Article

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One Pot Cascade Synthesis of Mono- and Di-Substituted Piperidines and Pyrrolidines using Carboxylic Acid Reductase (CAR), ω-Transaminase (ω-TA) and Imine Reductase (IRED) Biocatalysts

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ABSTRACT

Access to enantiomerically pure chiral mono- and di-substituted piperidines and pyrrolidines has been achieved using a biocatalytic cascade involving carboxylic acid reductase (CAR), ω-transaminase (ω-TA) and imine reductase (IRED) enzymes. Starting from keto acids or keto

aldehydes, substituted piperidine or pyrrolidine frameworks can be generated in high conversion, *ee* and *de*, in one pot, with each biocatalyst exhibiting chemo-, regio- and/or stereoselectivity during catalysis. The study also includes a systematic investigation of the effect of the position of a methyl group ring substituent on the IRED-catalyzed reduction of a chiral imine. Analysis of the selectivity observed in these reactions revealed an interesting balance between substrate versus enzyme control; the configuration of the products obtained were rationalized based on minimizing 1,3- or 1,2-steric interactions with incoming NADPH.

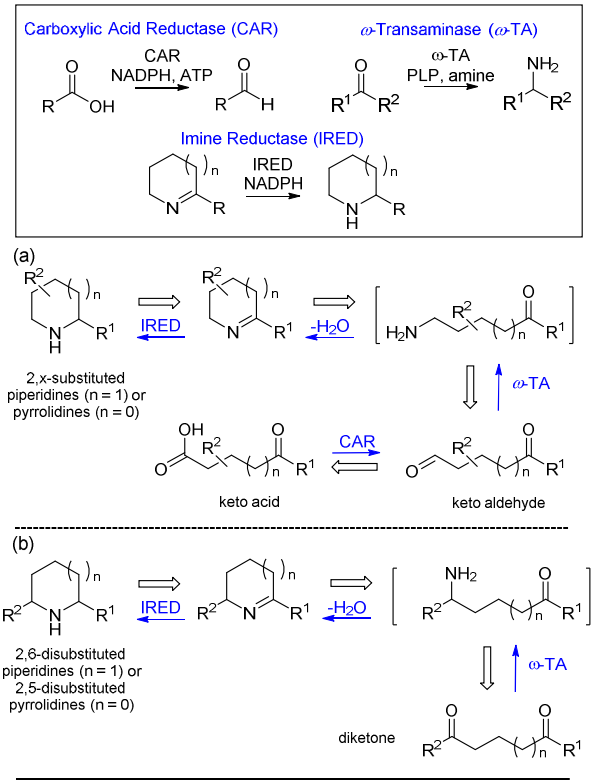
KEYWORDS Biocatalysis, carboxylic acid reductase, ω-transaminase, imine reductase, piperidines, pyrrolidines

INTRODUCTION

Chiral substituted piperidines and pyrrolidines are important architectures that are found in many biologically active natural products and pharmaceuticals. Further substitution of these templates and concomitant addition of asymmetric centers gives rise to more structural complexity, generating increasingly attractive scaffolds to explore chemical space in medicinal chemistry.[1] However, controlling the formation of two or more stereogenic centers, to access a single desired piperidine/pyrrolidine stereoisomer, is often challenging.[2–5] Current asymmetric approaches that have been developed include those based upon diastereoselective metalation/cross-coupling,[6–9] ring-closing reactions,[10–12] pyridine reductions[13–16] and chemo-enzymatic synthesis.[17–21] Alternative strategies involve the synthesis of the required diastereomers as racemates followed by kinetic resolution to generate the single enantiomer products.[22,23] However, the recent emergence of new toolboxes of engineered biocatalysts, with

broad substrate coverage, offers the prospect of constructing such targets via multi-enzyme catalysis using simple and inexpensive precursors.

Biocatalytic retrosynthetic analysis[24] suggested that the substituted piperidine/pyrrolidine skeleton could be prepared from the corresponding keto acid via a sequence involving carboxylic acid reductase (CAR), ω-transaminase (ω-TA) and imine reductase (IRED) (Scheme 1a). Implicit in the design of this sequence is the expected exquisite chemoselectivity of each individual biocatalyst, which could allow for a one-pot reaction sequence owing to the orthogonal reactivity of each enzyme. We envisaged employing an IRED as the key biocatalyst



Scheme 1. Biocatalytic retrosynthesis and forward routes to (a) 2,x-disubstituted piperidines ($x \neq 6$) and pyrrolidines ($x \neq 5$) and (b) 2,6-disubstituted piperidines and 2,5-disubstituted pyrrolidines.

for establishing the C-N bond stereochemistry of the product (Scheme 1a). Adaptation of this sequence (Scheme 1b) would also provide a route to the corresponding 2,6-di-substituted piperidines/2,5-di-substituted pyrrolidines from the corresponding diketones. In this sequence, there is a requirement for both regioselective and stereoselective transamination in order to differentiate between two similar ketones and also establish the initial stereogenic center.

CARs have recently gained interest as biocatalysts due to their ability to selectively and efficiently reduce a broad range of carboxylic acids to aldehydes,[25–27] a feat that is very difficult to accomplish cleanly and in high yield using traditional chemical methods. CARs are both ATP- and NADPH-dependent and hence are best used as whole-cell biocatalysts, with the addition of glucose to allow for intracellular regeneration of cofactors by endogenous enzymes. For maximum activity, co-expression of a 4'-phosphopantetheinyl transferase is required to post-translationally modify the native CAR apo-enzyme, by adding a 4'-phosphopantetheine arm that is required to transfer acyl-AMP intermediates between adenylation and reduction domains within the enzyme.[25,28]

ω-Transaminases are well-established biocatalysts that convert ketones or aldehydes into chiral primary amines, typically with high *ee*, at the expense of a suitable amine donor such as alanine or isopropylamine.[29–31] The ability of these enzymes to distinguish between two ketones, with regioselective amination at the least hindered carbonyl, has been demonstrated by Kroutil and co-workers and ourselves previously.[17–21] Furthermore, the synthetic utility and robustness of these enzymes has already led to their incorporation as key biocatalysts in a number of different cascade systems.[31]

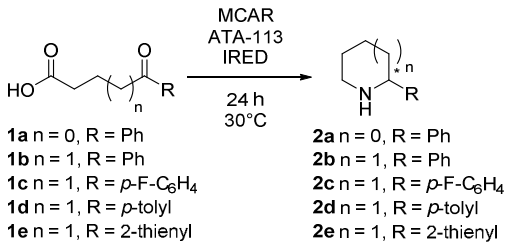
Imine reductases (IREDs) have been shown to be useful biocatalysts for generating chiral mono-substituted piperidines and pyrrolidines from chemically synthesized cyclic imines.[32–42] These NADPH-dependent enzymes typically accept a broad range of prochiral cyclic imines which are reduced to the corresponding chiral amines, often with high conversion and *ee*.

RESULTS AND DISCUSSION

In order to initially test the feasibility of the envisaged cascade, five simple keto acid substrates **1a-1e** were examined. The bacterial CAR from *Mycobacterium marinum* (MCAR), which has been reported to reduce various medium to long chain fatty acids,[27] was employed as a whole-cell biocatalyst in *E. coli*. In addition, the gene for a 4'-phosphopantetheinyl transferase (Sfp) from *Bacillus subtilis* was co-expressed within the cell as previously described.[27] A commercially available transaminase from Codexis, ATA-113, was used with racemic DL-alanine supplied as the amine donor for the amination reaction. To ensure full conversion of the intermediate aldehyde, a glucose dehydrogenase (GDH)/lactate dehydrogenase (LDH) system, which has previously been reported,[43,44] was also implemented to shift the transaminase equilibrium. Finally, two enantiocomplementary whole-cell IRED biocatalysts were employed in the cascade reactions in order to access both enantiomers of the amine products: the (*R*)-IRED from *Streptomyces* sp GF3587[34,37,45] and the (*S*)-IRED from *Streptomyces* sp GF3546.[34,36,38,42]

Table 1 shows the results of biotransformations carried out as one-pot cascade processes. Rapid and complete consumption of the starting keto acid was observed in all cases (24 h), with subsequent reduction of the imine to the amine product with modest to excellent *ee* values that are consistent with our previously reported work with these IREDs.[37,38] No transamination of the ketone carbonyl was observed, as predicted, which would have afforded the regioisomeric imine.

Table 1. CAR-TA-IRED Cascade Synthesis of 2-Substituted Piperidines



Substrate	*Conv. to imine/%*	*IRED*	*Conv. to **2**/%*	*ee/% (abs config)*
1a	>98	(*R*)-IRED	87	17 (*S*)
1a	>98	(*S*)-IRED	34	75 (*R*)
1b	>98	(*R*)-IRED	>98	38 (*S*)
1b	>98	(*S*)-IRED	>98	93 (*R*)
1c	>98	(*R*)-IRED	97	90 (*S*)
1c	>98	(*S*)-IRED	98	96 (*R*)
1d	>98	(*R*)-IRED	>98	>98 (*S*)
1d	>98	(*S*)-IRED	>98	94 (*R*)
1e	>98	(*R*)-IRED	>98	71 (n.d.)[a]
1e	>98	(*S*)-IRED	89	74 (n.d.)[a]

Reaction conditions: 5 mM substrate , 75 mg/mL MCAR wet whole cells, 50 mg/mL IRED wet whole cells, 2.5 mg/mL ATA-113, 1 mg/mL GDH, 0.5 mg/mL LDH, 250 mM DL-alanine, 100 mM glucose, 1.5 mM NAD^+, 1 mM PLP, 500 mM pH 7.0 NaP_i buffer, 500 µL reaction

volume, 30°C, 250 rpm, 24 h. Conversions and *ee* values were determined by GC-FID or HPLC analysis on a chiral stationary phase with absolute configuration determined by comparison to literature retention times.[37] (a) Absolute configuration not determined, however, (*R*)- and (*S*)-IRED afforded opposite enantiomers.

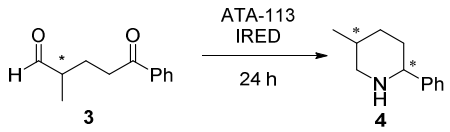
In the absence of the GDH/LDH system, conversion through to the final amine product was achieved, however, some over-reduction of the keto acid to the keto alcohol was observed (see Supporting Information). This suggests that, without a strong driving force for the transaminase reaction, the keto aldehyde can be intercepted and reduced by endogenous reductases in the whole-cell before the desired transamination. Some keto alcohol by-product was also observed in reactions with **1a** despite employing the GDH/LDH system, however, for substrates **1b-e** no keto alcohol was detected.

The use of isopropylamine as an alternative amine donor for the transaminase was tested with substrate **1b**, however, lower conversions were obtained. Furthermore, a CAR orthologue from *Nocardia iowensis* (NCAR)[46,47] was tested in the cascade with substrates **1b-1d**, which led to similar or slightly lower conversions in some cases and so was not pursued further. Substrate loading (up to 50 mM) was also investigated which saw a decrease in conversion as substrate concentration increased (see Supporting Information). Higher catalyst loading in this case may be required to achieve greater conversion.

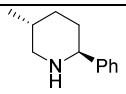
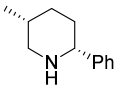
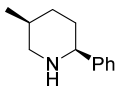
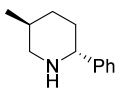
Having established the viability of the cascade for 2-substituted piperidines, we turned our attention to disubstituted analogues. Initially, the formation of 5-methyl-2-phenylpiperidine **4** was investigated, since both enantiomers of the required keto aldehyde substrate **3** could be prepared based on the report by Gellman *et al*..[48] Aldehydes (*R*)- and (*S*)-**3** were subjected to the cascade conditions employing ATA-113 and either (*R*)- or (*S*)-IRED (Table 2). In all cases,

conversion of the starting material to the imine intermediate was complete and subsequent conversion to the product piperidine by the IRED was good to excellent. It was observed that the chiral center alpha to the aldehyde functionality can racemize in buffer, however, this was minimized in the cascade as upon transamination and formation of the imine, this stereocenter is configurationally stable.

Table 2. Synthesis of 2,5-Disubstituted Piperidines



3 **4**

Substrate	*IRED*	*Product*	*Conv./%*	*de/%*	*ee/%*
(*R*)-**3**	(*R*)-IRED	 (2*S*,5*R*)-**4**	83	72	88
(*R*)-**3**	(*S*)-IRED	 (2*R*,5*R*)-**4**	95	>98	>98
(*S*)-**3**	(*R*)-IRED	 (2*S*,5*S*)-**4**	96	29	>98
(*S*)-**3**	(*S*)-IRED	 (2*R*,5*S*)-**4**	45	79	>98

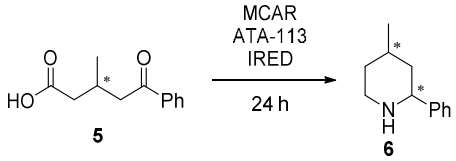
Reaction conditions: 5 mM substrate, 2.5 mg/mL ATA-113, 1 mM PLP, 250 mM DL-alanine, 45 mg/mL IRED wet whole cells, 100 mM glucose, 100 mM pH 7.0 NaP_i buffer, 500 μL reaction volume, 30°C, 250 rpm, 24 h. Conversions, *de* and *ee* values were determined by GC-FID analysis on a chiral stationary phase.

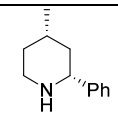
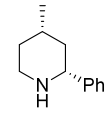
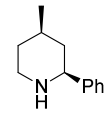
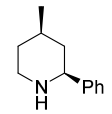
Interestingly, the pre-existing stereogenic center present in the imine intermediate did not affect the stereoselectivity of the IRED reduction, i.e. the (*R*)-IRED yielded the product with a C-2-(*S*)-stereocenter and the (*S*)-IRED affording the C-2-(*R*)-isomer.

All four possible stereoisomers of 5-methyl-2-phenylpiperidine **4** could thus be achieved using this cascade, with the best result being the formation of (2*R*,5*R*)-**4** in excellent conversion and *de* by employing (*S*)-IRED (Table 2, Entry 2). Compound (2*R*,5*R*)-**4** is noteworthy as it is the minor kinetic product upon reduction of the corresponding imine with $NaBH_4$ and, therefore, the cascade offers a convenient route to this diastereomer.

Access to the regioisomeric compounds 4-methyl- and 3-methyl-2-phenylpiperidine (**6** and **10** respectively) was then investigated. The corresponding keto acids were used as substrates and the keto aldehydes generated *in situ* by means of the full CAR-TA-IRED cascade (Table 3). Complete consumption of the starting material was observed after 24 h, with only amine product and unreacted imine intermediates detected. Interestingly, for these compounds, the inherent selectivity of the IRED was overridden by the existing chirality of the imine, with only the *cis*-diastereomer observed in high conversion and *de* independent of whether the (*R*)- or (*S*)-IRED was used. This element of substrate control favored the more thermodynamically stable product with both ring substituents equatorial.

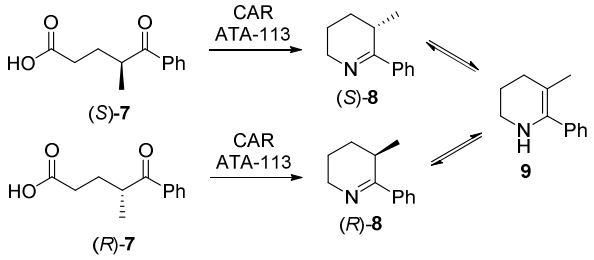
Table 3. Synthesis of 2,4-Disubstituted Piperidines



Substrate	*IRED*	*Product*	*Conv./%*	*de/%*	*ee/%*
(*R*)-**5**	(*R*)-IRED	 (2*R*,4*S*)-**6**	69	87	>98
(*R*)-**5**	(*S*)-IRED	 (2*R*,4*S*)-**6**	>98	95	>98
(*S*)-**5**	(*R*)-IRED	 (2*S*,4*R*)-**6**	74	92	>98
(*S*)-**5**	(*S*)-IRED	 (2*S*,4*R*)-**6**	91	71	>98

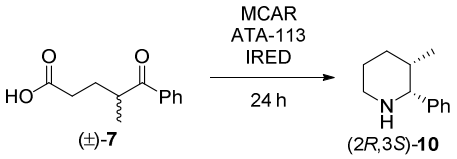
Reaction conditions: 5 mM substrate , 75 mg/mL MCAR wet whole cells, 50 mg/mL IRED wet whole cells, 2.5 mg/mL ATA-113, 1 mg/mL GDH, 0.5 mg/mL LDH, 250 mM DL-alanine, 100 mM glucose, 1.5 mM NAD^+, 1 mM PLP, pH 500 mM pH 7.0 NaP_i buffer, 500 µL reaction volume, 30°C, 250 rpm, 24 h. Conversions, *de* and *ee* values were determined by GC-FID analysis on a chiral stationary phase.

For keto acid **7** we observed racemization of the pre-existing stereogenic center at C-4 once it had entered the cascade, likely due to the equilibrium of the imine intermediate with its enamine tautomer (Scheme 2). As a consequence, supplying either a single enantiomer or the racemic keto acid into the cascade furnished an identical product distribution. Under these conditions a chiral imine intermediate is generated that can equilibrate its enantiomers, allowing the IREDs to catalyze a dynamic kinetic resolution (DKR) process (Table 4).



Scheme 2. Racemization of (*S*) or (*R*)-**7** by imine-enamine tautomerization

Table 4. Synthesis of 2,3-Disubstituted Piperidines



Substrate	*IRED*	*Conv./%*	*de/%*	*ee/%*
(±)-**7**	(*R*)-IRED	89	95	18
(±)-**7**	(*S*)-IRED	86	81	81

Reaction conditions: 5 mM substrate, 75 mg/mL MCAR wet whole cells, 50 mg/mL IRED wet whole cells, 2.5 mg/mL ATA-113, 1 mg/mL GDH, 0.5 mg/mL LDH, 250 mM DL-alanine, 100 mM glucose, 1.5 mM NAD^+, 1 mM PLP, 500 mM pH 7.0 NaP_i buffer, 500 µL reaction

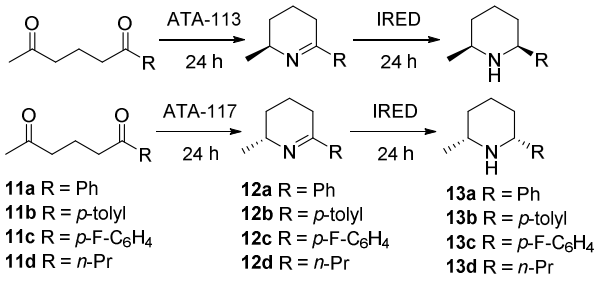
volume, 30°C, 250 rpm, 24 h. Conversions, *de* and *ee* values were determined by GC-FID analysis on a chiral stationary phase.

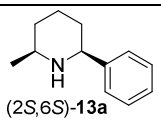
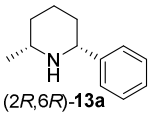
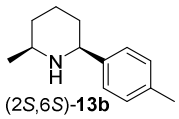
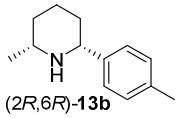
Remarkably, in all cases the *cis*-diastereomer was the major product and was obtained in high conversion and in good to high *de*. Starting with a single enantiomer of **7** afforded comparable results (see Supporting Information).

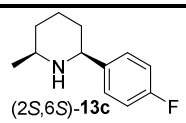
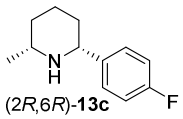
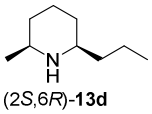
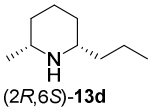
For the synthesis of 2,6-disubstituted piperidines, a panel of 1,5-diketones (Table 5) were treated with either the (*S*)-selective ATA-113 or the enantiocomplementary (*R*)-selective ATA-117.[49] In these cases, the transaminases were used to establish the first stereogenic center by transformation of an achiral starting material. As previously described,[18–20] the transaminases catalyzed the amination of the less-hindered carbonyl exclusively, producing the disubstituted chiral imine in very high *ee*. Initial results suggested that the presence of IRED-containing whole-cells in a one-pot reaction cascade inhibited conversion of the starting diketone by the transaminase enzymes. Therefore, the procedure was optimized to allow complete consumption of the diketone starting material by the transaminase, before sequential addition of the IRED whole-cell biocatalysts after 24 h in the same pot.

The 2,6-disubstituted piperideines proved to be challenging substrates for imine reduction, therefore the IRED cell loading was increased to 200 mg/mL in order to achieve higher conversions. Substrate control was once again apparent, as seen for the 2,4-disubstituted piperidines; the more stable 1,3-diequatorial product was formed regardless of the IRED used. This outcome was particularly pronounced with bulky aromatic-substituted piperideines, which were converted to their corresponding amines in high *de*. In cases where the inherent

Table 5. Synthesis of 2,6-Disubstituted Piperidines



Substrate	*ATA*	*Conv. to **12**/%*	*IRED*	*Amine product*	*Conv./%*	*de/%*	*ee/%*
11a	113	>98	(*R*)-IRED	 (2*S*,6*S*)-**13a**	81	>98	>98
11a	117	>98	(*S*)-IRED	 (2*R*,6*R*)-**13a**	32	>98	>98
11b	113	>98	(*R*)-IRED	 (2*S*,6*S*)-**13b**	71	>98	>98
11b	117	>98	(*S*)-IRED	 (2*R*,6*R*)-**13b**	61	>98	>98

11c	113	>98	(*R*)-IRED	 (2*S*,6*S*)-**13c**	42	>98	>98
11c	117	>98	(*S*)-IRED	 (2*R*,6*R*)-**13c**	63	>98	>98
11d	113	>98	(*R*)-IRED	 (2*S*,6*R*)-**13d**	>98	95	>98
11d	117	>98	(*R*)-IRED	 (2*R*,6*S*)-**13d**	21	13	>98

Reaction conditions: 5 mM substrate, 2.5 mg/mL ATA-113 or ATA-117, 1 mg/mL GDH, 0.5 mg/mL LDH, 250 mM L-alanine (with ATA-113) or D-alanine (with ATA-117), 100 mM glucose, 1.5 mM NAD^+, 1 mM PLP, 100 mM pH 7.0 NaP_i buffer, 500 µL reaction volume, 30°C, 250 rpm, 24 h followed by addition of 200 mg/mL IRED wet whole cells and incubation at 30°C, 250 rpm for 24 h. Conversions, *de* and *ee* values were determined by GC-FID analysis on a chiral stationary phase.

stereoselectivity of the IREDs and the imine enantiomer matched to afford a *cis*-diastereomer product, higher conversions and *de* of the amine were achieved. In this respect, the (*R*)-IRED was best matched with ATA-113 in order to furnish the highest conversions of the starting imine whereas the (*S*)-IRED was paired more effectively with ATA-117 (Table 5). For further information on all ATA-IRED combinations see Supporting Information. Substrate **11d** with ATA-113 and the (*R*)-IRED afforded the natural product (-)-dihydropinidine (2*S*,6*R*)-**13d** in excellent conversion and *de*. Unusually, following transamination of diketone **11d** by ATA-117,

the (*R*)-IRED showed low conversion to the product amine (2*R*,6*S*)-**13d** in only 13% *de*, giving rise to a larger proportion of the *trans*-diastereomer product in this case.

To test the synthetic utility of these cascade reactions, preparative-scale reactions were carried out (Table 6). Substrates were chosen to exemplify the formation of mono- and di-substituted piperidines starting from a keto acid, keto aldehyde or diketone via the CAR-TA-IRED or TA-IRED cascades. The isolated product yields demonstrate that these cascades can be successfully employed on synthetically relevant scale to afford compounds that are difficult to prepare by traditional chemical means.

Table 6. Preparative-Scale Synthesis Employing the CAR-TA-IRED or TA-IRED Cascade

Substrate	*Enzyme Cascade*	*Product*	*Isolated yield/% (Quantity)*
1b	MCAR, ATA-113, (*S*)-IRED	(*R*)-**2b**	83 (70 mg)
(*R*)-**3**	ATA-113, (*S*)-IRED	(2*R*,5*R*)-**4**	79 (73 mg)
(±)-**7**	MCAR, ATA-113, (*S*)-IRED	(2*R*,3*S*)-**10**	76 (65 mg)
(*S*)-**15**[a]	(*R*)-IRED	(2*S*,6*S*)-**13a**	92 (46 mg)
11d	ATA-113, (*R*)-IRED	(2*S*,6*R*)-**13d**	90 (51 mg)[b]

Reactions were carried out as per the analytical-scale reactions as described above. Crude product *de*s and *ee*s matched those obtained in the analytical-scale reactions. (a) (*S*)-**15** was prepared from **11a** and ATA-113 and isolated (93% yield). (b) Isolated as hydrochloride salt.

The observed stereoselectivities of the IRED-catalysed reduction of the chiral piperideine substrate panel can be tentatively rationalized as shown in Figure 1. For mono-substituted 2-phenylpiperideine, we suggest that there is a preferred binding orientation that allows hydride delivery from NADPH to one face of the prochiral imine, resulting in the observed stereochemistry of the product. Assuming that the lowest energy pathway operates, the hydride is predicted to attack antiperiplanar with respect to the σ-C-H bond vicinal to the imino functional group, as described by Yamamoto *et al.*[50,51] and based on the theoretical predictions by Houk *et al*.[52] and Cieplak.[53] For all of the disubstituted compounds, the corresponding chiral imines would also presumably prefer to bind in the same orientation at the enzyme active-site. However, if this binding mode places the methyl group on the ring in a pseudo-axial position, such that there is a steric 1,3-interaction with the incoming hydride from NADPH, it is presumably more favorable for the substrate to enter the active site in a 180° rotated orientation. An associated ring-flip of the imine would also be expected assuming hydride delivery via the lowest energy nucleophilic attack trajectory. In this new binding mode, hydride is delivered to the opposite face of the imine as there is no longer an unfavorable 1,3- steric interaction. This situation applies for one enantiomer of the 2,6- and 2,4-piperideines with each IRED and explains why, in these cases, the inherent selectivity of the enzyme is overridden. For the 2,3-piperideine **8**, which can tautomerize via the enamine **9**, the lowest energy route minimizes 1,2-steric interactions between the methyl group and the incoming hydride, resulting in the *cis*-diastereomer as the major product.

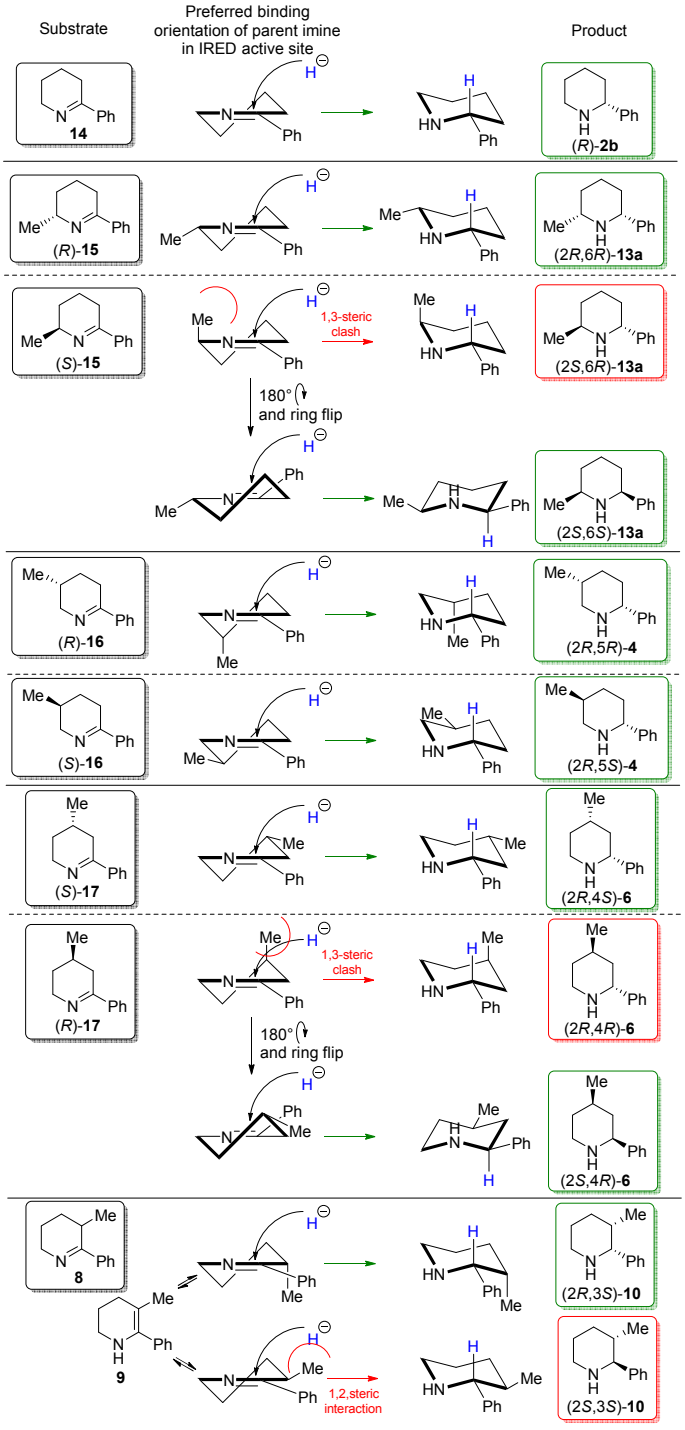
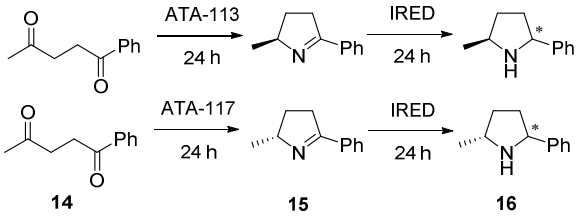


Figure 1. Proposed rationale for selectivity of IRED-catalysed reduction of chiral imines. Hydride (blue) represents NADPH as hydride source. Lowest energy routes represented by green arrows and higher energy routes indicted by red arrows. Major products are in green boxes and minor products are in red boxes.

In contrast to the piperideine ring systems, investigations into the effect of a pre-existing stereogenic center on the reduction of 2,5-disubstituted pyrrolines by IREDs revealed a less predictable pattern (Table 7).

Table 7. Synthesis of 2,5-Disubstituted Pyrrolidines



Entry	*ATA*	*IRED*	*Conv. to **15**/%*	*Amine product*	*Conv./%*	*de/%*	*ee/%*
1	113	(*R*)-IRED	>98	(2*S*,5*R*)-**16**	11	63	>98
2	113	(*S*)-IRED	>98	(2*S*,5*S*)-**16**	17	65	>98
3	117	(*R*)-IRED	>98	(2*R*,5*S*)-**16**	95	97	>98
4	117	(*S*)-IRED	>98	(2*R*,5*S*)-**16**	68	>98	>98

Reaction conditions: 5 mM substrate, 2.5 mg/mL ATA-113 or ATA-117, 1 mg/mL GDH, 0.5 mg/mL LDH, 250 mM L-alanine (with ATA-113) or D-alanine (with ATA-117), 100 mM glucose, 1.5 mM NAD^+, 1 mM PLP, 100 mM pH 7.0 NaP_i buffer, 500 µL reaction volume,

30°C, 250 rpm, 24 h followed by addition of 200 mg/mL IRED wet whole cells and incubation at 30°C, 250 rpm for 24 h. Conversions, *de* and *ee* values were determined by GC-FID analysis on a chiral stationary phase.

Taking the ATA-113 product (*S*)-**15** as a substrate, the IREDs displayed modest conversion of the chiral imine, giving the corresponding amines in moderate *de* (63–65%). When compared to the IRED reductions of mono-substituted 2-phenylpyrroline, substrate (*S*)-**15** appeared to override the enzyme's inherent selectivity, producing pyrrolidines of the opposite configuration.

When the opposite enantiomer (*R*)-**15** was treated with the IREDs, higher levels of conversion were obtained and remarkably both enzymes furnished amines of the same configuration in high *de* (97->98%) (Table 7, entries 3 and 4). It is interesting to note that although there appears to be a preference for the (*R*)-enantiomer as a substrate for both enzymes, it is clear that more subtle and complex binding interactions are in play.

CONCLUSIONS

In summary, a versatile and highly efficient one-pot cascade route to chiral piperidines and pyrrolidines has been successfully demonstrated utilizing CAR, ω-TA and IRED biocatalysts to afford valuable chiral amines from simpler, acyclic starting materials. Disubstituted compounds can also be accessed using this approach, with one stereogenic center being set by an IRED and the other derived from the substrate or generated using an ω-TA. In certain cases, chiral imine substrates can override inherent IRED selectivity, providing an insight into substrate binding and substituent effects with these enzymes which has not been investigated previously.

ASSOCIATED CONTENT

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Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org. Experimental procedures and methods, substrate synthesis and isolated product characterization, analytical data.

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ABBREVIATIONS

PLP (pyridoxal 5'-phosphate)

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