

University of Groningen

Exploiting Catalytic Promiscuity for Biocatalysis

Miao, Yufeng

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:
2015

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Miao, Y. (2015). *Exploiting Catalytic Promiscuity for Biocatalysis: Carbon-Carbon Bond Formation by a Proline-Based Tautomerase*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen.

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Exploiting Catalytic Promiscuity for Biocatalysis
Carbon-Carbon Bond Formation by a Proline-Based Tautomerase

Yufeng Miao
2015

The research described in this thesis was carried out in the Department of Pharmaceutical Biology (Groningen Research Institute of Pharmacy, University of Groningen, The Netherlands) and was financially supported by the European Research Council under the European Community's Seventh Framework Programme (FP7/2007-2013)/ERC Grant agreement n° 242293.

The research work was carried out according to the requirements of the Graduate School of Science, Faculty of Mathematics and Natural Sciences, University of Groningen, The Netherlands.

Printing of this thesis was financially supported by the University Library and the Graduate School of Science, Faculty of Mathematics and Natural Sciences, University of Groningen, The Netherlands.

ISBN: 978-94-6182-563-6 (printed version)

ISBN: 978-94-6182-566-7 (electronic version)

Layout and Printing: Off Page, www.offpage.nl

Cover design: Yufeng Miao, Off Page

Copyright © 2015 Yufeng Miao. All rights are reserved. No part of this thesis may be reproduced or transmitted in any form or by any means without the prior permission in writing of the author.

Cover pictures: original paintings by Puek Schut

Front cover: '*4-OT in a Flask*'

Back cover: '*Tulip*'

Invitation card: '*4-OT in a Flask*'



university of
 groningen

Exploiting Catalytic Promiscuity for Biocatalysis
Carbon-Carbon Bond Formation by a Proline-Based Tautomerase

PhD thesis

to obtain the degree of PhD at the
University of Groningen
on the authority of the
Rector Magnificus Prof. E. Sterken
and in accordance with
the decision by the College of Deans.

This thesis will be defended in public on

Friday 5 June 2015 at 14.30 hours

by

Yufeng Miao

born on 29 November 1982
in Jiangsu, China

Supervisors

Prof. G.J. Poelarends

Prof. W.J. Quax

Assessment committee

Prof. M.J.E.C. van der Maarel

Prof. F.J. Dekker

Prof. U. Hanefeld

“It is a slightly arresting notion that if you were to pick yourself apart with tweezers, one atom at a time, you would produce a mound of fine atomic dust, none of which had ever been alive but all of which had once been you.”

Bill Bryson

A Short History of Nearly Everything

Paranimfen

Harshwardhan Poddar
Jan-Ytzen van der Meer

Table of Contents

	Aim and outline of this thesis	9
Chapter 1	Recent Developments in Enzyme Promiscuity for Carbon–Carbon Bond–Forming Reactions	13
Chapter 2	Promiscuous Catalysis of the Asymmetric Michael-Type Addition of Linear Aldehydes to β -Nitrostyrene by the Proline-Based Enzyme 4-Oxalocrotonate Tautomerase	27
Chapter 3	Biocatalytic Michael-type Additions of Acetaldehyde to Nitroolefins with the Proline-based Enzyme 4-Oxalocrotonate Tautomerase Yielding Enantioenriched γ -Nitroaldehydes	51
Chapter 4	<i>Practical method:</i> Asymmetric Michael-type Additions of Acetaldehyde to Nitroolefins Catalyzed by 4-Oxalocrotonate Tautomerase (4-OT) Yielding Valuable γ -Nitroaldehydes	85
Chapter 5	Asymmetric Michael-type Addition of Acetaldehyde to <i>meta</i> -, <i>para</i> -, and <i>ortho</i> -Substituted β -Nitrostyrene Derivatives by 4-Oxalocrotonate Tautomerase	97
Chapter 6	Inter- and Intramolecular Aldol Reactions Catalyzed by a Highly Promiscuous Proline-based Tautomerase	167
Chapter 7	Summary and future perspectives	205
	Nederlandse samenvatting	213
	Dankwoord	219



Aim and outline of this thesis

The history of enzymes as catalysts in organic synthesis dates back to 1858, when Louis Pasteur achieved the first kinetic resolution by treating a solution of racemic ammonium tartrate with a mold of *Penicillium glaucum* to enrich (-)-tartaric acid. However, the low accessibilities and relatively poor performances (i.e. narrow substrate scope, low activity and/or stereoselectivity) of enzymes under non-physiological conditions (when compared to other methodologies) limited their catalytic applications in the past. Due to the invention of recombinant DNA technology and the development of directed evolution techniques in the last few decades, nowadays enzymes can be produced in large quantities with tailor-made properties. In addition, the rapidly growing number of publicly available sequences in gene banks and protein databases has significantly expanded the enzyme toolbox for practical applications. These developments make enzymes increasingly appealing catalysts for use in organic synthesis.

In particular, enzymes that can efficiently catalyze important carbon-carbon (C-C) bond-forming reactions, like the Aldol, Michael, Henry, Mannich, Diels-Alder, and Knoevenagel reactions, are of great interest for synthetic applications. The development of enzymatic systems for these types of reactions is highly challenging but also very attractive, because enzymes can generally be used under mild reaction conditions, could provide high selectivity (i.e. chemo-, regio-, and stereoselectivity), and, in some cases, may even catalyze reactions that are otherwise not possible.

Although enzymes are known for their high specificities, many existing enzymes were found to catalyze reactions other than their biologically relevant ones. This remarkable property is defined as catalytic promiscuity. Enzyme promiscuity is thought to play a key role in natural enzyme evolution and has become an important source for new enzymatic activities that can serve as starting points for laboratory evolution of novel biocatalysts. A number of enzymatic systems have been established for synthetically useful C-C bond-forming reactions with satisfactory efficiency and stereoselectivity. Notably, several of these methodologies involve the use of catalytically promiscuous enzymes since enzymes that naturally catalyze these reactions are rare or have not been identified. In **Chapter 1**, we review recent advances in enzyme promiscuity for C-C bond-forming reactions, with a focus on enzymes that exhibit high enantioselectivities.

Recently, we have established a biocatalytic methodology for asymmetric Michael-type addition of acetaldehyde to *trans*-nitrostyrene using the proline-based enzyme 4-oxalocrotonate tautomerase (4-OT). This catalytic promiscuity of 4-OT was discovered via an envisioned catalytic mechanism: the N-terminal proline (Pro-1) of 4-OT forms an enamine intermediate with acetaldehyde, which acts as a nucleophile and adds to the double bond of *trans*-nitrostyrene yielding the corresponding Michael adduct. In **Chapter 2**, we describe the enamine donor scope of the 4-OT catalyzed Michael-type addition reaction. Surprisingly, linear aldehydes ranging from acetaldehyde to octanal are all accepted by 4-OT as enamine donors and added to the electrophile *trans*-nitrostyrene to form the corresponding Michael adducts.

Inspired by the success in extending the enamine donor scope of the 4-OT catalyzed Michael-type addition reaction, we further investigated the electrophile (i.e. Michael acceptor) scope of this synthetically useful reaction. In **Chapter 3**, we report the asymmetric Michael-type addition of acetaldehyde to a series of nitroolefins yielding enantioenriched γ -nitroaldehydes, which are valuable precursors of several marketed γ -aminobutyric acid (GABA)-based pharmaceuticals such as phenibut, baclofen and pregabalin. This novel enzymatic methodology is as efficient as the most potent organocatalytic procedure for this type of reaction and provides a greener alternative for the synthesis of GABA derivatives.

Although the 4-OT methodology for asymmetric Michael-type addition reactions is still far from industrial application, it is a so far unique approach for this type of reaction because the additions are enzyme-catalyzed, proceed in aqueous media, with high stereoselectivity, and include a broad range of substrates. In **Chapter 4**, we describe practical methods for the asymmetric synthesis of two important γ -nitroaldehydes using 4-OT. The protocol includes the expression and purification procedures for 4-OT, procedures for the preparative scale synthesis of the γ -nitroaldehydes using 4-OT, analytical methods for monitoring reaction progress and product characterization, as well as the materials and equipment that are required.

As the 4-OT catalyzed asymmetric Michael-type addition of acetaldehyde to nitroolefins gives convenient access to valuable chiral precursors for GABA derivatives, we are interested in further improving this biocatalytic approach by both protein and substrate engineering. In **Chapter 5**, we describe the 4-OT catalyzed Michael-type addition of acetaldehyde to a number of *ortho*-, *meta*-, and *para*-substituted β -nitrostyrene derivatives. We report that different electron donating (e.g. hydroxyl and methoxyl groups) and electron withdrawing (e.g. chloro and nitro groups) substitutions at the aromatic ring of the nitroolefin substrate can significantly influence the catalytic rates and enantioselectivity of the 4-OT catalyzed Michael-type addition reactions.

In addition to the Michael-type additions, we previously discovered that 4-OT promiscuously catalyzes the aldol condensation of acetaldehyde with benzaldehyde to yield cinnamaldehyde. Mechanism-inspired engineering provided an active site mutant (F50A) of 4-OT with strongly enhanced aldol condensation activity. In **Chapter 6**, we report that 4-OT and the 4-OT F50A mutant accept various carbonyl compounds as substrates for both inter- and intramolecular aldol reactions.

In **Chapter 7**, we summarize the work described in this thesis and present some future perspectives of this study.



