

# Table of contents

List of publications, awards, invention disclosures, conference talks .....	I
Abstract .....	IV
Zusammenfassung .....	VI
List of scheme, figures and tables .....	X
List of abbreviations .....	XIII
<b>1. Introduction .....</b>	<b>1</b>
1.1 The impact of chirality in organic compounds .....	1
1.2 Production of optically pure compounds .....	2
1.3 Potential of industrial biotechnology .....	3
1.3.1 Enzymes in asymmetric synthesis .....	4
1.4 'Green' reductive amination – an "aspirational reaction" .....	5
1.4.1 Chemical synthesis of chiral amines .....	6
1.4.2 Biocatalytic synthesis of chiral amines .....	8
1.4.3 $\omega$ -Transaminases for the asymmetric synthesis of chiral amines .....	9
1.4.3.1 Taxonomy of transaminases .....	10
1.4.3.2 Reaction mechanism and stereoselectivity .....	11
1.4.3.3 Screening for $\omega$ -transaminase activity .....	13
1.4.3.4 History, availability and substrate range of $\omega$ -transaminases .....	15
1.4.3.5 Reaction equilibrium – a challenge for $\omega$ -transaminases reactions .....	18
1.5 Chiral vicinal amino alcohols – valuable products from inexpensive starting materials .....	20
1.5.1 Synthetic strategies for valuable vicinal alcohols .....	20
1.5.2 Synthetic routes towards chiral vicinal amino alcohols - chemical and enzymatic methods .....	21
1.5.3 Nor(pseudo)ephedrine – interesting compounds with challenging synthetic strategies .....	23
1.5.4 Nor(pseudo)ephedrine synthesis – what we can learn from nature .....	26
1.5.5 Availability of enzymes for biocatalytic synthesis of nor(pseudo)ephedrine .....	28
1.6 Enzymatic cascade reactions – advantages and challenges .....	33
1.6.1 Enzyme (tandem) cascades: cascade designs and reaction modes .....	34
1.6.2 Easy and efficient multi-parameter optimisation .....	36

<b>2. Aim of the work</b> .....	38
<b>3. Publications</b> .....	40
3.1 TTC-based screening assay for $\omega$ -transaminases: a rapid method to detect reduction of 2-hydroxy ketones .....	41
3.2 Two steps in one pot: Enzyme cascade for the synthesis of nor(pseudo)ephedrine from inexpensive starting materials .....	48
3.3 Efficient 2-step biocatalytic strategies for the synthesis of all nor(pseudo)ephedrine isomers .....	74
<b>4. Discussion</b> .....	95
Context of publications and invention disclosures .....	95
4.1 A novel screening assay for $\omega$ -transaminases .....	96
4.1.1 Characterisation of the TTC-based screening assay .....	97
4.1.2 Reliability of the TTC-based screening assay .....	99
4.1.3 Results of the TTC-based screening assay for (S)-selective $\omega$ -transaminases .....	100
4.1.4 "Reverse" TTC-based screening assay .....	105
4.1.5 Summary TTC-based screening assay for $\omega$ -transaminases .....	108
4.2 Combining lyases and $\omega$ -transaminases for the synthesis of nor(pseudo)ephedrine in two steps (strategy 1) .....	110
4.2.1 2-Step synthesis of (1R,2S)-norephedrine with $\alpha$ -methylbenzylamine as amine donor .....	111
4.2.2 2-Step synthesis of nor(pseudo)ephedrine with alanine as amine donor .....	114
4.2.3 2-Step synthesis of (1S,2S)-norpseudoephedrine and (1S,2R)-nor-ephedrine .....	125
4.3 Combining $\omega$ -transaminases and alcohol dehydrogenases for the synthesis of nor(pseudo)ephedrine in two steps (strategy 2) .....	127
<b>5. Conclusion and future perspectives</b> .....	132
<b>6. References</b> .....	135
<b>7. Acknowledgements</b> .....	145
<b>8. Appendix</b> .....	147