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Enzyme-Inspired Axially Chiral Pyridoxamines Armed with a Cooperative Lateral Amine Chain for Enantioselective Biomimetic Transamination

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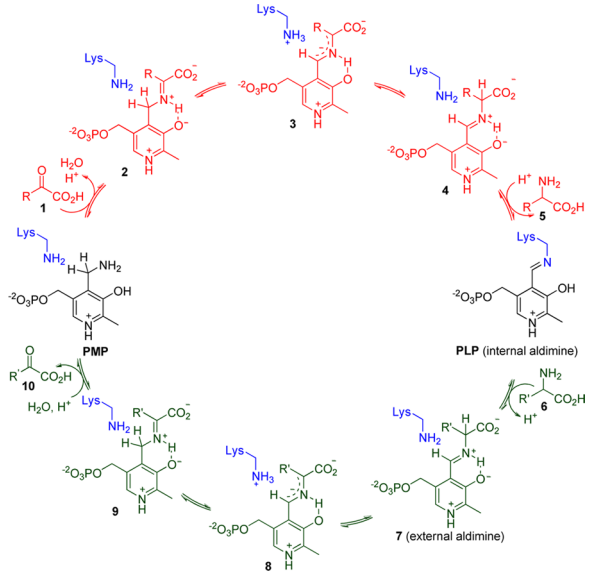
Supporting Information

ABSTRACT: Enzymatic transamination is catalyzed by pyridoxal/pyridoxamine, and it involves remarkable cooperative catalysis of a Lys residue in the transaminase. Inspired by transaminases, we developed a class of axially chiral pyridoxamines **11** bearing a lateral amine arm. The pyridoxamines exhibited high catalytic activity and excellent enantioselectivity in asymmetric transamination of α-keto acids, to give various α-amino acids in 67−99% yields with 83−94% ee's. The lateral amine arm likely participates in cooperative catalysis as the Lys residue does in biological transamination and has an important impact on the transamination in terms of activity and enantioselectivity.

Enzymatic transamination of α-keto acids is the most important process to access optically active α-amino acids in biological systems.[1] The process is catalyzed by pyridoxal/pyridoxamine phosphates and proceeds via a two-half-transamination pathway (Scheme 1).[1,2] The Lys residue of the transaminase plays a crucial role in the transamination.[3] The ε-NH_2 group of the Lys residue can act as an intramolecular base to deprotonate the imino C−H of ketimine **2** and the α C−H of the carboxylic group of aldimine **7**, respectively, to promote the 1,3-proton transfers from ketimine **2** to aldimine **4** and from aldimine **7** to ketimine **9**. Moreover, the Lys residue may assist the hydrolysis of Schiff bases such as aldimine **4** and ketimine **9**, to accelerate the transamination. The Lys effect has been supported by the fact that replacement or deletion of the Lys residue via mutagenesis resulted in a dramatic decrease (up to 10^6-fold) of transamination activity.[3c]

Asymmetric biomimetic transamination affords an intriguing strategy for chemical synthesis of various chiral amines and thus has attracted much attention since the 1970s.[4] The studies mainly include asymmetric transamination with stoichiometric chiral pyridoxamine analogues as amine sources,[5] catalytic asymmetric transamination in the presence of pyridoxal/pyridoxamine-based supramolecular bilayer assemblies[6] or semisynthetic transaminases,[7] and asymmetric 1,3-proton transfer of Schiff bases catalyzed/promoted by chiral bases[8] or Lewis acids.[9] Although stoichiometric asymmetric transamination has already been deeply studied mainly by Breslow[5b,c,e−g] and Kuzuhara,[5a,d] catalytic asymmetric transamination with chiral pyridoxal/pyridoxamine[10] molecules as the catalyst[11] has not yet been well developed.[12] As the pyridoxal/pyridoxamine catalyst is the key for the transformation, development of more efficient catalytic systems is crucial and highly desirable in this area.

Scheme 1. Enzymatic Transamination Involving Cooperative Catalysis of a Lys Residue



Inspired by the astonishing function displayed by the Lys residue (Scheme 1), it should be greatly anticipated to apply a similar cooperative-catalysis strategy into the development of asymmetric biomimetic transamination.[5c,7c] To continue to pursue asymmetric catalytic transamination with enhanced activity and excellent enantioselectivity,[12] we have designed axially chiral pyridoxamines **11** and pyridoxals **12** bearing a lateral amine arm (Figure 1).[13] The biaryl backbone was chosen because the catalyst could adjust its conformations by rotating around the biaryl axis, to meet different structural requirements of various transition states involved in transamination. The amine side arm was expected to mimic the Lys residue of transaminase to carry out cooperative catalysis, so that to achieve good catalytic

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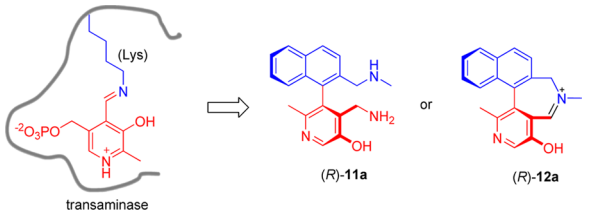
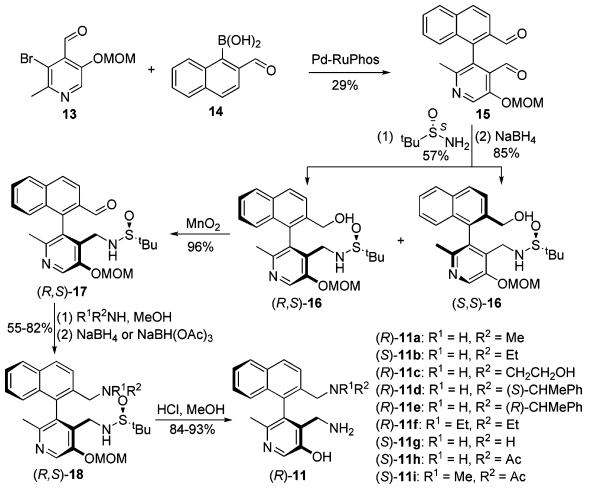


Figure 1. Transaminase-inspired design of axially chiral pyridoxamine/pyridoxal catalysts.

performance in asymmetric transamination. Herein we report our results on the subject.

The studies commenced with the synthesis of the chiral pyridoxamines **11** (Scheme 2). Suzuki cross-coupling of

Scheme 2. Synthesis of Chiral Pyridoxamines 11a–f[a]

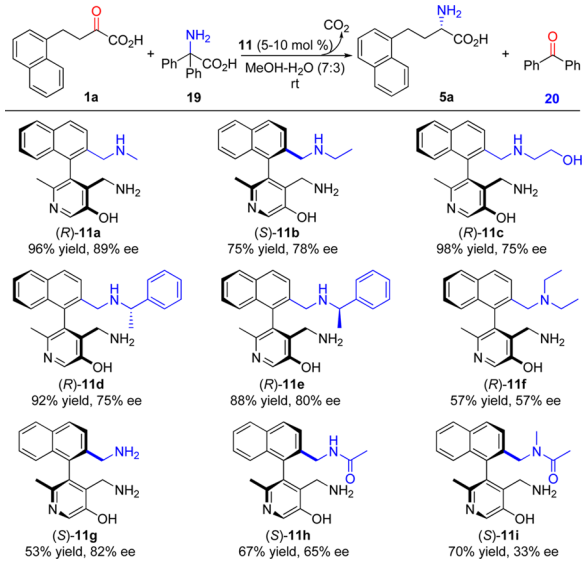


[a]For the modified synthesis of pyridoxamines **11g–i** from (*S*,*S*)-**16**, see Supporting Information (SI).

bromopyridine **13** and naphthalenylboronic acid **14** afforded biaryl dialdehyde **15** in a 29% yield. Treatment of **15** with 1 equiv of (*S*)-*tert*-butylsulfinamide and subsequent reduction with $NaBH_4$ gave a pair of TLC-separable diastereoisomers (*R*,*S*)-**16** and (*S*,*S*)-**16**. The enantiopure compound (*R*,*S*)-**16** or (*S*,*S*)-**16** underwent oxidation with MnO_2, reductive amination, and deprotection with acid to form the desired chiral pyridoxamines **11a–f** as HCl salts. Pyridoxamines **11g–i** were obtained from (*S*,*S*)-**16** by following modified synthetic procedures [Supporting Information (SI)]. The absolute configurations of the chiral pyridoxamines **11** were determined by X-ray analysis of the intermediate (*R*,*S*)-**17** (see SI).

The axially chiral pyridoxamines **11** were then tested in asymmetric transamination of α-keto acid **1a** with 2,2-diphenylglycine (**19**)[14,15] as the amine source (Table 1 and Table S1 in SI). A catalyst bearing a tertiary amine side arm (**11f**) is less enantioselective than those with a secondary (**11a–e**) or primary amine (**11g**) group on the side chain.[16] Introducing an acetyl group onto the nitrogen to diminish the basicity of the lateral amine resulted in a dramatic decrease of ee value and a longer reaction time to achieve similar yields (Table 1, **11h–i** vs **11a–g**). Pyridoxamine **11a** exhibited the best performance in terms of enantioselectivity and activity among the catalysts examined. Further studies showed that water is crucial for the reaction (Table S1, entry 13 vs 1 and 14–15) and a mixed system of MeOH and H_2O (8:2) was the choice of solvent (Table S1,

Table 1. Catalyst Screening for the Asymmetric Transamination[a]



[a]All reactions were carried out with **1a** (0.10 mmol), **19** (0.10 mmol), **11** (0.010 mmol for **11a–c** and **11f–i** and 0.0050 mmol for **11d–e**) in MeOH–H_2O (7:3, 1.0 mL) at rt for 12–65 h (12 h for **11a**, 24 h for **11b–g**, and 65 h for **11h–i**). The isolated yields were based on **1a**. The ee values were determined by chiral HPLC analysis of the corresponding methyl ester of **5a**.

entry 15). Addition of 1 equiv of acetic acid led to a slight increase in enantioselectivity for the transamination (Table S1, entry 17).

Under the optimal conditions, the substrate scope was then investigated for the asymmetric transamination (Table 2). In the presence of 5 mol % of pyridoxamine **11a**, various aliphatic (for **5b–f**), aromatic (for **5a** and **5g–i**), heteroaromatic (for **5j**), and heteroaliphatic (for **5k–o**) α-keto acids were all efficiently transaminated with 2,2-diphenylglycine (**19**) as the sacrificial amine source to give the corresponding chiral α-amino acids in high yields (67–99%) with excellent enantioselectivities (83–94% ee's). When chiral α-keto acids (for **5p–r**) were applied, high diastereoselectivities were observed in the transformation. Various functional groups such as C–C double bond (for **5c**, **5p**, and **5r**), C–C triple bond (**5d**), silyl group (**5l**), Cl (**5m**), Br (**5n**), and acetal (**5o**) were all well tolerated in the transamination due to the highly mild reaction conditions. However, sterically bulky α-keto acids such as 3,3-dimethyl-2-oxobutanoic acid and β,γ-unsaturated α-keto acids such as (*E*)-2-oxo-4-phenylbut-3-enoic acid are both not efficient substrates for the transamination.

In order to understand and identify the pathway of the pyridoxamine-catalyzed transamination, control experiments were carried out (Scheme 3). As expected, the reaction between stoichiometric chiral pyridoxamine (*R*)-**11a** and α-keto acid **1a** in MeOH–H_2O (8:2) did occur smoothly, to give α-amino acid **5a** in 54% yield and 85% ee along with the corresponding pyridoxal which were in situ converted into the internal iminium (*R*)-**12a**. On the other hand, the pyridoxamine (*R*)-**11a** can be regenerated in 94% yield by reaction of (*R*)-**12a** with **19** in MeOH/H_2O (8:2) in 2 h. These results imply a two-half-transamination mechanism for the reaction (Scheme 4). Pyridoxamine **11a** condensates with α-keto acid **1** to form ketimine **21**, which undergoes asymmetric 1,3-proton transfer via a delocalized azaallylanion, followed by hydrolysis of the aldimine **23**, to give amino acid **5** and the corresponding pyridoxal. The pyridoxal is in situ converted into

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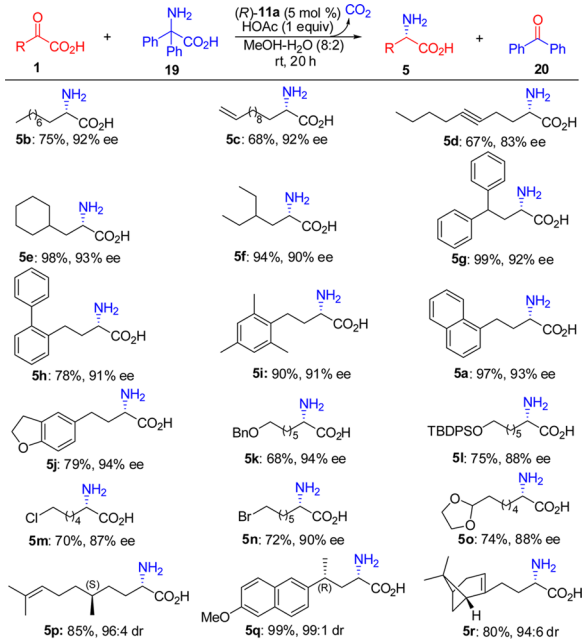
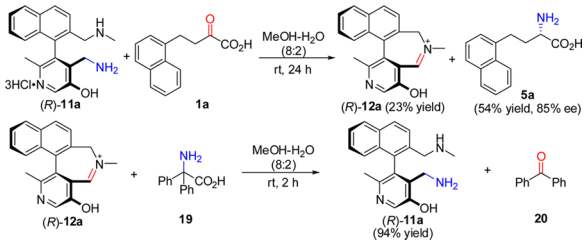
Table 2. Pyridoxamine 11a Catalyzed Asymmetric Transamination of α-Keto Acids[a]



[a]All reactions were carried out with **1** (0.10 mmol), **19** (0.10 mmol), **11a** (0.0050 mmol), and HOAc (0.10 mmol) in MeOH−H_2O (8:2, 0.50 mL) at rt for 20 h unless otherwise stated. For **5m**, **5n**, and **5p**, the reactions were conducted on double scale. The isolated yields were based on **1**. The ee's were determined by chiral HPLC analysis of the methyl ester for **5a** and the *N*-benzoyl methyl esters for **5b**−**o**. The dr values of **5p**−**r** were determined by HPLC analysis of the corresponding *N*-benzoyl methyl esters. The absolute configuration of **5e** was assigned as *S* by comparison of its optical rotation with the reported one (ref 17). The absolute configurations of other amino acids were proposed by analog.

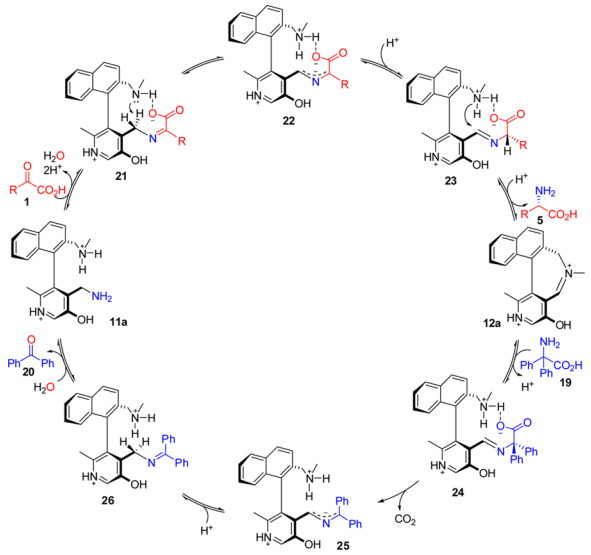
Scheme 3. Stoichiometric Half-Transaminations



the internal iminium **12a** via intramolecular condensation. The iminium **12a** then undergoes a reverse process, i.e. condensation with **19**, decarboxylative transamination,[18] and subsequent hydrolysis to reform pyridoxamine **11a**, completing a catalytic cycle for the transamination.

^{1}H NMR monitoring of the **11a**-catalyzed transamination of α-keto acid **1a** indicated that the protonated pyridoxamine is the resting state of the catalyst for the reaction (see SI). It has been reported that formation of a ketimine from pyridoxamine and α-keto acid is a rapid and reverse process with a low Schiff base formation constant at acidic conditions.[19] Therefore, deprotonation of the imino C−H of ketimine **21** to trigger asymmetric 1,3-proton transfer from ketimine **21** to aldimine **23** should be the rate-limiting step for the transamination.[12,19c,20] This is consistent with the side arm effect that pyridoxamines bearing a basic side arm such as **11a** and **11f** displayed obviously higher activity than

Scheme 4. Proposed Transamination Mechanism



those without a basic side chain such as **11h** (Figure 2). The amine group on the side arm can serve as an intramolecular base to

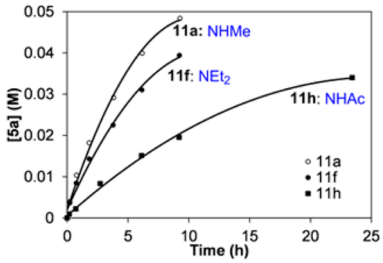


Figure 2. Plots of [**5a**] against reaction time for the transamination. The reactions were carried out with **1a** (0.033 mmol), **19** (0.033 mmol), and pyridoxamine (*R*)-**11a** (0.0066 mmol) in DMSO-d_6/H_2O (8:2) (0.50 mL) in an NMR tube at rt.

deprotonate the imino C−H of **21**, which promotes the 1,3-proton transfer and thus accelerates the transamination. Pyridoxamine **11a** is somewhat more active than **11f**, probably because the secondary amine (NHMe) in **11a** is sterically beneficial to access the imino C−H of **21** for deprotonation as compared to the tertiary amine (NEt_2) in **11f**. The NHMe group in **11a** also likely can promote hydrolysis of Schiff bases such as from aldimine **23** to iminium **12a** and amino acid **5**, as the Lys residue of transaminase behaves in enzymatic transamination.

The enantioselectivity of the transamination is generated during the asymmetric 1,3-proton transfer from ketimine **21** to aldimine **23** (Scheme 4). A possible transition state (**27**) has been tentatively proposed for understanding the origin of the chirality (Figure 3). The carboxylic group of α-keto acid is oriented toward

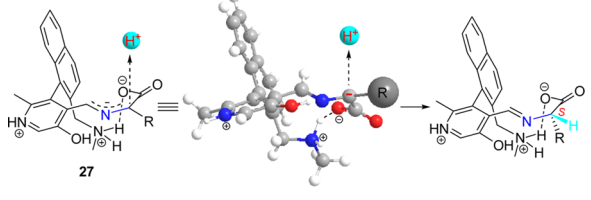


Figure 3. Proposed transition model for 1,3-proton transfer.

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the amine side chain probably due to acid−base and/or hydrogen bonding interactions. Protonation of the azaallylanion occurred at the α-C of the carboxylic group from the up side of the pyridine ring away from the lateral chain to give the α-amino acid with *S* configuration. For pyridoxamines with basic side arms such as **11a**, the acid−base attraction and hydrogen bonding formed between the lateral amine and the carboxylic acid of α-keto acid strengthen the orientation of the α-keto acid in the transition state **27**, thus resulting in high enantioselectivity in the transamination. For pyridoxamine **11h**, the hydrogen bonding between its NHAc chain and the α-keto acid likely accounts for the obviously higher enantioselectivity in the transamination as compared to pyridoxamine **11i** with an NMeAc side chain (Table 1, 65% ee vs 33% ee). The proposed transition model is also supported by transamination of **1a** with stoichiometric (*R*)-**11a** in the CD_3OD−D_2O (see SI). Deuterating the azaallylanion proceeded via a similar way to generate the corresponding α-deuterated amino acid **5a**-*d* with the same configuration (*S*).

In summary, we have developed a class of axially chiral pyridoxamines **11** bearing an amine side arm, which have successfully mimicked multiple parameters of transaminases including transamination activity, chiral environment, and cooperative catalysis of the Lys residue. The pyridoxamines displayed high catalytic activity and excellent enantioselectivity in asymmetric transamination of α-keto acids, to give a variety of optically active α-amino acids in 67−99% yields with 83−94% ee's under very mild conditions. Impressive effects of the side arm on activity and enantioselectivity were observed in the transamination.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b03930.

Crystallographic data (CIF)

Procedures for synthesis of **11** and **13**−**18** and transamination of α-keto acids, characterization data, NMR spectra, and X-ray data of (*R*,*S*)-**17** along with HPLC chromatograms (PDF)

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Notes

The authors declare no competing financial interest.

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