Advanced Synthesis & Catalysis

Accepted Article

Title: Imine Reductase-Catalyzed Enantioselective Reduction of Bulky α, β-Unsaturated Imines en Route to a Pharmaceutically Important Morphinan Skeleton

Authors: Peiyuan Yao, Zefei Xu, Shanshan YU, Qiaqing Wu, and Dunming Zhu

To be cited as: *Adv. Synth. Catal.* 10.1002/adsc.201801326

Link to VoR: http://dx.doi.org/10.1002/adsc.201801326

10.1002/adsc.201801326

FULL PAPER

DOI: 10.1002/adsc.201((will be filled in by the editorial staff))

Imine Reductase-Catalyzed Enantioselective Reduction of Bulky α, β-Unsaturated Imines en Route to a Pharmaceutically Important Morphinan Skeleton

Peiyuan Yao,[a,b,‡] Zefei Xu,[a,b,‡] Shanshan Yu,[b] Qiaqing Wu,[a,b,*] and Dunming Zhu[a,b,*]

[a] University of Chinese Academy of Sciences, 19(A) Yuquan Road, Shijingshan District, Beijing 100049, People's Republic of China

[b] National Engineering Laboratory for Industrial Enzymes, Tianjin Engineering Research Center of Biocatalytic Technology, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, 32 Xi Qi Dao, Tianjin Airport Economic Area, Tianjin 300308, People's Republic of China

[c] Guangdong Provincial Key Laboratory of Fermentation and Enzyme Engineering, South China University of Technology, Guangzhou 510006, People's Republic of China
Fax: (+86) 22-24828703; e-mail: zhu_dm@tib.cas.cn, wu_qq@ tib.cas.cn

‡ These authors contributed equally

Received: ((will be filled in by the editorial staff))

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/adsc.201######.((Please delete if not appropriate))

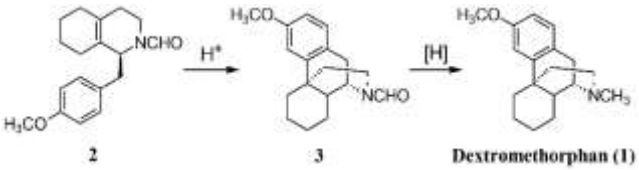
Abstract. The morphinan skeleton is an important sub-structure in many medicines such as dextromethorphan, and can be constructed from 1-benzyl-1,2,3,4,5,6,7,8-octahydroisoquinoline (1-benzyl-OHIQ) derivatives. 1-Benzyl-3,4,5,6,7,8-hexahydroisoquinolines (1-benzyl-HHIQs), the precursors of 1-benzyl-OHIQs, constitute a type of bulky α, β-unsaturated imines. Until now, the application of imine reductases (IREDs) to α, β-unsaturated imines has only rarely been reported. In this study, through evaluation of 48 IREDs, both enantiomers of 1-(4-methoxybenzyl)-1,2,3,4,5,6,7,8-octahydroisoquinoline (1-(4-methoxybenzyl)-OHIQ) were obtained in high yield and excellent optical purity. Among the enzymes, the most steric hindrance-tolerant IRED from *Sandarearacinus amylolyticus* (IR40) was able to convert various phenyl substituted 1-benzyl-HHIQ to the corresponding 1-benzyl-OHIQ derivatives with excellent enantiometric excess. These results provide an effective route to synthesize these important compounds via enantioselective reduction of bulky α, β-unsaturated imine precursors, which can be readily prepared from 2-(1-cyclohexenyl)ethylamine and corresponding aryl acetic acids.

Keywords: Imine reductase; α, β-unsaturated imine; enantioselectivity; chemoselectivity; biocatalysis; octahydroisoquinoline

Introduction

The isoquinoline nucleus is an important molecular scaffold in natural products and pharmacologically active compounds.[1] Within this class of compounds, the 1-benzyl-1,2,3,4,5,6,7,8-octahydroisoquinoline (1-benzyl-OHIQ) derivatives are of particular interest as starting materials in the synthesis of the morphinan skeleton. For example, dextromethorphan (**1**), which is widely used as a non-opioid antitussive for over 50 years and has anticonvulsant and neuroprotective properties,[2] could be prepared from (*S*)-1-(4-methoxybenzyl)-*N*-formyl-OHIQ (**2**) by Grewe cyclization and reduction of the *N*-formyl group (**Scheme 1**).[3] Therefore, the development of efficient methods for the synthesis of optically pure 1-benzyl-OHIQ derivatives is highly desired.

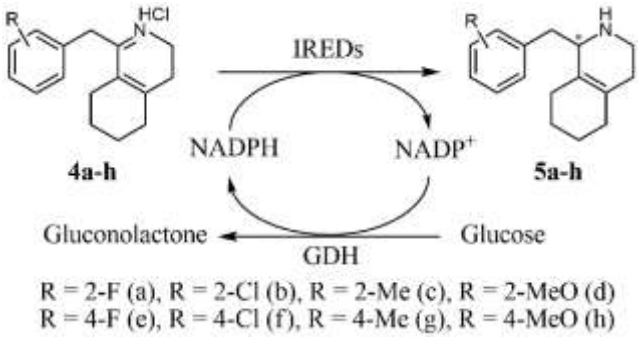
Various methods for the synthesis of these compounds have been previously reported. Kinetic resolution of the racemic amines has been used to access their optically pure forms,[4] but it is economically and ecologically unattractive due to the maximum yield of 50%. Transition-metal complexes such as iridium and ruthenium have been reported to catalyze the asymmetric transfer hydrogenation of the corresponding (*Z*)-enamide or iminium salts in high *ee* values and yields.[5] However, harsh reaction conditions such as high pressure and flammable hydrogen were used. As an alternative to traditional chemical methods, biocatalysis deserves special



Scheme 1. Synthesis of dextromethorphan (**1**).

attention because of its mild reaction conditions, nontoxicity and high stereoselectivity.[6] But biocatalytic methods for the synthesis of 1-benzyl-OHIQ derivatives are limited. Deracemization of 1-(4-methoxybenzyl)-OHIQ (**5h**) to (*S*)-**5h** in 78% isolated yield and 99% *ee* has been achieved in our laboratory by using the mutant Y321I of a cyclohexylamine oxidase (CHAO) from *Brevibacterium oxidans* IH-35A.[7] In this deracemization process, the chemical reducing agent (ammonia borane) was added in 8-fold excess amount. Recently, imine reductases (IREDs) have been shown to catalyze the asymmetric reduction of a wide range of five-, six-, and seven-membered cyclic imines with good conversions and high enantioselectivity.[8] However, the IRED-catalyzed chemo- and enantioselective reduction of α, β-unsaturated imine, especially the bulky 1-benzyl-3,4,5,6,7,8-hexahydroisoquinoline (1-benzyl-HHIQ) has been less developed. Turner *et al.* reported that 2-isopropenyl-3,4,5,6-tetrahydropyridine could be chemoselectively reduced by a IRED from *Streptomyces sp.* GF3587 at the carbon-nitrogen double bond with no detectable reduction of the alkene in 96% *ee*.[8i]

Herein, through systematically exploring the activity of a collection of 48 IREDs, we successfully identified a group of highly hindrance-tolerant enzymes that could efficiently convert a series of phenyl substituted 1-benzyl-HHIQ derivatives into the corresponding 1-benzyl-OHIQs with high enantioselectivity and conversion (**Scheme 2**).



Scheme 2. Asymmetric Reduction of α, β -unsaturated imines catalyzed by IREDs.

Results and Discussion

The protein sequences of the known pteridine reductase from *Leishmania major* (IR1)[9] and reductive aminase from *Aspergillus oryzae* (IR18)[10] were respectively used as a query sequence in Basic Local Alignment Search Tool (BLAST) searches. According to the sequence similarity, 48 IREDs were selected. These included 44 new enzymes and 4 previously disclosed ones (**Table S1** and **Figure S1** in the Supporting Information).[10-11] Genes encoding these IREDs were synthesized based on the codon bias of *E. coli*, cloned into pET28a or pET21a, and overexpressed in *E. coli* BL21 (DE3). The resulting cell-free extracts were used to examine their activity and enantioselectivity towards substrate **4h**, and the results are presented in **Table 1**.

As shown in **Table 1**, eighteen out of the 48 phylogenetically different IREDs were found to catalyze the asymmetric reduction of **4h** to give **5h**.

Table 1. Enantioselectivity of IREDs towards **4h**[a,b]

IRED	*ee*(%)	IRED	*ee*(%)	IRED	*ee*(%)
IR1	ND[c]	IR17	ND[c]	IR33	ND[c]
IR2	ND[c]	IR18	ND[c]	IR34	ND[c]
IR3	ND[c]	IR19	73(S)	IR35	95(R)
IR4	ND[c]	IR20	92(S)	IR36	97(R)
IR5	ND[c]	IR21	ND[c]	IR37	87(R)
IR6	ND[c]	IR22	91(S)	IR38	87(R)
IR7	ND[c]	IR23	90(S)	IR39	76(R)
IR8	ND[c]	IR24	38(S)	IR40	>99(R)
IR9	ND[c]	IR25	ND[c]	IR41	ND[c]
IR10	ND[c]	IR26	60(R)	IR42	ND[c]
IR11	ND[c]	IR27	ND[c]	IR43	ND[c]
IR12	ND[c]	IR28	ND[c]	IR44	>99(R)
IR13	ND[c]	IR29	ND[c]	IR45	84(R)
IR14	ND[c]	IR30	96(S)	IR46	ND[c]
IR15	ND[c]	IR31	35(S)	IR47	ND[c]
IR16	ND[c]	IR32	64(S)	IR48	71(R)

[a] The reaction mixture (1.0 mL) contained 10 μmol of substrate in 20 μL of DMSO, 5 U of GDH, 20 μmol of glucose, 0.5 mg of $NADP^+$, and 980 μL cell-free extracts of IREDs (50 mg wet cells weight) in sodium phosphate buffer (100 mM, pH 7.5), and was shaken at 30ºC, 200 rpm for 18 h. [b] Enantiomeric excess was determined by chiral HPLC analysis. [c] No desired product was detected.

IR19, IR20, IR22-IR24, and IR30-IR32 are *S*-selective, while IR26, IR35-IR40, IR44, IR45, and IR48 are *R*-selective towards **4h**. Among them, IR30 from *Sciscionella marina*, IR40 from *Sandaracinus amylolyticus* and IR44 from *Rhizobium sp.* LCM 4573 displayed the highest enantioselectivity with different stereopreferences. IREDs are sometimes classified into *R*-selective IRED and *S*-selective IRED according to their stereopreference. A conservation analysis of all residues in the proposed substrate binding cleft shows that the amino acid residues at positions 139 and 194 of *S*-selective IREDs are proline and phenylalanine, respectively, while *R*-selective IREDs have a hydrophobic residue (valine, threonine or isoleucine) at position 139 and a methionine or leucine at position 194.[12] The multiple sequence complete alignment of IREDs in this study reveals that the residue at position 139 is a valine for *S*-selective IREDs and a threonine for *R*-selective IREDs, except for the *R*-selective IRED IR26 from *Nocardiopsis chromatogenes* which has a valine at position 139. For the residue at position 194, all the active IREDs towards **4h** have a methionine or leucine (**Figure S2** in the Supporting Information). It is worth noting that the residue at position 225 (W210 for IR18) is conserved and differs in *R*- and *S*-selective IREDs, which has dramatic effects on the

stereoselectivity, as demonstrated in the reductive amination of several ketones.[10] Therefore, although some substrate-binding residues exert significant control on the stereoselectivity of IREDs, the connection of certain residues with a particular enantiopreference, and thus classification of these enzymes as (*R*) or (*S*) are not straightforward.[8a]

After evaluation of the enantioselectivity and relative activity, IR30 and IR40 with different stereopreferences were selected for further study. The enantioselectivity of IR30 towards **4h** (96% *ee* values) was not high enough, so we tried to optimize the reaction condition to improve its enantioselectivity. The effect of reaction pH on the enantioselectivity of **4h** (10 mM) to **5h** by using the cell-free extracts of 5.0 wt% wet cells was investigated at 25ºC (**Figure S3** in the Supporting Information). IR30 displayed activity from pH 5.5 to pH 9.5. As the pH value increased, the enantioselectivity became higher and higher. The optimal pH values was approximately 9.0 using tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) buffer (50 mM), and the *ee* value was 98%. The effect of reaction temperature was also examined. As shown in **Figure S4** in the Supporting Information, higher stereoselectivity was observed at lower reaction temperature. As the temperature increased from 20 to 37ºC, the *ee* values decreased from 98% to 96%, and dramatically dropped to 27% at 45ºC. Therefore, all subsequent biotransformation for IR30 was performed in Tris-HCl buffer (50 mM, pH 9.0) at 25ºC.

The N-terminal His_6-tagged IR30 and IR40 were purified by nickel-affinity chromatography (**Figure S5** in Supporting Information). The kinetic parameters of IR30 and IR40 towards **4h** were obtained by measuring the initial velocities of the enzymatic reaction at varied substrate concentrations and curve-fitting according to the Michaelis–Menten equation. The V_{max} of IR30 and IR40 towards **4h** proved to be 0.035 U/mg and 0.087 U/mg, respectively. The data of the catalytic rate (k_{cat}) and catalytic efficiency (k_{cat}/K_m) are summarized in **Table 2**. IR40 showed a 2.6-fold and 4.5-fold greater k_{cat} and k_{cat}/K_m, respectively, towards **4h** than IR30.

Table 2 Kinetics Parameters of IR30 and IR40 towards **4h**[a)]

IRED	V_{max} [μmol/min/mg]	k_{cat} [s^{-1}]	K_m [mM]	k_{cat}/K_m [s^{-1} mM^{-1}]
IR30	(3.5±0.1) ×10^{-2}	(1.8±0.1) ×10^{-2}	(33.3±2.4) ×10^{-2}	5.4 ×10^{-2}
IR40	(8.7±0.2) ×10^{-2}	(4.7±0.2) ×10^{-2}	(19.5±1.5) ×10^{-2}	24.1 ×10^{-2}

[a)] Kinetic parameters were determined at pH7.5 for IR40 and pH9.0 for IR30 using purified enzymes.

To further simplify the biotransformation process, we constructed two co-expression vectors pRSFduet-GDH-IR30 and pRSFduet-GDH-IR40 under the control of a strong inducible promoter (*T7*/*lac*) and all genes carried the same ribosome binding site (RBS). Then the recombinant plasmids pRSFduet-GDH-IR30 and pRSFduet-GDH-IR40 were transformed into *E. coli* BL21 (DE3) for overexpression. Analysis of the *E. coli* BL21(DE3) cells harboring pRSFduet-GDH-IR30 and pRSFduet-GDH-IR40 by protein gel electrophoresis of cell-free extracts showed that all three enzymes were expressed (**Figure S6** in the Supporting Information). The activities of co-expression strains were then measured. The activities of IR30 and GDH for pRSFduet-GDH-IR30 were 1.2 U/g wet cells and 740 U/g wet cells, while those of pRSFduet-GDH-IR40 were 1.0 U/g wet cells and 860 U/g wet cells, respectively.

Under the optimized conditions, preparative asymmetric reduction of **4h** to (*R*)- or (*S*)-**5h** was carried out by using 50 g/L of wet *E. coli* BL21(DE3) cells harboring pRSFduet-IR30-GDH at 10 mM substrate concentration or pRSFduet-IR40-GDH at 25 mM substrate concentration. **4h** was completely converted within 18h, affording (*S*)-**5h** in 74% yield and 98% *ee*, and (*R*)-**5h** in 80% yield and >99% *ee*, respectively. As such, the stereo-complementary **5h** were prepared in good yields and excellent optical purity by employing the enantio-complementary IREDs.

Table 3. Asymmetric Reduction of 1-benzyl-HHIQs (**4a-h**) catalyzed by IR30- and IR40[a)]

Entry	substrate	IRED	ee [%][b)]	isolated yield [%][c)]
1	**4a**	IR40-GDH	>99 (R)	78
2	**4b**	IR40-GDH	99 (R)	90
3	**4c**	IR40-GDH	98 (R)	73
4	**4d**	IR40-GDH	99 (R)	95
5	**4e**	IR40-GDH	99 (R)	85
6	**4f**	IR40-GDH	99 (R)	82
7	**4g**	IR40-GDH	97 (R)	47
8	**4h**	IR40-GDH	>99 (R)	80
9	**4f**	IR30-GDH	94 (S)	70
10	**4h**	IR30-GDH	98 (S)	74

[a)] The reaction mixture contained 10 mM substrate, 20 mM of glucose, 6 mg of $NADP^+$ and 2% (v/v) DMSO in 100 mL Tris HCl buffer (50 mM, pH 9.0) for IR30-GDH, and 25 mM substrate, 50 mM glucose, 12 mg $NADP^+$ and 2% (v/v) DMSO in 50 mL sodium phosphate buffer (100 mM, pH 7.5) for IR40-GDH. In all cases, 50 mg/mL wet cells was added and the reaction mixtures were protected by nitrogen and shaken at 25ºC, 200 rpm. [b)] Enantiomeric excess was determined by chiral HPLC analysis. The absolute configurations of the products were assigned by comparing the sign of the optical rotation with literature data. [c)] Isolated yield.

To investigate the substrate scope of whole cell biocatalysts carrying pRSFduet-GDH-IR30 or pRSFduet-GDH-IR40, the asymmetric reductions of **4a-4g** were also performed on a preparative scale

under the same conditions (**Table 3**). For pRSFduet-GDH-IR40, 25 mM substrates were totally converted within 18 h, affording the corresponding products in good yields (73-95%) except **4g** (47% isolated yield), and excellent enantioselectivities (97−99% *ee* values) (**Table 3**). These results suggest that IR40 is a steric hindrance-tolerant enzyme accepting a wide range of phenyl-substituted 1-benzyl-HHIQ derivatives as the substrate. However, for the reaction of 1-(*ortho*-substituted-benzyl)-HHIQs (**4a** – **4d**) with pRSFduet-GDH-IR30 as the biocatalyst, no desired product was detected. The enantioselectivity and activity of pRSFduet-GDH-IR30 were also not good towards the 1-(*para*-substituted-benzyl)-HHIQs **4e** and **4g**, and (*S*)-**5f** was isolated from the reaction mixture of **4f** in 70% yield and 94% *ee* (**Table 3**). These results indicate that the substituent on the phenyl ring of 1-benzyl-HHIQ derivatives greatly affect the activity and enantioselectivity of IRED IR30.

Conclusion

In summary, through identification and evaluation of a large collection of IREDs (44 new and 4 known IREDs), two IREDs with high stereospecificity and complementary enantiopreferences towards 1-benzyl-HHIQ derivatives have been identified. In particular, IR40 is able to efficiently convert *para*- and *ortho*-substituted 1-benzyl-HHIQs into the corresponding 1-benzy-OHIQ products with high yields and excellent enantioselectivity. The possible applications of IR30 and IR40 in the synthesis of the dextromethorphan intermediate (*S*)-**5h** and levallorphan intermediate (*R*)-**5h** was demonstrated at preparative-scale. This study provides a novel chemo-enzymatic approach to prepare these important compounds from readily available 2-(1-cyclohexenyl)ethylamine and corresponding aryl acetic acids. Further protein engineering of selected IREDs is underway in our laboratory to reveal the substrate-binding and stereoselective mechanisms, and to obtain highly active and enantioselective mutant IREDs towards these important substrates and other non-cyclic α, β-unsaturated imines.

Experimental Section

Materials

N-(2-cyclohexenylethyl)-2-arylacetamide (**6a-h**) was prepared from 2-(1-cyclohexenyl)ethylamine and corresponding aryl acetic acids by following the similar experimental procedure of literature.[13] 2-(1-cyclohexenyl)ethylamine was purchased from Heowns Biochem LLC. DifcoTM LB Broth, Miller (Luria-Bertani) was purchased from Becton Dickinson and Company. Ampicilin was purchased from Beijing Probe Bioscience Co., Ltd. Isopropyl β-D-1-thiogalactopyranoside (IPTG) was purchased from AMRESCO Inc. Other commercial chemicals were purchased from Aladdin Industrial Corporation, Sinopharm Chemical Reagent Co., Ltd and Shanghai Macklin Biochemical Co., Ltd. The restriction enzymes and other reagents for molecular biology were supplied by Fermentas (Germany) and TaKaRa (Japan). The HPLC analysis was performed on an Agilent 1200 series HPLC system. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III 600 MHz NMR spectrometer.

Synthesis, Cloning, and Expression of IREDs

The amino acid sequence of IR-1, 18, 19 and 35 are the same as the previously identified proteins PTR1,[9] AspRedAm Q240A,[10] 95% identity with IR-09,[11b] IR-01.[11b] Other IREDs were achieved from the National Center for Biotechnology Information Database based on homologous search of IR-1 and IR-18. The genes of these enzymes including 44 novel entities (**Table S1**) and 4 known IREDs were all synthesized by GENEWIZ (Genewiz Biotech Co., Ltd. China) with codons optimized for expression in *E. coli*. Synthetic IRED genes (with CAT and GAATTC in the 5'- and 3'- termini respectively of the deposited sequences) were further cloned into the *Nde*I/*Eco*RI sites of pET28a. and the resulting plasmids pET28a-IR1-48 were transformed into *E. coli* BL21 (DE3) for overexpression of *N*-terminal 6×His-tagged fusion proteins (**Table S1**). IR1 was cloned into the *Nde*I/*Xho*I sites of pET21a with His-6-tag in *N* terminal. The recombinant cells were cultivated in LB medium containing 50 μg/mL kanamycin at 37ºC with shaking at 180 rpm until OD600 nm reached 0.6−0.8, and then the IRED gene was induced by 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at 25ºC for 12 h. The cells were harvested by centrifugation (6000 *g*, 10 min, 4ºC), washed once by sodium phosphate buffer (100 mM, pH 7.5) and stored at -20°C for further use.

Screening of IREDs' Enantioselectivity with Crude Enzyme

20 μL of 1-(4-methoxybenzyl)-3,4,5,6,7,8-hexahydro isoquinoline hydrochloride (**4h)** in DMSO (500 mM, 10 mM of final concentration), 20 mM of glucose, 0.5 mg of $NADP^+$ and 2 mg of GDH (2.5 U/mg) were added to the cell-free extracts of IREDs (50 mg wet weight) in 980 μL of sodium phosphate buffer (100 mM, pH 7.5) in 2 mL Eppendorf tube. The mixture was shaken at 200 rpm and 30°C on an orbital shaker for 18 h. Then the reaction mixture was carefully adjusted to pH 10-11 with 5.0 M NaOH. The aqueous layer was extracted with 1.0 mL of *tert*-butyl methyl ether and the phase separation was facilitated by centrifugation (12000 *g*, 1 min). The organic phase was collected, dried over anhydrous sodium sulphate and analyzed by chiral HPLC.

Determination of Specific activities and Kinetic Constants

The N-terminal His_6-tagged IR30 and IR40 were purified from the clarified lysate by nickel-affinity chromatography and analyzed by SDS-PAGE (**Figure S5**). Specific activities and kinetic parameters for substrates were determined using purified enzyme on PolarStar Omega 96 well plate reader by monitoring the decrease of NADPH at 340 nm (ε= 6220 L $M^{-1}cm^{-1}$) at 28ºC. The reaction volume (200 μL) contained buffer and 150-200 μg pure enzyme, 0.5 mg/mL NADPH, 15%(v/v) dimethylsulfoxide and imine substrate. Tris-HCl buffer (100 mM pH8.5) and sodium phosphate buffer (100 mM, pH 7.5) were used for IR-30 and IR-40, respectively. The reaction was performed by adding the enzyme to the mixture. One unit of imine reductase is defined as the amount of protein that oxidizes 1 μmol NADPH per minute. Kinetic parameters were deduced by non-linear regression analysis based on Michaelis–Menten kinetics using the program Origin 9.0. All activities were measured in triplicates and error bars indicate the standard deviation.

Asymmetric Reduction of 1-benzyl-3,4,5,6,7,8-hexahydroisoquinoline derivatives Using Recombinant Cells of IR30-GDH and IR40-GDH

General procedure for the asymmetric reduction of 1-benzyl-3,4,5,6,7,8-hexahydroisoquinoline derivatives was carried out as follows: the wet cell pellets of IR30-GDH (5.0 g) or IR40-GDH (2.5 g) was resuspended in 100 mL of 2% (v/v) DMSO and Tris HCl buffer (50 mM, pH 9.0) or 50 mL 2% (v/v) DMSO and sodium phosphate buffer (100 mM, pH 7.5). 10 or 25 mM substrate (due to the instability in air, the HCl salts of the substrates were used), 20 mM or 50 mM of glucose, 12 mg or 6 mg of $NADP^+$ were added to the reaction mixture. The mixture was protected by nitrogen and shaken at 200 rpm and 25°C on an orbital shaker for 18 h. Then the reaction mixture was carefully adjusted to pH 10-11 with 5.0 M NaOH. The aqueous layer was extracted three times with 100 mL of ethyl acetate and the phase separation was facilitated by centrifugation (6000 *×g*, 15 min). The combined organic layer was dried over anhydrous sodium sulfate and filtered. Removal of the solvent and purification by column chromatography over silica-gel with appropriate mixture of dichloromethane and methanol gave the product. The absolute configurations for the products were determined by comparison of its optical rotation with the literatures.

Acknowledgements

This work was financially supported by Youth Innovation Promotion Association of the Chinese Academy of Sciences (Grant No. 2016166), and Tianjin Municipal Science and Technology Commission (15PTGCCX00060 and 15PTCYSY00020). We thank Professor Peter C. K. Lau for his constructive suggestions on the manuscript.

References

[1] a) M. Chrzanowska, A. Grajewska, M. D. Rozwadowska, *Chem. Rev.* **2016**, *116*, 12369-12465; b) Z. Qing, P. Yang, Q. Tang, P. Cheng, X. Liu, Y. Zheng, Y. Liu, J. Zeng, *Curr. Org. Chem.* **2017**, *21*, 1920-1934.

[2] a) C. P. Taylor, S. F. Traynelis, J. Siffert, L. E. Pope, R. R. Matsumoto, *Pharmacol. Ther.* **2016**, *164*, 170-182; b) A. Siu, R. Drachtman, *CNS Drug Rev.* **2007**, *13*, 96-106; c) A. A. Weinbroum, V. Rudick, G. Paret, R. Ben-Abraham, *Can. J. Anesth.* **2000**, *47*, 585-596; d) J. L. Bem, R. Peck, *Drug Saf.* **1992**, *7*, 190-199; e) L. Nguyen, K. L. Thomas, B. P. Lucke-Wold, J. Z. Cavendish, M. S. Crowe, R. R. Matsumoto, *Pharmacol. Ther.* **2016**, *159*, 1-22; f) L. P. H. Yang, E. D. Deeks, *Drugs* **2015**, *75*, 83-90; g) K. P. Garnock-Jones, *CNS Drugs* **2011**, *25*, 435-445.

[3] a) T. Kumaraguru, N. W. Fadnavis, *Org. Process Res. Dev.* **2014**, *18*, 174-178; b) A. I. Meyers, T. R. Bailey, *J. Org. Chem.* **1986**, *51*, 872-875.

[4] a) A. Brossi, O. Schnider, *Helv. Chim. Acta* **1956**, *39*, 1376-1386.; b) M. Murakami, S. Kawahara, N. Inukai, N. Nagano, H. Iwamoto, H. Ida, *Chem. Pharm. Bull. (Tokyo)* **1972**, *20*, 1706-1710.

[5] a) E. A. Broger, W. Burkart, M. Hennig, M. Scalone, R. Schmid, *Tetrahedron: Asymmetry* **1998**, *9*, 4043-4054; b) M. Kitamura, Y. Hsiao, R. Noyori, H. Takaya, *Tetrahedron Lett.* **1987**, *28*, 4829-4832; c) B. Heiser, E. A. Broger, Y. Crameri, *Tetrahedron: Asymmetry* **1991**, *2*, 51-62; d) M. Kitamura, Y. Hsiao, M. Ohta, M. Tsukamoto, T. Ohta, H. Takaya, R. Noyori, *J. Org. Chem.* **1994**, *59*, 297-310.

[6] a) G. Torrelo, U. Hanefeld, F. Hollmann, *Catal. Lett.* **2015**, *145*, 309-345; b) U. T. Bornscheuer, G. W. Huisman, R. J. Kazlauskas, S. Lutz, J. C. Moore, K. Robins, *Nature* **2012**, *485*, 185-194; c) M. T. Reetz, *J. Am. Chem. Soc.* **2013**, *135*, 12480-12496; d) M. Honig, P. Sondermann, N. J. Turner, E. M. Carreira, *Angew. Chem. Int. Ed.* **2017**, *56*, 8942-8973; e) R. de Souza, L. S. M. Miranda, U. T. Bornscheuer, *Chem. Eur. J.* **2017**, *23*, 12040-12063; f) A. Wells, H. P. Meyer, *ChemCatChem* **2014**, *6*, 918-920.

[7] G. Li, P. Yao, P. Cong, J. Ren, L. Wang, J. Feng, P. C. K. Lau, Q. Wu, D. Zhu, *Sci. Rep.* **2016**, *6*, 24973.

[8] a) J. Mangas-Sanchez, S. P. France, S. L. Montgomery, G. A. Aleku, H. Man, M. Sharma, J. I. Ramsden, G. Grogan, N. J. Turner, *Curr. Opin. Chem. Biol.* **2017**, *37*, 19-25; b) M. Lenz, N. Borlinghaus, L. Weinmann, B. M. Nestl, *World J. Microbiol. Biotechnol.* **2017**, *33*, 199; c) G. Grogan, N. J. Turner, *Chem. Eur. J.* **2016**, *22*, 1900-1907; d) J. H. Schrittwieser, S. Velikogne, W. Kroutil, *Adv. Synth. Catal.* **2015**, *357*, 1655-1685; e) J. Zhu, H. Tan, L. Yang, Z. Dai, L. Zhu, H. Ma, Z. Deng, Z. Tian, X. Qu, *ACS Catal.* **2017**, *7*, 7003-7007; f) H. Li, P. Tian, J. Xu, G. Zheng, *Org. Lett.* **2017**, *19*, 3151-3154; g) S. P. France, G. A. Aleku, M. Sharma, J. Mangas-Sanchez, R. M. Howard, J. Steflik, R. Kumar, R. W. Adams, I. Slabu, R. Crook, G. Grogan, T. W. Wallace, N. J. Turner, *Angew. Chem. Int. Ed.* **2017**, *56*, 15589-15593; h) H. Li, G. Zhang, L. Li, Y. Ou, M. Wang, C. Li, G. Zheng, J. Xu, *ChemCatChem* **2016**, *8*, 724-727; i) S. Hussain, F. Leipold, H. Man, E. Wells, S. P. France, K. Mulholland, G. Grogan, N. J. Turner, *ChemCatChem* **2015**, *7*, 579-583; j) K. Mitsukura, T. Kuramoto, T. Yoshida, N. Kimoto, H. Yamamoto, T. Nagasawa, *Appl. Microbiol. Biotechnol.* **2013**, *97*, 8079-86; k) F. Leipold, S. Hussain, D. Ghislieri, N. J. Turner, *ChemCatChem* **2013**, *5*, 3505-3508; l) K. Mitsukura, M. Suzuki, S. Shinoda, T. Kuramoto, T. Yoshida, T. Nagasawa, *Biosci., Biotechnol., Biochem.* **2011**, *75*, 1778-1782.

[9] A. W. Schüttelkopf, L. W. Hardy, S. M. Beverley, W. N. Hunter, *J. Mol. Biol.* **2005**, *352*, 105-16.

[10] G. A. Aleku, S. P. France, H. Man, J. Mangas-Sanchez, S. L. Montgomery, M. Sharma, F. Leipold, S. Hussain, G. Grogan, N. J. Turner, *Nat. Chem.* **2017**, *9*, 961-969.

[11] a) D. G. Gourley, A. W. Schuttelkopf, G. A. Leonard, J. Luba, L. W. Hardy, S. M. Beverley, W. N. Hunter, *Nat. Struct. Biol.* **2001**, *8*, 521-525; b) G. D. Roiban, M. Kern, Z. Liu, J. Hyslop, P. L. Tey, M. S. Levine, L. S. Jordan, K. K. Brown, T. Hadi, L. A. F. Ihnken, M. J. B. Brown, *ChemCatChem* **2017**, *9*, 4475-4479.

[12] S. Fademrecht, P. N. Scheller, B. M. Nestl, B. Hauer, J. Pleiss, *Proteins Struct. Funct. Bioinform.* **2016**, *84*, 600-610.

[13] M. Bala, P. K. Verma, N. Kumar, B. Singh, *Synth. Commun.* **2015**, *45*, 847-856.

10.1002/adsc.201801326

FULL PAPER

Imine Reductase-Catalyzed Enantioselective Reduction of Bulky α, β-Unsaturated Imines en Route to a Pharmaceutically Important Morphinan Skeleton

Adv. Synth. Catal. **Year**, *Volume*, Page – Page

Peiyuan Yao,[a,b,‡] Zefei Xu,[a,b,‡] Shanshan Yu,[b] Qiaqing Wu,[a,b,*] and Dunming Zhu[a,b,*]