (Scheme 1, route a).[3,4] An important requirement is to find a catalyst that specifically reduces the imine bond but not the carbonyl to the corresponding alcohol.[5]

Biocatalysis is increasingly applied in developing green chemistry for chemical synthesis at the industrial scale.[6–8] Enzymes often provide excellent stereo- and regioselectivity and facilitate to work under mild and aqueous conditions.



Scheme 1 Enzymatic approaches provide a "green" alternative for synthesis of chiral amines. Route (a): Imine hydrogenation requires multiple steps. Route (b): Enzymatic preparation of amines with imine reductase (IRED), amine transaminase (ATA) and amine dehydrogenase (ADH). Only IREDs are suitable for obtaining *sec*-amines in one step. Some IREDs also accept ammonia to yield primary amines.

Compared to many other catalysts, enzymes can be produced from renewable resources. Toxic reagents or catalysts containing heavy metals are avoided, as well as extensive protection and deprotection steps, which otherwise guarantee regioselectivity. Shorter routes offer savings of reagents and solvents that otherwise are consumed during intermediate product isolation and purification steps, which also often reduce the overall yield.

Many established enzymatic routes for the synthesis of chiral amines start from the amine racemate,[9–13] but more interesting are enzymes that transform prochiral carbonyl compounds into amines: transaminases, amine dehydrogenases, and imine reductases (IREDs) (Scheme 1, route b).

Transaminases transfer an amino group from a donor substrate to an acceptor compound utilizing the cofactor pyridoxal-5-phosphate (PLP).[14] This type of reaction enables kinetic resolution of a racemic amine as well as asymmetric

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synthesis from a ketone substrate.[15] Dehydrogenases catalyze the NAD(P)H-dependent reductive amination of carbonyl compounds, usually α-keto acids, but also ketones.[16] However, especially for secondary and tertiary amines, asymmetric synthesis is quite challenging due to the natural catalytic limitations of these enzymes. Transaminases are limited to the transfer of a (primary) amino group because an *N*-alkyl substituent of a secondary amine substrate would cause a steric clash with the 3′-OH-group of the PLP cofactor when the planar quinoid intermediate is formed during catalysis. Also amine dehydrogenases[17] are currently limited to ammonia as a nitrogen donor due to steric restrictions of the ammonia binding pocket of the enzymes. Recently, several groups investigated asymmetric reductive amination catalyzed by IREDs[18–20] (Scheme 2a). Besides reduction of cyclic imine substrates,[21–26] IREDs also reduce acyclic imines[27–29] that have been formed in small quantities by condensation of a ketone and an amine directly in the aqueous reaction medium.[27] This facile reaction setup is possible due to the outstanding chemoselectivity of the IREDs that reduce selectively the imine, but not the excess ketone substrate. Thus, this one-step enzymatic conversion represents an interesting alternative and bears large potential as an environmentally benign approach. Due to the unfavorable equilibrium of imine formation under aqueous conditions, reductive amination turned out to be challenging. The feasibility of reductive amination was demonstrated recently at the analytical scale.[28,29] In our joint research consortium focusing on IREDs,[27] suitable enzymes from Roche's in-house collection were identified that convert several model ketones such as (cyclo)hexanone with methyl- or butylamine or ammonia. After optimizing the reaction conditions, increased conversions and reduced enzyme loads allowed the preparative scale conversion of 1% (w/v) substrate to the corresponding amines with high yields and enantiomeric/diastereomeric excess.[27] This finding provides a new biocatalytic opportunity for the straightforward synthesis of chiral secondary amines.

In this study, we wanted to investigate the scope of IREDs as catalysts for reductive amination for the preparation of pharmaceutically relevant amines. We have selected three conceivable candidates which are used in the application of treatment of Parkinson's disease,[30] rasagiline, selegiline and pramipexole (Scheme 2b).

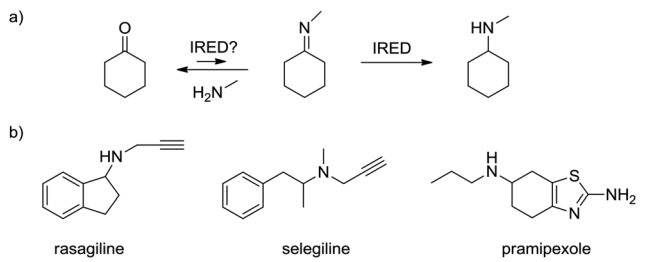
Especially the tertiary amine selegiline is challenging, as it requires the acceptance of a secondary amine by the IRED, which has not yet been shown so far.

Results and discussion

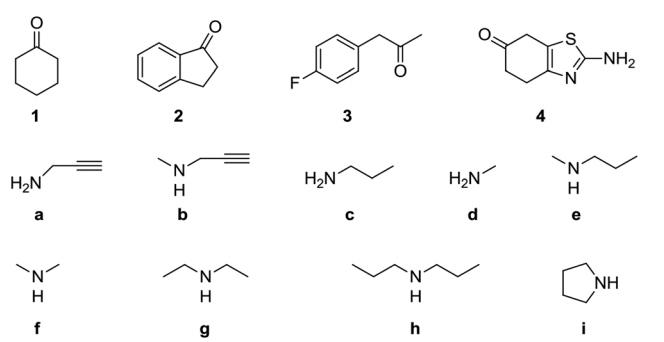
We screened the IRED library provided by Roche,[21] and enzymes from our group[31] (enzymes summarized in Table S1‡) for conversion of the corresponding ketones (for selegiline we used 4-fluoro-phenyl acetone due to the chemical regulations) and amines to yield the desired pharmaceutical products. Only for two IREDs out of this enzyme library, we found an initial activity giving a conversion of 21% for IR-14 (91% ee (*R*)-rasagiline) and 12% for IR-Sip (71% ee (*S*)-enantiomer of rasagiline).

As for most of the enzymes these substrate combinations did not yield detectable product formation, we were interested whether the corresponding ketone or amine substrates are causing the limitation. The ketones **2–4** (Scheme 3) are larger compared to the successfully converted ketones reported recently.[27] It was also shown that larger amines such as butylamine are only accepted by few IREDs. Besides propylamine **c**, amines **a** and **b** (Scheme 3) might be difficult substrates because of steric demands (rigid geometry at the triple bond, secondary amine). In our previous study, the combination of cyclohexanone and methylamine was especially well accepted by almost all IREDs investigated in the library.[27] Thus, we decided to combine the challenging ketones **2–4** with methylamine **d** and to react the desired amines **a–c** with cyclohexanone **1** to explore the enzyme's limitations.

In our screening we employed the 14 most promising IREDs. When using the well-accepted model ketone cyclohexanone, we identified various enzymes that accepted propargyl- and propylamine to yield the secondary amines **1a** and **1c** with high conversion (Table 1). Most interestingly, a significant formation of the tertiary amine **1b** with >20% conversion could be observed by two IREDs. When we reacted the pharmaceutically relevant ketones **2** and **3** with methylamine, we obtained conversions of up to 70% for 1-indanone and >80%

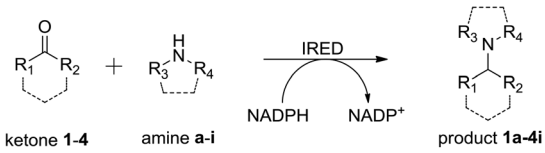


Scheme 2 (a) IRED-catalyzed reductive amination of the model substrates cyclohexanone and methylamine. Whether the imine forms in the bulk solution or inside the active site of the enzyme is not yet known. (b) Three pharmaceutical target molecules are subjects of this study.



Scheme 3 Selected compounds for the substrate screening of IREDs. **1–4**: ketone substrates, **a–i**: amine substrates.

Table 1 Screening results for the reductive amination with IREDs



ketone **1-4** amine **a-i** product **1a-4i**

Screening 1[a] IRED	Apparent product formation[b] [%] 1a	1b	1c	2d	3d	4d[c]
IR-10	41	0	2	0	0	0
IR-11	72	0	59	29	0	0
IR-13	7	0	6	0	0	0
IR-14	>95	44	>95	12	33	0
IR-19	26	3	60	0	0	0
IR-20	82	0	25	42	0	0
IR-22	80	0	32	14	10	0
IR-23	>95	0	>95	23	73	0
IR-4	56	0	25	3	14	0
IR-5	36	0	47	64	0	0
IR-15	>95	2	>95	11	0	0
IR-Sip	>95	23	>95	72	>80	0
IR-32	85	7	87	9	53	0
IR-33	85	0	93	0	28	0

Screening 2[d]	1b	1e	1f	1g	1h	1i
IR-14	83	9	47	0	0	>95
IR-Sip	67	7	55	0	0	>95

[a] IRED screening 1: Cyclohexanone with three amines **1a–1c**; and methylamine with three ketone substrates **2d–4d**. Screening conditions: 500 mM amine, 10 mM ketone, pH 9.5, 0.5 mg mL^{-1} purified enzyme, 0.5 mM NADPH, 0.1 mg mL^{-1} glucose dehydrogenase (Codexis GDH-105), 60 mM D-glucose, 20 h, 30 °C, stopped with sodium hydroxide (end concentration 1.7 M). [b] Apparent conversion was calculated based on the peak areas of product and substrate. [c] Substrate precipitated during time, kinetic ^{1}H-NMR measurements showed keto–enol tautomerization and a decrease of substrate concentration to 79% during 20 h (ESI Fig. S3a–S3d). [d] IRED screening 2: Cyclohexanone with six secondary amines **b**, **e–i**. Compared to screening 1, the reaction time was elongated to 90 h.

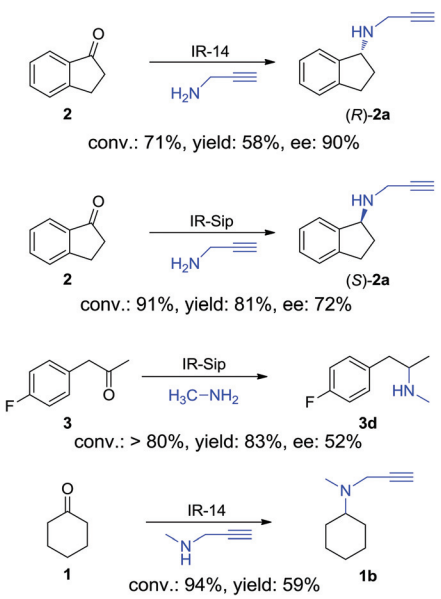
for 4-fluoro-phenyl acetone, in contrast to product **4d**, which could not be detected and thus ketone **4** seems not to be a substrate for the IREDs investigated. This experiment shows that although an IRED might accept a desired ketone or amine (if combined with well-known model substrates), this does not guarantee that arbitrary combinations of these (accepted) substrates are converted efficiently.

The formation of the tertiary amine **1b** demonstrates for the first time that IREDs also accept secondary amines as nucleophiles. As asymmetric synthesis of tertiary amines is challenging, we explored the amination of cyclohexanone **1** using the two enzymes (IR-14, IR-Sip) that were positive hits for the formation of the tertiary amine product **1b** and selected the secondary amines **e–i** as model substrates. The reaction conditions were similar to the first screening except for a prolonged reaction time (4 d). Compared to the conversion with amine **b**, the analogous saturated amine **e** showed a significantly lower conversion. How the triple bond of substrate **b** increases the activity of the IRED is not understood at the moment. Substrate amines **g** and **h** having both alkyl substituents larger than a methyl group were not accepted. In contrast, we were pleased to find nearly quantitative conversion with pyrrolidine **i**. This calls for a systematic in-depth characterization of secondary amines as substrates, which is planned for a future study.

Next, we conducted preparative scale experiments for the asymmetric reductive amination. Of special interest was the synthesis of rasagiline considering the pharmaceutical relevance of this compound. To increase the initial conversion of only 21%, we optimized the reaction conditions in small-scale experiments: ketone and amine concentrations and enzyme concentrations were varied as described in detail in the ESI (Fig. S4–S7‡). Under the optimal conditions, rasagiline **2a** was obtained with 86% conversion employing IR-Sip. Furthermore, preparative-scale experiments were performed to yield 4-fluoro methamphetamine **3d** and the tertiary amine *N*-methyl-*N*-propargyl cyclohexanamine **1b**. For rasagiline **2a**, two reactions employing IR-14 and IR-Sip yielded both enantiomers (Scheme 4).

The products were precipitated as HCL-salt, yielding 70 mg (58%) for (*R*)-**2a** with 90% ee; 98 mg (81%) for (*S*)-**2a** with 72% ee; 101 mg (83%) for **3d** with 52% ee and 73 mg (59%) for **1b**. This one-step approach is an interesting alternative to recently reported 3–5 step syntheses relying on the preparation of enantiopure precursors 1-aminoindane or indane-1-ol followed by an alkynylation step. These precursors can be prepared by (dynamic)[32] kinetic resolution,[33] or asymmetric synthesis *via* C–H-amination.[34]

Synthesis approaches for chiral primary amines featuring an outstanding eco-efficiency were developed by utilizing amine transaminases, for instance the synthesis of sitagliptin[35] or nor(pseudo)ephedrine.[36,37] Imine reductases clearly



Scheme 4 Preparative-scale reductive aminations catalyzed by IREDs. Reaction conditions: 100 mg substrate (1-indanone, 4-fluoro-phenyl acetone or cyclohexanone, 10 mM), amine buffer pH 9.5 (propargylamine, *N*-methyl propargylamine 0.4 M; methylamine 1 M), 1.5 mg mL^{-1} purified IRED, 7 days at 30 °C.

have similar potential for the synthesis of secondary and tertiary amines, if the following issues are optimized in future studies: identifying or creating IREDs that (i) are highly active and stable to decrease the enzyme load and (ii) convert high ketone concentrations with preferentially one equivalent of the amines. Increasing efficiency was also a challenge when IREDs were first investigated for reduction of cyclic imines. The characterization of a large number of IREDs showed that specific activities and enantioselectivities vary considerably. A few enzymes could be identified that are highly active, *e.g.* the IRED from *Amycolatopsis orientalis*[26] having a 100-fold higher activity in the formation of (*S*)-amines compared to the first discovered (*S*)-selective IRED from *Streptomyces* GF3546.[38]

Compared to imine reduction, where already high product concentrations of up to 480 mM can be reached,[24,27] ongoing enzyme and reaction engineering efforts for the reductive amination will increase the eco-efficiency of this one-step route. Protein engineering will be the method of choice to broaden the substrate specificity towards bulky ketones and larger amines. Especially the potential of IREDs to synthesize chiral tertiary amines will be further exploited and optimized, as the number of possible (pharmaceutical) targets is large. Tertiary amines are even more diverse in their molecular architecture compared to secondary amines and thus it is important to identify or create a toolbox of enzymes having the desired substrate specificities.

Conclusion

We explored IREDs for the synthesis of pharmaceutical compounds and demonstrated the synthesis of both enantiomers of rasagiline and for the first time the ability of IREDs to synthesize tertiary amines. This paves the way for further developing the IRED toolbox for the eco-efficient one-step synthesis of pharmaceuticals containing chiral secondary and tertiary amines.

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