Chemoenzymatic Asymmetric Synthesis of Pyridine-Based α-Fluorinated Secondary Alcohols

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Dedicated to Professor Udo Kragl on the occasion of his 60th birthday.

Fluoro-substituted and heteroaromatic compounds are valuable intermediates for a variety of applications in pharma- and agrochemistry and synthetic chemistry. This study investigates the chemoenzymatic preparation of chiral alcohols bearing a heteroaromatic ring with an increasing degree of fluorination in α-position. Starting from readily available picoline derivatives growing interest for pharmaceutically- and agrochemical relevant compounds.[1] For example, the CHF₂ moiety is a valuable target as it is a bioisostere of hydroxy, thiol and amide groups.[2]

To synthesize such chiral alcohols typically prochiral ketones are converted via an asymmetric reduction reaction to the desired chiral alcohol. Frequently used catalysts for this kind of transformation are homogeneous catalysts such as transition metal complexes.[3] In contrast, biotransformations became a powerful alternative and such versatile biological catalysts frequently outperform classical chemical ketone reduction reactions under specifically mild reaction conditions and avoid the involvement of potentially toxic metals in the synthesis of the desired drug.[4] Aside kinetic resolutions with hydrolases, alcohol dehydrogenases (ADH) or keto reductases (KRED) have been reported, which similarly catalyze highly regioselective the enantioselective reduction reaction and are available with both (R)- and (S)-enantioselectivity.[5] However, within the focus on α-halogenation typically only substrates with classical benzylic derivates were reported,[6,7] whereas the multi-step synthesis of molecules bearing heteroaromatic structural motifs are very rarely investigated.

The aim of this study is to investigate a straight-forward synthetic approach to close this gap towards chiral secondary alcohols bearing a pyridine ring and different grades of α-halogenation in close proximity to the chiral center. This was achieved through a straight-forward two-step chemoenzymatic approach (Table 1). The proposed synthetic route starts from readily available picoline derivatives forming the prochiral α-halogenated ketones 1 forming the prochiral α-halogenated ketones 2. The subsequent enantioselective reduction of the formed carbonyl group to the chiral alcohol 3 was investigated exemplarily with the alcohol dehydrogenase from Lactobacillus kefir.

Introduction

Enantiopure heteroaromatic alcohols are valuable compounds for a series of applications in pharma- and agrochemistry. Especially compounds with interesting structural features, e.g. a chiral secondary alcohol function in combination with a pyridine side chain, are important for many pharmacological relevant compounds[1] and ligands for catalysts of asymmetric synthesis.[2] Major examples include the use as glucocorticoid mimetics for the treatment of allergic, immune or inflammatory disorders, rheumatic diseases or help to overcome organ transplant rejection.[5] Nevertheless, besides their desired anti-inflammatory effects glucocorticoids suffer from harmful side effects like alterations in electrolyte or fluid balance, edema till development of diabetes mellitus or osteoporosis.[4] The research aims to reduce these adverse effects by introducing new mimetics with equal potential concerning the anti-inflammatory effects combined with a reduced rate of adverse effects. A special option is the use of fluorinated structural motifs, as these enable beneficial biological properties with a prochiral α-halogenated acyl moieties were introduced with excellent selectivity and 64–95 % yield. The formed carbonyl group was subsequently reduced to the corresponding alcohols using the alcohol dehydrogenase from Lactobacillus kefir, yielding an enantiomeric excess of 95– > 99% and up to 98% yield.

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Results

Chemical synthesis of heteroaromatic prochiral ketones

In the first step the ortho- or para-positioned methyl group of picoline derivatives were converted to the corresponding ketones by the use of the bases n-butyllithium (nBuLi), lithium diisopropyl amide (LDA) or pyridine to form the exocyclic deprotonated intermediate. The different α-halogenated acyl moieties were introduced through the use of different esters or dimethylacetamide for simple methyl ketones (DMA) (Table 2). The straight-forward one pot synthesis was followed by a workup consisting of an aqueous extraction, while in some examples an additional column chromatography was required.

All ketones could be synthesized in satisfying isolated yields from 64% up to 95%. This shows that the applied procedure can in generally be used to form any grade of α-halogenation in methyl ketones with such a heteroaromatic side chain.

Table 2. Base-induced conversion of picolines 1a–m to the corresponding prochiral ketones.

| 2  | Base | Ester/DMA | Yield [%] | 2  | Base | Ester/DMA | Yield [%] |
|----|------|----------|-----------|----|------|----------|-----------|
| a  | LDA  | DMA      | 65        | h  | Pyridine | TFAA      | 64[a]     |
| b  | LDA  |         | 95        | i  | nBuLi   | CCF<sub>3</sub>OC<sub>2</sub>H<sub>3</sub> | 84        |
| c  | LDA  |         | 84        | j  | nBuLi   | DMA       | 90        |
| d  | LDA  |         | 81        | k  | nBuLi   | F<sub>3</sub>COC<sub>2</sub>H<sub>3</sub> | 90        |
| e  | nBuLi| DMA      | 84        | l  | nBuLi   | DMA       | 82        |
| f  | nBuLi|         | 95        | m  | nBuLi   | F<sub>3</sub>COC<sub>2</sub>H<sub>3</sub> | 91        |
| g  | nBuLi|         | 72        |     |         |           |           |

LDA = lithium diisopropyl amide; nBuLi = n-butyllithium; DMA = dimethylacetamide; TFAA = trifluoroacetic anhydride. [a] Synthesized according to Kawase et al.[12]
During all conversions no issues in terms of regioselectivity or nucleophilic attack of the butylanion or a second lithiated picoline moiety were noticed. In addition, the obtained prochiral ketones can easily be converted to the racemic secondary alcohols rac 3a–3m by an excess of sodium borohydride in methanol. The respective yields are between 65% and 99% after aqueous workup with an optional subsequent column chromatography (see supporting document).

Enzymatic conversion to enantiopure secondary alcohols

The subsequent enzymatic conversion was performed in a monophasic reaction system consisting of phosphate buffer pH 7.0 containing 0.1 mM MgCl₂ and the dissolved substrate. In case the substrate was not soluble in the buffer system a two-phase system of additional 50 % (v/v) MTBE was used as a two-phase system.[19] As a biocatalyst, the ADH from Lactobacillus kefir was chosen, as it exhibits exceptionally high enantioselectivity for classical methyl ketones[14] aside the well-studied ADHs from Lactobacillus brevis and Rhodococcus ruber.[13] The final reaction system, including the substrate coupled cofactor regeneration of NAD⁺ via isopropanol, is shown in Table 3. The reaction system was heated to 30 °C within 15 min and then the reaction time of 48 h was started by an addition of the enzyme. The crude product mixture was purified by aqueous workup followed by column chromatography. The obtained results show that almost half of the investigated substrates were successfully transformed into the respective chiral alcohols.

The simple methyl ketones 3a and 3e were converted to the enantiopure (R)-alcohol with 93 and 36 % yield, respectively. With an increasing degree of fluorination in α-position, from CH₃F to CHF₃, successful conversions were obtained with 36–98 % yield and high enantiomeric excess. It is also visible that 2-picoline derivatives 3a–c allowed higher conversions compared to their 4-substituted analogues 3e–g, which highlights the preference of the applied ADH. The reason for this behavior seems to be based on the chelating effect of the β-enaminketones due to their one electron donor ability. This might also deactivate the enzyme by chelating the central magnesium ion. In addition, a trifluoromethyl group in α-position to the carbonyl group was not accepted for both isomers 3d and 3h. Such a behavior was expected as lower activities were reported for trifluoromethyl acetophenone with the closely related ADH from Lactobacillus brevis.[10] An identical result was found for the even larger difluoromonochloro group (3i), which was not converted by the applied ADH. In contrast, unexpectedly the introduction of further methyl substituents at the heterocycle (3j–m) caused a full loss of enzymatic activity, which was not found in general for substituted acetophenone derivatives with such ADHs. This is probably based on size restrictions within the active site due to the additionally methyl group and the corresponding size increase of the heterocyclic domain. Finally, all obtained chiral alcohols show no decomposition or racemization over time when stored below 0 °C.

Table 3. Enzymatic reduction of the picoline-based ketones to the corresponding chiral alcohols.

| 3  | Yield [%] | e.e.[a] [%] | [α]D[23]° | 3  | Yield [%] | e.e.[a] [%] | [α]D[23]° |
|----|-----------|-------------|-----------|----|-----------|-------------|-----------|
| a  | 93        | > 99(R)     | −15.84(28)| h  | 0         |             |           |
| b  | 98        | n.d.        | +23.69(26)| i  | 0         |             |           |
| c  | 95        | > 99(S)     | +1.04(27) | j  | 0         |             |           |
| d  | 0         |             |           | k  | 0         |             |           |
| e  | 36        | > 99(R)     | −37.73(27)| l  | 0         |             |           |
| f  | 70        | 95(S)       | −17.17(28)| m  | 0         |             |           |
| g  | 60        | n.d.        | +13.21(22)| n  | 0         |             |           |

[a] Absolute configuration in parenthesis based on comparison of GC spectra with the corresponding racemic alcohol. [b] 1.0 M in chloroform, temperature (°C) in parenthesis. [c] 1.0 M in dichloromethane.
synthetic potential and limits of this chemoenzymatic pathway, while substitutions at the heterocycle were not accepted as a substrate by the applied alcohol dehydrogenase. The results expand the product library with valuable heterocyclic alcohols as well as investigating the limits of the α-fluorination in this conversion.

In summary, it was successfully shown that α-halogenation can easily be used to convert even complex heterocyclic substrates with high yield to the corresponding enantiopure compounds.

Experimental Section

Chemicals

All required chemicals were obtained from commercial sources and used as received. Dry solvents were bought from Acros and used as received. The alcohol dehydrogenase from Lactobacillus kefiri (lyophilized lysate) was obtained from Evocatal, Düsseldorf, Germany (now evoxx technologies GmbH).

GC-analysis

The enantiomeric excess of corresponding alcohols was analyzed by comparison of the racemic mixture with the enantioenriched alcohol. For analysis a HP 1100 with Chiralyser, DAD and RI Detector by comparison of the racemic mixture with the enantioenriched

Representative synthesis of α-halogenated chiral alcohols (3)

The enzymatic synthesis of the α-halogenated chiral alcohols was performed in 33 mM phosphate buffer or an aqueous two-phase system (ATPS) consisting of methyl tert-butyl ether (MTBE) and 33 mM phosphate buffer pH 7.0 with 1 mM MgCl₂ at 30°C in a ratio of 1:1. After the addition of the substrate (0.15 M) and the enzyme (78 U, 1.0 mg ml⁻¹), NADP⁺ (0.5 mM) as cofactor and isopropanol (2.25 M) for cofactor regeneration were added. The reaction mixture was heavily stirred over 48 h and monitored by TLC. For the monophosphonic reaction the reaction products were extracted after 48 h into MTBE. The respective organic layer was washed two times with 10 ml of water, dried with Na₂SO₄ and eventually evaporated in vacuo. The crude product was further purified by flash chromatography.

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Conflict of Interest

The authors declare no conflict of interest.

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[1] a) J. Deeter, J. Frazier, G. Staten, M. Staszak, L. Weigel, Tetrahedron Lett. 1990, 31, 7101–7104; b) H. Waldmann, Tetrahedron Lett. 1989, 30, 3057–3058; c) D. G. Wishka, D. R. Graber, E. P. Seest, L. A. Dolak, F. Han, W. Watt, J. Morris, J. Org. Chem. 1998, 63, 7851–7859; d) A. T. Ormos, C. S. of Oliveira, K. T. Andrade, M. G. Capeletto, RSC Adv. 2015, 5, 103563–103565.

[2] a) S. Kawano, M. Horiyawa, Y. Yasohara, J. Hasegawa, Biocis. Biotecnol. Biochem. 2003, 67, 809–814; b) R. Sablon, J. A. Osborn, Tetrahedron Lett. 1996, 37, 4937–4940; c) G.-M. Chen, H. C. Brown, P. V. Ramachandran, J. Org. Chem. 1999, 64, 721–725.

[3] Y. Bekkali, R. Betagéri, T. A. Gilmore, M. G. Cardozo, T. M. Kirani, D. Kuzmich, J. R. Proudfoot, H. Takahashi, D. Thomas, J. Wang et al., Glucoconidic mimetics, methods of making them, pharmaceutical compositions, and uses there of WO 03082280A1.

[4] Glucoconidic (Eds.: N. J. Goulding, R. J. Flower), Birkhäuser, Basel, 2001.

[5] a) Y. Zhou, J. Wang, Z. Gu, S. Wang, W. Zhu, J. L. Aceñ, A. V. Salosoohonok, K. Izawa, H. Liu, Chem. Rev. 2016, 116, 422–518; b) J. Wang, M. Sánchez-Rosello, J. L. Aceñ, C. Del Pozo, A. E. Soroschinsky, S. Fustero, V. A. Salosoohonok, H. Liu, Chem. Rev. 2014, 114, 2432–2506; c) K. Müller, C. Faeh, F. Diederich, Science 2007, 317, 1881–1886; d) F. Giornal, S. Pazenon, L. Rofefeld, N. Lui, J. P. Vorst, F. R. Leroux, J. Fluorine Chem. 2013, 152, 2–11.

[6] a) S. Sadhuhusun, J. Santhi, B. Baire, Chem. Eur. J. 2020, 26, 7145–7155; b) D. M. Carminati, J. Decaes, S. Couve-Bonnier, P. Joubart, R. Fasan, Angew. Chem. Int. Ed. 2021, 60, 7072–7076.

[7] a) J. Tauber, L. A. Schwartz, M. J. Krische, Org. Process Res. Dev. 2019, 23, 730–736; b) C. Batisse, M. F. Céspedes Dávila, M. Castello, A. Messara, B. Vivet, G. Marciniak, A. Panossian, G. Hanquet, F. R. Leroux, Tetrahedron 2019, 75, 3063–3079; c) R. Noyori, T. Okuhama, Angew. Chem. Int. Ed. 2001, 40, 40–73; Angew. Chem. 2001, 113, 40–75; d) C. Li, X. Lu, M. Wang, L. Zhang, J. Jiang, S. Yan, Y. Yang, Y. Zhao, L. Zhang, Tetrahedron 2017, 114, 7715–7730.
Lett. 2020, 61, 152356; e) W.-Y. Shen, Y.-Y. Li, Z.-R. Dong, J.-X. Gao, Synthesis 2009, 2009, 2413–2417; f) Ohkuma, Koizumi, Yoshida, Noyori, Org. Lett. 2000, 2, 1749–1751.

[8] a) Biocatalysis for Green Chemistry and Chemical Process Development (Eds.: J. Tao, R. J. Kazlauskas), Wiley, Hoboken, 2011; b) K. Faber, Biotransformations in Organic Chemistry, Springer, Cham, 2018; c) M. T. Reetz, J. Am. Chem. Soc. 2013, 135, 12480–12496; d) B. M. Nestl, B. A. Nebel, B. Hauer, Curr. Opin. Chem. Biol. 2011, 15, 187–193; e) U. T. Bornscheuer, G. W. Huisman, R. J. Kazlauskas, S. Lutz, J. C. Moore, K. Robins, Nature 2012, 485, 185–194.

[9] a) F. Z. Ibn Majdoub Hassani, S. Amzazi, J. Kreit, I. Lavandera, ChemCatChem 2020, 12, 832–836; b) J. Fan, Y. Peng, W. Xu, A. Wang, J. Xu, H. Yu, X. Lin, Q. Wu, Org. Lett. 2020, 22, 5446–5450; c) D. Alsafadi, S. Alsalman, F. Paradisi, Org. Biomol. Chem. 2017, 15, 9169–9175; d) A. A. Koesoema, D. M. Standley, S. Ohshima, M. Tamura, T. Matsuda, Green Chem. 2011, 13, 2285; f) F. G. Mutti, A. Orthaber, J. H. Schrittwieser, J. G. de Vries, R. Pietschnig, W. Kroutil, Chem. Commun. 2010, 46, 8046–8048; g) S. Kara, D. Spickermann, A. Weckbecker, C. Leggewie, I. W. C. E. Arends, F. Hollmann, ChemCatChem 2014, 6, 973–976.

[10] C. Rodríguez, W. Borzęcka, J. H. Sattler, W. Kroutil, I. Lavandera, V. Gotor, Org. Biomol. Chem. 2014, 12, 673–681.

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