Synthesis of stereoenriched piperidines via chemo-enzymatic dearomatization of activated pyridines

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Abstract

The development of efficient and sustainable methods for the synthesis of nitrogen heterocycles is an important goal for the chemical industry. In particular, substituted chiral piperidines are prominent targets due to their prevalence in medicinally relevant compounds and their precursors. A potential biocatalytic approach to the synthesis of this privileged scaffold would be the asymmetric dearomatization of readily assembled activated pyridines. However, nature has yet to yield a suitable biocatalyst specifically for this reaction. Here, by combining chemical synthesis and biocatalysis, we present a general chemo-enzymatic approach for the asymmetric dearomatization of activated pyridines for the preparation of substituted piperidines with precise stereochemistry. The key step involves a stereoselective one-pot amine oxidase/ene imine reductase cascade to convert *N*-substituted tetrahydropyridines to stereo-defined 3- and 3,4-substituted piperidines. This chemo-enzymatic approach has proved useful for key transformations in the syntheses of the antipsychotic drugs Preclamol and OSU-6162, as well as for the preparation of two important intermediates in synthetic routes of the ovarian cancer monotherapeutic Niraparib.

One-Sentence Summary

A biocatalytic cascade for the asymmetric synthesis of *N*-heterocyclic therapeutic precursors including Niraparib.

Introduction

The ubiquity of saturated nitrogen heterocycles (*N*-heterocycles) in natural products and pharmaceuticals continues to drive the development of innovative strategies for their efficient synthesis(*1*, *2*). In particular, chiral piperidines are much sought after structures due to their prevalence as scaffolds in a range of bioactive molecules including market approved active pharmaceutical ingredients (APIs)(*3*). Nature provides highly efficient biocatalysts for the biosynthesis of *N*-heterocycles(*4*, *5*), offering high enantio- and regio-selectivity under benign conditions. These biocatalysts have previously enabled the development of one-pot cascade reactions to access stereo-enriched 2-, 2,6- and 2,3-substituted piperidines(*6*–*10*). However, the translation of these methods to the corresponding stereoenriched 3-

substituted and 3,4-substituted scaffolds, the core of many important therapeutic compounds (Fig. 1A), remains challenging due to difficulties in stereoselectivity control combined with limited availability of suitable starting materials.

Asymmetric chemical synthetic approaches for the preparation of 3-substituted and 3,4-disubstituted piperidines include those based on metalation/cross-coupling(*11–14*), Grignard Michael addition(*15*), ring-closure(*16*), and transition-metal-catalyzed dearomatization of pyridines(*17–20*). However, limitations are associated with all of these approaches, including high reaction temperatures, sensitivity to moisture, lack of availability of starting materials and the use of expensive non-commercial chiral ligands(*11*, *21*). Among reported methods, the catalytic asymmetric dearomatization of pyridines is achieved by quaternization-activation of the pyridine nitrogen, permitting access to mild reduction methods to chiral piperidines (Fig. 1B, left)(*17*). Whilst nature has yielded pyridine synthases to prepare pyridines(*22*), an effective biocatalyst for their dearomatization has yet to be discovered. With this in mind, we sought to combine mild chemical reduction of pyridiniums to tetrahydropyridines (THPs) with the exquisite stereoselectivity of a biocatalytic cascade to reduce the final C=C bond as an efficient strategy for asymmetric dearomatization of activated 3- and 3,4-substituted pyridines (Fig. 1C). Biocatalysts with broad substrate scope for the reduction of C=C bonds require the conjugation of the alkene to an electron withdrawing group. Recently, C=C bonds conjugated to C=N bonds have been shown to undergo full reduction to amines through the combination of ene-reductases (EREDs) and imine reductases (IREDs)(*6*), as well as the newly discovered ene imine reductase (EneIREDs)(*23*). We reasoned that biocatalytic oxidation, using an amine oxidase (AmOx), of the THP *in situ* would generate the corresponding dihydropyridiniums (DHPs), generating an activated C=C bond conjugated to the C=N bond, which could then be reduced with these biocatalysts to generate a cascade to the desired 3- and 3,4-substituted piperidines. This cascade would complement previously reported amine oxidase AmOx-IRED deracemisation processes in which only amine oxidation and C=N bond reduction take place(*24*, *25*).

Results and discussion

A series of substituted *N*-alkyl THPs **1b**-**21b** was prepared in good yields (50-90%) from activated pyridines (**1a-21a**) using $NaBH_4$ as previously reported(*26*). Initially, we explored the conversion of THPs to piperidines using AmOxs in combination with EREDs or EneIREDs (see supplementary materials 2.1.; Figs. S1 to S5 for the complete list of THPs screened). For the first step we tested AmOx variants that have been shown to be effective biocatalysts for the oxidation of *N*-alkyl THPs(*27*, *28*). The 6-hydroxy-D-nicotine oxidase (6-HDNO) variant, E350L/E352D(*29*), was found to be effective, with broad substrate scope, including oxidation of **1b**, a precursor to Preclamol. We next screened for activity for the second step, namely reduction of the C=C bond of the α,β-unsaturated iminium ion. Whereas the panel of EREDs displayed no activity, the EneIRED from an unidentified *Pseudomonas* sp. (EneIRED-01)(*23*), in combination with 6-HDNO, was effective at reducing a number of THPs and could be used to prepare piperidine (*R*)-**1c** in good yield and with excellent enantioselectivity (Table S1; entry 1-3, ≥42% yield, 96% ee).

Next, we set out to identify further EnelREDs that could also generate enantioenriched 3-substituted piperidines. By screening the recently reported metagenomic IRED collection(*30*), in combination with the 6-HDNO variant, we were able to quickly identify biocatalysts capable of generating either enantiomer of piperidine (*R*/*S*)-**1c** from THP **1b** (Table S2). From this screen we organized these EnelREDs into two groups: Series A (red: EnelREDs 01-04) that gave piperidine (*R*)-**1c** (Table S2 up to >99% ee), and Series B (blue: EnelREDs 05-09) that generated the enantiocomplementary piperidine (*S*)-**1c** (Table S2, up to 96% ee).

With effective EnelREDs for the preparation of both enantiomeric series, we probed the substrate scope of the 6-HDNO-EnelRED cascade (Fig. 2). Enzymes in Series A and B accepted a variety of aryl substituents at the C-3-position of the THP scaffold, affording products **1c**-**7c** in high yields, conversion, and enantioselectivity. 5-membered heterocyclic 3-substituents such as furan **8c** and thiophene **9c** were also well tolerated (≥62% yield, ≥86% ee). Sterically demanding substrates, *e.g.* containing a 2-naphthyl substituent **10b**, were also tolerated producing (*A*)-and (*B*)-**10c** in excellent yield and stereoselectivity (73% yield, >99% conv., ≥94% ee). Additionally, a variety of *N*-alkyl substituents were accepted forming the piperidine products **1c**-**12c** in good to excellent yields. Of these, *N*-allyl **3c**-**4c** and *N*-propargyl **12c** piperidines provide useful synthetic handles that can be easily removed or further functionalized.

More hindered 3,4-disubstitituted THPs could also be reduced using the cascade, resulting in the formation of the substrate dependent *cis* or *trans* isomer. These included substrates in which both substituents were simple alkyl (3,4-dimethyl) **17b** or part of a fused bi-cyclic ring system (octahydroisoquinoline) **18b**. We then probed the tolerance for a combination of alkyl and aryl substituents at C-3 and C-4. 3-Phenyl-4-methyl disubstituted compounds **19b** and **20b** provided the corresponding piperidines (*A*)-**19c** and (*A*)-**20c** in excellent yields and diastereoselectivity (*cis* major; >96:4 dr; ≥87% yield). The system also tolerated the isomeric 3-methyl-4-phenyl disubstituted THP **21c**. In addition to the inversion of stereochemical outcome at the C-3 position (Series A *vs* Series B), we also discovered some EnelREDs in Series B that provided an inverted diastereomeric configuration of the 3,4-disubstitituted piperidines **17c-20c** compared to Series A.

To probe the mechanism of the 6-HDNO-EnelRED cascade, the conversion of THP **6b** to piperidine **6c** was investigated by *in situ* ^{19}F NMR reaction monitoring (Fig. 3A). After <5 min, formation of piperidine **6c** was apparent and after 60 min the THP **6b** was completely consumed. In the absence of the EnelRED, 6-HDNO catalyzed the previously reported aromatization of THP **6b** to the corresponding pyridinium ion **6a** (Fig. S6, >99% conversion after 24 h)(*28*). As expected, no direct reduction of the THP **6b** to piperidine **6c** was observed in the absence of 6-HDNO suggesting that a transiently generated dihydropyridinium (DHP) is the substrate for the EnelRED reduction.

Using the cascade with THP-**10b** we were able to isolate the enamine 1**0e** before full conversion to the piperidine **10c** (see supplementary materials 4.1., 63% yield) which strongly suggested the participation of this compound in the reaction pathway. Accordingly, enamine **10e** was converted to piperidine **10c** using EnelRED-01 alone, in excellent

yield and high enantioselectivity, equivalent to the full cascade with THP-**10b** (Fig. 3B, top; 88% yield, 94% ee). Deuterium labelling experiments were also implemented to further elucidate the mechanism of the EneIRED enamine reduction step. Carrying out the reduction of enamine **10e** using EneIRED-01, in the presence of GDH and *D*-glucose-1-d_1 to generate deuterated NAD(P)D *in situ*, C-2-mono-deuterated **10c-d_1** was formed (80% deuterium incorporation, 82% yield). This suggests that the enamine intermediate **10e** may undergo protonation to the iminium before NAD(P)H hydride delivery (Fig. 3B, bottom and see supplementary materials 4.2.).

A proposed mechanism for the 6-HDNO-EneIRED cascade is outlined in Fig. 3C. For illustrative purposes, we have depicted the transformation of THP-**3b** to (*S*)-**3c** as this product is used in the subsequent synthesis of the key intermediate for Niraparib described below. Initially, 6-HDNO oxidation of THP-**3b** results in the activation of the THP C=C bond for EneIRED-catalyzed asymmetric conjugate reduction of DHP-**3d** at the expense of NADPH to generate enamine **3e** (Cycle 1; step iii). This intermediate **3e** is expected to be in equilibrium with chiral iminium **3f** *via* a non-selective protonation in solution, which has been extensively documented (*9*, *31*, *32*). Depending on the EneIRED employed (Series A or B), the kinetic selective reduction of one enantiomer of chiral iminium **3f**, affords the enantioenriched piperidine **3c** as the final product via reduction with a second molecule NADPH (Cycle 2; step vii). *In situ* epimerization of the enantiomer in **3f** *via* enamine **3e**, enables a dynamic kinetic resolution (DKR) to occur to generate piperidine (*S*)-**3c** mediated by the EneIRED.

Predominantly, EneIREDs in Series A yielded (*R*)-piperidines whilst enzymes in Series B such as EneIRED-07 yielded the (*S*)-product. In order to gain insight into the mode of substrate binding in the active site we determined the structure of EneIRED-07 from *Micromonospora* sp. Rc5 to a resolution of 2.55 Å in complex with $NADP^+$ using X-ray crystallography. Crystals were obtained in the $P2_12_12$ space group and featured six molecules in the asymmetric unit, representing three dimers. EneIRED-07 displays the canonical fold observed for IREDs(*33*, *34*), with two monomers associating to form two active sites between the *N*-terminal Rossmann domain of one subunit and the *C*-terminal helical bundle of its neighbor (Fig. 3D).

Analysis using the DALI server(*35*) suggested that the IRED with the most closely related structure was the IRED from *Streptosporangium roseum* (PDB 5OCM) with an rmsd of 1.0 Å over 288 Cα atoms. Following building and refinement of the protein atoms clear omit density was observed in each active site corresponding to the cofactor $NADP^+$. The iminium intermediate (*S*)-**3f** the preferred enantiomer for EneIRED-07 imine reduction, was modelled into the active site using Autodock Vina (Fig. 3E)(*36*). The model suggests that the allyl group of (*S*)-**3f** is bound within a hydrophobic pocket formed by methionine residues M125, M183 and M214 at the rear of the active site as shown; the *para*-bromo-phenyl group projects towards the front of the active site bordered by L180, W184 and the $NADP^+$ cofactor. This ligand conformation places the electrophilic carbon approximately 3.6 Å from the $NADP^+$ pyridinium ring C4 atom, from which hydride is transferred. Modelling of the (*R*)-enantiomer of **3f** places the allyl group at the base of the active

site with less favorable interactions with hydrophilic residues Y225 and D238 (see supplementary materials 6.4, Fig. S13)

Finally we sought to apply the chemo-enzymatic dearomatization of activated pyridines to several target bioactive molecules (Fig. 4). First, we targeted the antipsychotic drug Preclamol. At preparative scale (1 mmol), THP-**1b** was converted to both (*R*)-(+)- and (*S*)-(-)-preclamol **22**, using EneIRED-01 and EneIRED-05 respectively. Both enantiomers were prepared in four steps from 3-(3-methoxyphenyl)pyridine and were obtained in ≥50% overall yield and with 96% ee (Fig. 4A and see supplementary materials 4.1.). Next, we carried out the three-step syntheses of both enantiomers of OSU6162 **2c**, using EneIRED-02 and EneIRED-06, and these were both accomplished in ≥36% overall yield and ≥92% ee (Fig. 4B).

To further demonstrate the application of the cascade we synthesized the two intermediates **23** and **24** *en route* to Niraparib (Fig. 4C), the first poly ADP ribose polymerase (PARP) inhibitor to be approved as a first-line monotherapeutic for the maintenance treatment of patients with advanced ovarian cancer(*37*). For route **I** we showed that commercially available 3-(4-bromophenyl)pyridine could be efficiently converted to piperidine (*S*)-**3c** in just three steps and 61% overall yield (99% ee). This was followed by deallylation and *N*-Boc-protection to yield (*S*)-**23** in 64% yield, a key intermediate in Merck's second-generation synthesis(*38*). Alternatively, in route **II**, by starting from commercially available 4-(pyridin-3-yl)aniline we converted pyridinium salt **4a** to (*S*)-**24** in 29% overall yield and with 93% ee, a key intermediate in Merck's first-generation synthesis(*39*). The general applicability of the method was also showcased by preparation of the corresponding (*R*)-enantiomers of both **23** and **24** in good yields and high enantioselectivity (see supplementary materials 4.1.).

Conclusion

In summary, we report the development of a versatile and highly efficient chemo-enzymatic dearomatization of activated pyridines for the preparation of stereo-enriched 3- and 3,4-disubstituted piperidines. The 6-HDNO-catalyzed oxidation of readily accessible THPs facilitates EneIRED-catalyzed conjugate reduction and iminium reduction to yield a broad range of chiral piperidines. The short syntheses of both enantiomers of Preclamol and OSU6162, as well as chiral precursors to Niraparib, highlight the flexibility and utility of the method presented, emphasizing the advantages of combining chemical synthesis with biocatalysis for developing new catalytic methods for the preparation of important chiral compounds. Furthermore, the increasing ability to systematically screen large panels of biocatalysts against new targets leads to the rapid identification of enzymes with applications in asymmetric synthesis.

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Competing interests: The authors declare no conflicts of interest.

Data and materials availability: All data are available in the main text or the supplementary materials. Crystallographic structure of EneIRED-07-$NADP^+$ has been deposited in the Protein Databank (PDB) with accession code 7OSN.

SUPPLEMENTARY MATERIALS

Materials and Methods

Supplementary Text

Figs. S1 to S59

Tables S1 to S12

References (*40–51*)

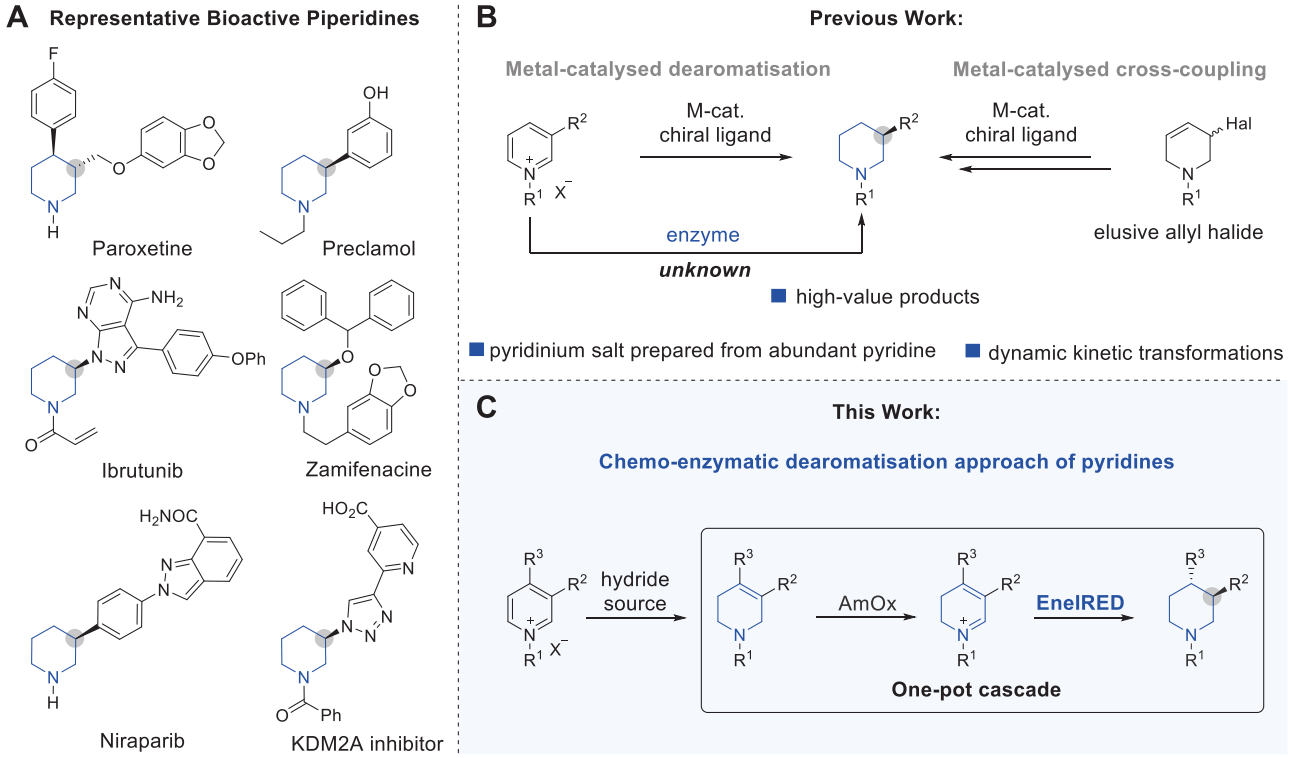

Figure 1: High value stereo-enriched 3- and 3,4-substituted piperidines and strategies for their synthesis. (**A**) Representative examples of biologically active chiral substituted piperidines. (**B**) Previous Work: Asymmetric transition metal-catalyzed synthesis of 3-substituted piperidines. (**C**) This Work: Chemo-enzymatic dearomatization of pyridines for the synthesis of chiral 3- and 3,4-substituted piperidines.

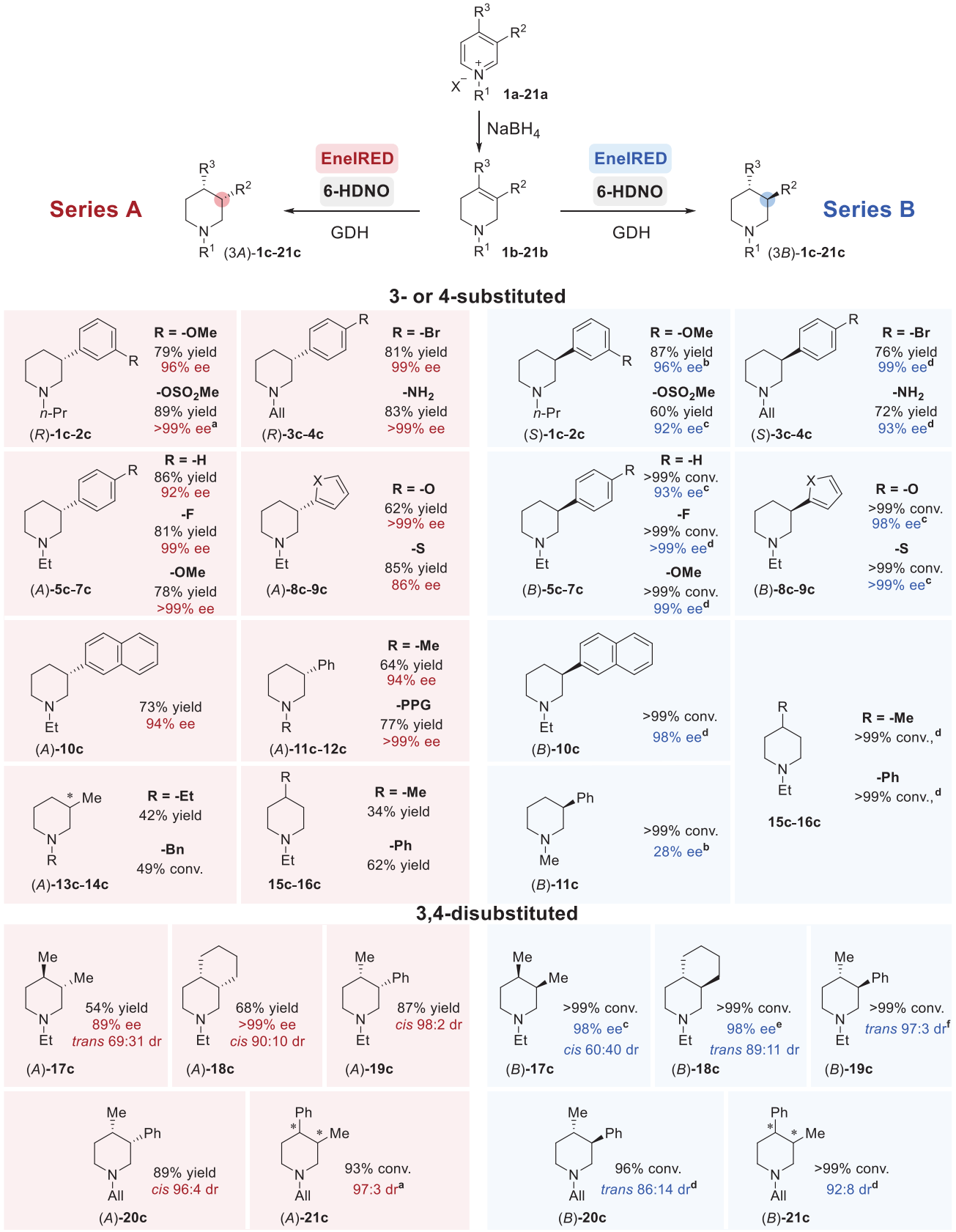

Figure 2: Scope of chemo-enzymatic dearomatization of activated pyridines. Series A and Series B provide (*R*)- and (*S*)-configuration respectively at C-3. Reaction conditions (preparative scale): purified 6-HDNO (1 mg/mL),

EnelRED CFE (10 mg/mL), THP (1 mmol), glucose (5 mmol), GDH CDX-901 (0.5 mg/mL), $NADP^+$ (0.5 mmol), DMSO (1% v/v), 100 mM KPi pH 7.0, 16-24 h, 30 °C, 200 rpm (analytical conditions): purified 6-HDNO (0.5 mg/mL), EnelRED CFE (4 mg/mL), 10 mM THP (5 µmol), 50 mM glucose (25 µmol), GDH CDX-901 (0.1 mg/mL), 0.5 mM $NADP^+$ (0.05 µM), 2-16 h). All examples use EnelRED-01 except; [a]EnelRED-02, [b]EnelRED-05, [c]EnelRED-06, [d]EnelRED-07, [e]EnelRED-08 and [f]EnelRED-09. See supplementary materials for more details.

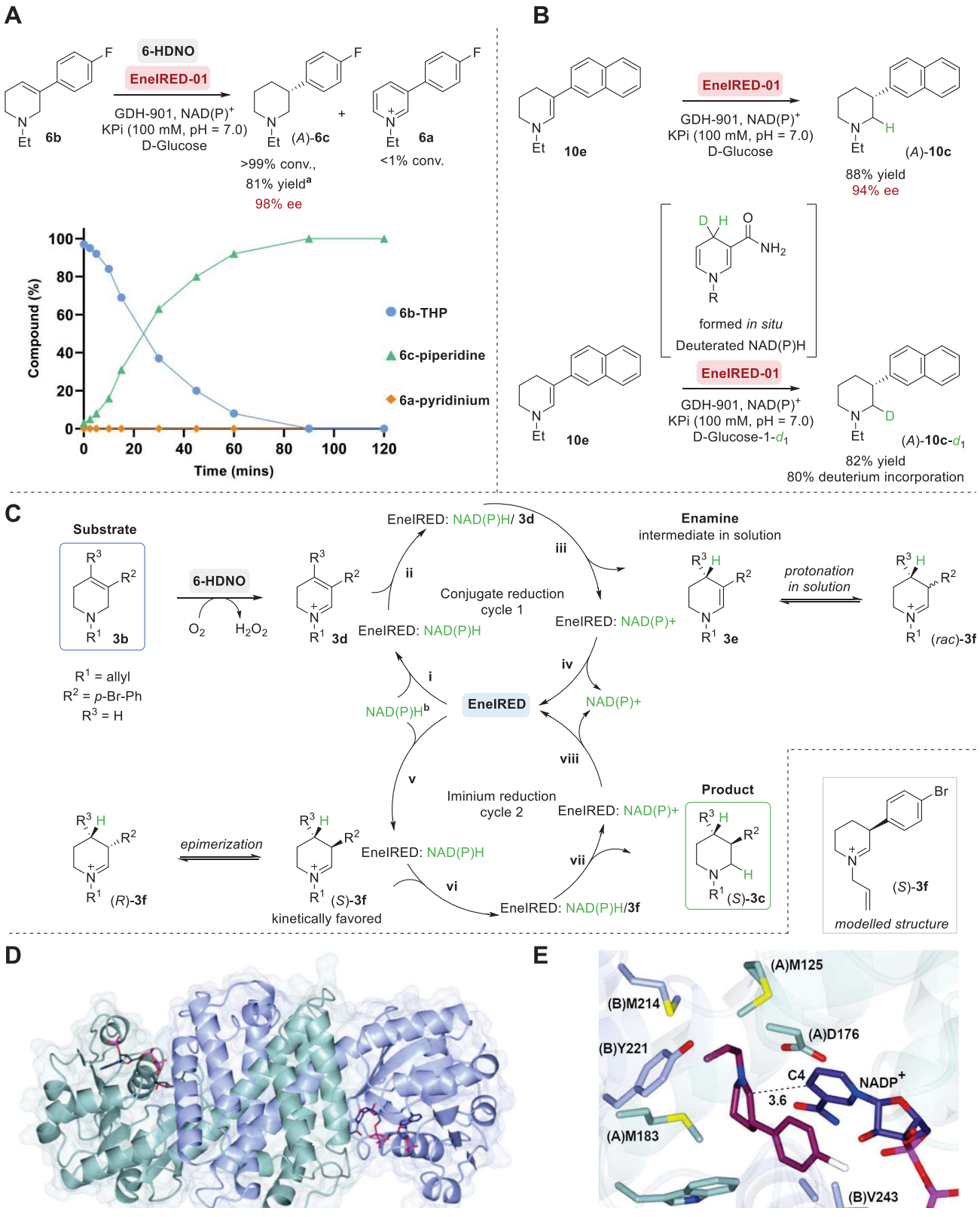

Figure 3: Mechanistic studies and proposed mechanism for the 6-HDNO-EneIRED cascade. (**A**) Kinetic profile from *in situ* ^{19}F NMR reaction monitoring of THP-**6b**. [a]Reactions run at 1 mmol. (**B**) An enamine **10e** is used to probe the role of this species as an intermediate in the cascade. (**C**) Proposed catalytic sequence for the AmOxEneIRED

biocatalytic cascade. [b]Two equivalents of NAD(P)H consumed to form piperidine (**D**) Structure of the dimer of EneIRED-07 in ribbon format with subunits shown in green and blue. $NADP^+$ can be seen bound at the two active sites. (**E**) Active site of EneIRED-07 with the (*S*)-enantiomer of iminium ion intermediate **3f** modelled into the active site. $NADP^+$ and (*S*)-**3f** are shown in cylinder format with carbon atoms in grey and purple respectively. The electrophilic carbon of the intermediate is 3.6 Å from the $NADP^+$ pyridinium ring C-4 atom, from which hydride is transferred.

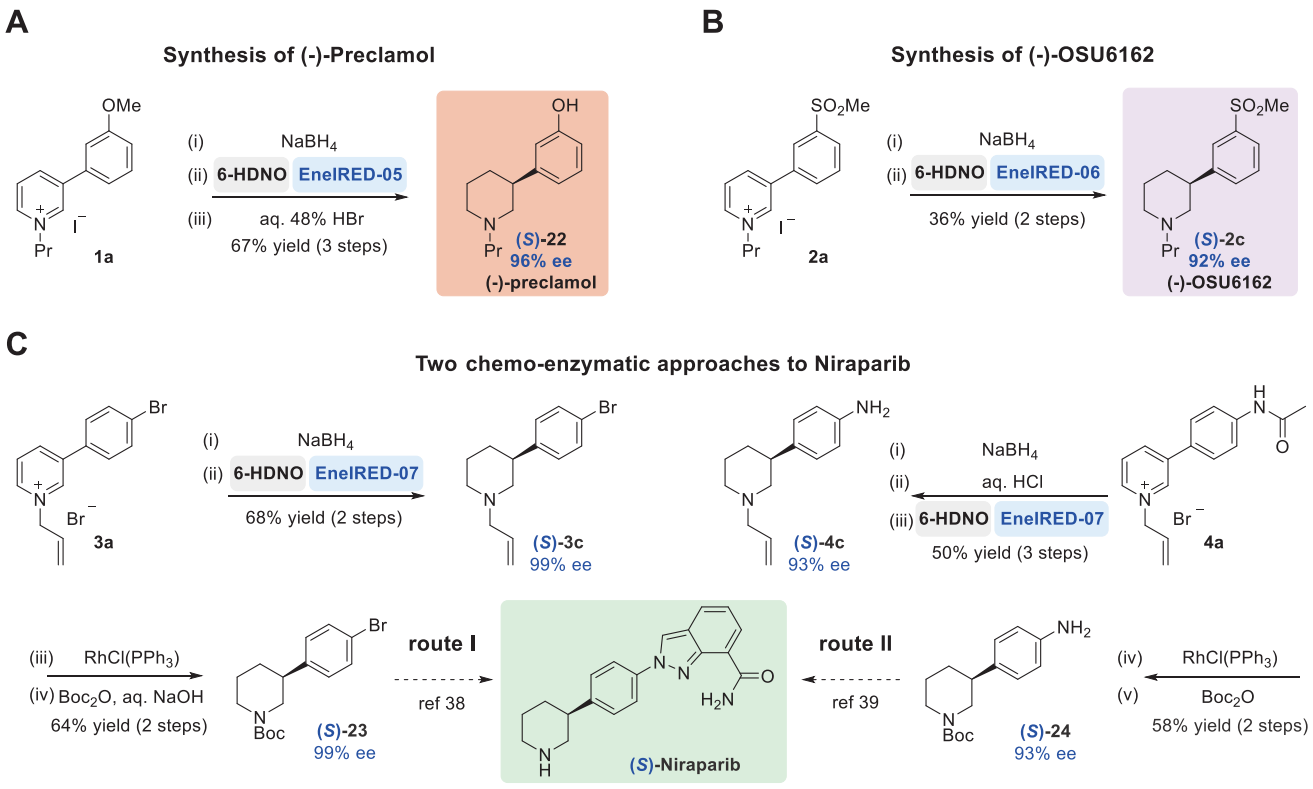

Figure 4: Application of the chemo-enzymatic dearomatization of pyridines for the preparation of APIs. (**A**) Synthesis of the anti-psychotic drug (-)-preclamol. (**B**) Synthesis of (-)-OSU6162. (**C**) Two synthetic routes to Niraparib.